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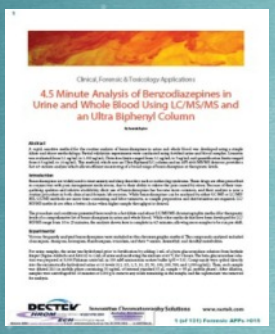
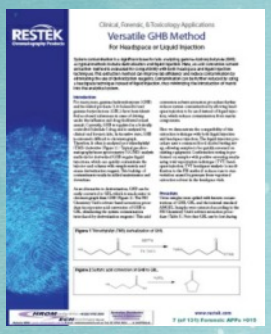
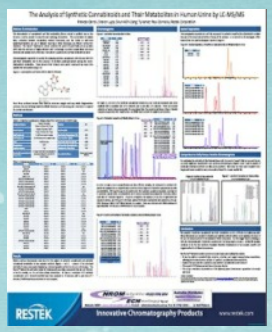
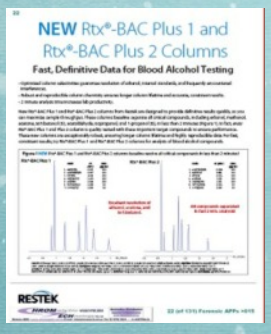


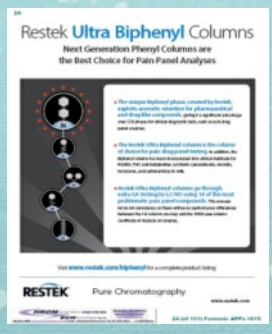





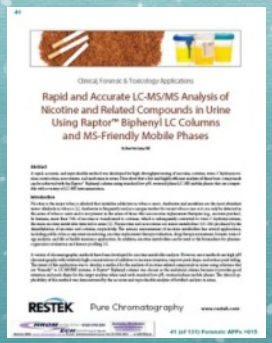




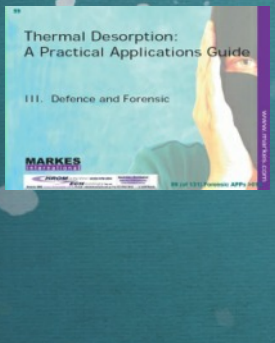



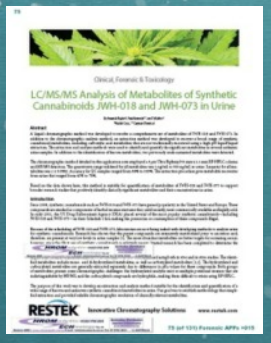






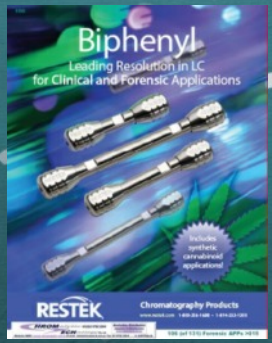



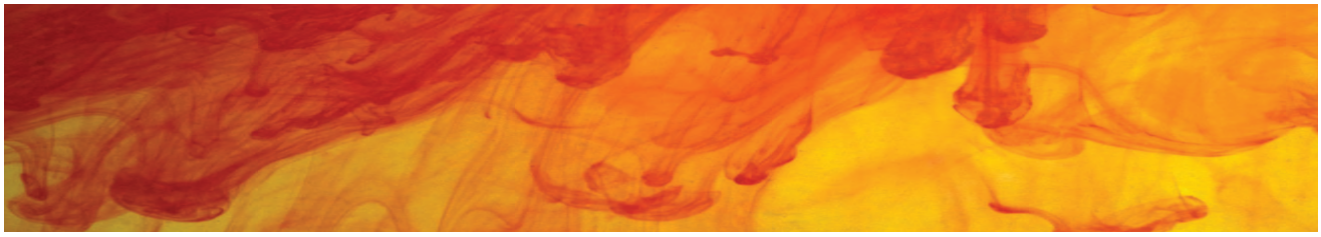












Clinical, Forensic & Toxicology Applications

# Urine and Whole Blood Using LC/MS/MS and an Ultra Biphenyl Column

By Amanda Rigdon


## Abstract

A rapid, sensitive method for the routine analysis of benzodiazepines in urine and whole blood was developed using a simple dilute-and-shoot methodology. Partial validation experiments were conducted using fortified urine and blood samples. Linearity was evaluated from 0.1 ng/mL to 1,000 ng/mL. Detection limits ranged from 0.1 ng/mL to 5 ng/mL and quantification limits ranged from 0.5 ng/mL to 10 ng/mL. This method, which uses an Ultra Biphenyl LC column and an API 4000 MS/MS detector, provides a fast 4.5 minute analysis which allows efficient monitoring of a broad range of benzodiazepines at therapeutic levels.


## Introduction

Benzodiazepines are widely used to treat anxiety and sleep disorders such as restless leg syndrome. These drugs are often prescribed in conjunction with pain management medications, due to their ability to relieve the pain caused by stress. Because of their tranquilizing qualities and relative availability, illicit use of benzodiazepines has become more common, and their analysis is now a routine procedure in both clinical and forensic laboratories. While benzodiazepines can be analyzed by either GC/MS or LC/MS/MS, GC/MS methods are more time-consuming and labor-intensive, as sample preparation and derivatization are required. LC/MS/MS methods are often a better choice when higher sample throughput is desired.

The procedure and conditions presented here result in a fast dilute-and-shoot LC/MS/MS chromatographic method for therapeutic levels of a comprehensive list of benzodiazepines in urine and whole blood. While other methods that have been developed for LC/MS/MS range from 10 to 23 minutes, the analysis shown here is complete in 4.5 minutes, allowing more samples to be run per shift.

## Experimental

Various frequently analyzed benzodiazepines were included in this chromatographic method. The compounds analyzed included clonazepam, diazepam, lorazepam, flunitrazepam, triazolam, and their 7-amino, desmethyl, and desalkyl metabolites.

For urine samples, the urine was hydrolyzed prior to fortification by adding 1 mL of a beta-glucuronidase solution from keyhole limpet (Sigma-Aldrich cat.# G8132) to 1 mL of urine and incubating the mixture at 60 °C for 3 hours. The beta-glucuronidase solution was prepared at 5,000 Fishman units/mL in 100 mM ammonium acetate buffer (pH = 5.0). Compounds were spiked directly into the enzymatically-hydrolyzed urine at 11 levels (0.1, 0.5, 1, 5, 10, 25, 50, 100, 200, 500, and 1,000 ng/mL). Then, each sample was diluted 20:1 in mobile phase containing 30 ng/mL of internal standard (5 µL sample + 95 µL mobile phase). After dilution, samples were centrifuged for 10 minutes at 3,000 g to remove any solids remaining in the sample, and the supernatant was removed for analysis.

For blood samples, whole blood was collected offsite and diluted 1:1 with acetonitrile in order to precipitate proteins. After precipitation, the sample was centrifuged for 10 minutes at 3,000 g. Benzodiazepines were spiked into the precipitated whole blood at the same levels specified for urine. Then the fortified samples were diluted 10:1 in mobile phase containing 30 ng/mL internal standard (100 µL sample + 900 µL mobile phase) to yield an overall 20-fold dilution. Samples were then centrifuged for 10 minutes at 3,000 g, and the supernatant was removed for analysis.

All samples were analyzed in triplicate using a Shimadzu UFLCXR HPLC coupled with an AB SCIEX® API 4000 MS/MS operating in positive electrospray mode. A 50 mm x 2.1 mm ID x 5 µm Ultra Biphenyl column (cat.# 9109552) was chosen for analysis as it provides good chromatographic retention of both polar and nonpolar compounds, including the 7-amino metabolites and more nonpolar compounds such as diazepam. Additionally, the short column length allows for a fast analysis.

Precision and accuracy were assessed by comparing back-calculated benzodiazepine concentrations in the calibration solutions with the prepared concentrations. Back-calculated concentrations were determined using a 1/x weighted quadratic calibration curve for each compound.

## Results and Discussion

The method developed here allows for fast analysis of a comprehensive list of benzodiazepines and their metabolites using a simple dilute-and-shoot methodology. In addition to the fast sample preparation, this gradient chromatographic method is complete in only 4.5 minutes, allowing for retention of the hydrophilic 7-amino metabolites, as well as timely elution of late eluters like diazepam. Although benzodiazepines are commonly analyzed isocratically, the use of a gradient is beneficial for dilute-and-shoot biological samples. The use of a gradient analysis that varies from low to high organic mobile phase composition ensures that salts, proteins, and fats are eluted from the column during each run. This can extend column lifetime and improve method reproducibility.

### *Benzodiazepines in Urine*

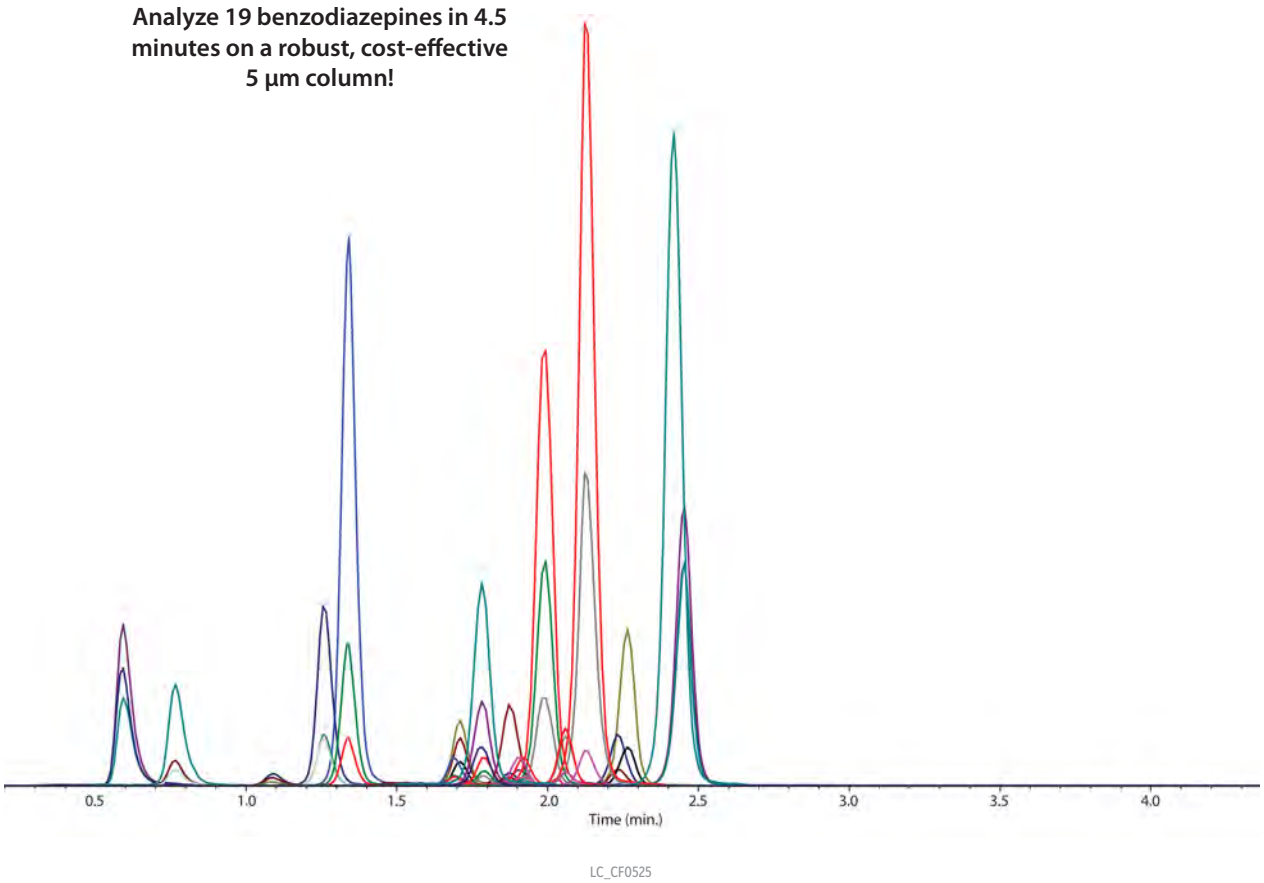
A representative chromatogram of benzodiazepines in dilute-and-shoot urine, along with the chromatographic and MS conditions used for analysis, are shown in Figure 1. Linearity, detection limit, precision, and accuracy data, as well as the expected ion ratios, are presented in Table I.

The limits of detection (LODs) for benzodiazepines in urine ranged from 0.1 ng/mL to 5 ng/mL. The requirement for LOD was a signal-to-noise value greater than 3:1. All limits of quantification (LOQs) were designated as the next calibrator above the LOD, and these levels exhibited a greater than 10:1 signal-to-noise value. R-squared values for all compounds were greater than or equal to 0.9977, demonstrating acceptable linear response across the test range.

Precision was based on relative standard deviations for triplicate injections of each calibrator. The precision range shown in Table I represents the low and high precision values across the calibration range for each analyte. Accuracy was determined using back-calculated values for triplicate injections of the calibrators. The accuracy range shown in Table I represents the low and high accuracy values across the calibration range for each analyte. Ion ratios are average ratios for each MRM over the calibration range.

Note that several compounds (7-aminoclonazepam, clonazepam, alprazolam, diazepam, lorazepam, temazepam, and nordiazepam) were not quantitated in the urine samples. However, data collected during method development and specificity studies indicate that this chromatographic method is suitable for urine analysis with the full list of benzodiazepines included in this application note.

**Figure 1** Benzodiazepines in hydrolyzed urine.




| | Peaks | RT (min.) | MRM1 | MRM2 | MRM3 |
|---|---|---|---|---|---|
| 1. | 7-Aminoclonazepam | 0.59 | 286.0/250.3 | 286.0/222.2 | 286.0/121.0 |
| 2. | 7-Aminoflunit azepam | 0.77 | 284.2/135.2 | 284.2/240.2 | 284.2/226.1 |
| 3. | Bromazepam | 1.10 | 315.9/182.0 | 315.9/209.1 | 315.9/260.9 |
| 4. | N-Desmethylflunit azepam | 1.71 | 300.0/254.1 | 300.0/225.2 | 300.0/198.0 |
| 5. | Nitrazepam | 1.69 | 282.1/236.1 | 282.1/207.2 | 282.1/180.0 |
| 6. | Lorazepam | 1.71 | 321.0/302.9 | 321.0/275.1 | 321.0/229.1 |
| 7. | 2-Hydroxyethylflu azepam | 1.79 | 333.1/246.1 | 333.1/166.0 | 333.1/109.1 |
| 8. | Nordiazepam | 1.78 | 271.0/140.0 | 271.0/208.0 | 271.0/225.9 |
| 9. | Estazolam | 1.88 | 294.9/267.1 | 294.9/241.0 | 294.9/138.0 |
| 10. | Desalkylflu azepam | 1.90 | 289.0/140.0 | 289.0/165.1 | 289.0/226.0 |
| 11. | Clonazepam | 1.92 | 315.9/270.2 | 315.9/214.1 | 315.9/207.1 |
| 12. | Alprazolam | 1.99 | 309.4/281.1 | 309.4/205.0 | 309.4/274.1 |
| 13. | Midazolam | 1.26 | 326.1/291.2 | 326.1/244.2 | 326.1/209.0 |
| 14. | Triazolam | 2.06 | 343.1/315.0 | 343.1/308.2 | 343.1/239.2 |
| 15. | Temazepam | 2.13 | 301.2/283.1 | 301.2/255.1 | 301.2/228.1 |
| 16. | Flurazepam | 1.34 | 388.1/315.1 | 388.1/288.2 | 388.1/317.1 |
| 17. | Flunitrazepam | 2.24 | 314.4/211.0 | 314.4/239.1 | 314.4/268.1 |
| 18. | Clobazam | 2.26 | 301.1/259.1 | 301.1/224.0 | 301.1/181.1 |
| 19. | Diazepam-D5 (IS) | 2.42 | 290.0/154.1 | — | — |
| 20. | Diazepam | 2.45 | 284.8/193.1 | 284.8/222.2 | 284.8/257.1 |

**Column** Ultra Biphenyl (cat.# 9109552)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 5 µm
Pore Size: 100 Å
Temp.: 40 °C
**Sample**
Diluent: Starting mobile phase + 30 ng/mL IS
Conc.: 100 ng/mL sample was diluted 20x prior to injection
Inj. Vol.: 30 µL
**Mobile Phase**

A: Water + 2 mM ammonium formate + 0.2% formic acid
B: Acetonitrile + 10% water + 2 mM ammonium formate + 0.2% formic acid

| Time (min.) | Flow (mL/min.) | %A | %B |
|---|---|---|---|
| 0 | 0.6 | 70 | 30 |
| 3.00 | 0.6 | 40 | 60 |
| 3.1 | 0.6 | 5 | 95 |
| 3.5 | 0.6 | 5 | 95 |
| 3.6 | 0.6 | 70 | 30 |
| 4.5 | stop | | |

**Detector** AB SCIEX API 4000 MS/MS
Model #: API 4000
Ion Source: TurboIonSpray®
Ion Mode: ESI+
Ion Spray Voltage: 2 kV
Curtain Gas: 40 psi (275.8 kPa)
Gas 1: 40 psi (275.8 kPa)
Gas 2: 40 psi (275.8 kPa)
Source Temp.: 600 °C
**Instrument** Applied Biosystems/MDS Sciex LC/MS/MS System
**Notes** MS/MS instrument was operated in scheduled MRM mode.

**Table I** Analytical results for benzodiazepines in hydrolyzed urine.

| Compound | Linearity (R^2) | LOD (ng/mL) | Precision (RSD) | Accuracy (%) | Quant. MRM | Qual. 1 MRM | Ion Ratio 1 (Quant./Qual.) | Qual. 2 MRM | Ion Ratio 2 (Quant./Qual.) |
|---|---|---|---|---|---|---|---|---|---|
| Flurazepam | 0.9991 | 0.1 | 3.0 - 14.6 | 92.3 - 113.0 | 388.1/315.1 | 388.1/288.2 | 12 | 388.1/317.1 | 4 |
| Triazolam | 0.9991 | 0.5 | 1.4 - 15.3 | 90.2 - 128.7 | 343.1/308.2 | 343.1/315.0 | 0.9 | NA* | NA |
| 7-Aminoflunitrazepam | 0.999 | 5 | 2.7 - 7.7 | 91.3 - 117.0 | 284.2/135.2 | 284.2/240.2 | 6.5 | 284.2/226.1 | 4.2 |
| Bromazepam | 0.9982 | 1 | 2.0 - 10.6 | 87.9 - 126.7 | 284.2/135.2 | 284.2/240.2 | 0.3 | 284.2/226.1 | 0.5 |
| N-Desmethylflunitrazepam | 0.9977 | 5 | 3.3 - 27.3 | 87.4 - 117.2 | 300.0/198.0 | 300.0/254.1 | 0.3 | 300.0/225.2 | 1 |
| 2-Hydroxyethylflurazepam | 0.9993 | 1 | 1.4 - 19.9 | 90.7 - 107.9 | 333.1/246.1 | 333.1/166.0 | 1.7 | 333.1/109.1 | 0.5 |
| Desalkylflurazepam | 0.9982 | 5 | 3.5 - 16.5 | 96.5 - 126.0 | 289.0/165.1 | 289.0/140.1 | 0.3 | 289.0/226.0 | 0.5 |
| Midazolam | 0.999 | 0.5 | 1.4 - 22.5 | 89.9 - 126.7 | 326.1/209.0 | 326.1/291.2 | 0.3 | 326.1/244.2 | 0.9 |
| Estazolam | 0.9993 | 1 | 1.5 - 8.8 | 92.3 - 116.3 | 294.9/267.1 | 294.9/241.0 | 6.9 | 294.9/138.0 | 9.6 |
| Clobazam | 0.999 | 1 | 1.6 - 12.9 | 91.2 - 124.0 | 301.1/259.1 | 301.1/224.0 | 3.8 | NA | NA |
| Flunitrazepam | 0.9992 | 1 | 1.1 - 8.7 | 90.5 - 174.0 | 314.4/239.1 | 314.4/211.0 | 3.7 | 314.4/268.1 | 0.3 |
| Nitrazepam | 0.9995 | 5 | 1.2 - 18.1 | 87.8 - 120.8 | 282.1/236.1 | 282.1/207.2 | 3.3 | 282.1/180.0 | 2.8 |

* In cases where ion ratios for an MRM showed unacceptable (>25%) variability, the transition was not used.

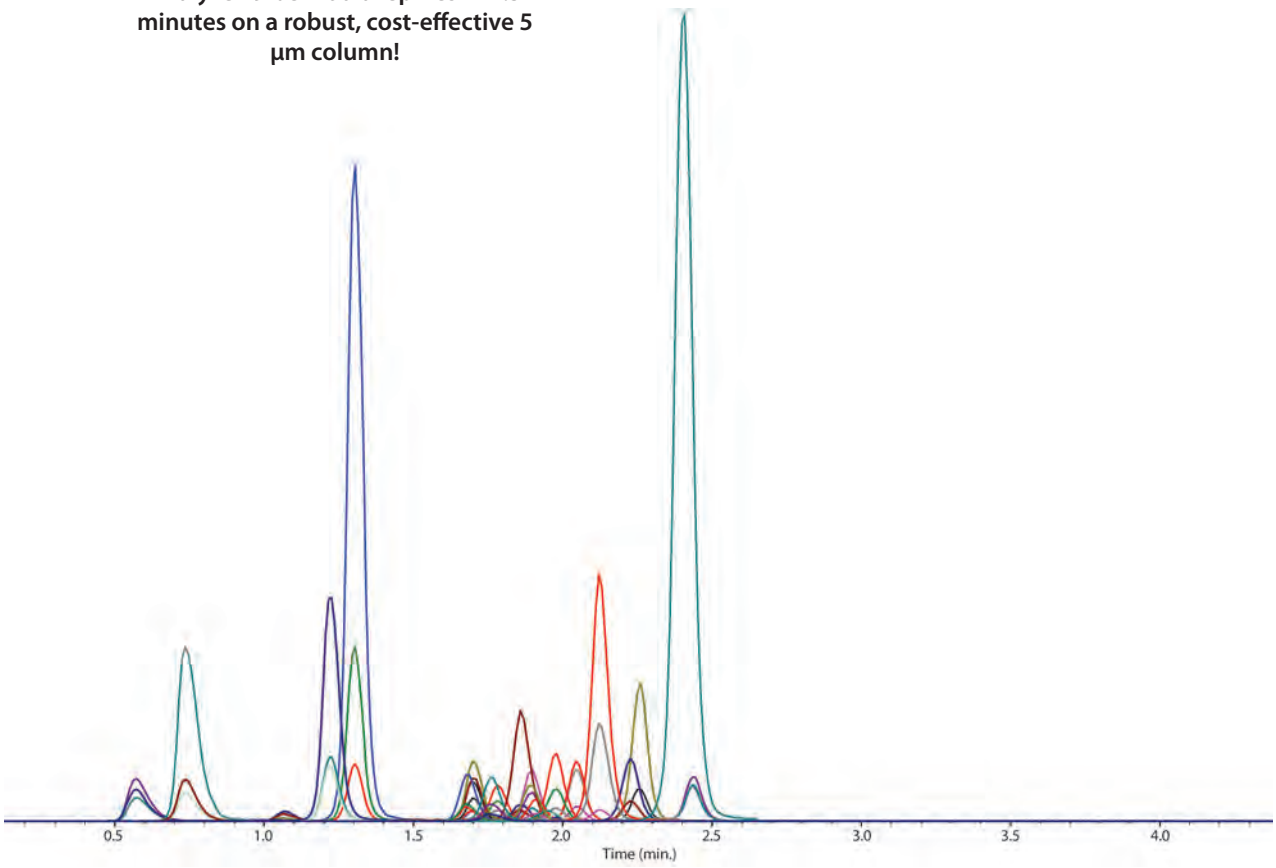
*Benzodiazepines in Whole Blood*

Figure 2 shows representative chromatography for benzodiazepines in dilute-and-shoot whole blood. Linearity, LOD, precision, and accuracy data were assessed using the same criteria and approach that was used for the urine samples. Results, along with the expected ion ratios, are presented in Table II. LODs for the dilute-and-shoot whole blood samples ranged from 0.1 ng/mL to 5 ng/mL. R-squared values of greater than or equal to 0.9992 were obtained for all compounds.

This method allows the determination of benzodiazepines in biological matrices from low levels to above therapeutic levels with a fast analysis time of just 4.5 minutes. The data shown here are for samples with a 20-fold dilution factor, if lower LOQs are desired, additional extraction and/or concentration steps may be employed using the same chromatographic method.

**Figure 2** Benzodiazepines in whole blood.

**Analyze 19 benzodiazepines in 4.5 minutes on a robust, cost-effective 5 µm column!**




| | Peaks | RT (min.) | MRM1 | MRM2 | MRM3 |
|---|---|---|---|---|---|
| 1. | 7-Aminoclonazepam | 0.57 | 286.0/250.3 | 286.0/222.2 | 286.0/121.0 |
| 2. | 7-Aminoflunit azepam | 0.74 | 284.2/135.2 | 284.2/240.2 | 284.2/226.1 |
| 3. | Bromazepam | 1.07 | 315.9/182.0 | 315.9/209.1 | 315.9/260.9 |
| 4. | N-Desmethylflunit azepam | 1.70 | 300.0/254.1 | 300.0/225.2 | 300.0/198.0 |
| 5. | Nitrazepam | 1.68 | 282.1/236.1 | 282.1/207.2 | 282.1/180.0 |
| 6. | Lorazepam | 1.70 | 321.0/302.9 | 321.0/275.1 | 321.0/229.1 |
| 7. | 2-Hydroxyethylflu azepam | 1.78 | 333.1/246.1 | 333.1/166.0 | 333.1/109.1 |
| 8. | Nordiazepam | 1.76 | 271.0/140.0 | 271.0/208.0 | 271.0/225.9 |
| 9. | Estazolam | 1.86 | 294.9/267.1 | 294.9/241.0 | 294.9/138.0 |
| 10. | Desalkylflu azepam | 1.90 | 289.0/140.0 | 289.0/165.1 | 289.0/226.0 |
| 11. | Clonazepam | 1.91 | 315.9/270.2 | 315.9/214.1 | 315.9/207.1 |
| 12. | Alprazolam | 1.98 | 309.4/281.1 | 309.4/205.0 | 309.4/274.1 |
| 13. | Midazolam | 1.22 | 326.1/291.2 | 326.1/244.2 | 326.1/209.0 |
| 14. | Triazolam | 2.05 | 343.1/315.0 | 343.1/308.2 | 343.1/239.2 |
| 15. | Temazepam | 2.12 | 301.2/283.1 | 301.2/255.1 | 301.2/228.1 |
| 16. | Flurazepam | 1.30 | 388.1/315.1 | 388.1/288.2 | 388.1/317.1 |
| 17. | Flunitrazepam | 2.23 | 314.4/211.0 | 314.4/239.1 | 314.4/268.1 |
| 18. | Clobazam | 2.26 | 301.1/259.1 | 301.1/224.0 | 301.1/181.1 |
| 19. | Diazepam-D5 (IS) | 2.40 | 290.0/154.1 | — | — |
| 20. | Diazepam | 2.44 | 284.8/193.1 | 284.8/222.2 | 284.8/257.1 |

**Column** Ultra Biphenyl (cat.# 9109552)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 5 µm
Pore Size: 100 Å
Temp.: 40 °C
**Sample**
Diluent: Starting mobile phase + 30 ng/mL IS
Conc.: 25 ng/mL sample was diluted 20x prior to injecton
Inj. Vol.: 30 µL
**Mobile Phase**

A: Water + 2 mM ammonium formate + 0.2% formic acid
B: Acetonitrile + 10% water + 2 mM ammonium formate + 0.2% formic acid

| Time (min.) | Flow (mL/min.) | %A | %B |
|---|---|---|---|
| 00.6 | 70 | 30 | |
| 3.00 | 0.6 | 40 | 60 |
| 3.1 | 0.6 | 5 | 95 |
| 3.5 | 0.6 | 5 | 95 |
| 3.6 | 0.6 | 70 | 30 |
| 4.5 | stop | | |

**Detector** AB SCIEX API 4000 MS/MS
Model #: API 4000
Ion Source: TurboIonSpray®
Ion Mode: ESI+
Ion Spray Voltage: 2 kV
Curtain Gas: 40 psi (275.8 kPa)
Gas 1: 40 psi (275.8 kPa)
Gas 2: 40 psi (275.8 kPa)
Source Temp.: 600 °C
Mode: MRM
**Instrument** Applied Biosystems/MDS Sciex LC/MS/MS System
**Notes** MS/MS instrument was operated in scheduled MRM mode.

**Table II** Analytical results for benzodiazepines in whole blood.

| Compound | Linearity (R^2) | LOD (ng/mL) | Precision (RSD) | Accuracy (%) | Quant. MRM | Qual. 1 MRM | Ion Ratio 1 (Quant./Qual.) | Qual. 2 MRM | Ion Ratio 2 (Quant./Qual.) |
|---|---|---|---|---|---|---|---|---|---|
| Flurazepam | 0.9994 | 0.1 | 0.8 - 20.1 | 91.5-118.3 | 388.1/315.1 | 388.1/288.2 | 11.2 | 388.1/317.1 | 3.8 |
| Triazolam | 0.9994 | 0.5 | 1.0 - 15.2 | 92.4-109.5 | 343.1/308.2 | 343.1/315.0 | 1.1 | 343.1/239.2 | 4.5 |
| 7-Aminoclonazepam | 0.9996 | 0.5 | 1.0 - 5.1 | 94.0 - 115.3 | 286.0/222.2 | 286.0/250.3 | 1.7 | 286.0/121.0 | 1.3 |
| 7-Aminoflunitrazepam | 0.9996 | 0.5 | 0.8 - 12.2 | 93.4 - 110.0 | 284.2/135.2 | 284.2/240.2 | 6.5 | 284.2/226.1 | 4.5 |
| Bromazepam | 0.9996 | 1 | 0.5 - 29.7 | 80.8 - 107.0 | 284.2/135.2 | 284.2/240.2 | 1.4 | 284.2/226.1 | 3.4 |
| N-Desmethylflunitrazepam | 0.9996 | 1 | 1.4 - 10.2 | 90.4 - 116.0 | 300.0/198.0 | 300.0/254.1 | 0.25 | 300.0/225.2 | 1.05 |
| 2-Hydroxyethylflurazepam | 0.9992 | 1 | 0.7 - 36.7 | 91.9 - 110.7 | 333.1/246.1 | 333.1/166.0 | 1.6 | 333.1/109.1 | 0.5 |
| Desalkylflurazepam | 0.9993 | 5 | 1.6 - 10.7 | 88.5 - 116.3 | 289.0/165.1 | 289.0/140.1 | 0.25 | 289.0/226.0 | 0.5 |
| Midazolam | 0.9993 | 0.5 | 1.2 - 14.6 | 90.4 - 114.4 | 326.1/209.0 | 326.1/291.2 | 0.2 | 326.1/244.2 | 0.8 |
| Estazolam | 0.9992 | 1 | 1.0 - 6.3 | 90.2 - 113.3 | 294.9/267.1 | 294.9/241.0 | 6.9 | 294.9/138.0 | 9.9 |
| Clobazam | 0.9992 | 1 | 0.5 - 3.9 | 92.1 - 115.7 | 301.1/259.1 | 301.1/224.0 | 4 | 301.1/181.1 | 40 |
| Alprazolam | 0.9992 | 1 | 1.0 - 8.2 | 92.0 - 119.7 | 309.4/281.1 | 309.4/205.0 | 2.1 | 309.4/274.1 | 4.6 |
| Clonazepam | 0.9994 | 5 | 0.6 - 13.2 | 93.0 - 107.6 | 315.9/270.2 | 315.9/214.1 | 3.4 | NA* | NA* |
| Diazepam | 0.9993 | 0.5 | 0.2 - 8.6 | 89.9 - 119.3 | 284.8/193.1 | 284.8/222.2 | 1.3 | 284.8/257.1 | 1.3 |
| Flunitrazepam | 0.9994 | 1 | 0.4 - 14.9 | 91.5 - 114.2 | 314.4/239.1 | 314.4/211.0 | 3.6 | 314.4/268.1 | 0.3 |
| Lorazepam | 0.9997 | 1 | 0.3 - 19.7 | 90.9 - 115.0 | 321.0/302.9 | 321.0/275.1 | 0.6 | 321.0/229.1 | 1.9 |
| Nitrazepam | 0.9995 | 1 | 1.0 - 14.6 | 92.8 - 112.1 | 282.1/236.1 | 282.1/207.2 | 3.4 | 282.1/180.0 | 2.7 |
| Temazepam | 0.9994 | 0.5 | 0.9 - 11.0 | 92.2 - 107.6 | 301.2/255.1 | 301.2/283.1 | 2.5 | 301.2/228.1 | 20 |
| Nordiazepam | 0.9993 | 1 | 0.9 - 12.2 | 93.3 - 106.3 | 371.0/140.0 | 271.0/208.0 | 2.5 | 271.0/225.9 | 5.3 |

* In cases where ion ratios for an MRM showed unacceptable (>25%) variability, the transition was not used.

## Conclusions

The chromatographic method presented here allows for fast analysis of a comprehensive list of benzodiazepines at or below therapeutic levels by LC/MS/MS in both urine and whole blood samples. The total run time for this method is only 4.5 minutes using a 5 µm Ultra Biphenyl HPLC column. The fast analysis time and simple dilute-and-shoot approach used here present an opportunity for labs to increase sample throughput by replacing more time-consuming methods.

## Acknowledgements

Restek would like to acknowledge Mike Coyer and Northern Tier Research for their contributions to this study.



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# Versatile GHB Method

## For Headspace or Liquid Injection

System contamination is a significant issue for labs analyzing gamma-hydroxybutyrate (GHB) as typical methods include derivatization and liquid injection. Here, an acid conversion solvent extraction method is evaluated for compatibility with both headspace and liquid injection techniques. This extraction method can improve lab efficiency and reduce contamination by eliminating the use of derivatization reagents. Contamination can be further reduced by using a headspace technique instead of liquid injection, thus minimizing the introduction of matrix into the analytical system.

### Introduction

For many years, gamma-hydroxybutyrate (GHB) and its related products (1,4-butanediol and gamma-butyrolactone [GBL]) have been identified as abused substances in cases of driving under the influence and drug-facilitated sexual assault. Currently, GHB is regulated as a federally controlled Schedule I drug and is analyzed by clinical and forensic labs. In its native state, GHB is extremely difficult to chromatograph. Therefore, it often is analyzed as a trimethylsilyl (TMS) derivative (Figure 1).[1] Typical gas chromatography/mass spectrometry (GC/MS) analysis methods for derivatized GHB require liquid injections, which can quickly contaminate the injector and column with sample matrix and excess derivatization reagent. This buildup of contaminants results in added maintenance and downtime.

As an alternative to derivatization, GHB can be easily converted to GBL, which is much easier to chromatograph than GHB (Figure 2). The FBI Chemistry Unit's solvent-based extraction procedure incorporates acid conversion of GHB to GBL, eliminating the system contamination introduced by derivatization reagents.[2] This acid conversion solvent extraction procedure further reduces system contamination by allowing headspace injection to be used instead of liquid injection, which reduces contamination from matrix components.

Here we demonstrate the compatibility of this extraction technique with both liquid injection and headspace injection. The liquid injection procedure uses a common blood alcohol testing set-up, allowing samples to be quickly screened on existing equipment. Confirmation testing is performed on samples with positive screening results using total vaporization technique (TVT) headspace injection. TVT headspace analysis (a modification to the FBI method) reduces run-to-run variation caused by pressure from vaporized extraction solvent in the headspace vials.

### Procedure

Urine samples were spiked with known concentrations of GHB, GBL, and the internal standard AMGBL. Samples were extracted according to the FBI Chemistry Unit's solvent extraction procedure (Table I). Note that GBL can be lost during

**Figure 1** Trimethylsilyl (TMS) derivatization of GHB.


**Figure 2** Sulfuric acid conversion of GHB to GBL.

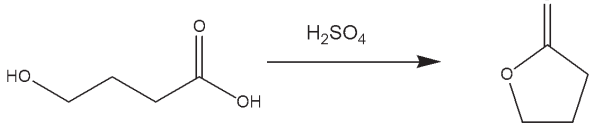
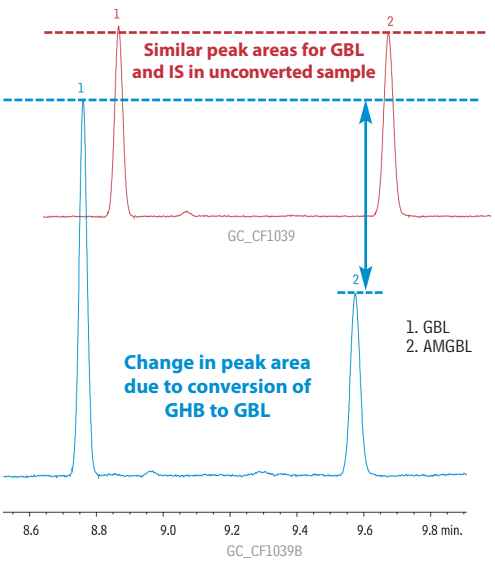


**Table I** Acid conversion solvent extraction procedure from FBI method for GHB analysis.

1. Add 1mL of sample (urine) to each tube.
2. Add 50µL of internal standard (50µg/mL AMGBL in methanol).
3. Add 150µL concentrated sulfuric acid.
4. Vortex and allow tubes to sit 5 minutes. Add 5mL methylene chloride to each tube. Shake by hand or on a mechanical shaker for 10 minutes to extract.
5. Centrifuge samples at 3,000 rpm for 5 minutes.
6. Transfer bottom (methylene chloride) layer to a clean test tube for drying.
7. Concentrate samples to ~100µL at 30°C under nitrogen. Use a gentle gas flow to prevent the loss of GBL.
8. For headspace analysis, inject 15µL of sample into a capped headspace vial.
9. For liquid injection, transfer extract to a limited volume insert.

**Note:** To demonstrate the effectiveness of the acid conversion of GHB to GBL, a second set of tubes was prepared according to Table I, except step 3 (acid conversion) was omitted.

**Figure 3** GBL peak area increases in acid treated samples due to conversion of GHB to GBL.




| | |
|---|---|
| Column: | Rtx®-BAC1, 30m, 0.32mm ID, 1.80µm (cat.# 18003) |
| Sample: | urine spiked with 50µg/mL each GHB, GBL, and AMGBL (IS), extracted according to procedure in Table I, and analyzed using headspace (total vaporization technique) |
| | A: unconverted |
| | B: converted with sulfuric acid |
| Inj.: | 1mL headspace injection, split (10:1), 1mm split inlet liner (cat.# 20972) |
| Inj. temp.: | 200°C |
| Carrier gas: | helium, constant flow |
| Flow rate: | 1.0mL/min. |
| Headspace conditions: | |
| | Equilibration temp.: 100°C |
| | Equilibration time: 10 min. |
| | Injection volume: 1mL |
| Oven temp.: | 50°C (hold 3 min.) to 150°C @ 20°C/min. (hold 7 min.) |
| Det.: | FID @ 240°C |
| Hydrogen: | 40mL/min. |
| Air: | 400mL/min. |
| Makeup: | 40mL/min. |

sample evaporation, so care must be taken when concentrating samples. Extracts were analyzed first by liquid injection/FID as a high-throughput screening method. Then, samples with positive screening results were analyzed by TVT headspace/MS for confirmation.

Equipment used for method evaluation included Shimadzu and Agilent GCs, an Overbrook syringe headspace autosampler, and a Tekmar HT3 headspace loop/trap autosampler. An Rtx®-BAC1 column (30m, 0.32mm, 1.8µm) and an Rtx®-5MS column (30m, 0.25mm, 0.25µm) were used for the GC/FID screening analysis and the GC/MS confirmation analysis, respectively.

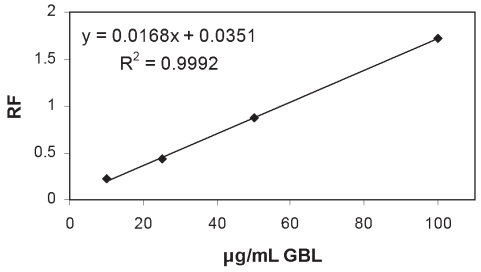
## Results and Discussion

First, conversion of GHB to GBL was verified by comparison of peak heights from converted and unconverted samples (Figure 3). Note, the unconverted sample shows similar levels of GBL and internal standard; whereas, in the converted sample, GBL levels are significantly higher than AMGBL levels. This difference in peak height represents the conversion of GHB to GBL in the acid conversion solvent extraction procedure. Conversion efficiency is maintained across the concentration range shown in Figure 4. Linearity was established for both unconverted (GBL only) and acid converted (GHB + GBL) samples analyzed by headspace/FID. This acid conversion solvent extraction procedure offers an effective alternative for labs currently using a derivatization procedure who wish to reduce system contamination and related maintenance resulting from the use of derivatization reagents.

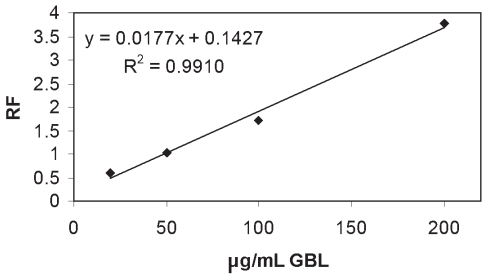
Next, recognizing that many labs currently use liquid injection systems, but are interested in headspace analysis as another means to reduce contamination, we evaluated the compatibility of the acid conversion solvent extraction samples with both liquid and headspace injection techniques. Linearity was established with both injection techniques using FID detection (Figure 5). Chromatographic evaluations were then done using both FID and MS detection to demonstrate versatility. Two system configurations where chosen to illustrate the range of analytical options that are compatible with the FBI acid conversion solvent extraction method: liquid injection/FID and headspace/MS. These analytical options can be used together as an FID screening and MS confirmation procedure, or they may be used independently, based on lab resources and requirements.

**Figure 4** Linear results were obtained for both unconverted and acid converted solvent extracted urine samples (headspace analysis/FID).

A. Unconverted (represents GBL content only)




B. Converted sample (represents GBL and GHB as GBL)



The liquid injection/FID analysis was done on a commonly used blood alcohol testing system using an Rtx®-BAC1 column (Figure 6). This configuration allows labs to use an existing set-up to quickly screen samples. Confirmation analysis can then be performed on positive samples using a headspace/MS system with an Rtx®-5MS column, as shown in Figure 7. The headspace injection was optimized using total vaporization technique (TVT), which eliminates matrix effects by completely vaporizing the sample. TVT headspace is especially useful for the analysis of volatile or difficult matrices, but the sample amount must be kept small (10-15µL) since the entire sample is in the gaseous phase in the headspace vial and higher pressure in the vial from larger sample volumes can cause irreproducible results. Both the liquid injection/FID and TVT headspace/MS systems provided excellent chromatographic results with short analysis times.

## Conclusions

Results demonstrate that the FBI acid conversion solvent extraction method is compatible with both headspace and liquid injection gas chromatographic systems, including existing blood alcohol testing systems. This method is advantageous compared to derivatization procedures, as it eliminates the use of derivatization reagents, which are a common system contaminant for GHB analysis. Contamination can be further reduced by using a TVT headspace procedure for analysis. The acid conversion solvent extraction procedure is a highly versatile method that should be considered by labs interested in reducing contamination resulting from either derivatization or liquid injection.

*References*

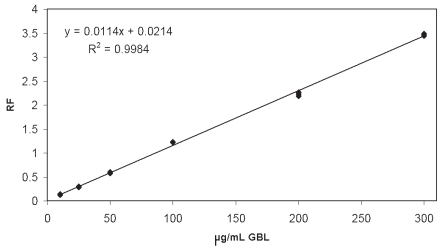
[1] A.A. Elian, Forensic Science International. 109 (2000) 183.

[2] M.A. LeBeau, M.A. Montgomery, M.L Miller, S.G. Burmeister, J. Anal. Toxicol. 24 (2000) 421.



**Figure 5** Linearity was established for both liquid injection and TVT headspace techniques (n=3, FID detection).

A. Liquid injection




B. TVT headspace injection

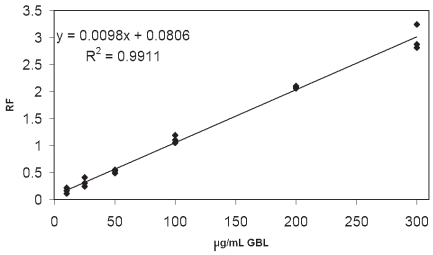
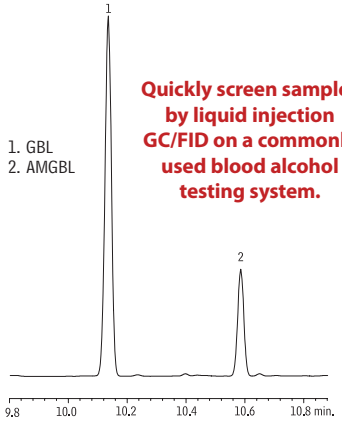


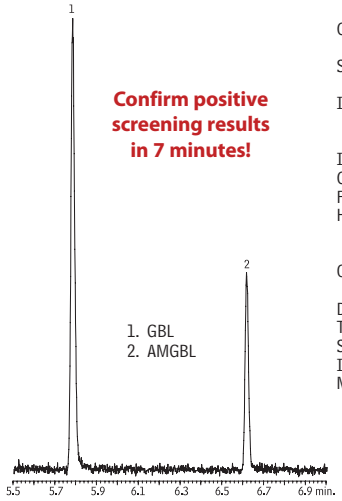

**Figure 6** Liquid injection/FID screening assay of 300µg/mL GHB in urine (acid conversion solvent extraction procedure, analyzed as GBL).




| | |
|---|---|
| Column: | Rtx®-BAC1, 30m, 0.32mm ID, 1.80µm (cat.# 18003) |
| Sample: | 300µg/mL GHB and 50µg/mL AMGBL in urine converted with sulfuric acid |
| Inj.: | 1µL liquid injection, split (10:1), 1mm split inlet liner (cat.# 20972) |
| Inj. temp.: | 200°C |
| Carrier gas: | helium, constant flow |
| Flow rate: | 1.0mL/min. |
| Oven temp.: | 50°C (hold 3 min.) to 150°C @ 20°C/min. (hold 7 min.) |
| Det.: | FID @ 240°C |
| Hydrogen: | 40mL/min. |
| Air: | 400mL/min. |
| Makeup: | 40mL/min. |

GC_CF01040

**Figure 7** TVT headspace/MS confirmation analysis of 300µg/mL GHB in urine (acid conversion solvent extraction procedure, analyzed as GBL).




| | |
|---|---|
| Column: | Rtx®-5MS, 30m, 0.25mm ID, 0.25µm (cat.# 12623) |
| Sample: | 300µg/mL GHB, 50µg/mL AMGBL in urine, converted with sulfuric acid |
| Inj.: | 1mL manual headspace injection, split (10:1), 1mm split inlet liner (cat.# 20972) |
| Inj. temp.: | 200°C |
| Carrier gas: | helium, constant flow |
| Flow rate: | 1.0mL/min. |
| Headspace conditions: | Equilibration temp.: 100°C Equilibration time: 10 min. Injection volume: 1mL |
| Oven temp.: | 50°C (hold 3 min.) to 150°C @ 20°C/min. (hold 7 min.) |
| Det.: | MS |
| Transfer line temp.: | 280°C |
| Scan range: | 20-150m/z |
| Ionization: | EI |
| Mode: | scan |

GC_CF01038

## **Rtx®-BAC1** (proprietary Crossbond® phase)

- Application-specific columns for blood alcohol analysis—achieve baseline resolution in less than 3 minutes. Also excellent for abused inhalant anesthetics, γ-hydroxybutyrate (GHB)/γ-butyrolactone (GBL), glycols, and common industrial solvents.
- Stable to 260°C.

| ID | df (µm) | temp. limits | 30-Meter |
|---|---|---|---|
| 0.32mm | 1.80 | -20 to 240/260°C | 18003 |
| 0.53mm | 3.00 | -20 to 240/260°C | 18001 |

### similar **phases**

DB-ALC1, DB-ALC2

## **Rtx®-5 MS** (low-polarity phase; Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

- General purpose columns for drugs, solvent impurities, pesticides, hydrocarbons, PCB congeners or (e.g.) Aroclor mixes, essential oils, semivolatiles.
- Temperature range: -60°C to 350°C.
- Equivalent to USP G27 and G36 phases.

The 5% diphenyl/95% dimethyl polysiloxane stationary phase is the most popular GC stationary phase and is used in a wide variety of applications. All residual catalysts and low molecular weight fragments are removed from the Rtx®-5 polymer, providing a tight monomodal distribution and extremely low bleed.

| ID | df (µm) | temp. limits | 15-Meter | 30-Meter | 60-Meter |
|---|---|---|---|---|---|
| 0.25mm | 0.10 | -60 to 330/350°C | 12605 | 12608 | 12611 |
| | 0.25 | -60 to 330/350°C | 12620 | 12623 | 12626 |
| | 0.50 | -60 to 330/350°C | 12635 | 12638 | 12641 |
| | 1.00 | -60 to 325/350°C | 12650 | 12653 | |
| 0.32mm | 0.10 | -60 to 330/350°C | 12606 | 12609 | 12612 |
| | 0.25 | -60 to 330/350°C | 12621 | 12624 | 12627 |
| | 0.50 | -60 to 330/350°C | 12636 | 12639 | 12642 |
| | 1.00 | -60 to 325/350°C | 12651 | 12654 | |

### similar **phases**

DB-5, HP-5, HP-5MS, Ultra-2, SPB-5, Equity-5, MDN-5, CP-Sil 8 CB

**Note:** The DB-5MS is a silarylene based polymer equivalent to the Rxi-5Sil MS.

## Exempted Drug of Abuse Reference Materials

Concentration is µg/mL. Volume is 1mL/ampul.

| Compound | Solvent CAS# | Code | Conc. | cat.# (ea.) |
|---|---|---|---|---|
| γ-butyrolactone (GBL) | 96-48-0 | ACN | 1,000 | 34077 |
| α-methylene-γ-butyrolactone (AMGBL) | 547-65-9 | ACN | 1,000 | 34079 |

ACN = acetonitrile

## Silylation Derivatization Reagents

| Compound | CAS# | cat.# |
|---|---|---|
| **BSTFA w/1% TMCS (N,O-bis[trimethylsilyltrifluoroacetamide] w/1% trimethylchlorosilane)** | | |
| 10-pk. (10x1g) | 25561-30-2 | 35606 |
| 25g vial | 25561-30-2 | 35607 |

## 1mm Split Liners for Agilent GCs

| | ID* x OD & Length | qty. | cat.# |
|---|---|---|---|
| 1mm Split | 1.0mm x 6.3mm x 78.5mm | ea. | 20972 |
| Siltek 1mm Split | 1.0mm x 6.3mm x 78.5mm | ea. | 20972-214.1 |

*Nominal ID at syringe needle expulsion point.

# The Analysis of Synthetic Cannabinoids and Their Metabolites in Human Urine by LC-MS/MS

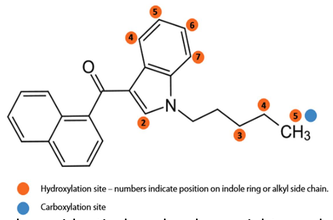
Frances Carroll, Sharon Lupo, Shun-Hsin Liang, Ty Kahler, Paul Connolly; Restek Corporation

## Abstract & Introduction

The determination of cannabinoids and their metabolites, from a natural or synthetic source, has become a routine analysis in many forensic toxicology laboratories. The optimization of analysis time, resolution between metabolites, method robustness, and the ability to add new analytes/metabolites are all of ultimate importance when developing an efficient method for validation. The Raptor™ Biphenyl LC column combines the speed of superficially porous particles (SPP) with the resolution of highly selective USLC® technology to produce simple dilute and shoot methods with analysis times of less than 7 minutes for cannabinoids and their metabolites in urine.

Chromatographic separation is essential for analyzing synthetic cannabinoids JWH-018 and JWH-073 and their metabolites due to the presence of multiple positional isomers among the mono-hydroxylated metabolites. These isomers form because each parent compound has many sites available for hydroxylation (Figure 1).

**Figure 1: Hydroxylation and carboxylation sites for JWH-018**




Since these positional isomers have identical molecular weights and very similar fragmentation patterns, they are indistinguishable by MS/MS detectors and chromatographic resolution is required for positive identification.

## Methods

**Table 1: Analytical Conditions for Waters Xevo® TQ-S with Acquity UPLC® I-Class**

| | | | |
|---|---|---|---|
| **Analytical Column:** | Raptor™ Biphenyl 50 mm x 3.0 mm ID, 2.7 µm | **Polarity:** | ES+ |
| **Guard Column:** | Raptor™ Biphenyl EXP® 5 mm x 3.0 mm ID, 2.7 µm | **Capillary (kV):** | 0.70 |
| **Mobile phase A:** | 0.1 % Formic acid in water | **Desolvation Temperature (°C):** | 500 |
| **Mobile phase B:** | 0.1 % Formic acid in acetonitrile | **Cone Gas Flow (L/Hr):** | 150 |
| **Flow:** | 0.600 mL/min | **Desolvation Gas Flow (L/Hr):** | 1000 |
| **Temperature:** | 30°C | **Collision Gas Flow (mL/min):** | 0.15 |
| **Injection volume:** | 2 µL | **Nebulizer Gas Flow (Bar):** | 7.00 |
| **Standard Concentration & preparation:** | A 5 ng/mL standard was prepared in urine and diluted 3x with 50:50 MeOH:Water | **Cone (V) & Collision:** | Optimized for each analyte |

**Table 2: Analyte transitions**

| Analyte | Precursor Ion | Product Ion Quantifier | Product Ion Qualifier |
|---|---|---|---|
| Pravadoline | 379.29 | 135.04 | 114.16 |
| AM2233 | 459.25 | 112.20 | 98.15 |
| JWH-200-d5 | 390.34 | 155.07 | NA |
| JWH-200 | 385.28 | 155.07 | 114.16 |
| WIN 55, 212 | 427.29 | 155.07 | 127.14 |
| JWH-073 N-butanoic acid | 358.27 | 155.08 | 127.11 |
| JWH-073 4-hydroxybutyl | 344.24 | 155.09 | 127.09 |
| JWH-018 N-pentanoic acid | 372.18 | 155.08 | 127.14 |
| JWH-018 5-hydroxypentyl-d5 | 363.50 | 155.08 | NA |
| JWH-018 5-hydroxypentyl | 358.27 | 155.08 | 127.11 |
| JWH-073 6-hydroxyindole | 344.24 | 155.09 | 127.09 |
| JWH-073 5-hydroxyindole-d7 | 351.21 | 155.07 | NA |
| JWH-073 5-hydroxyindole | 344.24 | 155.09 | 127.09 |
| JWH-073 7-hydroxyindole | 344.24 | 155.09 | 127.09 |
| JWH-018 6-hydroxyindole | 358.27 | 155.08 | 127.11 |
| JWH-018 5-hydroxyindole | 358.27 | 155.08 | 127.11 |
| JWH-018 7-hydroxyindole | 358.27 | 155.08 | 127.11 |
| RCS-4 | 322.27 | 135.12 | 77.09 |
| XLR-11 | 330.25 | 232.17 | 125.10 |
| JWH-015-d7 | 335.28 | 155.07 | NA |
| JWH-250 | 336.28 | 121.12 | 91.07 |
| JWH-015 | 328.26 | 155.07 | 127.13 |
| AM2201 | 360.26 | 155.07 | 127.14 |
| JWH-203 | 340.23 | 188.18 | 125.09 |
| JWH-073 | 328.26 | 155.07 | 127.13 |
| UR-144 | 312.32 | 214.17 | 125.1 |
| JWH-073 4-hydroxyindole | 344.24 | 155.09 | 127.09 |
| JWH-018-d9 | 351.34 | 155.07 | NA |
| JWH-018 | 342.27 | 155.08 | 127.11 |
| JWH-081 | 372.28 | 185.12 | 157.09 |
| JWH-018 4-hydroxyindole | 358.27 | 155.08 | 127.11 |
| JWH-122 | 356.29 | 169.12 | 141.11 |
| JWH-019 | 356.29 | 155.07 | 127.10 |
| JWH-210 | 370.31 | 183.12 | 153.26 |

## Results

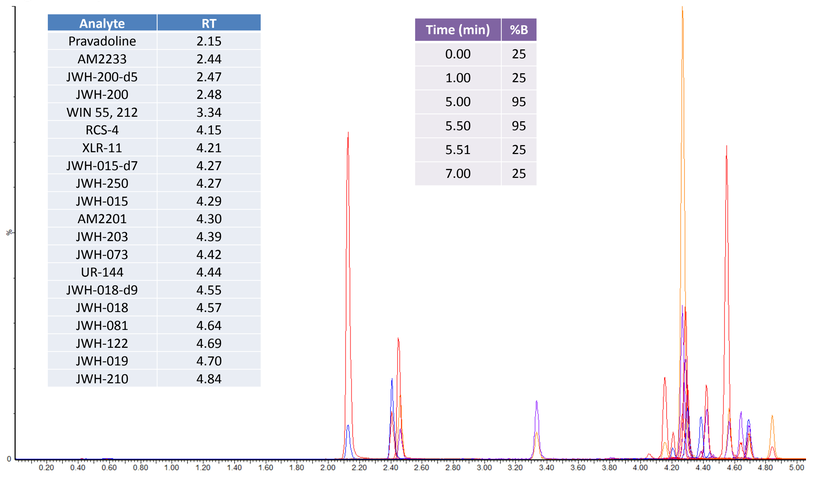
Initially, method development was done for the analysis of synthetic cannabinoids and synthetic cannabinoid metabolites in two separate methods (Figures 2 and 3). Because of the structural similarity of many compounds, developing a chromatographic method can be very challenging. The Raptor™ Biphenyl is particularly adept at retaining and separating compounds that are not retained, or hard to resolve, on C18 and other phenyl chemistries. In Figure 2, a mixture of 17 synthetic cannabinoids and 3 internal standards have been analyzed in 5.5 minutes, with a cycle time of 7 minutes (2 MRM transitions are shown for each analyte).

## Chromatograms

**Figure 2: Synthetic Cannabinoids in Urine**

| Analyte | RT |
|---|---|
| Pravadoline | 2.15 |
| AM2233 | 2.44 |
| JWH-200-d5 | 2.47 |
| JWH-200 | 2.48 |
| WIN 55, 212 | 3.34 |
| RCS-4 | 4.15 |
| XLR-11 | 4.21 |
| JWH-015-d7 | 4.27 |
| JWH-250 | 4.27 |
| JWH-015 | 4.29 |
| AM2201 | 4.30 |
| JWH-203 | 4.39 |
| JWH-073 | 4.42 |
| UR-144 | 4.44 |
| JWH-018-d9 | 4.55 |
| JWH-018 | 4.57 |
| JWH-081 | 4.64 |
| JWH-122 | 4.69 |
| JWH-019 | 4.70 |
| JWH-210 | 4.84 |

| Time (min) | %B |
|---|---|
| 0.00 | 25 |
| 1.00 | 25 |
| 5.00 | 95 |
| 5.50 | 95 |
| 5.51 | 25 |
| 7.00 | 25 |

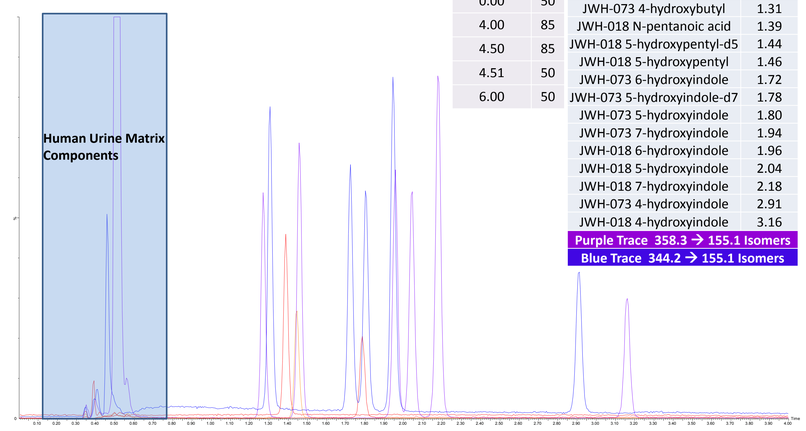


In Figure 3, a mixture of 12 synthetic cannabinoid metabolites and 2 internal standards have been analyzed with an analysis time of 4.5 minutes, and a cycle time of 6 minutes. There is increased retention of early-eluting compounds. By separating them from matrix interference there is limited ionization suppression (for simplification, one MRM trace is shown for analytes and isomers).

**Figure 3: Synthetic Cannabinoid Metabolites in Urine**

| Time (min) | %B |
|---|---|
| 0.00 | 50 |
| 4.00 | 85 |
| 4.50 | 85 |
| 4.51 | 50 |
| 6.00 | 50 |

| Analyte | RT |
|---|---|
| JWH-073 N-butanoic acid | 1.27 |
| JWH-073 4-hydroxybutyl | 1.31 |
| JWH-018 N-pentanoic acid | 1.39 |
| JWH-018 5-hydroxypentyl-d5 | 1.44 |
| JWH-018 5-hydroxypentyl | 1.46 |
| JWH-073 6-hydroxyindole | 1.72 |
| JWH-073 5-hydroxyindole-d7 | 1.78 |
| JWH-073 5-hydroxyindole | 1.80 |
| JWH-073 7-hydroxyindole | 1.94 |
| JWH-018 6-hydroxyindole | 1.96 |
| JWH-018 5-hydroxyindole | 2.04 |
| JWH-018 7-hydroxyindole | 2.18 |
| JWH-073 4-hydroxyindole | 2.91 |
| JWH-018 4-hydroxyindole | 3.16 |
| Purple Trace 358.3 → 155.1 Isomers | |
| Blue Trace 344.2 → 155.1 Isomers | |

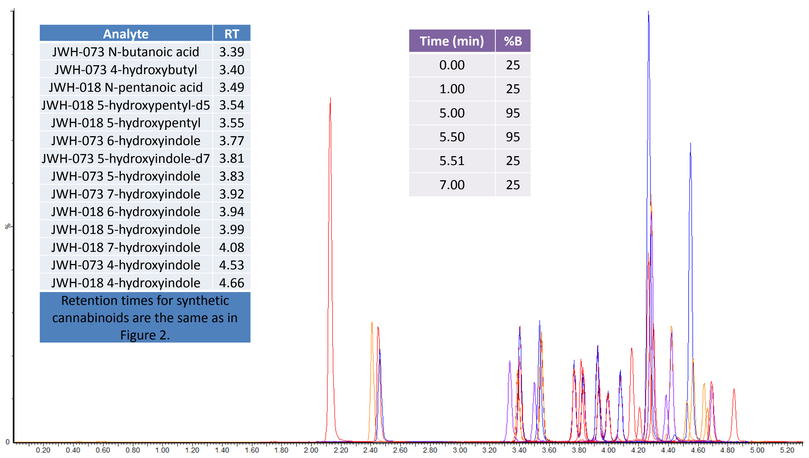



In order to make a more comprehensive and time efficient analysis, we attempted to combine the above two analyses into a comprehensive screen for both a large set of synthetic cannabinoids as well as metabolites. Utilizing the analytical conditions for the synthetic cannabinoid analysis, a mixture of 17 synthetic cannabinoids, 12 metabolites and 5 internal standards was analyzed in 5 minutes, with a cycle time of 7 minutes (Figure 4). 25 µL of a 10 ng/mL urine standard, 25 µL of a 25 ng/mL internal standard solution, and 350 µL of 0.1% formic acid in 50:50 water:acetonitrile were added to a 0.2 µm PVDF Thomson SINGLE StEP® Filter Vial prior to analysis. Data was collected with MRM windows of approximately ± 30 seconds (1 MRM shown for each analyte).

**Figure 4: Combined Analysis of Synthetic Cannabinoids and Metabolites in Urine**

| Analyte | RT |
|---|---|
| JWH-073 N-butanoic acid | 3.39 |
| JWH-073 4-hydroxybutyl | 3.40 |
| JWH-018 N-pentanoic acid | 3.49 |
| JWH-018 5-hydroxypentyl-d5 | 3.54 |
| JWH-018 5-hydroxypentyl | 3.55 |
| JWH-073 6-hydroxyindole | 3.77 |
| JWH-073 5-hydroxyindole-d7 | 3.81 |
| JWH-073 5-hydroxyindole | 3.83 |
| JWH-073 7-hydroxyindole | 3.92 |
| JWH-018 6-hydroxyindole | 3.94 |
| JWH-018 5-hydroxyindole | 3.99 |
| JWH-018 7-hydroxyindole | 4.08 |
| JWH-073 4-hydroxyindole | 4.53 |
| JWH-018 4-hydroxyindole | 4.66 |
| Retention times for synthetic cannabinoids are the same as in Figure 2. | |

| Time (min) | %B |
|---|---|
| 0.00 | 25 |
| 1.00 | 25 |
| 5.00 | 95 |
| 5.50 | 95 |
| 5.51 | 25 |
| 7.00 | 25 |



## Isomer Separation

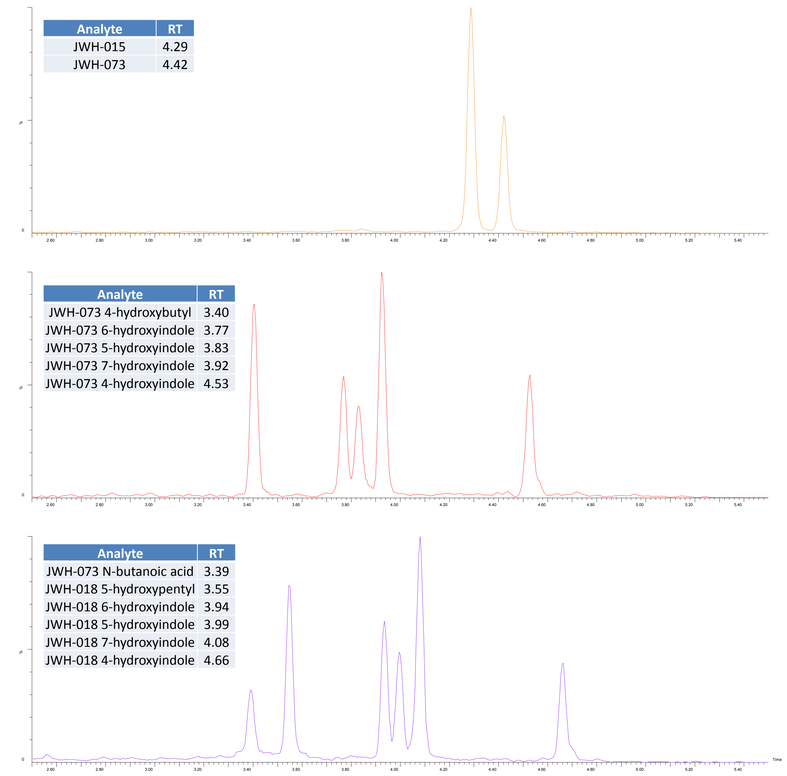
Chromatographic separation is a critical component to synthetic cannabinoid and metabolite analysis because of the structural similarities of many of the analytes. An extracted ion chromatogram of the isomers from the combined analysis is shown in Figure 5.

**Figure 5: Isomer Separation of Synthetic Cannabinoids and Metabolites in Urine**

| Analyte | RT |
|---|---|
| JWH-015 | 4.29 |
| JWH-073 | 4.42 |

| Analyte | RT |
|---|---|
| JWH-073 4-hydroxybutyl | 3.40 |
| JWH-073 6-hydroxyindole | 3.77 |
| JWH-073 5-hydroxyindole | 3.83 |
| JWH-073 7-hydroxyindole | 3.92 |
| JWH-073 4-hydroxyindole | 4.53 |

| Analyte | RT |
|---|---|
| JWH-073 N-butanoic acid | 3.39 |
| JWH-018 5-hydroxypentyl | 3.55 |
| JWH-018 6-hydroxyindole | 3.94 |
| JWH-018 5-hydroxyindole | 3.99 |
| JWH-018 7-hydroxyindole | 4.08 |
| JWH-018 4-hydroxyindole | 4.66 |



## Comparison to Fully Porous Particle Chromatograms

By combining the selectivity of the Biphenyl phase with the speed of superficially porous particles we can show significant improvement over previous methodologies. Figures 6 and 7 give examples of previously developed methods on fully porous particles. Note how run times were required to be longer and isomer resolution was not as easily achieved for an even smaller list.

**Figure 6: Synthetic Cannabinoids on Ultra Biphenyl 5 µm**

| Peaks | RT (min) | MRM1 | MRM2 | MRM3 |
|---|---|---|---|---|
| 1. WIN 48098 | 3.85 | 379.2/135.2 | 379.2/134.3 | 379.2/107.3 |
| 2. JWH-200 | 4.34 | 385.3/134.0 | 385.3/127.0 | 385.3/155.0 |
| 3. WIN 55212-2 | 5.81 | 427.2/155.1 | 427.2/127.1 | 427.2/100.1 |
| 4. AM-694 | 7.01 | 436.1/309.1 | 436.1/231.2 | -- |
| 5. JWH-015 | 7.23 | 328.3/155.1 | 328.3/200.1 | 328.3/200.1 |
| 6. JWH-250 | 7.25 | 336.3/121.1 | 336.3/91.0 | 336.3/144.0 |
| 7. JWH-073 | 7.48 | 328.2/127.1 | 328.2/155.2 | 328.2/199.9 |
| 8. JWH-018 | 7.75 | 342.3/127.1 | 342.2/155.1 | 342.3/144.9 |
| 9. JWH-081 | 7.88 | 372.2/185.2 | 372.2/157.1 | 372.2/144.1 |
| 10. JWH-019 | 8.01 | 356.3/127.2 | 356.3/155.0 | 356.3/144.2 |

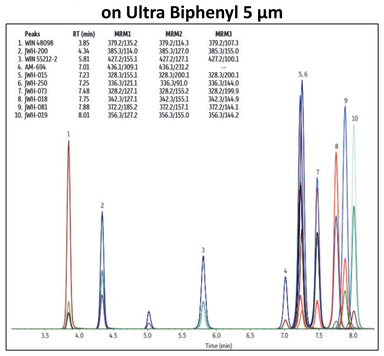
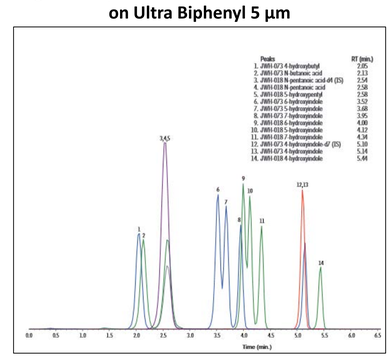


**Figure 7: Synthetic Cannabinoid Metabolites on Ultra Biphenyl 5 µm**

| Peaks | RT (min.) |
|---|---|
| 1. JWH-073 4-hydroxybutyl | 2.05 |
| 2. JWH-073 N-butanoic acid | 2.13 |
| 3. JWH-018 N-pentanoic acid-d9 (IS) | 2.54 |
| 4. JWH-018 N-pentanoic acid | 2.58 |
| 5. JWH-018 5-hydroxypentyl | 2.58 |
| 6. JWH-073 6-hydroxyindole | 3.52 |
| 7. JWH-073 5-hydroxyindole | 3.68 |
| 8. JWH-073 7-hydroxyindole | 3.95 |
| 9. JWH-018 6-hydroxyindole | 4.00 |
| 10. JWH-018 5-hydroxyindole | 4.12 |
| 11. JWH-018 7-hydroxyindole | 4.34 |
| 12. JWH-073 4-hydroxyindole-d7 (IS) | 5.38 |
| 13. JWH-073 4-hydroxyindole | 5.54 |
| 14. JWH-018 4-hydroxyindole | 5.94 |



## Conclusions

The analysis of synthetic cannabinoids and their metabolites can be a difficult and challenging task. Many laboratories are required to develop and validate methods without much guidance because of the lack of standardization for the analysis of these compounds. An even bigger issue is keeping up with the ever-growing list of synthetic cannabinoids illicit drug makers produce. LC-MS/MS analysis continues to be the best method of analysis, therefore development of an accurate, specific and rugged method is of ultimate importance.

The Raptor™ Biphenyl provides solutions to many issues surrounding this analysis:

- It has the ability to provide highly retentive, selective, and rugged reversed-phase separations, allowing for the simultaneous analysis of synthetic cannabinoids and metabolites.
- Analyte lists can easily be expanded as new synthetic cannabinoids are introduced.
- The speed of SPP allows analysis times to become shorter.
- The unique selectivity characteristics of the Biphenyl phase allows isomer separation to be easily achieved.

# Sensitive GC/MS Analysis for Drugs of Abuse

## 1ng Limit of Detection for Acidic/Neutral or Basic Drugs on New Rxi®-5ms Column

By Kristi Sellers, Clinical/Forensic Innovations Chemist

- New stationary phase, inert to acidic or basic drugs.
- Unique deactivation allows 1ng LOD.
- Column technology specially developed for GC/MS.

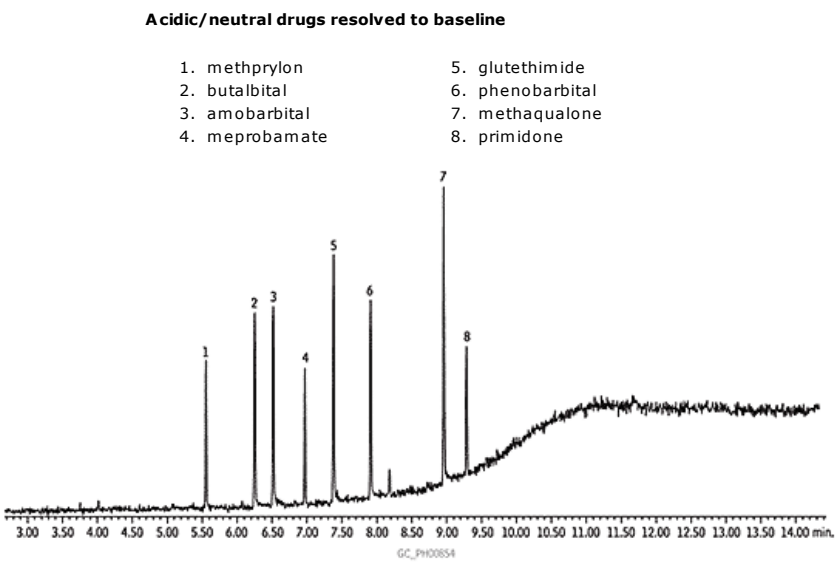
GC/MS is considered the standard for confirming the presence of abused drugs in body fluids, including acidic drugs (e.g., methaqualone), neutral drugs (e.g., phenobarbital), and basic drugs (e.g., methamphetamine). These methods are well established, and the positive identifications that mass spectral data generate are accepted as confirming evidence in courts of law. The accepted stationary phase for these analyses is a 5% phenyl / 95% methyl polysiloxane phase, because it provides the best selectivity for separating the drugs and their metabolites. Unfortunately, not all 5% phenyl columns provide the inertness needed to accurately quantify low concentrations of reactive acidic or basic drugs.

Now, Restek's R&D chemists have developed a new 5% phenyl stationary phase and a unique column deactivation technology specifically for GC/MS. The product of this combination - the Rxi®-5ms column - ensures enhanced inertness for acidic or basic compounds, while maintaining the selectivity of a conventional 5% phenyl column.

Using mixtures of underivatized acidic/neutral drugs and basic drugs, at an on-column concentration of 1ng for each drug, we evaluated a 30m, 0.25mm ID, 0.25µm Rxi®-5ms column for resolution and inertness. Figure 1 shows chromatography for acidic/neutral drugs and basic drugs analyzed by GC/MS. In either analysis, all compounds are resolved to baseline and exhibit symmetric peaks. Note that a Siltek® treated inlet liner contributes to these results: our unique Siltek® surface passivation process assures the liner will have the inertness needed for accurate low-level analyses of reactive acids or bases.

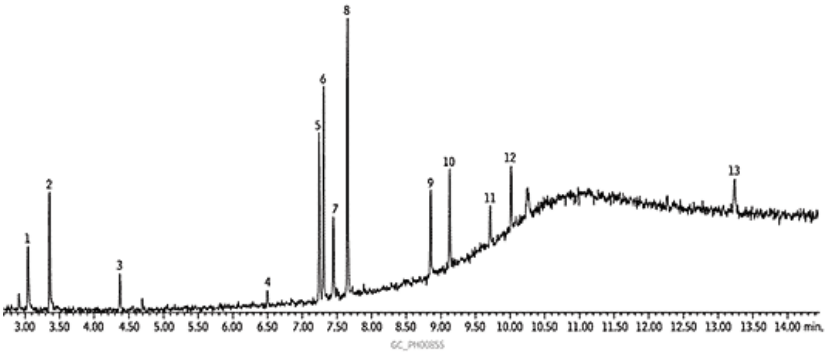
In combination, an Rxi®-5ms column and a Siltek® treated inlet liner represent a complete solution for analyzing acidic, neutral, and basic drugs by GC/MS.

**Figure 1** Analyze underivatized acidic drugs or basic drugs under the same conditions, using an Rxi®-5ms column.



**Sensitive analysis for basic drugs in free base form**

1. amphetamine
2. methamphetamine
3. nicotine
4. cotinine
5. caffeine
6. benzphetamine
7. ketamine
8. phencyclidine
9. methadone
10. cocaine
11. scopolamine
12. codeine
13. alprazolam




| | |
|---|---|
| Column: | Rxi®-5ms 30m, 0.25mm ID, 0.25µm (cat.# 13423) |
| Sample: | 10µg/mL each acidic/neutral and basic drugs in methanol<br>1.0µL split (10:1), 1ng each compound on column; |
| Inj.: | Siltek® treated 4mm single gooseneck inlet liner (cat # 20798-214.1) |
| Inj. temp.: | 250°C |
| Carrier gas: | helium, constant pressure |
| Flow rate: | 1mL/min. |
| Oven temp.: | 100°C to 300°C @ 20°C/min. (hold 5 min.) |
| Det.: | MS |
| Transfer line temp.: | 300°C |
| Scan range: | 35-550 amu |
| Ionization: | EI |
| Mode: | scan |

**RELATED SEARCHES**

*underivatized*, *acidic drugs*, *basic drugs*, *Rxi-5ms*

Rxi® GC Columns

# 3-IN-1 TECHNOLOGY

Highest Inertness • Lowest Bleed • Exceptional Reproducibility

## Rxi®-5Sil MS

### Assured Performance
for Forensic Applications

- Exceptional column inertness means greater certainty and lower detection limits.
- Versatile selectivity lets you keep analyzing samples instead of changing columns between methods.
- Robust, low-bleed phase results in better sensitivity and longer column lifetime.

RESTEK® Pure Chromatography www.restek.com

# Rxi®-5Sil MS Columns…

Whether analyzing postmortem samples or supporting athletic or workplace drug testing, toxicology labs are challenged with producing critical evidence that stands up under scrutiny. Increased pressure for fast, definitive results is driving labs to investigate standardized procedures and certifications aimed at reducing variability. GC column choice plays a vital role in data quality and using rugged, versatile Rxi®-5Sil MS capillary columns is an easy way to improve chromatography performance and simplify lab operations.

For years, "5" type (5% diphenyl/95% dimethyl polysiloxane) columns have been recognized as the column of choice for analyzing drugs of abuse, because they offer higher selectivity and retention for functionalized compounds than "1" type columns (100% dimethyl polysiloxane). While the selectivity of 5 type columns has many forensic applications, column performance can vary significantly among these columns. Some 5 type columns have inadequate deactivations, causing tailing peaks, or are poorly stabilized, resulting in high bleed levels, reduced sensitivity, and shorter column lifetimes. Rxi®-5Sil MS columns are based on a silarylene phase (Figure 1) that offers improved inertness and stability compared to typical 5 type columns.

Toxicology labs interested in improved data quality can increase confidence in results and reduce downtime by using Rxi®-5Sil MS columns. Exceptional inertness increases accuracy and precision at trace levels, while ruggedness assures low bleed and long column lifetime. As shown on the following pages, these versatile columns can improve lab efficiency and data quality for many different drugs of abuse, including cannabinoids, benzodiazepines, cocaine, opiates, and amphetamines.

**Figure 1:** Rxi®-5Sil MS columns: phase structure results in a more inert, low-bleed column with broad selectivity for a wide range of compounds.

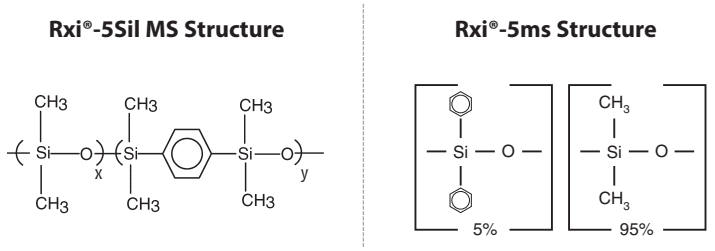
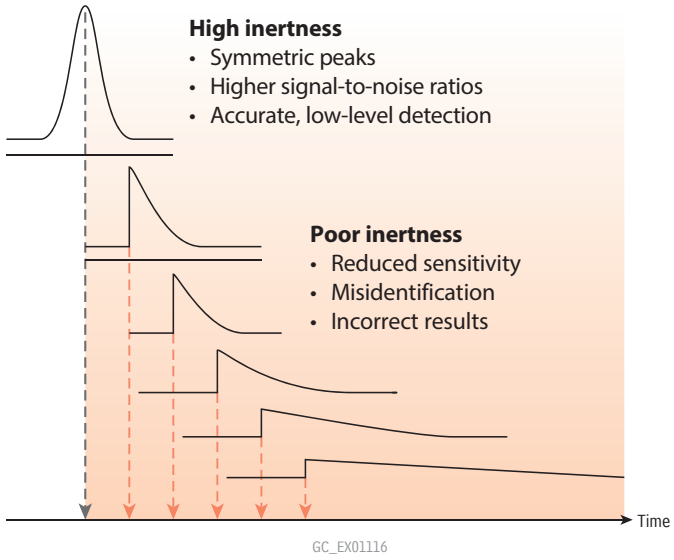



**Figure 2:** As column activity increases, signal decreases and retention time shifts.

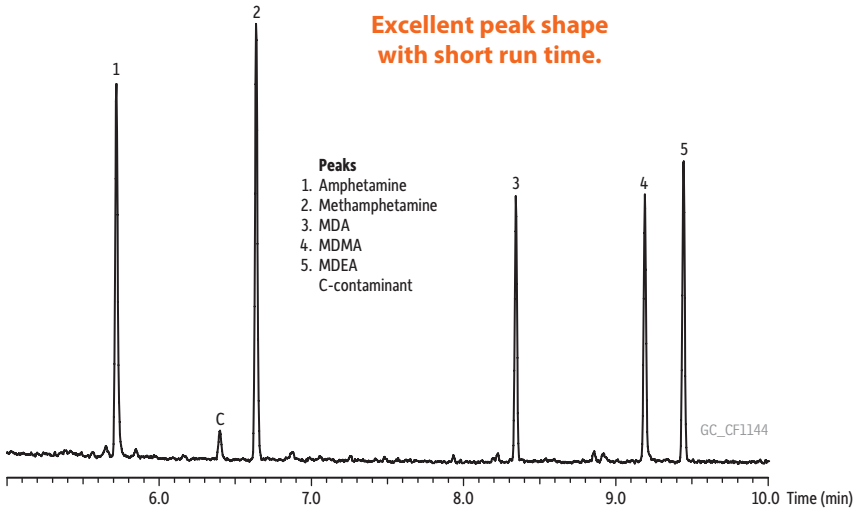



## Exceptional Inertness Means Greater Certainty and Lower Detection Limits

Column inertness improves peak shape, which greatly affects the signal-to-noise ratio and, therefore, analytical sensitivity. Rxi®-5Sil MS columns are exceptionally inert, ensuring symmetric peak shape and high response for a wide range of analyte chemistries. In addition to influencing signal-to-noise ratios, column inertness also affects retention time stability, which is an important factor for correct peak identification. Inertness is critical because peak tailing will increase as column activity increases, causing retention times to shift (Figure 2). Analyzing derivatized amphetamines or cocaine and its metabolites on highly inert Rxi®-5Sil MS columns results in symmetric peak shapes and excellent low-level response (Figures 3 and 4).

# Assured Performance for Forensic Applications

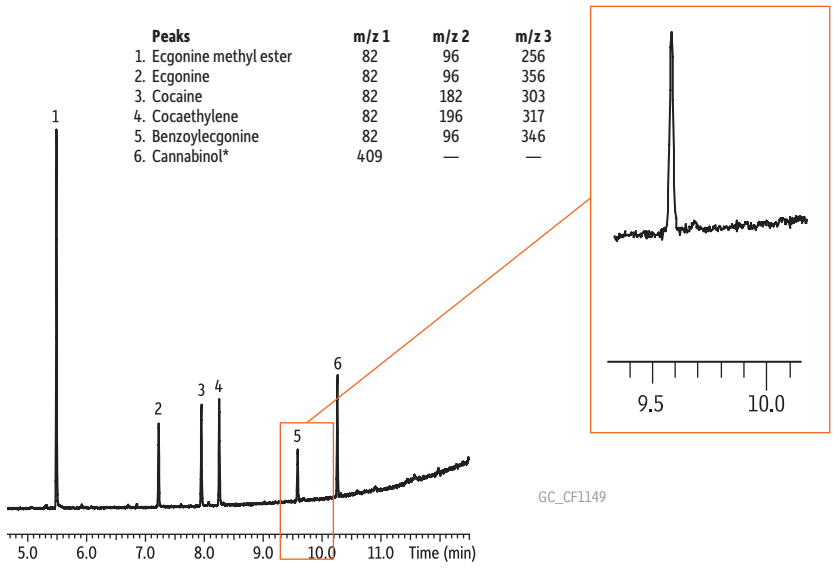
**Figure 3:** Robust, inert Rxi®-5Sil MS columns do not break down under harsh conditions, such as exposure to the derivatization reagents used in amphetamines analysis. Compounds shown are HFAA derivatives.




**Column:** Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623); **Sample:** 500 ng/mL HFAA derivatives in butyl chloride; **Injection:** Inj. Vol.: 1 µL splitless (hold 1 min); Liner: 3.5 mm splitless taper w/wool (cat.# 22286-200.1); Inj. Temp.: 250 °C; Purge Flow: 28 mL/min; **Oven:** Oven Temp: 75 °C to 300 °C at 15 °C/min; Carrier Gas; He, constant linear velocity, 45 cm/sec, 13.5 psi, 93.1 kPa @ 75 °C; **Detector:** MS, Scan; Transfer Line Temp.: 250 °C; Analyzer Type: Quadrupole; Source Temp.: 200 °C; Electron Energy: 70 eV; Solvent Delay Time: 4 min; Tune Type: PFTBA; Ionization Mode: EI; Scan Range: 40-300 amu; Scan Rate: 5 scans/sec; **Instrument:** Shimadzu 2010 GC & QP2010+ MS.

**Figure 4:** Low levels of derivatized cocaine and its metabolites can also be reliably separated on Rxi®-5Sil MS columns.

| Peaks | m/z 1 | m/z 2 | m/z 3 |
|---|---|---|---|
| 1. Ecgonine methyl ester | 82 | 96 | 256 |
| 2. Ecgonine | 82 | 96 | 356 |
| 3. Cocaine | 82 | 182 | 303 |
| 4. Cocaethylene | 82 | 196 | 317 |
| 5. Benzoylecgonine | 82 | 96 | 346 |
| 6. Cannabinol* | 409 | — | — |




**Column:** Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623); **Sample:** 100 ng/mL in butyl chloride; **Injection:** Inj. Vol.: 1 µL splitless (hold 1 min); Liner: single taper w/wool (cat.# 22286-200.1); Inj. Temp.: 250 °C; Purge Flow: 20 mL/min; **Oven:** Oven Temp: 100 °C to 200 °C at 30 °C/min to 300 °C at 15 °C/min; Carrier Gas: He, constant linear velocity, 40 cm/sec, 12.5 psi, 86.2 kPa @ 100 °C; **Detector:** MS, SIM; Transfer Line Temp.: 310 °C; Source Temp.: 250 °C; Solvent Delay Time: 4 min.; Tune Type: PFTBA; Ionization Mode: EI; **Instrument:** Shimadzu 2010 GC & QP2010+ MS; **Notes:** Samples were prepared as follows: Standards brought to dryness under nitrogen, then 50 µL, BSTFA + 1%TMCS (cat.# 35606) added. 50 µL pyridine was then added, and samples were incubated at 70 °C for 30 min. After incubation, samples were diluted with butyl chloride.

* Used as derivitazation check

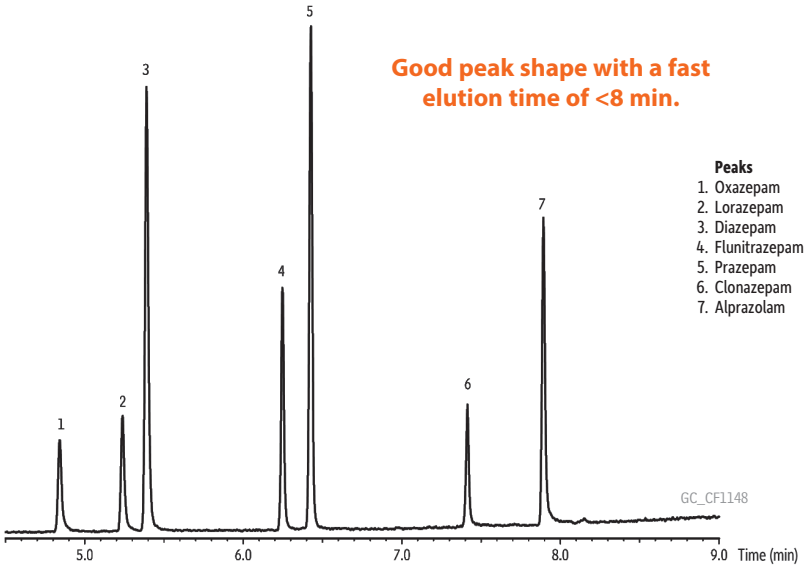
# Rxi®-5Sil MS Columns…

## Optimized Selectivity Lets You Keep Analyzing Samples Instead of Changing Columns Between Methods

While the inertness of Rxi®-5Sil MS columns exceeds typical 5 type columns, the selectivity is similar and is ideal for many toxicological applications. A wide range of analyte classes can be reliably separated on Rxi®-5Sil MS columns, including structurally-related compounds, such as benzodiazepines. Benzodiazepines are often analyzed on a fluorinated phase (e.g. Rtx®-200), but the selectivity of the Rxi®-5Sil MS column provides complete separation of all peaks of interest (Figure 5). Since a fluorinated column is no longer necessary, more time can be spent running samples with fewer time-consuming column changes between methods.

In addition to benzodiazepines, the selectivity of the Rxi®-5Sil MS column is also well-suited for the analysis of several common classes of drugs of abuse including cannabinoids, cocaine and its metabolites, opiates, and amphetamines. The Miami Dade Medical Examiner's Laboratory provides another example of how Rxi®-5Sil MS columns can simplify analyses and improve lab efficiency. The versatility and robustness of the Rxi®-5Sil MS column assisted the lab in streamlining operations by reducing time-consuming column changes and maintenance. One of the applications routinely run on this column is the analysis of opiates (Figure 6). The selectivity of the Rxi®-5Sil MS column gives excellent separation between all compounds, and very low limits of detection are achieved since bleed is minimal. In addition, the column stands up extremely well to the derivatization reagents used prior to analysis, further increasing throughput by reducing instrument downtime for maintenance. The Rxi®-5Sil MS column also produces excellent chromatography for cannibinoids (Figure 7).

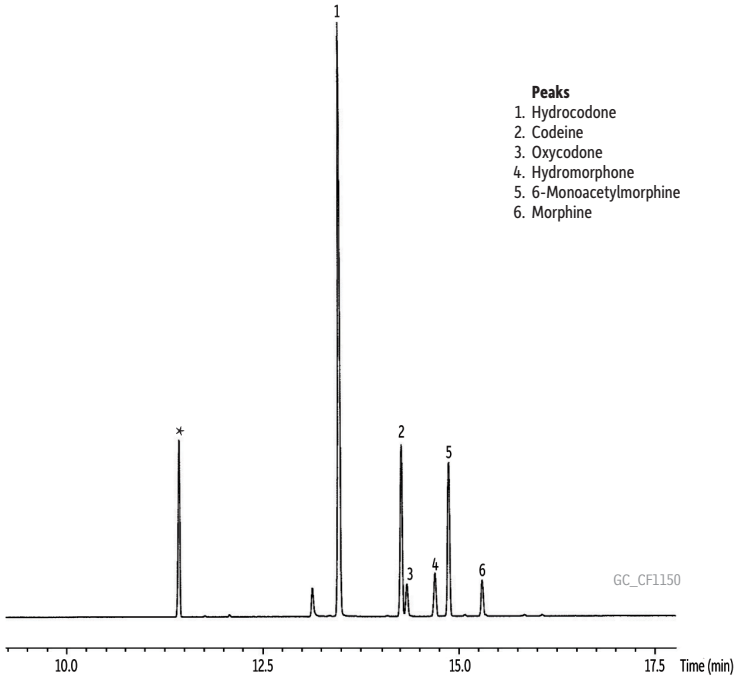
**Figure 5:** No need to change columns to analyze benzodiazepines—Rxi®-5Sil MS columns give excellent separation of structurally-related benzodiazepines.




**Column:** Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623); **Sample:** 15 µg/mL in butyl chloride; **Injection:** Inj. Vol.: 1 µL splitless (hold 1 min); Liner: 3.5 mm splitless taper w/wool (cat.# 22286-200.1); Inj. Temp.: 280 °C; Purge Flow: 32.2 mL/min (20:1 split); **Oven:** Oven Temp: 200 °C to 330 °C at 15 °C/min (hold 3 min); **Carrier Gas:** He, constant linear velocity, 50 cm/sec, 23.7 psi, 163.4 kPa @ 200 °C; **Detector:** MS, Scan; Transfer Line Temp: 280 °C; Analyzer Type: Quadrupole; Source Temp.: 200 °C; Electron Energy: 70 eV; Solvent Delay Time: 4 min; Tune Type: PFTBA; Ionization Mode: EI; Scan Range: 50-350 amu; Scan Rate: 5 scans/sec; **Instrument:** Shimadzu 2010 GC & QP2010+ MS

# Assured Performance for Forensic Applications

**Figure 6:** Analysis of derivatized opiates on an Rxi®-5Sil MS column performed by the Miami Dade Medical Examiner's lab.



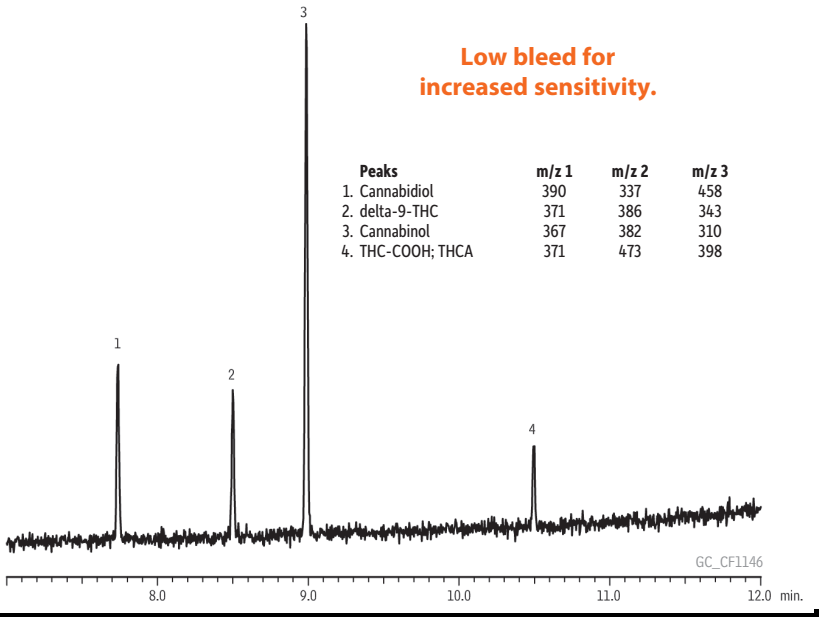

**Peaks**
1. Hydrocodone
2. Codeine
3. Oxycodone
4. Hydromorphone
5. 6-Monoacetylmorphine
6. Morphine

| | |
|---|---|
| **Column** | Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623) |
| **Sample** | |
| Diluent: | Ethyl acetate |
| Conc.: | 100 ng/mL propionic anhydride derivatives |
| **Injection** | |
| Inj. Vol.: | 1 µL splitless (hold 1 min) |
| Liner: | 4 mm splitless taper w/wool (cat.# 22405) |
| Inj. Temp.: | 250 °C |
| Purge Flow: | 100 mL/min |
| **Oven** | |
| Oven Temp: | 65 °C (hold 1 min) to 315 °C at 15 °C/min |
| **Carrier Gas** | He, constant flow |
| Flow Rate: | 1 mL/min |
| Linear Velocity: | 35 cm/sec @ 65 °C |
| **Detector** | MS |
| Mode: | SIM |
| Transfer Line Temp.: | 250 °C |
| Analyzer Type: | Quadrupole |
| Solvent Delay Time: | 7 min |
| Tune Type: | PFTBA |
| Ionization Mode: | EI |
| **Notes** | Opiates were spiked into a blood sample and extracted by SPE, then derivatized with propionic anhydride. |

Acknowledgement
Data courtesy of Miami Dade County Medical Examiner Department



**Figure 7:** High signal response due to column inertness and efficiency, combined with low bleed, results in maximum sensitivity for derivatized cannabinoids (50 ng/mL).




| Peaks | m/z 1 | m/z 2 | m/z 3 |
|---|---|---|---|
| 1. Cannabidiol | 390 | 337 | 458 |
| 2. delta-9-THC | 371 | 386 | 343 |
| 3. Cannabinol | 367 | 382 | 310 |
| 4. THC-COOH; THCA | 371 | 473 | 398 |

| | |
|---|---|
| **Column** | Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623) |
| **Sample** | |
| Diluent: | Ethyl acetate |
| Conc.: | 50 ng/mL TMS derivatives |
| **Injection** | |
| Inj. Vol.: | 1 µL splitless (hold 1 min.) |
| Liner: | 3.5 mm splitless taper w/wool (cat.# 22286-200.1) |
| Inj. Temp.: | 250 °C |
| Purge Flow: | 21.4 mL/min |
| **Oven** | |
| Oven Temp: | 150 °C to 330 °C at 15 °C/min (hold 3 min) |
| **Carrier Gas** | He, constant linear velocity |
| Linear Velocity: | 40 cm/sec, 13.8 psi, 95.1 kPa @ 150 °C |
| **Detector** | MS |
| Mode: | SIM |
| SIM Program: | 390, 337, 458, 367, 382, 310, 371, 386, 343, 473, 398 m/z |
| Transfer Line Temp.: | 280 °C |
| Analyzer Type: | Quadrupole |
| Source Temp.: | 200 °C |
| Solvent Delay Time: | 4 min |
| Tune Type: | PFTBA |
| Ionization Mode: | EI |
| Instrument | Shimadzu 2010 GC & QP2010+ MS |

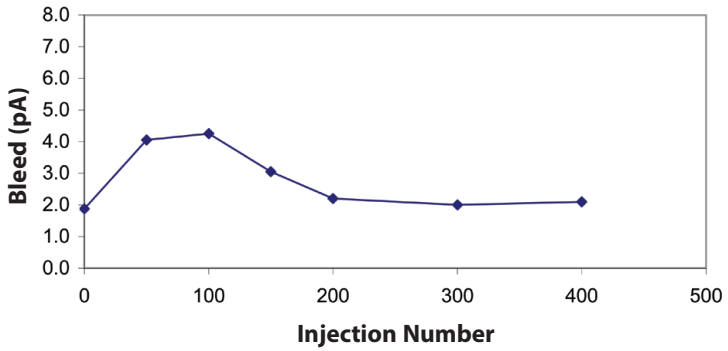
# Rxi®-5Sil MS Columns…

## Robust, Low-Bleed Phase Results in Better Sensitivity and Longer Column Lifetime

Many drug assays require that compounds be derivatized prior to analysis. Derivatization not only allows for GC analysis of compounds not otherwise amenable to gas chromatography, it also helps to produce unique, high molecular weight fragments that assist with GC-MS quantitation. While derivatization has its advantages, derivatization reagents and their byproducts are extremely harsh and can reduce column lifetimes by damaging the stationary phase. Phase damage usually manifests as increased bleed and tailing of active compounds. The unique Rxi®-5Sil MS stationary phase, with its embedded arylene groups, provides a more rigid matrix that is less likely to be damaged by derivatization reagents or their byproducts.

As a test of column lifetime, an Rxi®-5Sil MS column was subjected to repeated injections of high concentration HFAA, a harsh derivatization reagent, as well as prolonged exposure to the column's maximum operational temperature during each injection. Throughout lifetime testing, column bleed and inertness were tested by analyzing a mixture of active test compounds that tail severely on less inert columns. After 400 injections, no change in bleed or inertness was observed (Figures 8 and 9). The enhanced stability of Rxi®-5Sil MS columns reduces phase bleed, resulting in longer column lifetimes and improved performance with sensitive mass spectrometry detectors.

**Figure 8:** Low column bleed results in long column lifetimes, saving labs replacement costs.

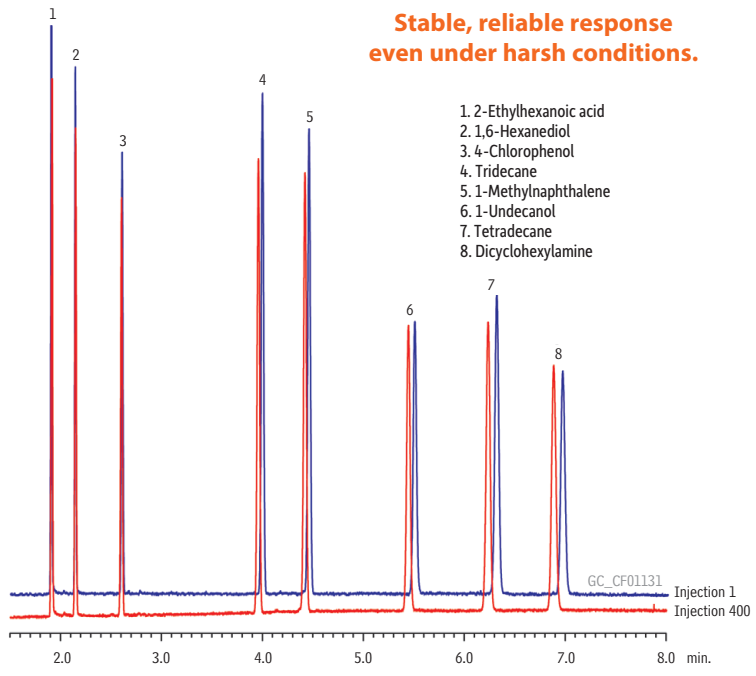



Column bleed over 400 injections of HFBA derivatization reagent. Column was held at the maximum isothermal temperature.

## Conclusion

Rxi®-5Sil MS columns are ideal for toxicology labs interested in improving data quality by increasing certainty and reducing downtime. These columns have similar selectivity to conventional 5 type columns, but are significantly more inert and robust. Rxi®-5Sil MS columns provide more accurate trace-level results and reduced downtime for column changes, offering labs a valuable tool for improving methods for the routine analysis of drugs of abuse.

**Figure 9:** Rugged Rxi®-5Sil MS columns produce consistent retention times, even after 400 injections of derivatization reagent.




**Column:** Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623); **Sample:** Column test mix (cat.# 35226); **Inj.:** 1.0 µL split (split ratio 1:60), 4 mm recessed single taper (cat.# 20983); Inj. temp.: 250 °C; **Carrier gas:** helium, constant pressure; Linear velocity: 36 cm/sec @ 125 °C; Oven temp.: 125 °C; **Det:** FID @ 320 °C; Instrument: Agilent 6890

# Assured Performance for Forensic Applications

## Restek's low-bleed MS columns exceed requirements of the most sensitive mass spectrometers.

**Rxi®-5Sil MS Columns** (fused silica)
(low polarity phase; Crossbond® 1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane)

- Engineered to be a low-bleed GC-MS column.
- Excellent inertness for active compounds.
- General-purpose columns—ideal for GC-MS analysis of drugs of abuse.
- Temperature range: -60 °C to 320/350 °C.

The Rxi®-5Sil MS stationary phase incorporates phenyl groups in the polymer backbone. This improves thermal stability, reduces bleed, and makes the phase less prone to oxidation. Rxi®-5Sil MS columns are ideal for GC-MS applications requiring high sensitivity, including use in ion trap systems.

| ID | df | temp. limits | 15-Meter cat.# | 30-Meter cat.# |
|---|---|---|---|---|
| 0.25 mm | 0.25 µm | -60 to 320/350 °C | 13620 | 13623 |
| | 0.50 µm | -60 to 320/350 °C | 13635 | 13638 |
| 0.32 mm | 0.25 µm | -60 to 320/350 °C | 13621 | 13624 |
| | 0.50 µm | -60 to 320/350 °C | | 13639 |

### similar **phases**

DB-5ms, DB-5msUI, VF-5ms, CP-Sil 8 CB, ZB-5msi, Rtx-5Sil MS

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**Rxi®-5Sil MS with Integra-Guard®**

- Extend column lifetime.
- Eliminate leaks with a built-in retention gap.
- Inertness verified by isothermal testing.

| Description | qty. | cat.# |
|---|---|---|
| 15 m, 0.25 mm ID, 0.25 µm Rxi-5Sil MS w/10 m Integra-Guard Column | ea. | 13620-127 |
| 30 m, 0.25 mm ID, 0.25 µm Rxi-5Sil MS w/5 m Integra-Guard Column | ea. | 13623-124 |
| 30 m, 0.25 mm ID, 0.25 µm Rxi-5Sil MS w/10 m Integra-Guard Column | ea. | 13623-127 |
| 15 m, 0.25 mm ID, 0.50 µm Rxi-5Sil MS w/5 m Integra-Guard Column | ea. | 13635-124 |
| 30 m, 0.25 mm ID, 0.50 µm Rxi-5Sil MS w/5 m Integra-Guard Column | ea. | 13638-124 |
| 30 m, 0.25 mm ID, 0.50 µm Rxi-5Sil MS w/10 m Integra-Guard Column | ea. | 13638-127 |
| 30 m, 0.32 mm ID, 0.50 µm Rxi-5Sil MS w/5 m Integra-Guard Column | ea. | 13639-125 |

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**Sky® 4.0 mm ID Single Taper Inlet Liner** w/ Wool
For Agilent GCs equipped with split/splitless inlets


| ID x OD x L | qty. | cat.# |
|---|---|---|
| Single Taper, Sky Technology, Borosilicate Glass with Quartz Wool | | |
| 4.0 mm x 6.5 mm x 78.5 mm | ea. | 23303.1 |
| 4.0 mm x 6.5 mm x 78.5 mm | 5-pk. | 23303.5 |
| 4.0 mm x 6.5 mm x 78.5 mm | 25-pk. | 23303.25 |

**Sky® 4.0 mm ID Single Taper Inlet Liner**
For Agilent GCs equipped with split/splitless inlets


| ID x OD x L | qty. | cat.# |
|---|---|---|
| Single Taper, Sky Technology, Borosilicate Glass | | |
| 4.0 mm x 6.5 mm x 78.5 mm | ea. | 23302.1 |
| 4.0 mm x 6.5 mm x 78.5 mm | 5-pk. | 23302.5 |
| 4.0 mm x 6.5 mm x 78.5 mm | 25-pk. | 23302.25 |

## Recommended for Split Injection

**Sky® 4.0 mm ID Precision® Inlet Liner** w/ Wool
For Agilent GCs equipped with split/splitless inlets


| ID x OD x L | qty. | cat.# |
|---|---|---|
| Precision, Sky Technology, Borosilicate Glass with Quartz Wool | | |
| 4.0 mm x 6.3 mm x 78.5 mm | ea. | 23305.1 |
| 4.0 mm x 6.3 mm x 78.5 mm | 5-pk. | 23305.5 |
| 4.0 mm x 6.3 mm x 78.5 mm | 25-pk. | 23305.25 |

**Sky® 4.0 mm ID Cyclo Inlet Liner**
For Agilent GCs equipped with split/splitless inlets


| ID x OD x L | qty. | cat.# |
|---|---|---|
| Cyclo, Sky Technology, Borosilicate Glass | | |
| 4.0 mm x 6.3 mm x 78.5 mm | ea. | 23312.1 |
| 4.0 mm x 6.3 mm x 78.5 mm | 5-pk. | 23312.5 |
| 4.0 mm x 6.3 mm x 78.5 mm | 25-pk. | 23312.25 |

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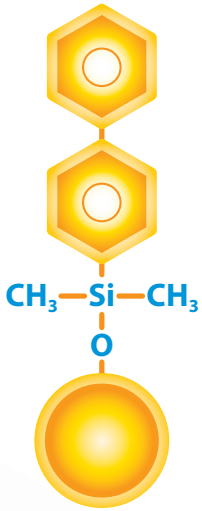
**The established choice for bioanalytical testing since 2005**

- Separates compounds that other phenyl and C18 chemistries can't.
- Allows the use of simple, MS-friendly mobile phases.
- Restek's most popular LC phase (also available on fully porous silica).

**Also available in acid-resistant ARC-18 for large, multiclass lists by MS**

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Lit. Cat.# CFBR1302A-UNV


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# **NEW** Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2 Columns

## Fast, Definitive Data for Blood Alcohol Testing

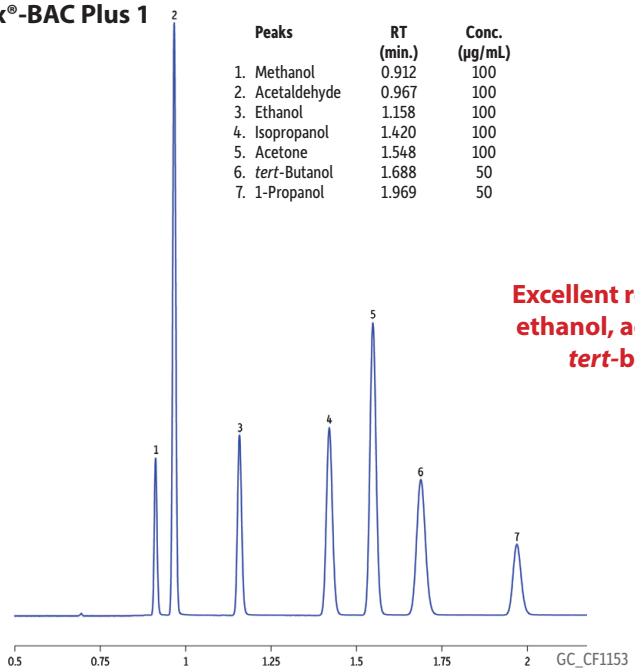
- Optimized column selectivities guarantee resolution of ethanol, internal standards, and frequently encountered interferences.
- Robust and reproducible column chemistry ensures longer column lifetime and accurate, consistent results.
- 2 minute analysis time increases lab productivity.

New Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2 columns from Restek are designed to provide definitive results quickly, so you can maximize sample throughput. These columns baseline separate all critical compounds, including ethanol, methanol, acetone, *tert*-butanol (IS), acetaldehyde, isopropanol, and 1-propanol (IS), in less than 2 minutes (Figure 1). In fact, *every* Rtx®-BAC Plus 1 and Plus 2 column is quality tested with these important target compounds to ensure performance. These new columns are exceptionally robust, ensuring longer column lifetime and highly reproducible data. For fast, consistent results, try Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2 columns for analysis of blood alcohol compounds.

**Figure 1 NEW** Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2 columns baseline resolve all critical components in less than 2 minutes!

**Rtx®-BAC Plus 1**

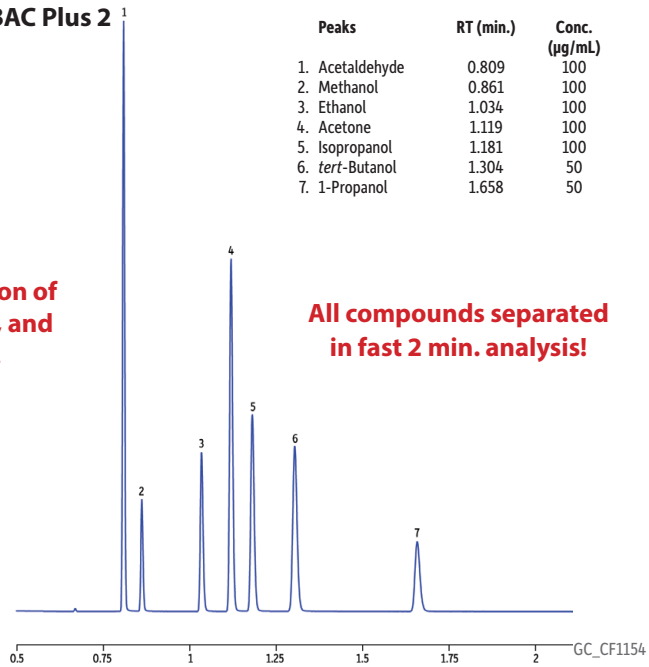
| Peaks | RT (min.) | Conc. (µg/mL) |
|---|---|---|
| 1. Methanol | 0.912 | 100 |
| 2. Acetaldehyde | 0.967 | 100 |
| 3. Ethanol | 1.158 | 100 |
| 4. Isopropanol | 1.420 | 100 |
| 5. Acetone | 1.548 | 100 |
| 6. *tert*-Butanol | 1.688 | 50 |
| 7. 1-Propanol | 1.969 | 50 |




**Excellent resolution of ethanol, acetone, and *tert*-butanol.**

**Rtx®-BAC Plus 2**

| Peaks | RT (min.) | Conc. (µg/mL) |
|---|---|---|
| 1. Acetaldehyde | 0.809 | 100 |
| 2. Methanol | 0.861 | 100 |
| 3. Ethanol | 1.034 | 100 |
| 4. Acetone | 1.119 | 100 |
| 5. Isopropanol | 1.181 | 100 |
| 6. *tert*-Butanol | 1.304 | 50 |
| 7. 1-Propanol | 1.658 | 50 |




**All compounds separated in fast 2 min. analysis!**

**Column**: Rtx®-BAC Plus 1, 30 m, 0.32 mm ID, 1.8 µm (cat.# 18004); Rtx®-BAC Plus 2, 30 m, 0.32 mm ID, 0.6 µm (cat.# 18006); **Sample:** Diluent: Water; **Injection:** headspace-loop split (split ratio 50:1); Liner: 1 mm ID straight inlet liner (cat.# 20972); **Headspace-Loop:** Inj. Port Temp.: 200 °C; Instrument: Tekmar HT3; Inj. Time: 3 min.; Transfer Line Temp.: 125 °C; Valve Oven Temp.: 125 °C; Standby fl w rate: 50 mL/min; Sample Temp.: 60 °C; Sample Equil. Time: 5 min.; Vial Pressure: 30 psi; Pressurize Time: 1 min.; Loop Pressure: 20 psi; Loop Fill Time: 1 min.; **Oven:** Oven Temp: 40 °C (hold 3 min.); **Carrier Gas:** He, constant fl w; Linear Velocity: 80 cm/sec. @ 40 °C; **Detector:** FID @ 240 °C; Make-up Gas Flow Rate: 30 mL/min.; Make-up Gas Type: $N_2$; **Instrument:** Agilent/HP6890 GC

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# **NEW** Columns and Standards for Blood Alcohol Testing

## Rtx®-BAC Plus 1/Rtx®-BAC Plus 2 Columns

- Optimized column selectivities guarantee resolution of ethanol, internal standards, and frequently encountered interferences.
- Robust and reproducible column chemistry ensures longer column lifetime and consistent results.

### **Rtx®-BAC Plus 1 Columns** (fused silica)

| ID | df | temp. limits | 30-Meter |
|---|---|---|---|
| 0.32mm | 1.80µm | -20 to 240/260°C | 18004 |
| 0.53mm | 3.00µm | -20 to 240/260°C | 18005 |

### **Rtx®-BAC Plus 2 Columns** (fused silica)

| ID | df | temp. limits | 30-Meter |
|---|---|---|---|
| 0.32mm | 0.6µm | -20 to 240/260°C | 18006 |
| 0.53mm | 1.0µm | -20 to 240/260°C | 18007 |

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## Blood Alcohol Resolution Control Standards

- Use to verify the retention time for each compound normally included in a blood alcohol test, and to verify that the compounds are resolved from and do not interfere with one another.
- Includes 1-propanol or *tert*-butanol internal standard.
- Intended for qualitative use only.

### **BAC Resolution Control Standard n-P** (6 components)

acetaldehyde
acetone
ethanol (BAC)
methanol
1-propanol
2-propanol (isopropanol)

100mg/dL each in water, 1mL/ampul

cat.# 36010 (ea.)

No data pack available.

### **BAC Resolution Control Standard t-B** (6 components)

acetaldehyde
acetone
*tert*-butanol (TBA)
ethanol (BAC)
methanol
2-propanol (isopropanol)

100mg/dL each in water, 1mL/ampul

cat.# 36011 (ea.)

No data pack available.

## **Blood Alcohol Standards** (Calibration)

- NIST-traceable ethanol calibration standards.
- Calibration mixtures ranging from 0.010g/dL to 0.40g/dL in water.
- Datapack and Certificate of Analysis for each standard available on our website.

| Compound | | qty. | cat.# |
|---|---|---|---|
| 0.010g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36276 |
| 0.015g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36232 |
| 0.02g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36233 |
| 0.025g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36234 |
| 0.04g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36235 |
| 0.05g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36257 |
| 0.08g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36262 |
| 0.1g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36236 |
| 0.15g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36237 |
| 0.16g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36417 |
| 0.2g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36238 |
| 0.3g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36239 |
| 0.4g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36266 |



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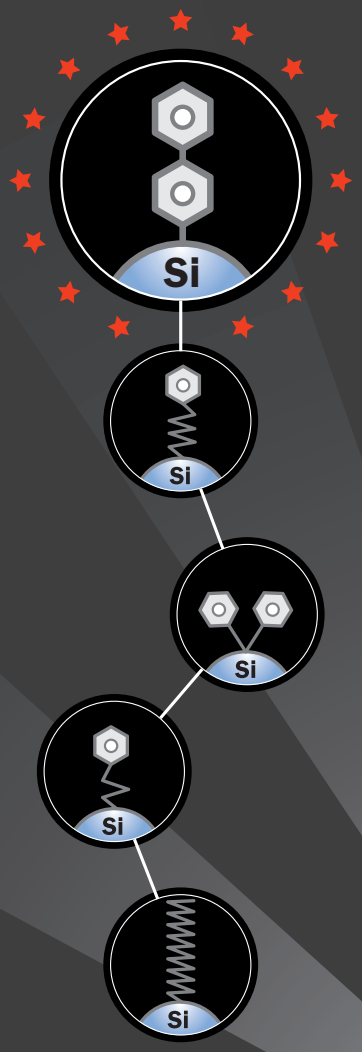
# Restek **Ultra Biphenyl** Columns

## Next Generation Phenyl Columns are the Best Choice for Pain Panel Analyses




★ **The unique Biphenyl phase, created by Restek, exploits aromatic retention for pharmaceutical and drug-like compounds,** giving it a significant advantage over C18 phases for clinical diagnostic tests, such as pain drug panel analyses.

★ **The Restek Ultra Biphenyl column is the column of choice for pain drug panel testing.** In addition, the Biphenyl column has been incorporated into clinical methods for NSAIDs, THC and metabolites, synthetic cannabinoids, steroids, hormones, and sulfonamides in milk.

★ **Restek Ultra Biphenyl columns go through extra QA testing by LC/MS using 18 of the most problematic pain panel compounds.** This ensures lot-to-lot consistency so there will be no performance differences between the 1st column you buy and the 100th (see column Certificate of Analysis on reverse).

Visit **www.restek.com/biphenyl** for a complete product listing

RESTEK® Pure Chromatography

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Every Restek Ultra Biphenyl column comes with a lot-specific Certificate of Analysis to ensure lot-to-lot consistency. QA testing includes pain panel compounds run under LC/MS conditions.

# LC Certificate of Analysis

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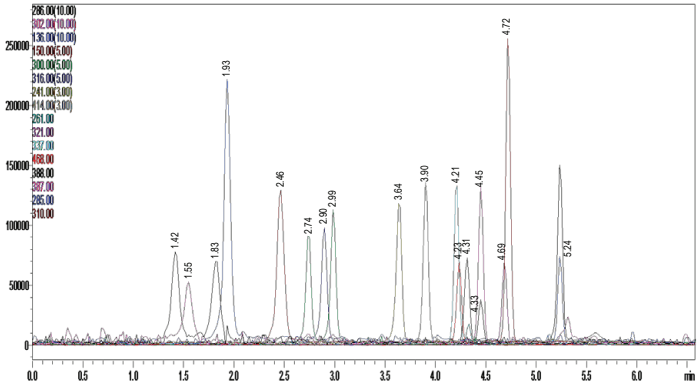
Lot Performance Report

Ultra Biphenyl 5um Lot# 110224R




| Compound | Expected m/z | Expected Retention * | Minimum Retention | Maximum Retention | Result* for Lot# 110224R | |
|---|---|---|---|---|---|---|
| Amphetamine | 136 | 1.93 | 1.68 | 2.18 | **1.93** | PASS |
| Buprenorphine | 468 | 4.22 | 3.97 | 4.47 | **4.23** | PASS |
| Carisoprodol | 261 | 4.36 | 4.11 | 4.61 | **4.33** | PASS |
| Codeine | 300 | 2.73 | 2.48 | 2.98 | **2.74** | PASS |
| Diazepam | 285 | 5.24 | 4.99 | 5.49 | **5.24** | PASS |
| Fentanyl | 337 | 4.19 | 3.94 | 4.44 | **4.21** | PASS |
| Flurazepam | 388 | 4.29 | 4.04 | 4.54 | **4.31** | PASS |
| Hydrocodone | 300 | 2.98 | 2.73 | 3.23 | **2.99** | PASS |
| Hydromorphone | 286 | 1.82 | 1.57 | 2.07 | **1.83** | PASS |
| Lorazepam | 321 | 4.72 | 4.47 | 4.97 | **4.69** | PASS |
| Meprobamate** | 241** | 3.66 | 3.41 | 3.91 | **3.64** | PASS |
| Methadone | 310 | 4.69 | 4.44 | 4.94 | **4.72** | PASS |
| Methamphetamine | 150 | 2.45 | 2.20 | 2.70 | **2.46** | PASS |
| Morphine | 286 | 1.42 | 1.17 | 1.67 | **1.42** | PASS |
| Norbuprenorphine | 414 | 3.88 | 3.63 | 4.13 | **3.90** | PASS |
| Oxycodone | 316 | 2.89 | 2.64 | 3.14 | **2.90** | PASS |
| Oxymorphone | 302 | 1.55 | 1.30 | 1.80 | **1.55** | PASS |
| Sufentanil | 387 | 4.43 | 4.18 | 4.68 | **4.45** | PASS |

* Expected retention and results for the lot tested are based on an analysis using a Shimadzu Prominence UFLC-XR with an MS detector along with proprietary method parameters. Actual retention times obtained may vary depending on the specific instrument manufacturer, model, and system configuration used.

** [M+H] of meprobamate is 219; however, dimer with formate is the predominant signal. An m/z = 241 is used for detection.

Approved By: Date: April 12, 2011

RESTEK QA APPROVED

Restek certifies that this product complies with all quality specifications. It was produced in accordance with the most current edition of the Restek Corporation Quality System Manual. Restek Corporation's quality system is registered to ISO 9001:2008 (BSI certificate # FM 80397).

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ISO 9001 · 17025 · Guide 34

**Lit. Cat.# GNTS1377**

# Reliably Confirm Cannabinoids by GC-MS

## Using a 12m x 0.20mm ID 0.33µm Rxi®-5ms Column

by Kristi Sellers, Clinical/Forensic Innovations Chemist

- Baseline resolution for all major metabolites.
- Ultra-low bleed at 300°C, for accurate data.
  Bake column at 340°C, to remove derivatization by-products and prolong column life.

Marijuana is one of the most abused substances in the United States. Its common abuse stems from its widespread availability and because it is inexpensive, compared to other abused substances such as cocaine and heroin. Marijuana use typically is determined by screening for its major metabolite in urine, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (Δ9-carboxy-THC), using an immunoassay. When screening results are positive, gas chromatography/mass spectrometry (GC/MS) is employed for confirmation. Marijuana use also can be determined by analyzing other sample matrices, such as blood, hair, oral fluid, or body tissues but, again, positive results require GC/MS confirmation.[1]

GC/MS confirmation methods require sample clean-up and derivatization of target analytes, and call for a capillary GC column that can produce reliable identification and quantification results. Δ9-carboxy-THC is the primary target in GC/MS confirmation analysis, but other marijuana metabolites present in samples include cannabinol, cannabidiol, 11-hydroxy-Δ9-tetrahydrocannabinol (Δ9-hydroxy-THC), Δ9-tetrahydrocannabinol (Δ9-THC), and Δ8-tetrahydrocannabinol (Δ8-THC). Further, a guard column typically is recommended for this analysis, to prevent non-volatile residue in the sample matrix from contaminating the analytical column. The guard column should have an internal diameter approximately equal to that of the analytical column, to minimize changes in flow rate.

For the analysis we show in this article, we used MTBSTFA (N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide) to derivatize the target compounds.[2] The analytical column we chose is our new 12m x 0.20mm ID x 0.33µm Rxi™-5ms column (5% diphenyl / 95% dimethylpolysiloxane stationary phase). The small internal diameter makes this column compatible for use with mass spectrometers, because the column can be operated using a 1.0mL/min. flow rate. The short length produces analysis times of less than 15 minutes for the major metabolite, Δ9-carboxy-THC, which elutes last. Because the target compounds have relatively high molecular weights (310-358 amu, underivatized — see Figure 1), the GC oven must be programmed to a relatively high temperature, 300°C, to keep analysis time short.

The column and conditions we used ensure baseline resolution for all of the metabolites in Figure 2. Figure 2 also shows that the ultra-low bleed exhibited by the Rxi™-5ms column does not interfere with the analysis. The GC oven must heated to an even higher temperature between samples, 340°C, to bake sample matrix interferences and derivatization by-products from the system. Derivatization by-products can be seen in the baseline in Figure 2.

The results of this analysis demonstrate that a 12m x 0.20mm ID x0.33µm Rxi™-5ms column has the selectivity and inertness needed to provide baseline resolution, suitably short analysis times, and no interference from bleed at high temperature. We highly recommend it for this analysis.

**Figure 1** Cannabinoids have relatively high molecular weights, so high temperatures must be used in their analysis.

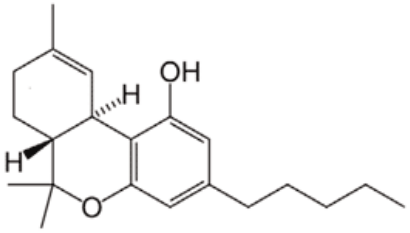
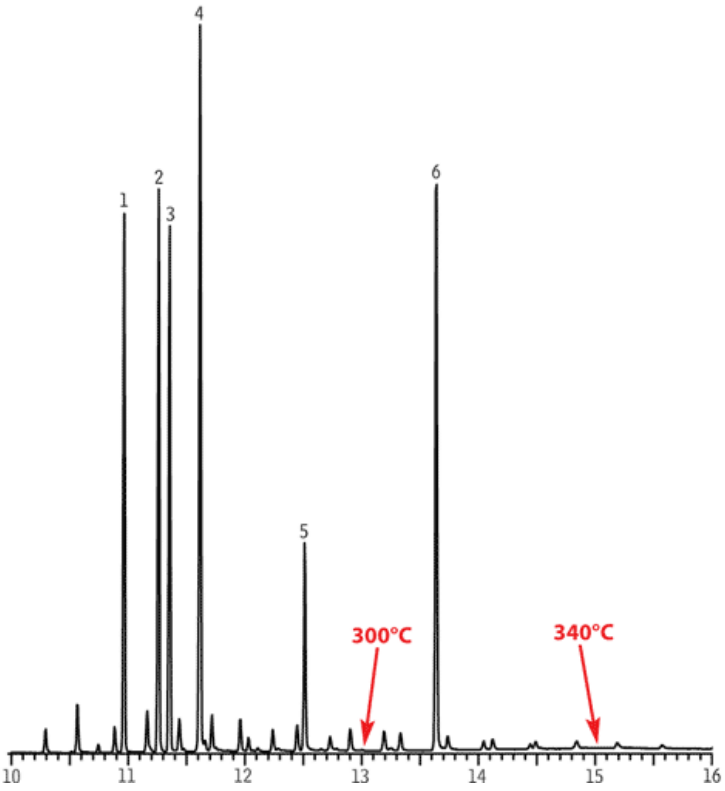


**Figure 2** A 12m x 0.20mm ID x 0.33µm Rxi™-5ms column provides baseline resolution and short analysis time for cannabinoids.

1. cannabidiol
2. $\Delta^8$-tetrahydrocannabinol
3. $\Delta^9$-tetrahydrocannabinol
4. cannabinol
5. 11-hydroxy-$\Delta^9$-tetrahydrocannabinol
6. 11-nor-$\Delta^9$-tetrahydrocannabinol carboxylic acid




GC_PH00891

| | |
|---|---|
| Column: | Rxi™-5ms 12m, 0.20mm ID, 0.33µm (cat.# 13497) |
| Sample: | 1000µg/mL each component in methanol |
| Inj.: | 1.0µL, split, split ratio 25:1, 4mm ID base-deactivated single gooseneck inlet liner w/wool (cat.# 20798-211.1) |
| Inj. temp.: | 250°C |
| Carrier gas: | helium, constant flow |
| Flow rate: | 1mL/min. |
| Oven temp.: | 40°C to 340°C @ 20°C/min. (hold 5 min.) |
| Det: | MS |
| Transfer line temp.: | 280°C |
| Scan range: | 100-550 amu |
| Ionization: | EI |
| Mode: | scan |

**References**

1. Smith, F. and J. Siegel *Handbook of Forensic Drug Analysis* Elsevier Academic Press, 2005, pp. 98-151.
2. Clouette, R., M. Jacob, P. Koteel, and M. Spain *Journal of Analytical Toxicology* 17 (1): 1-4 (Jan./Feb. 1993).

**RELATED SEARCHES**

*marijuana*, *cannabinoid metabolites*, *Rxi-5ms*, *THC*

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# Reduce Downtime and Cost of Materials with Rugged Rxi®-5Sil MS GC Columns

By Amanda Rigdon, Clinical/Forensic Innovations Chemist and Gary Stidsen, GC Columns Product Marketing Manager

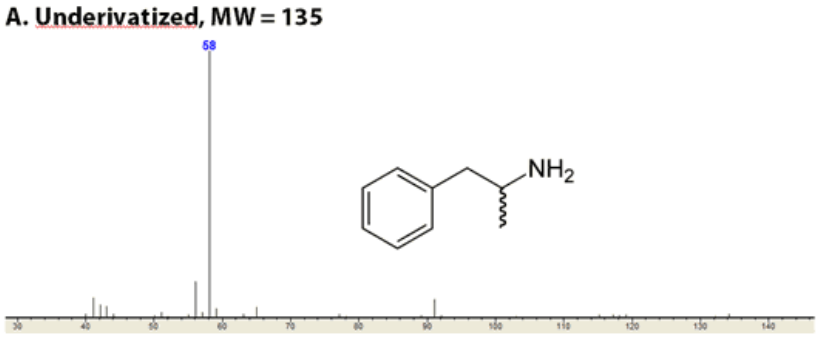
- Save costs with long column lifetime.
- Reduce downtime from column trimming and replacement.
- Improve peak shape for active compounds.

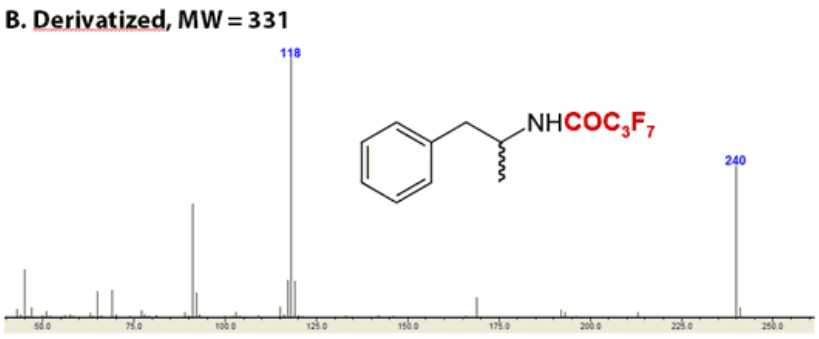
**Rugged Rxi®-5Sil MS columns produce consistent results, even under harsh conditions.**

When performing GC/MS analysis of drugs, many chemists choose to derivatize samples prior to analysis. Derivatization not only increases the volatility of some drug compounds, but it also reduces activity, resulting in improved peak shape and more accurate quantification. An additional advantage is that derivatized compounds have a higher molecular weight, thus producing more reliable mass spectra than underivatized compounds. Despite these benefits, derivatization reagents are often harsh and can damage analytical columns, leading to high bleed, significant reduction in retention times, and increased tailing for active compounds. Often, this damage is concentrated near the head of the column, so trimming a short length can improve results. However, trimming is a finite solution as repeated clipping ultimately results in decreased efficiency and shorter column lifetimes. Choosing a more rugged column, such as the Rxi®-5Sil MS column, is a better alternative. The Rxi®-5Sil MS column is extremely stable and holds up to harsh treatment, including repeated exposure to derivatization reagents.

The analysis of amphetamine illustrates the ruggedness of the arylene-based Rxi®-5Sil MS polymer. Amphetamine is typically derivatized, because the underivatized form is an active basic compound that produces only a few low molecular weight ions for monitoring. In contrast, upon derivatization, activity decreases, resulting in dramatically improved peak shape and more accurate quantitation. Additionally, several higher molecular weight ions are produced, which can be monitored for definitive identification (Figure 1).

**Figure 1** Derivatizing amphetamine results in more definitive identification by creating higher molecular weight ions.



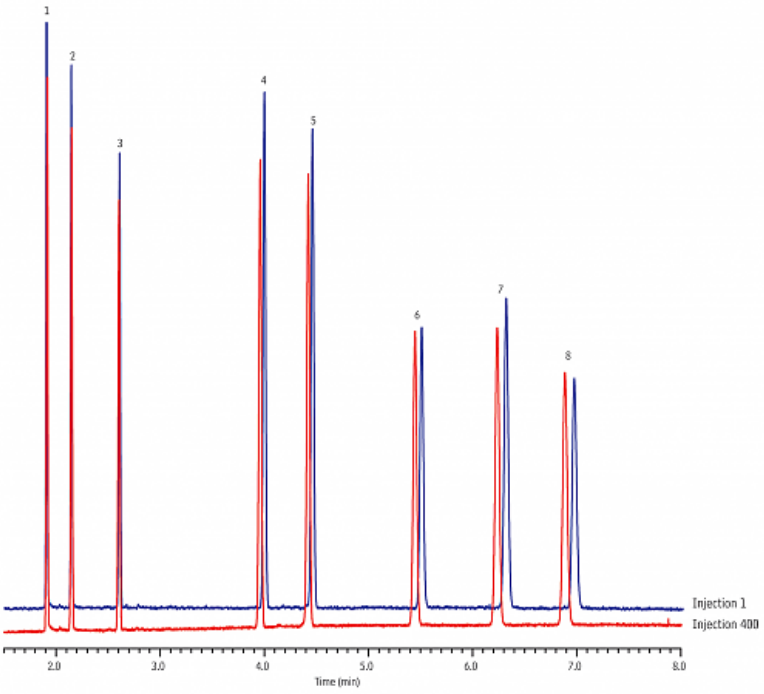

**Phase Stability Extends Column Lifetime**

In order to demonstrate the ruggedness of the Rxi®-5Sil MS column, 400 injections of heptafluorobutyric acid anhydride (HFBA) in butyl chloride were performed. HFBA is a very harsh derivatization reagent, and the concentration of reagent in the solvent was equivalent to that of a derivatized sample. Throughout the course of 400 injections, bleed, retention, and peak shape for active compounds was monitored by periodically injecting a column test mix containing active compounds (1,6-hexanediol, 4-chlorophenol, and dicyclohexylamine). Chromatographic results were remarkably consistent, even after 400 injections (Figure 2). Column bleed was monitored over the course of the experiment and remained below 5pA (Figure 3). The consistency of retention time data and low bleed levels demonstrate phase stability, which results in longer column lifetimes and reduced maintenance and replacement costs.

**Figure 2** Rugged Rxi®-5Sil MS columns produce consistent retention times, even after 400 injections of derivatization reagent.

**Peaks**

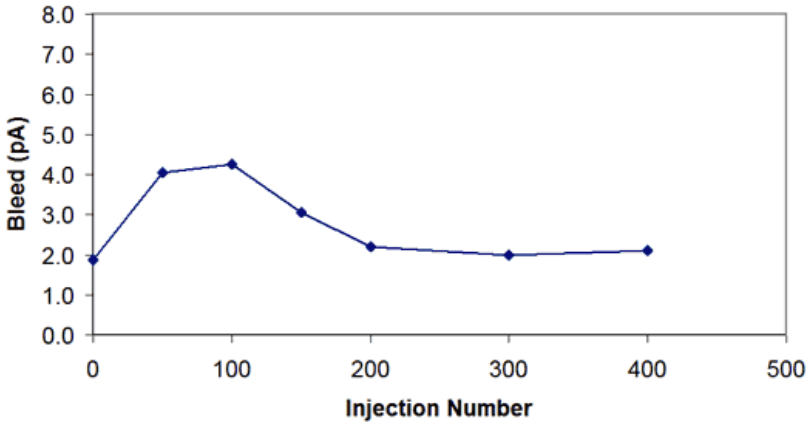
1. 2-Ethylhexanoic acid
2. 1,6-Hexanediol
3. 4-Chlorophenol
4. Tridecane
5. 1-Methylnaphthalene
6. 1-Undecanol
7. Tetradecane
8. Dicyclohexylamine




GC_CF01131

| | |
|---|---|
| **Column** | Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623) |
| **Sample** | Rxi -5Sil MS/XLB column test mix (cat.# 35226) |
| **Injection** | |
| Inj. Vol.: | 1.0 µL split (split ratio 60:1) |
| Liner: | 4 mm recessed single taper (cat.# 20983) |
| Inj. Temp.: | 250 °C |
| **Oven** | |
| Oven Temp.: | 125 °C |
| **Carrier Gas** | He, constant pressure |
| Linear Velocity: | 36 cm/sec @ 125 °C |
| **Detector** | FID @ 320 °C |
| **Instrument** | Agilent/HP6890 GC |

**Figure 3** Low column bleed results in long column lifetimes, saving labs replacement costs.



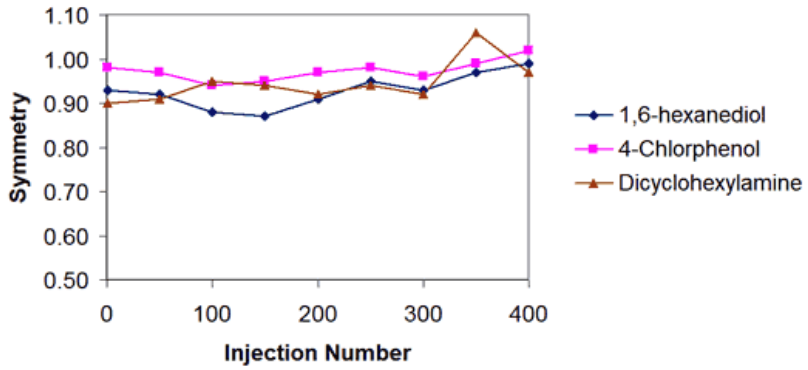

Column bleed over 400 injections of HFBA derivatization reagent.

**Symmetric Peaks for More Accurate Results**

Peak shape was also monitored to ensure column inertness was stable over time—an important factor in maintaining accuracy. Peaks for the active test probes were symmetric even after 400 injections, allowing easy identification and consistent integration (Figure 4). In a second experiment to complement the test probe results, underivatized amphetamine was injected onto a new Rxi®-5Sil MS column, an Rxi®-5Sil MS column after 400 injections of derivatization reagent, and a new competitor column of equivalent phase chemistry. Even though underivatized amphetamine is highly active, peak symmetry on the Rxi®-5Sil MS column was consistent and unaffected by exposure of the column to derivatization reagent (Figure 5).

Additionally, peak shape on both the exposed and unexposed Rxi®-5Sil MS column was better than that on the new competitor column (Figure 5).

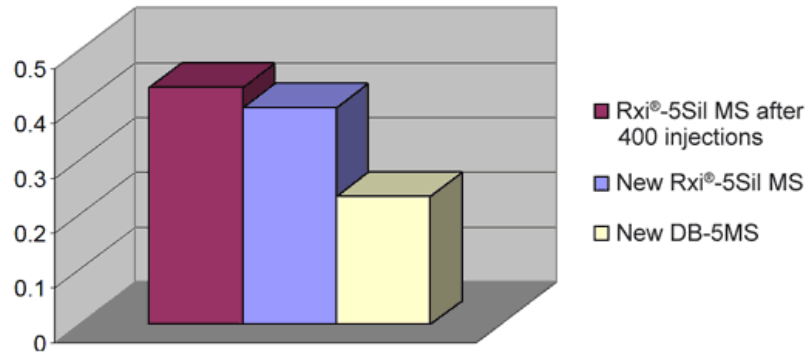
**Figure 4** Active probes show consistent, symmetric peak shape, demonstrating the inertness needed for accurate quantification.




Test probe symmetry over 400 injections of HFBA derivatization reagent.

Symmetry values <1 indicate peak tailing and values >1 indicate fronting.

**Figure 5** Peak symmetry for underivatized amphetamine is significantly better on an Rxi®-5Sil MS than on a competitor column, even after 400 injections of HFBA derivatization reagent.




Symmetry values <1 indicate peak tailing and values >1 indicate fronting.

The rugged arylene phase of the Rxi®-5Sil MS column results in highly stable performance, even under the most demanding of analytical conditions, and its exceptional inertness ensures good peak shape for reproducible quantitation. The stability of the Rxi®-5Sil MS column results in longer column lifetimes, reducing both downtime and replacement costs.

**RELATED SEARCHES**

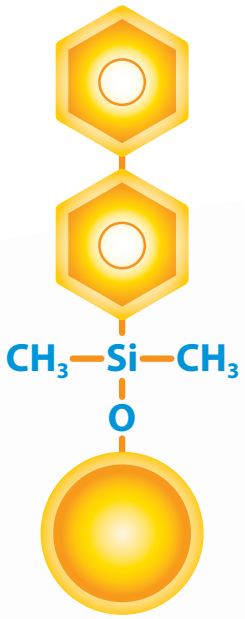
*amphetamine*, *derivitization*, *derivitize*, *HFBA*, *heptafluorobutyric acid anhydride*, *Rxi-5Sil MS*

Stationary Phase: **Biphenyl**

Raptor™
LC Columns

*Selectivity Accelerated*

# Fast, Rugged Raptor™ Columns with Time-Tested Selectivity





RESTEK® Pure Chromatography

www.restek.com/raptor

# The Raptor™ Biphenyl Column

With Raptor™ LC columns, Restek chemists became the first to combine the speed of superficially porous particles (also known as SPP or "core-shell" particles) with the resolution of highly selective USLC® technology. This new breed of chromatographic column allows you to more easily achieve peak separation and faster analysis times without expensive UHPLC instrumentation.

Our top priority when developing our new SPP line was to create a version of our innovative Biphenyl. The industry-leading Biphenyl is Restek's most popular LC stationary phase because it is particularly adept at separating compounds that are hard to resolve or that elute early on C18 and other phenyl chemistries. As a result, the rugged Raptor™ Biphenyl column is extremely useful for fast separations in bioanalytical testing applications like drug and metabolite analyses, especially those that require a mass spectrometer (MS). Increasing retention of early-eluting compounds can limit ionization suppression, and the heightened selectivity helps eliminate the need for complex mobile phases that are not well suited for MS detection.

In 2005, Restek was the first to bring you the benefits of the Biphenyl ligand, and we have the experience to maximize the SPP performance of this premier phenyl chemistry for today's challenging workflows.

## Column Description:


**Stationary Phase Category:**
Phenyl (L11)

**Ligand Type:**
Biphenyl

**Particle:**
2.7 µm or 5 µm superficially porous silica (SPP or "core-shell")

**Pore Size:**
90 Å

**Surface Area:**
150 $m^2/g$ (2.7 µm)
or 100 $m^2/g$ (5 µm)

**Recommended Usage:**
pH Range: 1.5–8.0

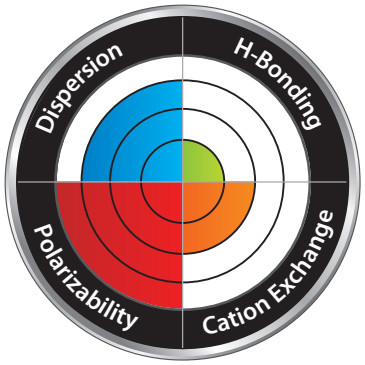
Maximum Temperature: 80 °C

Maximum Pressure: 600 bar / 8,700 psi (2.7 µm)
or 400 bar / 5,800 psi (5 µm)

**Properties:**
- Increased retention for dipolar, unsaturated, or conjugated solutes.
- Enhanced selectivity when used with methanolic mobile phase.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.

**Switch to a Biphenyl when:**
- Limited selectivity is observed on a C18.
- You need to increase retention of hydrophilic aromatics.

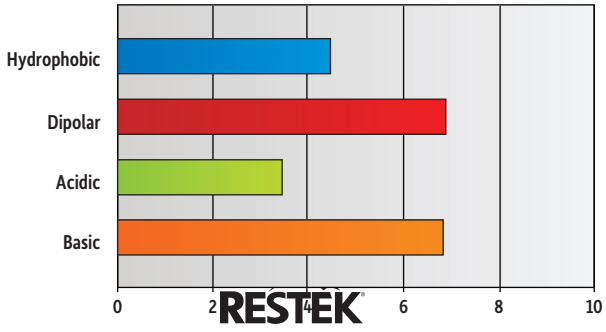
## Column Interaction Profile:

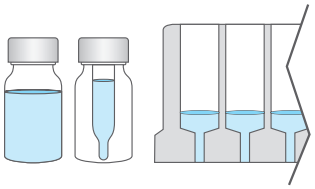

**Defining Solute Interactions:**
- Polarizability
- Dispersion

**Complementary Solute Interaction:**
- Cation exchange

## Solute Retention Profile:




**Target Analyte Structures:**
- Aromatic
- Dipolar



**Target Analyte Functionalities:**
- Hydrophilic aromatics
- Strong dipoles
- Lewis acids
- Dipolar, unsaturated, or conjugated compounds
- Fused-ring compounds with electron withdrawing groups

## More Aromatic Selectivity than Ordinary Phenyl-Hexyls

SPP core-shell columns commonly employ traditional phenyl-hexyl stationary phases, but the innovative Biphenyl ligand, developed by Restek's chemists, is the next generation of phenyl column chemistry. It provides greater aromatic selectivity than commercially available phenyl-hexyl columns [1] and a greater degree of dispersion than conventional phenyls. As a result, the Raptor™ Biphenyl allows you to more easily separate bioanalytical compounds like aromatics (Figures 1 and 2), which elute early or are hard to separate on C18 or other phenyl chemistries.

[1] In-house testing based on: M. R. Euerby, P. Petersson, W. Campbell, W. Roe, Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns containing phenyl moieties using principal component analysis, J. Chromatogr. A 1154 (2007) 138–151.

**Figure 1:** Raptor™ Biphenyl columns exhibit the highest aromatic selectivity compared to other SPP phenyl columns.

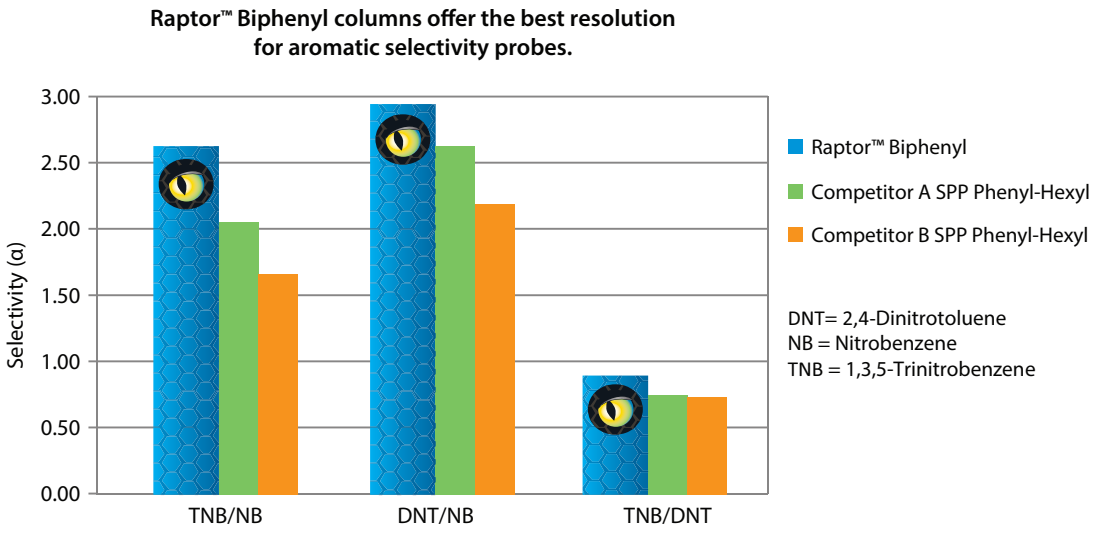
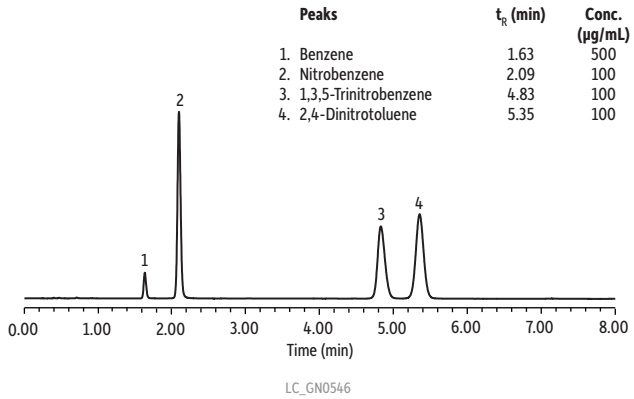



**Figure 2:** Raptor™ Biphenyl columns show increased retention for compounds containing electron withdrawing groups. Retention and elution order are dramatically different from a traditional C18.




**Column:** Raptor™ Biphenyl (cat.# 9309A55); Dimensions: 50 mm x 4.6 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Temp.: 40 °C; **Sample:** Diluent: acetonitrile; Conc.: 100-500 µg/mL; Inj. Vol.: 1 µL **Mobile Phase:** water: methanol (50:50); Flow: 1.2 mL/min; **Detector:** Waters Acquity® PDA @ 254 nm; **Instrument:** Waters Acquity® UPLC H-Class.

# The New Standard for Performance and Durability for SPP Core-Shell Columns

## Pressure Stability:

One of the greatest advantages of an SPP column is the ability to achieve fast, efficient separations by operating at higher linear velocities than are possible with a conventional fully porous particle column. However, these higher velocities can also result in higher back pressures. Raptor™ columns were designed to handle the increased pressures needed to achieve *Selectivity Accelerated*, and handle it far better than other SPP columns on the market (Figure 3).

## Reproducibility:

To help keep your productivity high and your lab budget low, we know that Raptor™ Biphenyl columns must produce exceptional selectivity and fast analysis times not just once, but every time. Ruggedness and repeatability are essential, which is why from the silica and the bonding technique, to the packing process and upgraded hardware, every decision that went into creating this column was made to ensure superlative reproducibility, from injection to injection (Figure 4) and from lot to lot (Figure 5). We also adopted new quality control (QC) specifications to guarantee the retention time stability you need for worry-free MRM analyses.

**Figure 3:** At high pressures, competitor phenyl-hexyl columns experience a quick and sharp drop-off in efficiency, but Raptor™ Biphenyl columns are unaffected to at least 3,000 injections.

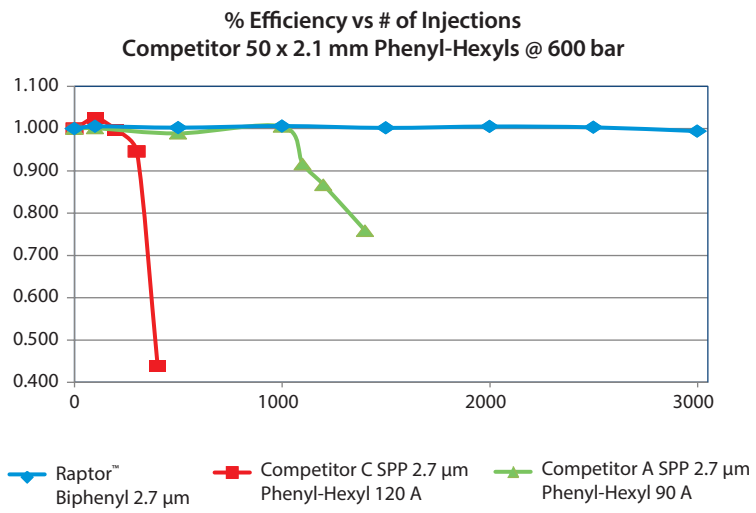
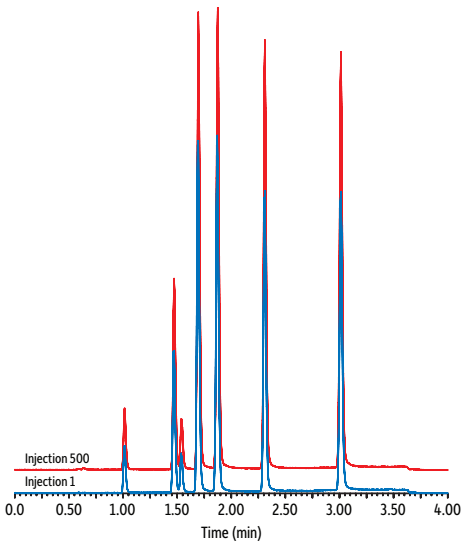


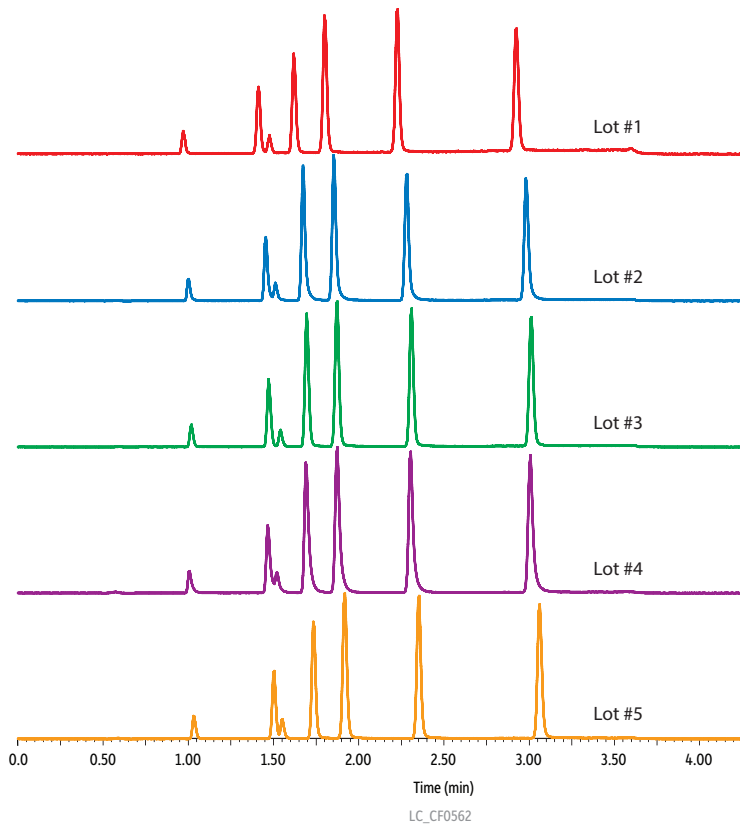

**Figure 4:** Even after hundreds of injections, a Raptor™ Biphenyl column will provide consistent, reliable data.




**Peaks**
1. Cortisol
2. 11-Deoxycortisol
3. Estradiol
4. Boldenone
5. Testosterone
6. Androstenedione
7. Progesterone

**Column:** Raptor™ Biphenyl (cat.# 9309A1E); Dimensions: 100 mm x 3.0 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Temp.: 30 °C; **Sample:** Diluent: initial mobile phase; Conc.: 50 ng/mL; Inj. Vol.: 5 µL **Mobile Phase:** A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile; **Gradient (%B):** 0.00 min (40%), 3.00 min (80%), 3.01 min (40%), 5.00 min (40%); **Flow:** 0.700 mL/min; **Detector:** Waters Xevo TQ-S; Ion Mode: ESI+; **Instrument:** Waters.

**Figure 5:** From one lot to the next, every Raptor™ Biphenyl column you purchase will perform the same.




**See Figure 4 for compound list and conditions.**

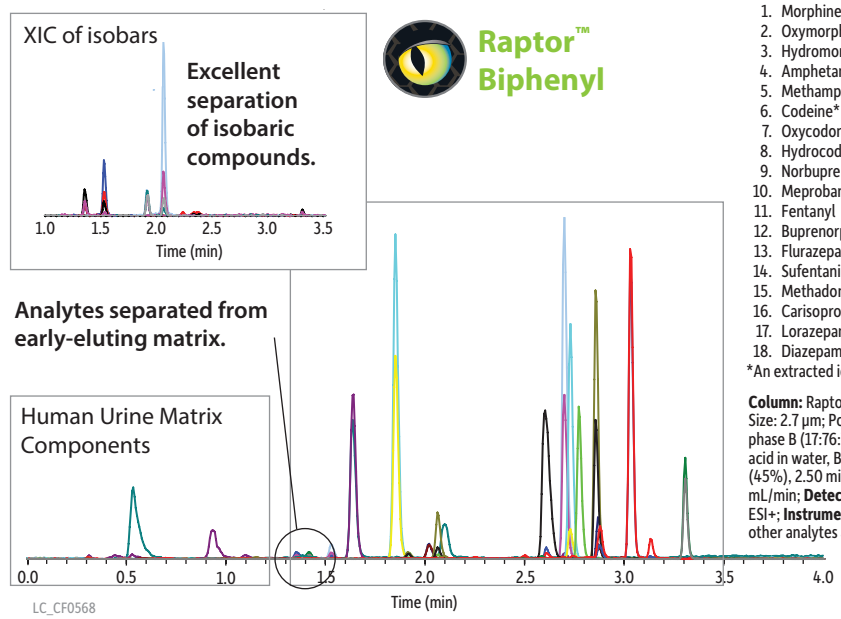
## Clinically Proven to Optimize Your Bioanalytical Workflows

For over a decade, the Restek® Biphenyl has been the column of choice for clinical testing because of its ability to provide highly retentive, selective, and rugged reversed-phase separations of drugs and metabolites. By bringing the speed of SPP to the Biphenyl family, the Raptor™ Biphenyl provides clinical labs with an even faster option for a wide variety of clinical assays.

### Rugged Pain Panels from Urine in Under 3.5 Minutes

Pain panels can be difficult to optimize and reproduce due to the limited selectivity of C18 and phenyl-hexyl phases, but not on the Raptor™ Biphenyl. Complete your pain panel analysis with a 5-minute cycle time and complete isobaric resolution using Raptor™ Biphenyl columns (Figure 6). Popular competitor columns offer tailing peaks, longer run times, and coelutions; the Raptor™ Biphenyl exhibits the selectivity and performance needed for this critical analysis.

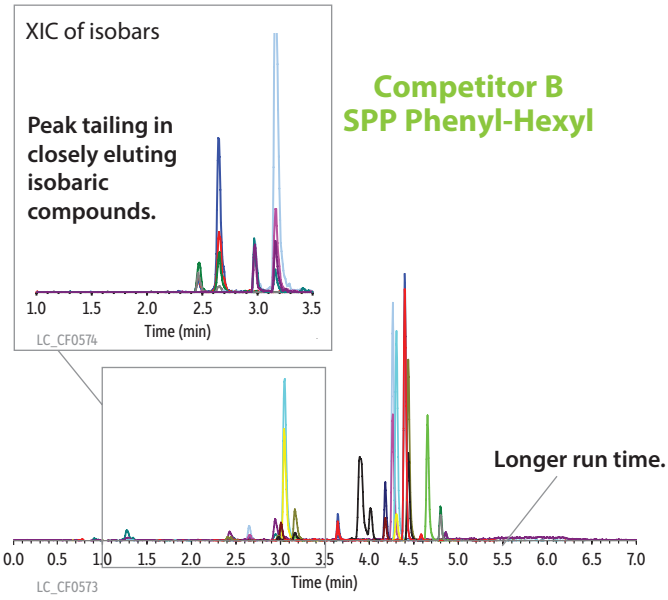
**Figure 6:** Raptor™ Biphenyl columns offer pain panel analyses with complete isobaric resolution in under 5 minutes!



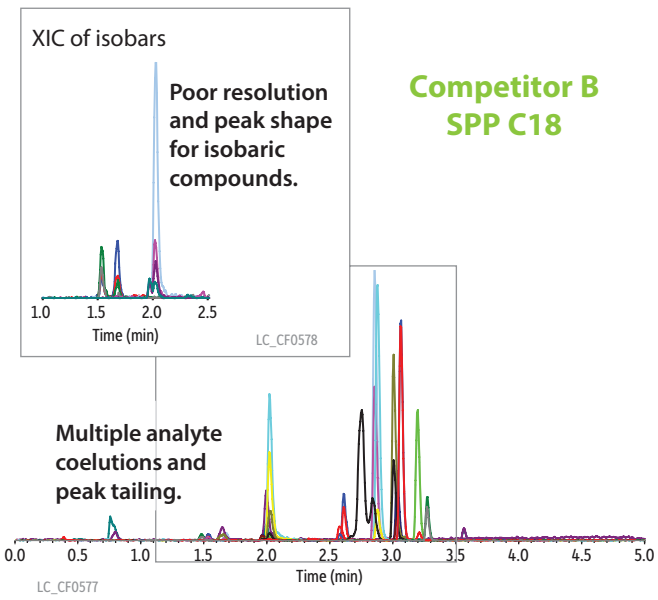

| | Peaks | $t_R$ (min) | Precursor ion | Product ion 1 | Product ion 2 |
|---|---|---|---|---|---|
| 1. | Morphine* | 1.34 | 286.2 | 152.3 | 165.3 |
| 2. | Oxymorphone | 1.40 | 302.1 | 227.3 | 198.2 |
| 3. | Hydromorphone* | 1.52 | 286.1 | 185.3 | 128.2 |
| 4. | Amphetamine | 1.62 | 136.0 | 91.3 | 119.2 |
| 5. | Methamphetamine | 1.84 | 150.0 | 91.2 | 119.3 |
| 6. | Codeine* | 1.91 | 300.2 | 165.4 | 153.2 |
| 7. | Oxycodone | 2.02 | 316.1 | 241.3 | 256.4 |
| 8. | Hydrocodone* | 2.06 | 300.1 | 199.3 | 128.3 |
| 9. | Norbuprenorphine | 2.59 | 414.1 | 83.4 | 101.0 |
| 10. | Meprobamate | 2.61 | 219.0 | 158.4 | 97.2 |
| 11. | Fentanyl | 2.70 | 337.2 | 188.4 | 105.2 |
| 12. | Buprenorphine | 2.70 | 468.3 | 396.4 | 414.5 |
| 13. | Flurazepam | 2.73 | 388.2 | 315.2 | 288.3 |
| 14. | Sufentanil | 2.77 | 387.2 | 238.5 | 111.3 |
| 15. | Methadone | 2.86 | 310.2 | 265.3 | 105.3 |
| 16. | Carisoprodol | 2.87 | 261.2 | 176.3 | 158.1 |
| 17. | Lorazepam | 3.03 | 321.0 | 275.4 | 303.1 |
| 18. | Diazepam | 3.31 | 285.1 | 193.2 | 153.9 |

*An extracted ion chromatogram (XIC) of the isobars is presented in the inset.

**Column:** Raptor™ Biphenyl (cat.# 9309A5E); Dimensions: 50 mm x 3.0 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Temp.: 30 °C; **Sample:** Diluent: urine:mobile phase A:mobile phase B (17:76:7); Conc.: 10-100 ng/mL; Inj. Vol.: 10 µL **Mobile Phase:** A: 0.1% formic acid in water, B: 0.1% formic acid in methanol; **Gradient (%B):** 0.00 min (10%), 1.50 min (45%), 2.50 min (100%), 3.70 min (100%), 3.71 min (10%) 5.00 min (10%); **Flow:** 0.6 mL/min; **Detector:** AB SCIEX API 4000™ MS/MS; Ion Source: TurboIonSpray®; Ion Mode: ESI+; **Instrument:** API LC-MS/MS; **Notes:** Lorazepam was prepared at 100 ng/mL; all other analytes are 10 ng/mL.




**Column:** Competitor B SPP C18; Dimensions: 50 mm x 4.6 mm ID; Particle Size: 2.6 µm; Pore Size: 100 Å; Temp.: 22 °C; **Sample:** Diluent: urine:mobile phase A:mobile phase B (17:76:7); Conc.: 10-100 ng/mL; Inj. Vol.: 10 µL **Mobile Phase:** A: 10 mM ammonium formate in water, B: 0.1% formic acid in methanol; **Gradient (%B):** 0.00 min (5%), 4.00 min (100%), 5.00 min (100%), 5.10 min (5%), 7.00 min (5%); **Flow:** 0.6 mL/min; **Detector:** AB SCIEX API 4000™ MS/MS; Ion Source: TurboIonSpray®; Ion Mode: ESI+; **Instrument:** API LC-MS/MS; **Notes:** Lorazepam was prepared at 100 ng/mL; all other analytes are 10 ng/mL. **Note:** Column and conditions used were specifically recommended or published by the manufacturer for this assay.

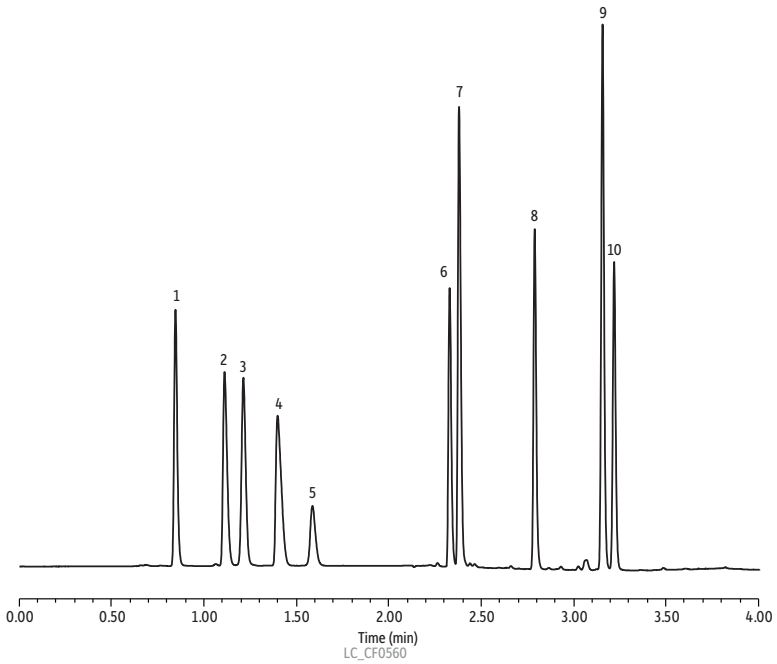



**Column:** Competitor B SPP C18; Dimensions: 50 mm x 3.0 mm ID; Particle Size: 2.6 µm; Pore Size: 100 Å; Temp.: 25 °C; **Sample:** Diluent: urine:mobile phase A:mobile phase B (17:76:7); Conc.: 10-100 ng/mL; Inj. Vol.: 10 µL **Mobile Phase:** A: 10 mM ammonium formate in water, B: 0.1% formic acid in methanol; **Gradient (%B):** 0.00 min (5%), 3.00 min (100%), 4.00 min (100%), 4.10 min (5%), 5.00 min (5%); **Flow:** 0.5 mL/min; **Detector:** AB SCIEX API 4000™ MS/MS; Ion Source: TurboIonSpray®; Ion Mode: ESI+; **Instrument:** API LC-MS/MS; **Notes:** Lorazepam was prepared at 100 ng/mL; all other analytes are 10 ng/mL. **Note:** Column and conditions used were specifically recommended or published by the manufacturer for this assay.

## Catecholamines and NSAIDs Without Ion Pairing, HILIC, or Complex Mobile Phases

Analyzing catecholamine compounds can be problematic by liquid chromatography and often forces chemists to turn to aqueous normal phase / HILIC or ion-pairing reagents that are not well suited for mass spectrometry (MS). Raptor™ Biphenyl columns easily retain and separate these difficult compounds using simple, MS-friendly mobile phases in a time frame that maximizes your productivity (Figure 7). Raptor™ Biphenyl also offers fast, efficient analysis of nonsteroidal anti-inflammatory drugs (NSAIDs) with LC-MS friendly solvents.

**Figure 7:** Separate catecholamine and other neurotransmitter compounds fast without ion pairing or HILIC.

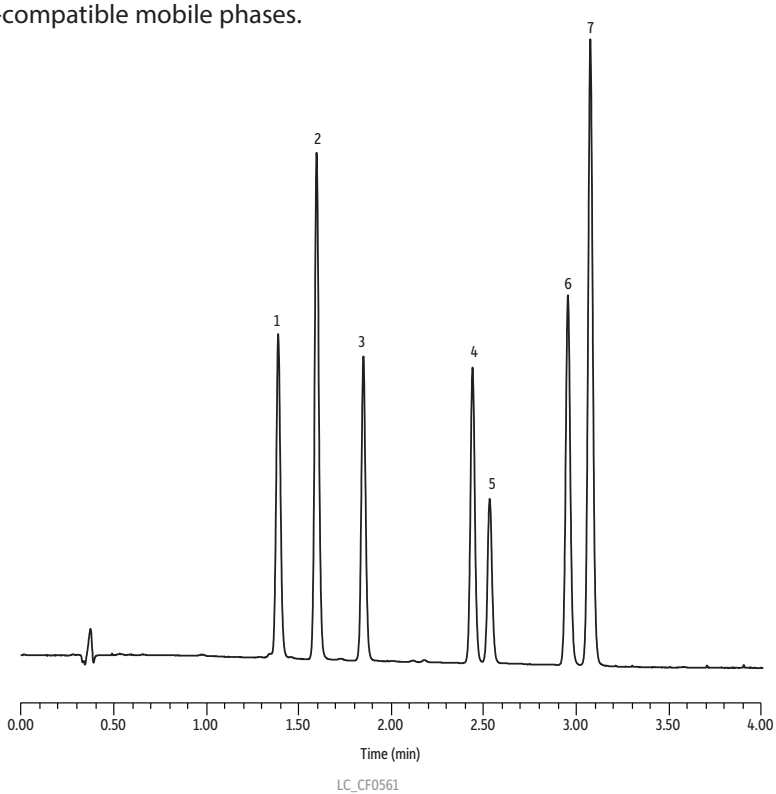



| Peaks | $t_R$ (min) |
|---|---|
| 1. Norepinephrine | 0.85 |
| 2. Epinephrine | 1.11 |
| 3. L-Dihydroxyphenylalanine | 1.21 |
| 4. Dopamine | 1.40 |
| 5. Tyrosine | 1.59 |
| 6. 3-Methoxytyramine* | 2.33 |
| 7. Serotonin | 2.38 |
| 8. (3,4-Dihydroxyphenyl)acetic acid* | 2.79 |
| 9. 5-Hydroxyindoleacetic acid** | 3.16 |
| 10. Homovanillic acid* | 3.22 |

* Dopamine metabolite, ** serotonin metabolite

**Column:** Raptor™ Biphenyl (cat.# 9309A62); Dimensions: 150 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Temp.: 30 °C; **Sample:** Diluent: water; Conc.: 50 µg/mL; Inj. Vol.: 1 µL **Mobile Phase:** A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile; **Gradient (%B):** 0.00 min (0%), 4.00 min (70%), 4.01 min (0%), 5.00 min (0%); **Flow:** 0.4 mL/min; **Detector:** Waters Acquity® PDA @ 280 nm; **Instrument:** Waters Acquity® UPLC H-Class.

**Figure 8:** Nonsteroidal anti-inflammatory drugs (NSAIDs) are also easily resolved with Raptor™ Biphenyl using UV- and MS-compatible mobile phases.



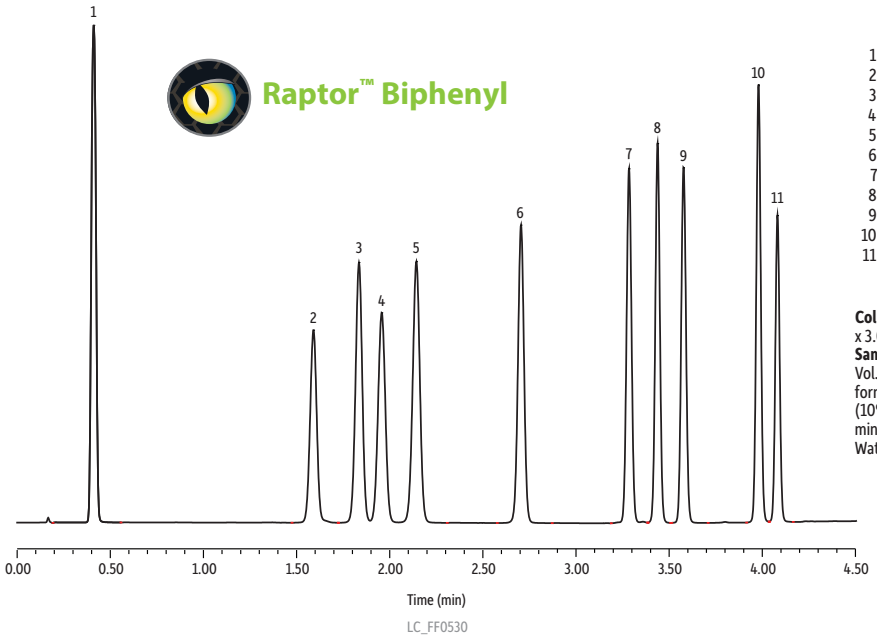

| Peaks | $t_R$ (min) | Conc. (µg/mL) |
|---|---|---|
| 1. Piroxicam | 1.39 | 25 |
| 2. Sulindac | 1.60 | 25 |
| 3. Naproxen | 1.85 | 25 |
| 4. Fenoprofen | 2.44 | 100 |
| 5. Ibuprofen | 2.54 | 200 |
| 6. Diclofenac | 2.96 | 25 |
| 7. Indomethacin | 3.08 | 25 |

**Column:** Raptor™ Biphenyl (cat.# 9309A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Temp.: 40 °C; **Sample:** Diluent: water:acetonitrile (65:35); Conc.: 25-200 µg/mL; Inj. Vol.: 2 µL **Mobile Phase:** A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile; **Gradient (%B):** 0.00 min (35%), 4.00 min (60%), 4.01 min (35%), 5.00 min (35%); **Flow:** 0.6 mL/min; **Detector:** Waters Acquity® PDA @ 270 nm; **Instrument:** Waters Acquity® UPLC H-Class.

## Fast Analysis of Sulfur Antibiotics Without Coelutions

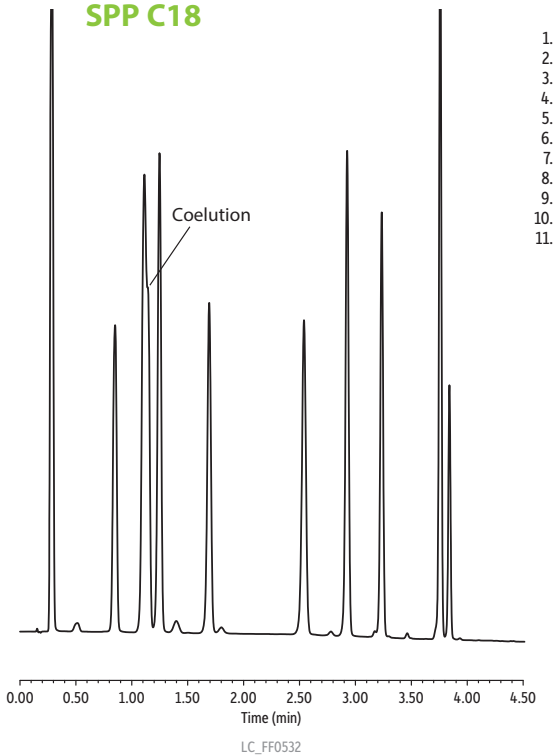
Even with high-efficiency UHPLC particles, C18 and ordinary phenyl columns fail to achieve baseline separation of sulfonamides. Not only does the Raptor™ Biphenyl have the selectivity to easily and completely separate these difficult compounds (Figure 9), it does so in well under 5 minutes!

**Figure 9:** Sulfonamides pose no problems for analysis, even at high linear velocities. Increased retention of early-eluting sulfanilamide also helps limit ionization suppression.

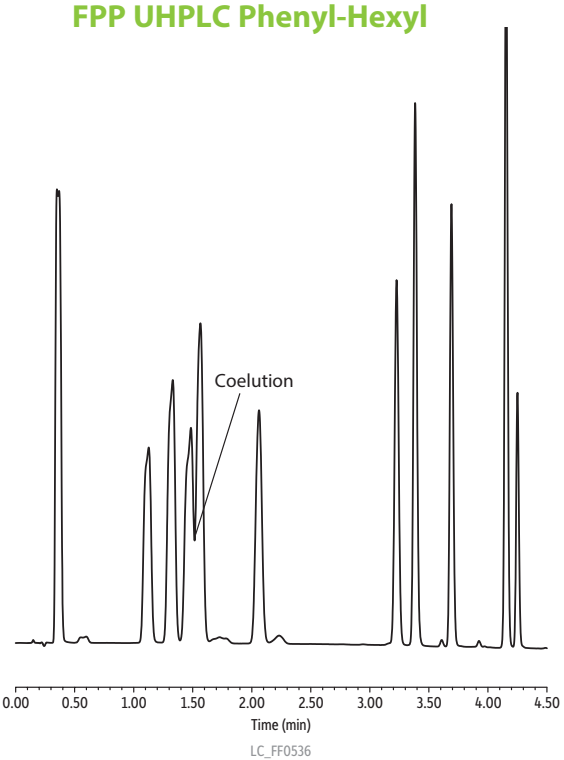



| Peaks | $t_R$ (min) |
|---|---|
| 1. Sulfanilamide | 0.41 |
| 2. Sulfadiazine | 1.59 |
| 3. Sulfapyridine | 1.84 |
| 4. Sulfathiazole | 1.96 |
| 5. Sulfamerazine | 2.14 |
| 6. Sulfamethazine | 2.71 |
| 7. Sulfachlorpyridazine | 3.29 |
| 8. Sulfadoxine | 3.44 |
| 9. Sulfisoxazole | 3.58 |
| 10. Sulfadimethoxine | 3.98 |
| 11. Sulfaquinoxaline | 4.08 |

**Column:** Raptor™ Biphenyl (cat.# 9309A5E); Dimensions: 50 mm x 3.0 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Temp.: 20 °C; **Sample:** Diluent: 0.1% formic acid in water; Conc.: 50 µg/mL; Inj. Vol.: 5 µL **Mobile Phase:** A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile; **Gradient (%B):** 0.00 min (5%), 2.00 min (10%), 4.50 min (35%), 4.51 min (5%), 5.50 min (5%); **Flow:** 1.5 mL/min; **Detector:** Waters Acquity® PDA @ 260, 4.8 nm; **Instrument:** Waters Acquity® UPLC H-Class.




| Peaks |
|---|
| 1. Sulfanilamide |
| 2. Sulfadiazine |
| 3. Sulfapyridine |
| 4. Sulfathiazole |
| 5. Sulfamerazine |
| 6. Sulfamethazine |
| 7. Sulfachlorpyridazine |
| 8. Sulfadoxine |
| 9. Sulfisoxazole |
| 10. Sulfadimethoxine |
| 11. Sulfaquinoxaline |




**Column:** Competitor B SPP C18; Dimensions: 50 mm x 3.0 mm ID; Particle Size: 2.6 µm; Pore Size: 100 Å; Temp.: 20 °C; **Sample:** Diluent: 0.1% formic acid in water; Conc.: 50 µg/mL; Inj. Vol.: 5 µL **Mobile Phase:** A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile; **Gradient (%B):** 0.00 min (5%), 2.00 min (10%), 4.50 min (35%), 4.51 min (5%), 5.50 min (5%); **Flow:** 1.5 mL/min; **Detector:** Waters Acquity® PDA @ 260, 4.8 nm; **Instrument:** Waters Acquity® UPLC H-Class.

**Column:** Competitor D FPP Phenyl-Hexyl; Dimensions: 50 mm x 2.1 mm ID; Particle Size: 1.7 µm; Temp.: 20 °C; **Sample:** Diluent: 0.1% formic acid in water; Conc.: 50 µg/mL; Inj. Vol.: 5 µL **Mobile Phase:** A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile; **Gradient (%B):** 0.00 min (5%), 2.00 min (10%), 4.50 min (35%), 4.51 min (5%), 5.50 min (5%); **Flow:** 0.75 mL/min; **Detector:** Waters Acquity® PDA @ 260, 4.8 nm; **Instrument:** Waters Acquity® UPLC H-Class. **Note:** Flow rate scaled to particle size

# Accelerated Performance and Time-Tested Biphenyl Selectivity for Clinical Diagnostic, Pain, Pharma, and Environmental Labs

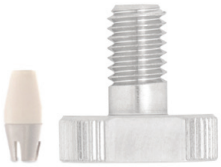
## Raptor™ Biphenyl LC Columns


| Length | 2.1 mm cat.# | 3.0 mm cat.# | 4.6 mm cat.# |
|---|---|---|---|
| **2.7 µm Columns** | | | |
| 30 mm | 9309A32 | 9309A3E | 9309A35 |
| 50 mm | 9309A52 | 9309A5E | 9309A55 |
| 100 mm | 9309A12 | 9309A1E | 9309A15 |
| 150 mm | 9309A62 | 9309A6E | 9309A65 |
| **5 µm Columns** | | | |
| 30 mm | — | 930953E | — |
| 50 mm | 9309552 | 930955E | 9309555 |
| 100 mm | 9309512 | 930951E | 9309515 |
| 150 mm | 9309562 | 930956E | 9309565 |
| 250 mm | — | — | 9309575 |

## EXP® Reusable Fittings for HPLC & UHPLC

for 10-32 fittings and 1/16" tubing

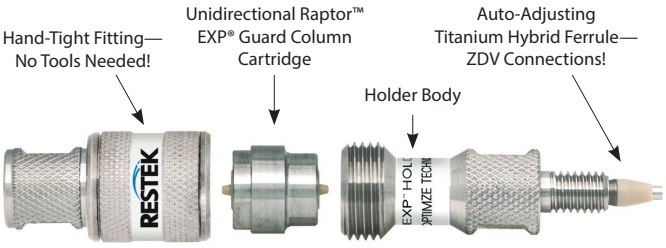
Effortlessly achieve 8,700+ psi HPLC seals by hand! (Wrench-tighten to 20,000+ psi.) Hybrid titanium/PEEK seal can be installed repeatedly without compromising your seal.



| Description | qty. | cat.# |
|---|---|---|
| EXP Hand-Tight Fitting (Nut w/Ferrule) | ea. | 25937 |
| EXP Hand-Tight Fitting (Nut w/Ferrule) | 10-pk. | 25938 |
| EXP Hand-Tight Nut (w/o Ferrule) | ea. | 25939 |

Hybrid Ferrule U.S. Patent No. 8201854, Optimize Technologies. Optimize Technologies EXP Holders are Patent Pending. Other U.S. and Foreign Patents Pending. The EXP, Free-Turn, and the Opti- prefix are registered trademarks of Optimize Technologies, Inc.

## Raptor™ EXP® Guard Cartridges




Protect your investment and extend the life of our already-rugged LC columns and change guard column cartridges by hand without breaking fluid connections—no tools needed!

### EXP® Direct Connect Holder

| Description | qty. | cat.# |
|---|---|---|
| EXP Direct Connect Holder for EXP Guard Cartridges (includes hex-head fitting & 2 ferrules) | ea. | 25808 |

### Raptor™ EXP® Guard Column Cartridges

| Description | Particle Size | qty. | 5 x 2.1 mm cat.# | 5 x 3.0 mm cat.# | 5 x 4.6 mm cat.# |
|---|---|---|---|---|---|
| Raptor Biphenyl EXP Guard Cartridge | 2.7 µm | 3-pk. | 9309A0252 | 9309A0253 | 9309A0250 |
| Raptor Biphenyl EXP Guard Cartridge | 5 µm | 3-pk. | 930950252 | 930950253 | 930950250 |

Maximum cartridge pressure: 600 bar / 8,700 psi (2.7 µm) or 400 bar / 5,800 psi (5 µm)

Raptor™ SPP LC columns combine the speed of SPP with the resolution of USLC® technology. Learn more at **www.restek.com/raptor**

Experience *Selectivity Accelerated.* Order the Raptor™ Biphenyl today at **www.restek.com/raptor**

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Clinical, Forensic & Toxicology Applications

# Rapid and Accurate LC-MS/MS Analysis of Nicotine and Related Compounds in Urine Using Raptor™ Biphenyl LC Columns and MS-Friendly Mobile Phases

By Shun-Hsin Liang, PhD


## Abstract

A rapid, accurate, and reproducible method was developed for high-throughput testing of nicotine, cotinine, *trans*-3'-hydroxycotinine, nornicotine, norcotinine, and anabasine in urine. Data show that a fast and highly efficient analysis of these basic compounds can be achieved with the Raptor™ Biphenyl column using standard low-pH, reversed-phase LC-MS mobile phases that are compatible with a variety of LC-MS instrumentation.


## Introduction

Nicotine is the major tobacco alkaloid that underlies addiction in tobacco users. Anabasine and anatabine are the most abundant minor alkaloids in tobacco [1]. Anabasine is frequently used as a unique marker for recent tobacco use as it can only be detected in the urine of tobacco users and is not present in the urine of those who use nicotine replacement therapies (e.g., nicotine patches). In humans, more than 70% of nicotine is transformed to cotinine, which is subsequently converted to *trans*-3'-hydroxycotinine, the main nicotine metabolite detected in urine [2]. Nornicotine and norcotinine are minor metabolites (0.5–2%) produced by the demethylation of nicotine and cotinine, respectively. The urinary measurement of nicotine metabolites has several applications, including public tobacco exposure monitoring, nicotine replacement therapy evaluation, drug therapy assessment, forensic toxicology analysis, and life or health insurance application. In addition, nicotine metabolites can be used as the biomarkers for pharmacogenomics evaluation and disease profiling [3].

A variety of chromatographic methods have been developed for nicotine metabolite analysis. However, most methods use high-pH chromatography with relatively high concentrations of additives to increase retention, improve peak shape, and reduce peak tailing. The intent of this application was to develop a method for the analysis of nicotine-related compounds in urine using solutions that are "friendly" to LC-MS/MS systems. A Raptor™ Biphenyl column was chosen as the analytical column because it provides good retention and peak shape for the target analytes when used with standard low-pH, reversed-phase mobile phases. The clinical applicability of this method was demonstrated by the accurate and reproducible analysis of fortified analytes in urine.

## Experimental

*Instrument and Analytical Conditions*

The instrument and analytical conditions are listed in Table I. The analyte MRMs are shown in Table II.

**Table I:** Analytical Conditions for Waters Xevo™ TQ-S with Acquity UPLC®

| | | |
|---|---|---|
| **Analytical Column** | Raptor Biphenyl 5 µm<br>100 mm x 2.1 mm (cat.# 9309512) | |
| **Guard Column** | Raptor Biphenyl 5 µm<br>5 µm, 5 mm x 2.1 mm (cat.# 930950252)<br>EXP® direct connect holder (cat.# 25808) | |
| **Injection Volume** | 5 µL | |
| **Mobile Phase A** | 0.1% Formic acid, 5 mM ammonium formate in water | |
| **Mobile Phase B** | 0.1% Formic acid in methanol | |
| **Gradient** | **Time (min)** | **%B** |
| | 0.0 | 10 |
| | 1.0 | 10 |
| | 2.0 | 30 |
| | 3.0 | 70 |
| | 3.01 | 10 |
| | 5.0 | 10 |
| **Flow Rate** | 0.4 mL/min | |
| **Column Temp.** | 30 °C | |
| **Ion Mode** | Positive ESI | |
| **Capillary Voltage** | 1.0 kV | |
| **Gas Flow** | 1,000 (L/Hr) desolvation | |
| | 150 (L/Hr) cone | |
| | 7.0 (bar) nebulizer | |
| **Desolvation Temp.** | 450 °C | |

**Table II:** Analyte MS/MS Transitions

| Analyte | Precursor Ion | Product Ion Quantifier | Product Ion Qualifier |
|---|---|---|---|
| **Nornicotine** | 149.10 | 80.05 | 105.89 |
| **Nornicotine-D4** | 153.17 | 84.03 | - |
| **Norcotinine** | 163.09 | 80.05 | 135.10 |
| **Norcotinine-$^{13}C_3$** | 166.22 | 80.05 | - |
| **Nicotine** | 163.15 | 132.10 | 117.07 |
| **Nicotine-D4** | 167.16 | 136.64 | - |
| **Cotinine** | 177.12 | 98.07 | 146.09 |
| **Cotinine-D3** | 180.20 | 101.07 | - |
| ***trans*-3'-Hydroxycotinine** | 193.12 | 80.05 | 134.12 |
| ***trans*-3'-Hydroxycotinine-D3** | 196.19 | 80.05 | - |
| **Anabasine** | 163.13 | 91.63 | 120.12 |
| **Anabasine-D4** | 167.20 | 96.19 | - |

*Sample/Calibration Standard Preparation*

Blank urine from an unexposed non-tobacco user was fortified to prepare calibration standards and fortified QC samples. A 5,000 ng/mL standard mix was prepared in urine and diluted with urine to make calibration standards at 2, 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, and 5,000 ng/mL (only continine and *trans*-3'-hydroxycotinine were tested at 2,500 and 5,000 ng/mL levels). Three levels of QC samples (7.5, 75, and 750 ng/mL for nicotine, nornicotine, norcotinine, and anabasine; 75, 750, and 10,000 ng/mL for cotinine and *trans*-3'-hydroxycotinine) were prepared in urine for accuracy and precision testing. The 10,000 ng/mL QC sample for cotinine and *trans*-3'-hydroxycotinine was diluted 5-fold in water before the sample preparation procedure. Analyses were performed on three different days.

All fortified standards and QC samples were processed according to the following liquid-liquid extraction (LLE) procedure.

1. Mix a 250 µL aliquot of urine with 40 µL of internal standard solution (250 ng/mL in methanol) and 50 µL of 5 N sodium hydroxide in a 4 mL glass vial.

2. Extract by adding 1.5 mL of 50:50 methylene chloride:diethyl ether and stirring for 1.5 minutes.

3. Centrifuge at 4,000 rpm for 5 minutes, then transfer 1 mL of the organic phase to a 1.5 mL HPLC vial and mix with 10 µL of 0.25 N hydrochloric acid.

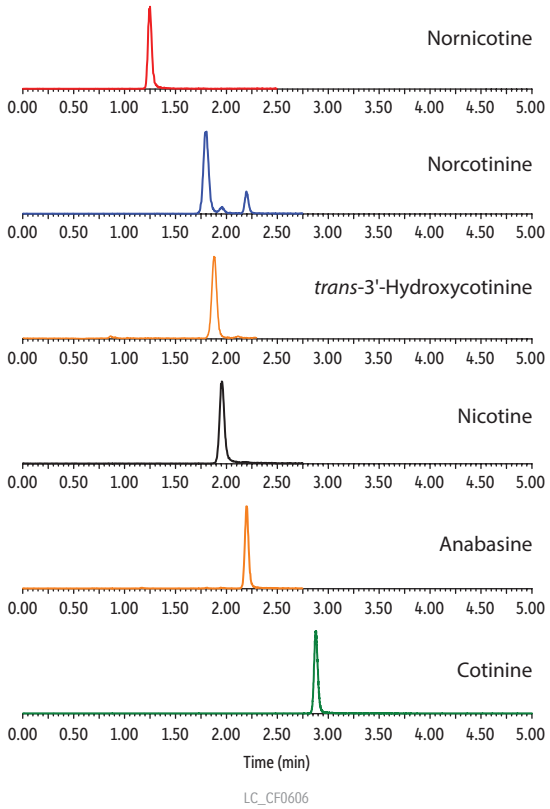
4. Evaporate to dryness at 35 °C under a gentle stream of nitrogen.

5. Reconstitute the dried extract with 200 µL of water.

## Results and Discussion

Since nicotine, norcotinine, and anabasine all share the same mass transitions, it is necessary to chromatographically separate these three compounds for accurate quantitation. As shown in Figure 1, baseline resolution was obtained for nicotine, norcotinine, and anabasine with the Raptor™ Biphenyl column. Simultaneous analysis of all six analytes was performed with a fast 3-minute gradient and a 5-minute total analysis time for each injection. Note that good peak shape was obtained for all analytes with the use of standard, low-pH, reversed-phase mobile phases. The Raptor™ Biphenyl column allowed an MS-friendly mobile phase to be used without any evidence of the peak tailing that is often observed in the analysis of these basic compounds, unless high-pH mobile phases with higher concentrations of additives are used.

**Figure 1:** Good chromatographic separations and peak shapes for nicotine-related compounds were achieved using the Raptor™ Biphenyl column and a simple, low pH, MS-friendly mobile phase.




| Peaks | tR (min) | Precursor Ion | Product Ion |
|---|---|---|---|
| 1. Nornicotine | 1.25 | 149.10 | 80.05 |
| 2. Norcotinine | 1.81 | 163.09 | 80.05 |
| 3. *trans*-3'-Hydroxycotinine | 1.88 | 193.12 | 80.05 |
| 4. Nicotine | 1.95 | 163.15 | 132.10 |
| 5. Anabasine | 2.20 | 163.13 | 91.63 |
| 6. Cotinine | 2.88 | 177.12 | 98.07 |

**Column**
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 5 µm
Pore Size: 90 Å
Guard Column: Raptor™ Biphenyl EXP® cartridges 5 mm, 2.1 mm ID, 5 µm (cat.# 930950252)
Temp.: 30 °C

**Sample**
Diluent: Water
Conc.: 25 ng/mL (fortified human urine)
Inj. Vol.: 5 µL

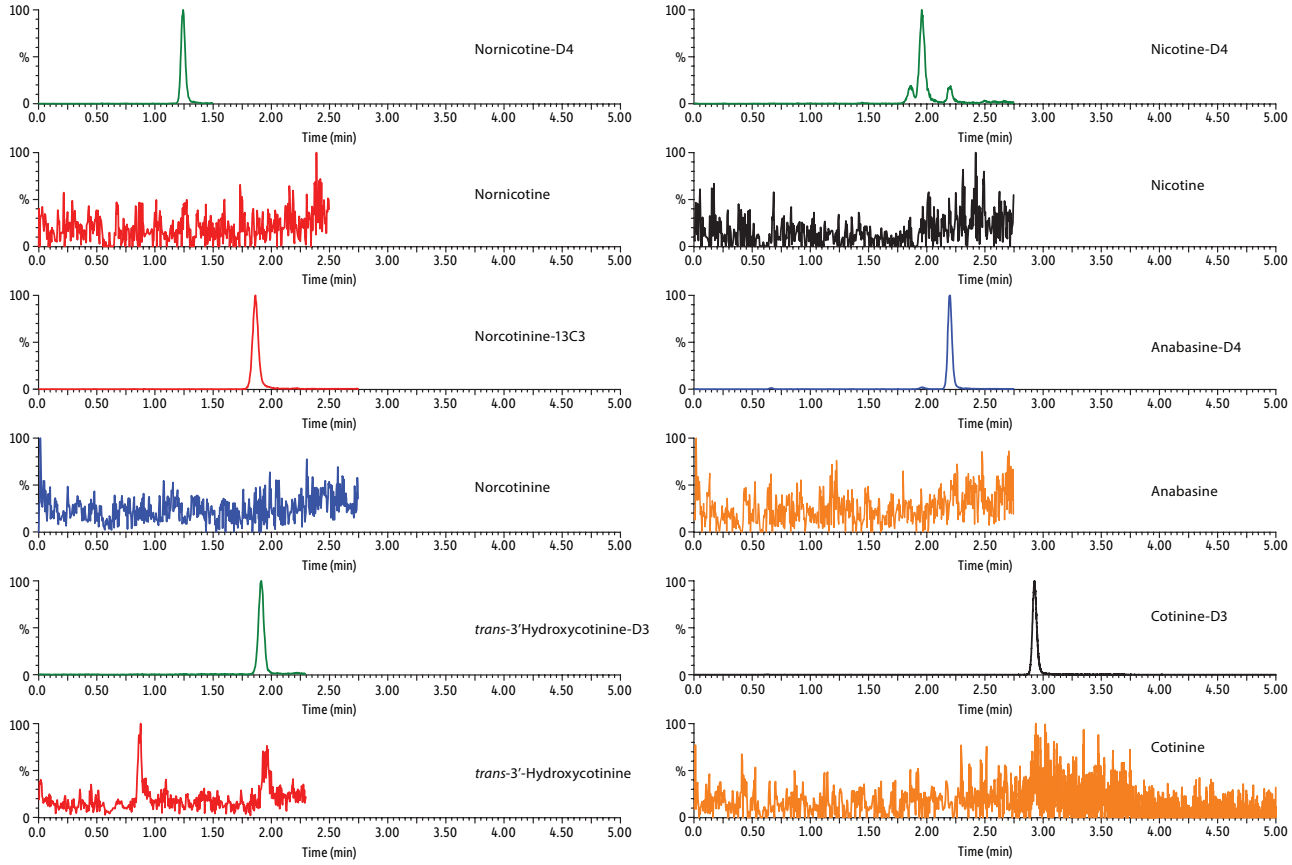
**Mobile Phase**
A: 0.1% Formic acid, 5 mM ammonium formate in water
B: 0.1% Formic acid in methanol

| Time (min) | Flow (mL/min) | %A | %B |
|---|---|---|---|
| 0.00 | 0.4 | 90 | 10 |
| 1.00 | 0.4 | 90 | 10 |
| 2.00 | 0.4 | 70 | 30 |
| 3.00 | 0.4 | 30 | 70 |
| 3.01 | 0.4 | 90 | 10 |
| 5.00 | 0.4 | 90 | 10 |

**Detector** MS/MS
Ion Mode: ESI+
Mode: MRM
**Instrument** UHPLC
**Notes** The urine from a non-tobacco user was fortified with analytes to a final concentration of 25 ng/mL and prepared by a liquid-liquid extraction method. A 250 µL urine sample was mixed with 50 µL of 5N NaOH and extracted with 1.5 mL of 50/50 methylene chloride/diethyl ether. A portion of organic phase (1.0 mL) was dried and reconstituted with 200 µL of water for analysis.

Analysis of blank urine samples spiked with internal standards and subjected to the extraction procedure confirmed there were no significant matrix interferences for the target analytes (Figure 2). Analysis of the calibration standards established the linear ranges for each compound as 2-1,000 ng/mL (nicotine, nornicotine, norcotinine, and anabasine); 5-5,000 ng/mL (cotinine); and 10-5,000 ng/mL (*trans*-3'-hydroxycotinine). To achieve detection across these wide concentration ranges, a detuning of the MS detector was necessary for all analytes in order to avoid saturation of the MS signal at the highest concentration. It was determined that a 1/x weighted quadratic regression gave the best and most consistent fit for all analytes, except anabasine. For anabasine, a $1/x^2$ weighted linear regression provided the best fit. As shown in Figure 3, $r^2$ was ≥ 0.995 for all analytes, and the percent deviation was within 15% of the nominal concentration (≤ 20% for the lowest concentrated standard). Based on the signal-to-noise value for the 10 ng/mL standard, the LLOQ is estimated to be 0.4 ng/mL for all analytes.

**Figure 2:** Chromatographic analysis of blank urine fortified with internal standards confirmed that no significant matrix interferences were present in the samples.



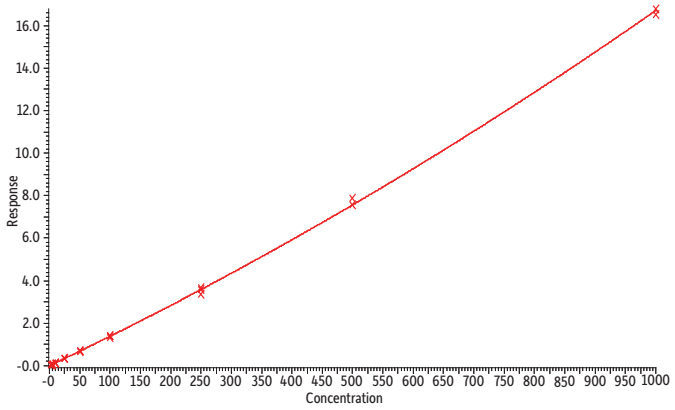

| | |
|---|---|
| **Column** | Raptor™ Biphenyl (cat.# 9309512) |
| Dimensions: | 100 mm x 2.1 mm ID |
| Particle Size: | 5 µm |
| Pore Size: | 90 Å |
| Guard Column: | Raptor™ Biphenyl EXP® guard cartridge 5 mm, 2.1 mm ID, 5 µm (cat.# 930950252) |
| Temp.: | 30 °C |
| **Sample** | |
| Diluent: | Water |
| Conc.: | Blank human urine with internal standards |
| Inj. Vol.: | 5 µL |
| **Mobile Phase** | |
| A: | 0.1% Formic acid, 5 mM ammonium formate in water |
| B: | 0.1% Formic acid in methanol |

| Time (min) | Flow (mL/min) | %A | %B |
|---|---|---|---|
| 0.00 | 0.4 | 90 | 10 |
| 1 | 0.4 | 90 | 10 |
| 2 | 0.4 | 70 | 30 |
| 3 | 0.4 | 30 | 70 |
| 3.01 | 0.4 | 90 | 10 |
| 5 | 0.4 | 90 | 10 |

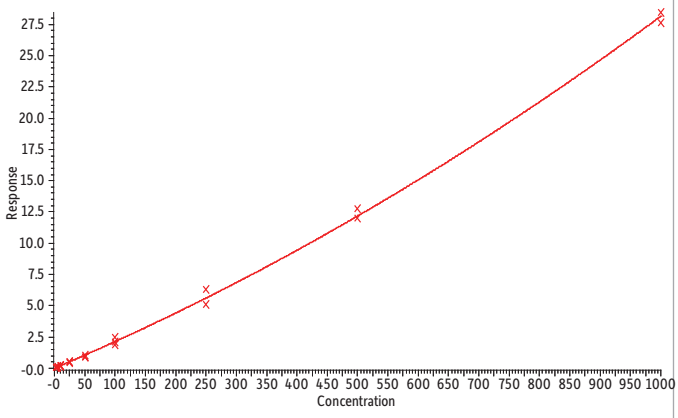
| | |
|---|---|
| **Detector** | MS/MS |
| Ion Mode: | ESI+ |
| Mode: | MRM |
| **Instrument** | UHPLC |
| **Notes** | Samples were processed with a liquid-liquid extraction (LLE) procedure. An aliquot of 250 µL urine was mixed with 40 µL of internal standard solution (250 ng/mL in methanol) and 50 µL of 5N sodium hydroxide in a 4 mL glass vial. Extraction was performed by adding 1.5 mL of 50:50 methylene chloride:diethyl ether and stirring for 1.5 minutes. After centrifugation at 4,000 rpm for 5 minutes, 1 mL of the organic phase was transferred to a 1.5 mL HPLC vial and mixed with 10 µL of 0.25 N hydrochloric acid before evaporating to dryness at 35 °C under a gentle stream of nitrogen. The dried extract was reconstituted with 200 µL of water. |

**Figure 3:** Good linear responses were obtained for all analytes using weighted regression.

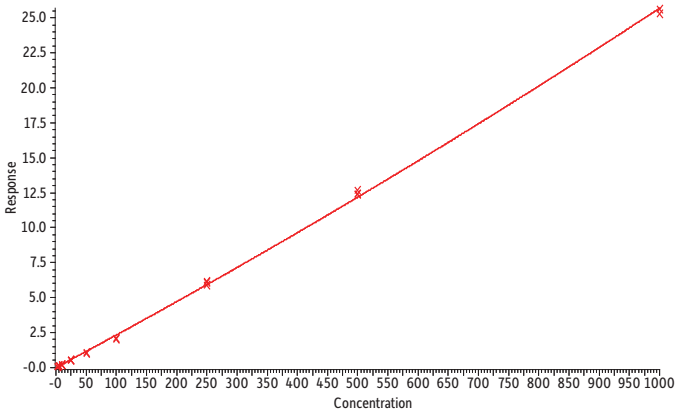
**A. Nornicotine, $r^2 = 0.999$**



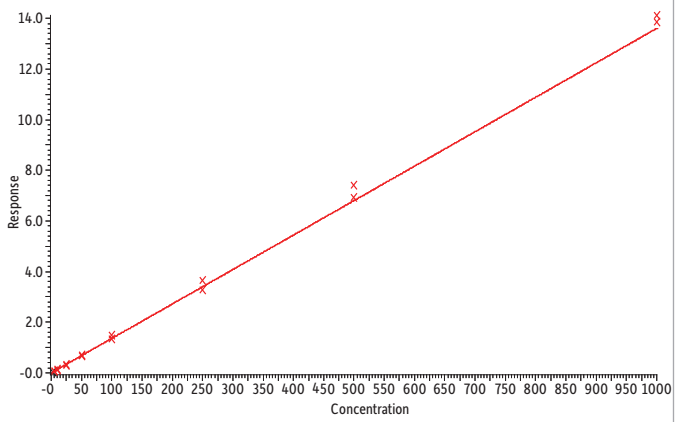

**D. Nicotine, $r^2 = 0.997$**



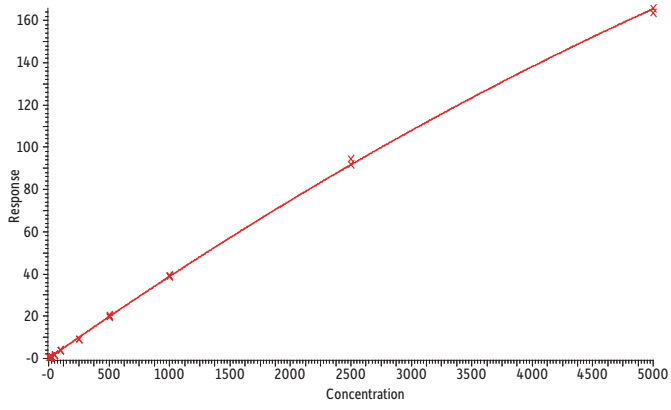

**B. Norcotinine, $r^2 = 0.999$**




**E. Anabasine, $r^2 = 0.995$**




**C. *trans*-3′-Hydroxycotinine, $r^2 = 0.999$**




**F. Cotinine, $r^2 = 0.999$**

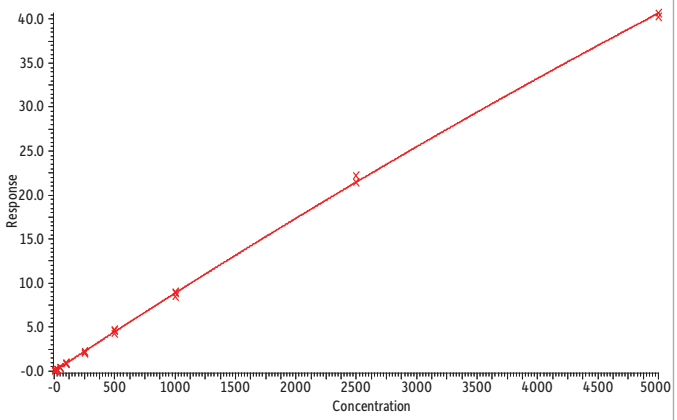
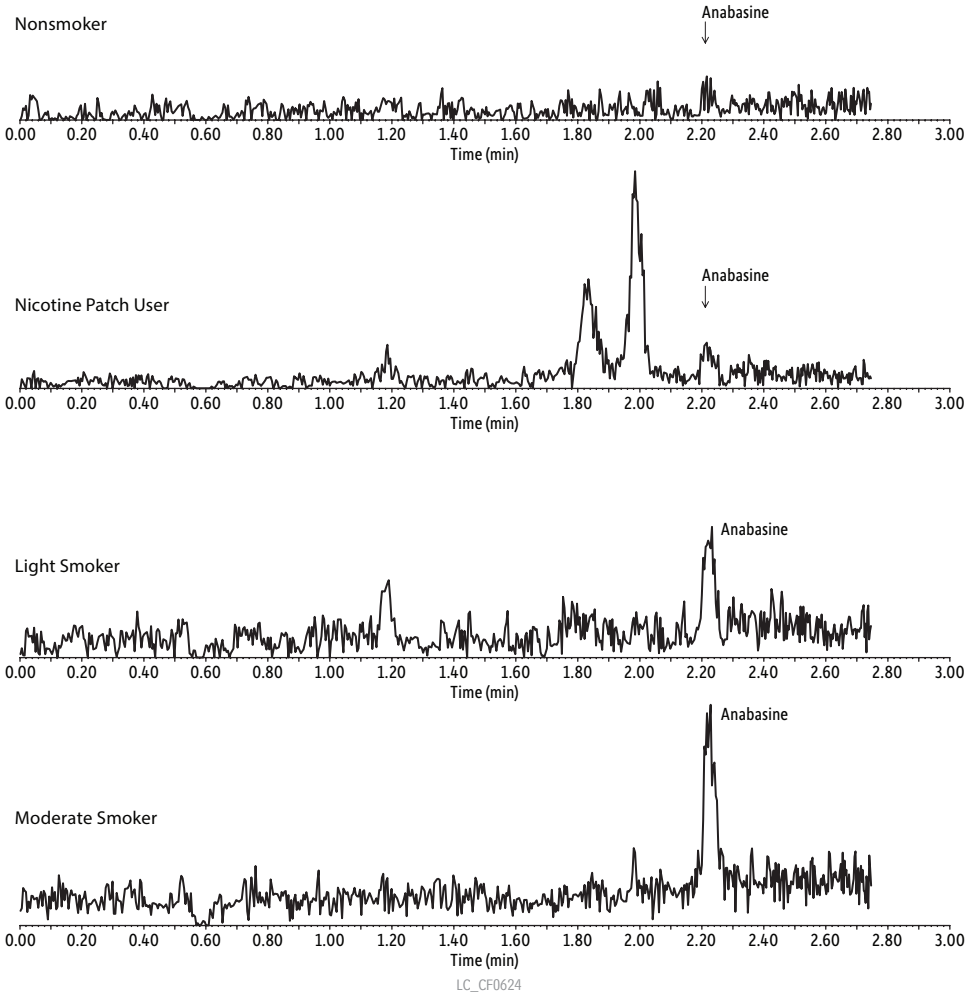


**Figure 4:** Specific detection of anabasine made it possible to distinguish different types of tobacco/nicotine product use.




| | |
|---|---|
| **Column** | Raptor™ Biphenyl (cat.# 9309512) |
| Dimensions: | 100 mm x 2.1 mm ID |
| Particle Size: | 5 µm |
| Pore Size: | 90 Å |
| Guard Column: | Raptor™ Biphenyl EXP® guard cartridge 5 mm, 2.1 mm ID, 5 µm (cat.# 930950252) |
| Temp.: | 30 °C |
| **Sample** | |
| Diluent: | Water |
| Conc.: | Unknown concentration in human urine sample |
| Inj. Vol.: | 5 µL |
| **Mobile Phase** | |
| A: | 0.1% Formic acid, 5 mM ammonium formate in water |
| B: | 0.1% Formic acid in methanol |

| Time (min) | Flow (mL/min) | %A | %B |
|---|---|---|---|
| 0.00 | 0.4 | 90 | 10 |
| 1 | 0.4 | 90 | 10 |
| 2 | 0.4 | 70 | 30 |
| 3 | 0.4 | 30 | 70 |
| 3.01 | 0.4 | 90 | 10 |
| 5 | 0.4 | 90 | 10 |

| | |
|---|---|
| **Detector** | MS/MS |
| Ion Source: | Waters Zspray™ ESI |
| Ion Mode: | ESI+ |
| **Instrument** | UHPLC |
| **Notes** | Samples were processed with a liquid-liquid extraction (LLE) procedure. An aliquot of 250 µL urine was mixed with 40 µL of internal standard solution (250 ng/mL in methanol) and 50 µL of 5N sodium hydroxide in a 4 mL glass vial. Extraction was performed by adding 1.5 mL of 50:50 methylene chloride:diethyl ether and stirring for 1.5 minutes. After centrifugation at 4,000 rpm for 5 minutes, 1 mL of the organic phase was transferred to a 1.5 mL HPLC vial and mixed with 10 µL of 0.25 N hydrochloric acid before evaporating to dryness at 35 °C under a gentle stream of nitrogen. The dried extract was reconstituted with 200 µL of water. |

Method accuracy was demonstrated using percent recovery and these values fell well within 10% of the nominal concentration for all QC levels (93.8–104.9%). Good method precision was also obtained, as indicated by %RSD ranges of 0.6-8.2% and 1.4-9.9% for intraday and interday results respectively. The interday accuracy and precision data are summarized in Table III.

The minor tobacco alkaloid, anabasine, can only be detected in the urine of current tobacco users; it is not present in the urine of non-smokers or nicotine patch/gum users. Using the method established here, specific and sensitive urinary analysis of anabasine was used to distinguish different types of tobacco users. As shown in Figure 4, different levels of anabasine were detected from a light smoker, a moderate smoker, and a non-smoking nicotine patch user.

**Table III:** Results from fortified samples demonstrate the method was both accurate and precise for all compounds at all QC levels.

| | QC-1 (7.5 ng/mL) | | | QC-2 (75 ng/mL) | | | QC-3 (750 ng/mL) | | | QC-4 (10,000 ng/mL) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Avg. Conc. (ng/mL) | Avg. Accuracy | %RSD | Avg. Conc. (ng/mL) | Avg. Accuracy | %RSD | Avg. Conc. (ng/mL) | Avg. Accuracy | %RSD | Avg. Conc. (ng/mL) | Avg. Accuracy | %RSD |
| Nicotine | 7.520 | 100.3 | 4.2 | 71.52 | 95.4 | 8.1 | 779.0 | 103.9 | 6.6 | - | - | - |
| Nornicotine | 7.568 | 100.9 | 7.6 | 75.63 | 100.8 | 6.6 | 754.9 | 100.7 | 3.8 | - | - | - |
| Norcotinine | 7.546 | 100.6 | 5.3 | 75.08 | 100.1 | 9.9 | 786.5 | 104.9 | 4.0 | - | - | - |
| Anabasine | 7.181 | 95.8 | 3.4 | 73.53 | 98.0 | 4.9 | 778.1 | 103.7 | 3.8 | - | - | - |
| Cotinine | - | - | - | 73.82 | 98.4 | 6.3 | 774.6 | 103.3 | 4.7 | 10,076 | 100.8 | 2.3 |
| *trans*-3'-Hydroxycotinine | - | - | - | 72.31 | 96.4 | 2.7 | 730.0 | 97.3 | 1.4 | 9,379 | 93.8 | 4.6 |

## Conclusions

While many current methods for the analysis of nicotine-related compounds require the use of high-pH mobile phases with additives that may damage the analytical system, the method established here using the Raptor™ Biphenyl column produced good results using a simple, MS-friendly mobile phase. The method provided excellent performance for the simultaneous analysis of nicotine, two major metabolites (cotinine and *trans*-3'-hydroxycotinine), two minor metabolites (nornicotine and norcotinine), and a minor tobacco alkaloid, anabasine, in human urine. Accurate and reproducible analysis was achieved in less than 5 minutes of chromatographic analysis time, making the column and method well suited to low-cost, high-throughput analysis of nicotine-related compounds.

## References

[1] P. Jacob, L. Yu, A.T. Shulgin, N.L. Benowitz, Minor tobacco alkaloids as biomarkers for tobacco use: comparison of users of cigarettes, smokeless tobacco, cigars, and pipes, Am J Public Health, 89 (5) (1999) 731–736.

[2] N.L. Benowitz, J. Hukkanen, P. Jacob, Nicotine chemistry, metabolism, kinetics and biomarkers, Handb Exp Pharmacol, 192 (2009) 29–60.

[3] K.S. Derby, K.C.C. Caberto, S.G. Carmella, A.A. Franke, S.S. Hecht, Nicotine metabolism in three ethnic/racial groups with different risks of lung cancer, Cancer Epidemiol Biomarker Prev, 17 (2008) 3,526–3,535.

# Rapid Analysis of Steroid Hormones by GC/MS

## Using the New Rxi®-1ms Column

By Kristi Sellers, Clinical/Forensic Innovations Chemist

- Resolve 6 common steroid hormones in less than 25 minutes.
- Ultra-low bleed column greatly reduces background interferences.
- Stable performance at 300°C or above.

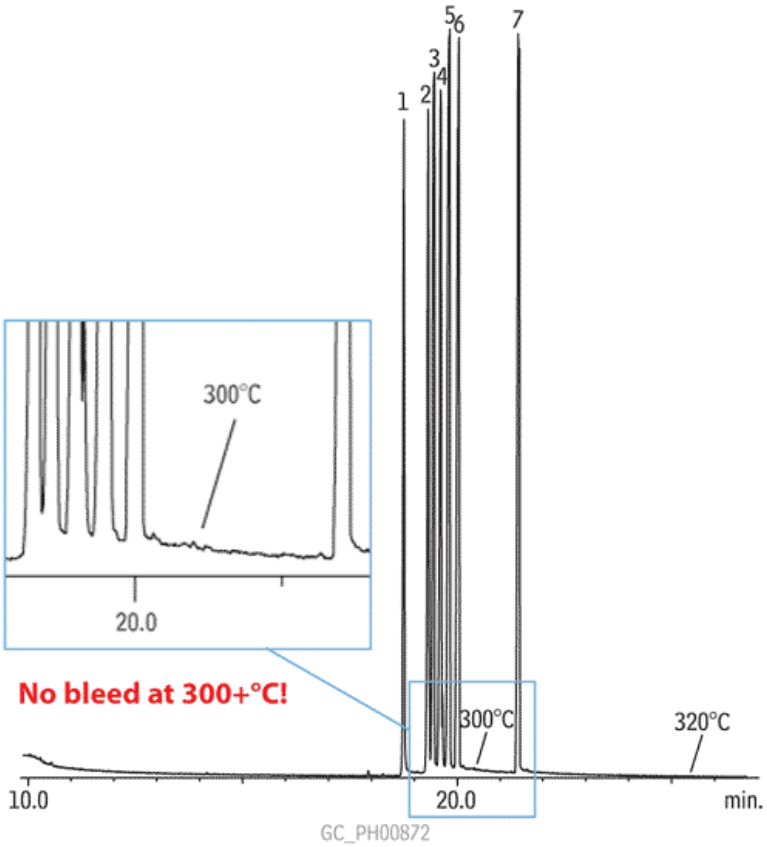
Determinations of urinary steroid hormones are widely used for diagnosing and monitoring many health conditions, including bio-identical hormone replacement, menopause, Cushing's syndrome, Addison's disease, adrenal fatigue, and others.[1] Many clinical laboratories use gas chromatography and mass spectrometry (GC/MS) as the primary analytical method for identification and quantification. A capillary GC column with a thin film (0.25µm or less) of 100% dimethylpolysiloxane is the column of choice for many analysts, because this stationary phase has the highest operating temperature available. Temperatures exceeding 300°C are required to elute the high molecular weight (250-400 Dalton) hormones in a reasonable analysis time while maintaining Gaussian peak shape and resolution. A phase film thickness of 0.25µm or less minimizes column bleed at these high temperatures. Also, in order to provide reliable quantification, the column must exhibit the inertness necessary to produce symmetric peaks and reproducible results.

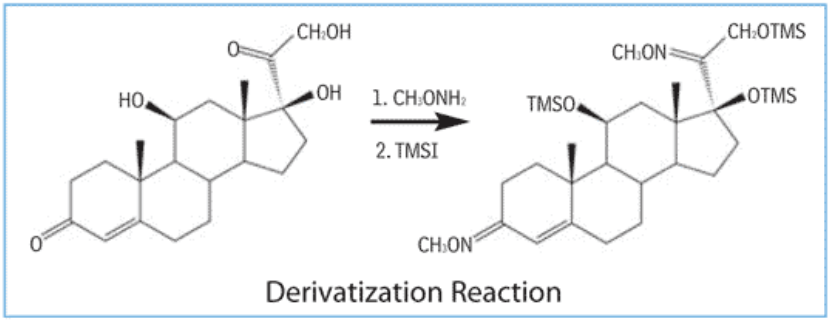
Our new Rxi®-1ms column, designed for GC-MS applications, provides the ultra-low bleed and exceptional inertness needed for analyzing urinary steroid hormones. For this application we derivatized six sex hormones, using methoxylamine HCl and trimethylsilyl imidazole to improve chromatography. Figure 1 shows this variety of derivatized steroid sex hormones, analyzed in less than 25 minutes by using an Rxi®-1ms column. Note that these compounds elute at temperatures near or above 300°C and that bleed from the Rxi®-1ms column is negligible at these temperatures. The Rxi®-1ms column exhibits the inertness needed to produce Gaussian peaks and excellent resolution.

Because GC/MS analysis of urinary steroid hormones is a demanding application, it is important to use the lowest bleed, most inert column available. The new Rxi®-1ms column meets these requirements better than any column we have tested, and we recommend it as the column of choice for this application.

**Figure 1** Negligible bleed, Gaussian peaks, and fast results characterize analyses of derivatized steroids on an Rxi®-1ms column.

1. androsterone
2. dehydroepiandrosterone (DHEA)
3. 17-α-estradiol
4. estrone
5. 17-β-estradiol
6. testosterone
7. derivatization by-product




| | |
|---|---|
| Condition: | Rxi™-1ms 30m, 0.25mm ID, 0.25µm (cat.# 13323)<br>100µg/mL each hormone in methanol or ethanol; compounds derivatized using 2% methoxylamine HCl ($CH_3ONH_2$) in pyridine, then |
| Sample: | N-trimethylsilylimidazole (TMSI), then analyzed<br>1.0µL splitless (hold 0.5 min.), 3.5mm single gooseneck inlet liner (cat.# |
| Inj.: | 20961) |
| Inj. temp.: | 250°C |
| Carrier gas: | helium, constant flow |
| Flow rate: | 1mL/min. |
| Oven temp.: | 100°C to 320°C @ 10°C/min. (hold 10 min.) |
| Det: | MS: Shimadzu 17A with QP5000 |
| Transfer line temp.: | 280°C |
| Scan range: | 40-700 amu |
| Ionization: | EI |
| Mode: | scan |

**References**

1. www.meridianvalley lab.com./steroid_dept.html

**RELATED SEARCHES**

*Rxi-1ms*, *steroid hormones*, *GC/MS*, *urine*

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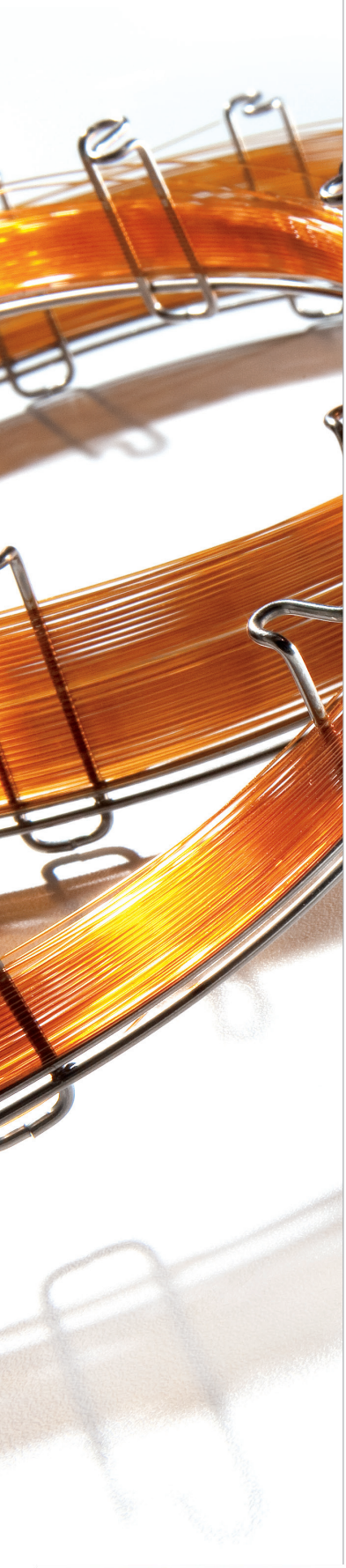
# NEW Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2 Columns

## Advanced Technology for Fast, Reliable Measurement of Alcohol in Blood

- Optimized column selectivities guarantee resolution of ethanol, internal standards, and common interferences.
- Quality testing of every column with target analytes ensures reliable performance.
- 2 minute analysis time increases lab productivity.

# New Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2 Columns

## Advanced Technology for Fast, Reliable Measurement of Alcohol in Blood



Getting accurate, reliable results quickly is critical for labs analyzing blood alcohol concentration (BAC). New Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2 columns provide definitive data in a fast, 2-minute analysis, so you can be certain of your results and maximize sample throughput.

Blood alcohol content is often determined using headspace injection and dual column GC-FID analysis. While this is a relatively straightforward procedure, column choice plays a major role in data quality and reliability. In order to produce accurate results, the primary and confirmation columns must fully separate target analytes from all interferences and produce symmetrical peaks. Due to deficiencies in selectivity and inertness, coelution and tailing peaks are observed on competitor columns, which make confident reporting of target alcohols difficult.

### Best Overall Performance

New Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2 columns have optimized column selectivities, which result in optimized resolution of all target compounds, as well as the retention time and elution order changes necessary for confirmation. These new columns outperform other blood alcohol column pairs and ensure baseline separation of all critical compounds, including ethanol, methanol, acetone, *tert*-butanol (IS), acetaldehyde, isopropanol, and 1-propanol (IS), in less than 2 minutes (Figure 1). The separation of *tert*-butanol is especially notable as it has recently been adopted as an internal standard for BAC headspace analysis, but coelutes with acetone on some columns.

In addition to being fully separated, target compounds exhibit excellent peak symmetry on Rtx®-BAC Plus columns, allowing more accurate integration. In contrast, alcohol peaks on competitor columns display tailing, which can make integration difficult. As shown in Table I and Figure 1, the Rtx®-BAC Plus columns produce the best peak shape for ethanol among the columns tested.

### Quality Counts

*Every* Rtx®-BAC Plus 1 and Plus 2 column is conditioned at maximum isothermal temperature and quality tested with the analytes shown in Figure 1 to ensure reliable separations of all target compounds. Quality specifications for retention and efficiency ensure consistent column-to-column performance, and tightly controlled manufacturing results in robust columns with long lifetimes. The maximum temperature of these 100% chemically bonded columns is 260 °C.

Compared to other columns, new Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2 columns offer the best overall performance for blood alcohol analysis. These columns provide fully separated, highly symmetrical peaks in a fast 2-minute analysis, allowing analysts to determine blood alcohol concentration quickly and report results with certainty.

**Table I:** Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2 columns exhibit both baseline resolution of critical compounds and excellent peak shape compared to other blood alcohol columns available.

| Column | Resolution (Methanol/Acetaldehyde) | Resolution (Acetone/*tert*-Butanol) | USP Tailing Factor (Ethanol) |
|---|---|---|---|
| Rtx®-BAC Plus 1 | - | 3.5 | 1.287 |
| DB®-ALC1 | - | 0.2 | 1.470 |
| ZB BAC 1 | - | 3.4 | 1.852 |
| Rtx®-BAC Plus 2 | 4.4 | - | 1.089 |
| DB®-ALC2 | 4.6 | - | 1.445 |
| ZB BAC 2 | 2.8 | - | 2.085 |

**Figure 1:** Rtx®-BAC Plus columns provide the best overall performance for determining blood alcohol concentration. Highly symmetrical, baseline separated peaks provide fast and definitive results for all target compounds.

## Primary Analysis

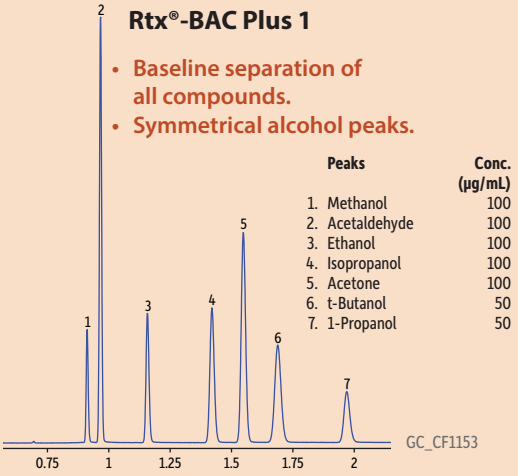
## Confirmational Analysis

RESTEK

### Rtx®-BAC Plus 1

- Baseline separation of all compounds.
- Symmetrical alcohol peaks.

| Peaks | Conc. (µg/mL) |
|---|---|
| 1. Methanol | 100 |
| 2. Acetaldehyde | 100 |
| 3. Ethanol | 100 |
| 4. Isopropanol | 100 |
| 5. Acetone | 100 |
| 6. t-Butanol | 50 |
| 7. 1-Propanol | 50 |

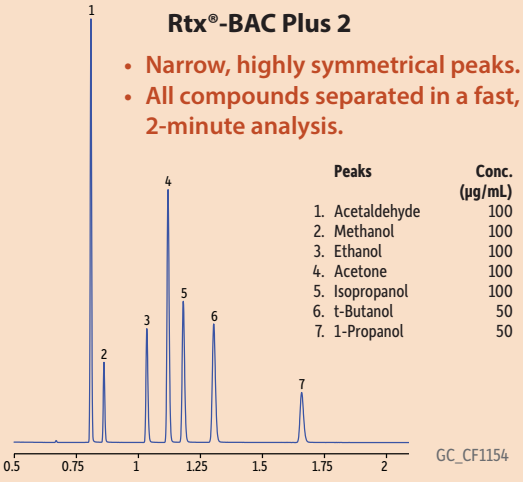



GC_CF1153

### Rtx®-BAC Plus 2

- Narrow, highly symmetrical peaks.
- All compounds separated in a fast, 2-minute analysis.

| Peaks | Conc. (µg/mL) |
|---|---|
| 1. Acetaldehyde | 100 |
| 2. Methanol | 100 |
| 3. Ethanol | 100 |
| 4. Acetone | 100 |
| 5. Isopropanol | 100 |
| 6. t-Butanol | 50 |
| 7. 1-Propanol | 50 |

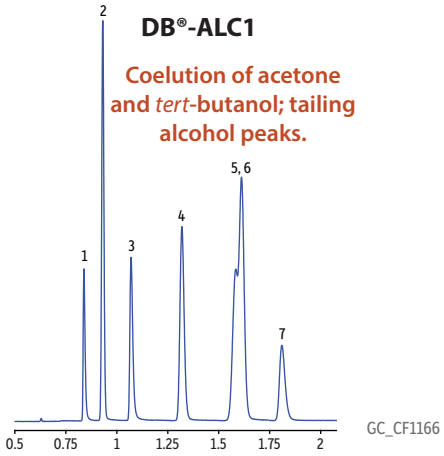



GC_CF1154

AGILENT

### DB®-ALC1

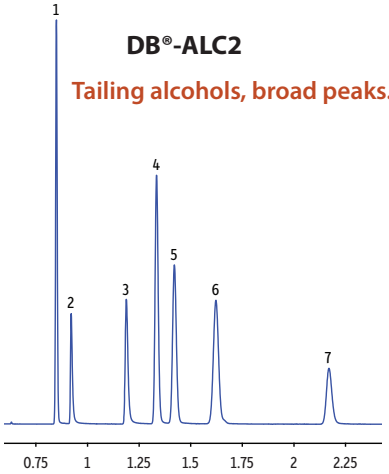
Coelution of acetone and *tert*-butanol; tailing alcohol peaks.




GC_CF1166

### DB®-ALC2

Tailing alcohols, broad peaks.

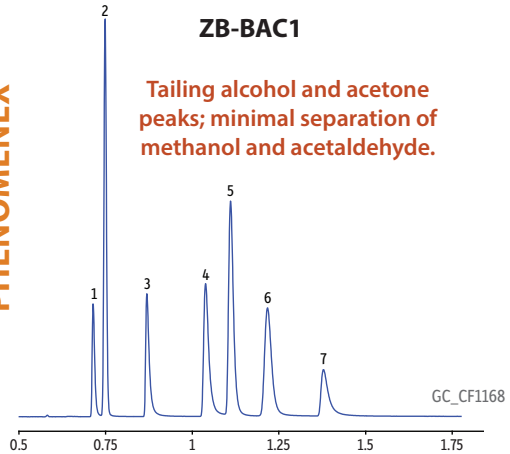



GC_CF1167

PHENOMENEX

### ZB-BAC1

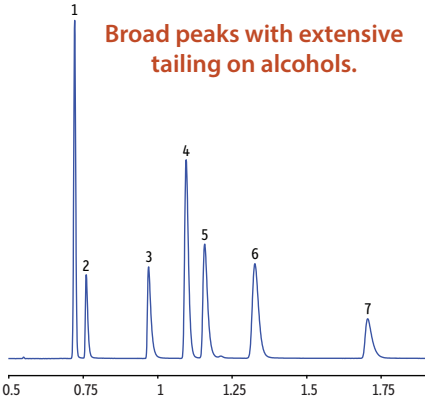
Tailing alcohol and acetone peaks; minimal separation of methanol and acetaldehyde.




GC_CF1168

### ZB-BAC 2

Broad peaks with extensive tailing on alcohols.




GC_CF1169

Columns: Rtx®-BAC Plus 1, 30 m, 0.32 mm ID, 1.8 µm (cat.# 18004); Rtx®-BAC Plus 2, 30 m, 0.32 mm ID, 0.6 µm (cat.# 18006); DB®-ALC2 and ZB-BAC2, 30 m, 0.32 mm ID, 1.2 µm; DB®-ALC1 and ZB-BAC1, 30 m, 0.32 mm ID, 1.2 µm; Sample: 50 µL each of BAC resolution control standard n-P (cat.# 36010) and BAC resolution control standard t-B (cat.# 36011) diluted in 900 µL water in a 20 mL headspace vial; Injection: Headspace-loop split (split ratio 50:1); Liner: 1 mm ID straight inlet liner (cat.# 20972); Headspace-Loop: Inj. Port Temp.: 200 °C; Instrument: Tekmar HT3; Transfer Line Temp.: 125 °C; Valve Oven Temp.: 125 °C; Standby Flow Rate: 50 mL/min; Sample Temp.: 60 °C; Sample Equil. Time: 5 min; Vial Pressure: 30 psi; Loop Pressure: 20 psi; Loop Fill Time: 1 min; Oven: 40 °C (hold 3 min); Carrier Gas He, constant flow; Linear Velocity: 80 cm/sec @ 40 °C; Detector FID @ 240 °C; Make-up Gas: Nitrogen, 30 mL/min; Instrument Agilent/HP6890 GC; Acknowledgement: Headspace concentrator courtesy of Teledyne Tekmar, Mason, OH.

# Other Applications

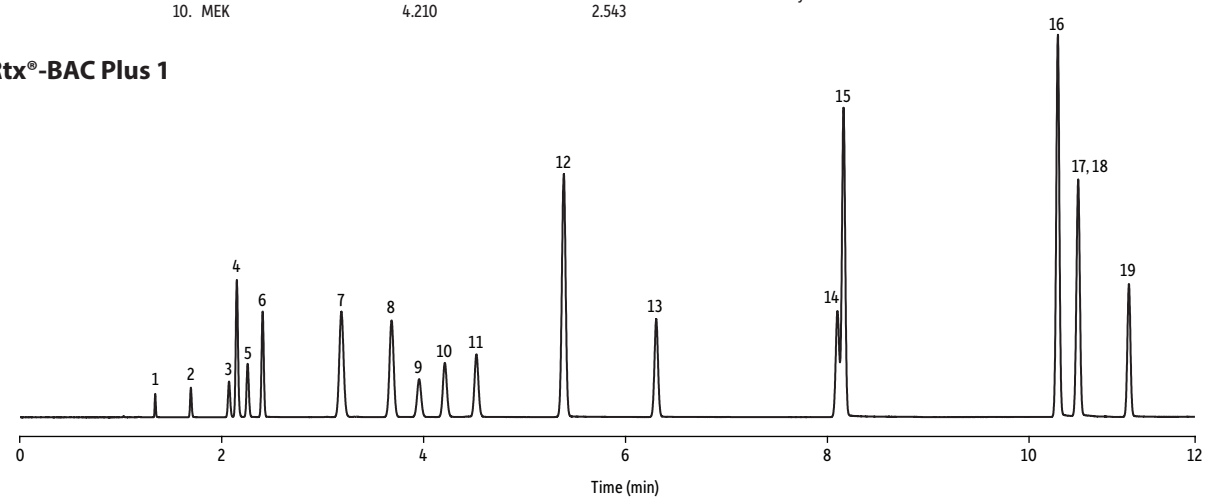
Rtx®-BAC Plus columns can be used for other low temperature applications for volatile organic compounds, due to their unique selectivity, retention, and inertness. For example, in the analysis of abused inhalants in Figure 2, most compounds are resolved between the columns with several elution order changes for confirmation. In addition, excellent peak shapes are observed. As shown in Figures 3 and 4, Rtx®-BAC Plus columns can also be used for single column applications, such as glycols or gamma-butyrolactone (GBL), which is a derivatized form of gamma-hydroxybutyrate (GHB). The low bleed character of these columns also makes them useful for mass spectrometry applications.

**Figure 2:** Dual column analysis of abused inhalants.

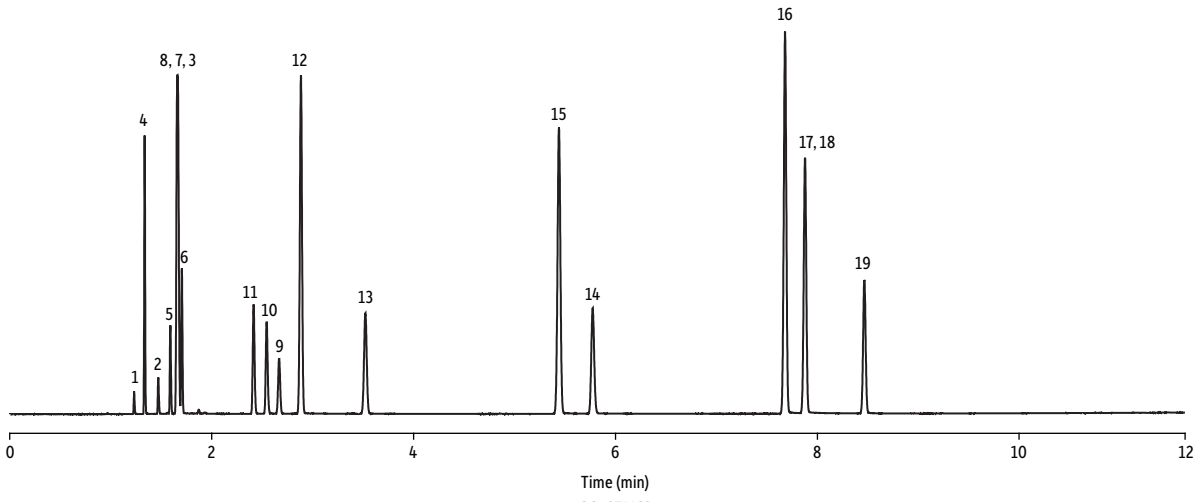
| Peaks | BAC Plus 1 RT (min) | BAC Plus 2 RT (min) |
|---|---|---|
| 1. Methanol | 1.340 | 1.230 |
| 2. Ethanol | 1.694 | 1.470 |
| 3. Isopropanol | 2.072 | 1.660 |
| 4. Diethyl ether | 2.149 | 1.334 |
| 5. Acetone | 2.256 | 1.589 |
| 6. Methylene chloride | 2.405 | 1.703 |
| 7. MTBE | 3.185 | 1.660 |
| 8. Hexane | 3.683 | 1.660 |
| 9. Chloroform | 3.956 | 2.667 |
| 10. MEK | 4.210 | 2.543 |

| Peaks | BAC Plus 1 RT (min) | BAC Plus 2 RT (min) |
|---|---|---|
| 11. Ethyl acetate | 4.54 | 2.414 |
| 12. Benzene | 5.390 | 2.883 |
| 13. Trichloroethylene | 6.306 | 3.522 |
| 14. MIBK | 8.101 | 5.775 |
| 15. Toluene | 8.162 | 5.440 |
| 16. Ethylbenzene | 10.286 | 7.681 |
| 17. *m*-Xylene | 10.487 | 7.879 |
| 18. *p*-Xylene | 10.487 | 7.879 |
| 19. *o*-Xylene | 10.990 | 8.467 |

**Rtx®-BAC Plus 1**




**Rtx®-BAC Plus 2**

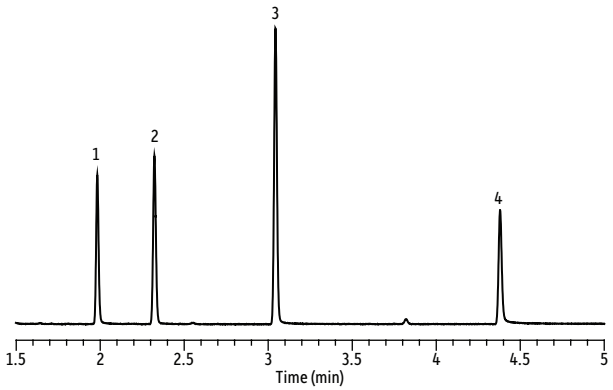



GC_CF1162

Columns: Rtx®-BAC Plus 1, 30 m, 0.32 mm ID, 1.8 µm (cat.# 18004) and Rtx®-BAC Plus 2, 30 m, 0.32 mm ID, 0.6 µm (cat.# 18006) using Rxi® guard column, 5 m, 0.32 mm ID (cat.# 10039) with Universal "Y" Press-Tight® connector (cat.# 20405-261); Sample: 50 µg/mL each inhalant in water; Injection: headspace-loop split (split ratio 50:1); Liner: 1 mm straight inlet liner (cat.# 20972); Headspace-Loop: Inj. Port Temp.: 220 °C; Instrument: Tekmar HT3; Inj. Time: 3 min; Transfer Line Temp.: 125 °C; Valve Oven Temp.: 125 °C; Sample Temp.: 70 °C; Sample Equil. Time: 5 min; Vial Pressure: 30 psi; Pressurize Time: 2 min; Loop Pressure: 20 psi; Loop Fill Time: 1 min; Oven Temp: 40 °C (hold 4 min) to 120 °C at 10 °C/min (hold 0 min); Carrier Gas: He, constant flow; Linear Velocity: 50 cm/sec; Detector: FID @ 240 °C; Make-up Gas: $N_2$, 30 mL/min; Instrument: Agilent/HP6890 GC; Notes The Rtx®-BAC Plus 1 and Plus 2 columns were connected to the injection port using a ~12 inch section of guard column between the injection port and the Universal Y Press-Tight® connector. Headspace concentrator courtesy of Teledyne Tekmar, Mason, OH.

**Figure 3:** Separation of glycols on the Rtx®-BAC Plus 1 column.

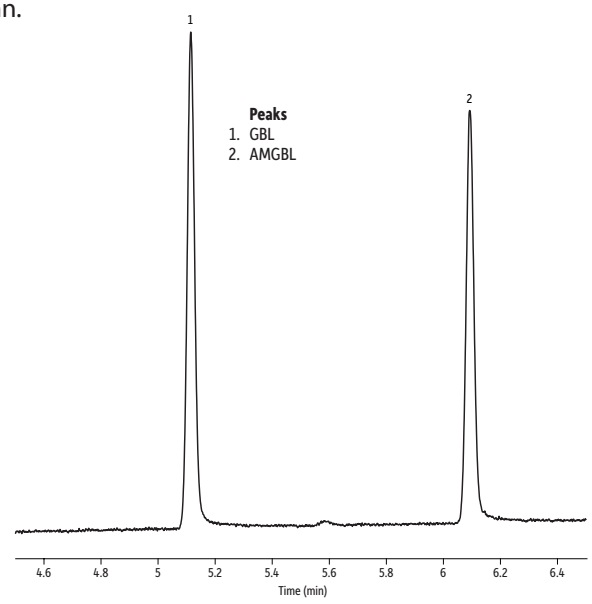
| Peaks | BAC Plus 1 RT (min) | BAC Plus 2 RT (min) |
|---|---|---|
| 1. Ethylene glycol | 1.983 | 2.207 |
| 2. Propylene glycol | 2.323 | 2.433 |
| 3. 1,3-Propanediol | 3.043 | 3.278 |
| 4. Diethylene glycol | 4.380 | 4.385 |




GC_CF1163

Columns: Rtx®-BAC Plus 1, 30 m, 0.32 mm ID, 1.8 µm (cat.# 18004); Sample: 200 µg/mL each glycol in methanol:water (40:60); Injection: 1 µL split (split ratio 20:1); Liner: Sky™ 4 mm straight inlet liner w/wool (cat.# 23300.1); Inj. Temp.: 260 °C; Oven Temp: 60 °C (hold 0 min) to 240 °C at 20 °C/min (hold 5 min); Carrier Gas: He, constant flow; Linear Velocity: 70 cm/sec; Detector: FID @ 240 °C; Make-up Gas: $N_2$, 30 mL/min; Instrument: Agilent/HP6890 GC; Notes: Injections were performed manually with a Merlin Microshot injector (cat.# 22229).

**Figure 4:** Gamma-butyrolactone (GBL) and internal standard alpha-methylene-gamma-butyrolactone (AMGBL) on an Rtx®-BAC Plus 1 column.




GC_CF1164

Columns: Rtx®-BAC Plus 1, 30 m, 0.32 mm ID, 1.8 µm (cat.# 18004); Sample: Gamma-butyrolactone (GBL) (cat.# 34077) and alpha-methylene-gamma-butyrolactone (AMGBL) (cat.# 34079); Injection: Headspace-loop split (split ratio 10:1); Liner: 1 mm ID straight inlet liner (cat.# 20972); Headspace-Loop: Inj. Port Temp.: 200 °C; Instrument: Tekmar HT3; Inj. Time: 1 min; Transfer Line Temp.: 125 °C; Valve Oven Temp.: 125 °C; Sample Temp.: 100 °C; Sample Equil. Time: 10 min; Vial Pressure: 30 psi; Pressurize Time: 2 min; Loop Pressure: 20 psi; Loop Fill Time: 1 min; Oven: 80 °C (hold 0 min) to 180 °C at 10 °/min (hold 0 min); Carrier Gas He, constant flow; Linear Velocity: 48 cm/sec; Detector FID @ 240 °C; Make-up Gas: Nitrogen, 30 mL/min; Instrument Agilent/HP6890 GC; Notes: Sample was prepared by injecting 1 µL of each standard into a capped headspace vial. The mass of each compound inside the headspace vial was 1 µg. Headspace concentrator courtesy of Teledyne Tekmar, Mason, OH.

## Looking for other applications?

- Search our chromatogram library by compound name, synonym, CAS# or keyword at **www.restek.com/chromatograms**
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# **NEW** Columns and Standards for Blood Alcohol Testing

## Rtx®-BAC Plus 1/Rtx®-BAC Plus 2 Columns

- Optimized column selectivities guarantee resolution of ethanol, internal standards, and frequently encountered interferences.
- Robust and reproducible column chemistry ensures longer column lifetime and consistent results.
- 2 minute analysis time increases lab productivity.
- Stable to 260 °C

### Rtx®-BAC Plus 1 Columns (fused silica)

| Description | temp. limits | cat.# |
|---|---|---|
| 30m, 0.32mm ID, 1.80µm | -20 to 240/260°C | 18004 |
| 30m, 0.53mm ID, 3.00µm | -20 to 240/260°C | 18005 |

### Rtx®-BAC Plus 2 Columns (fused silica)

| Description | temp. limits | cat.# |
|---|---|---|
| 30m, 0.32mm ID, 0.6µm | -20 to 240/260°C | 18006 |
| 30m, 0.53mm ID, 1.0µm | -20 to 240/260°C | 18007 |

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## Blood Alcohol Resolution Control Standards

- Use to verify the retention time for each compound normally included in a blood alcohol test, and to verify that the compounds are resolved from and do not interfere with one another.
- Includes 1-propanol or *tert*-butanol internal standard.
- Intended for qualitative use only.

### BAC Resolution Control Standard n-P (6 components)

acetaldehyde
acetone
ethanol (BAC)
methanol
1-propanol (*n*-propanol)
2-propanol (isopropanol)

100mg/dL each in water, 1mL/ampul

cat.# 36010 (ea.)

No data pack available.

### BAC Resolution Control Standard t-B (6 components)

acetaldehyde
acetone
*tert*-butanol (TBA)
ethanol (BAC)
methanol
2-propanol (isopropanol)

100mg/dL each in water, 1mL/ampul

cat.# 36011 (ea.)

No data pack available.

## Blood Alcohol Standards (Calibration)

- NIST-traceable ethanol calibration standards.
- Calibration mixtures ranging from 0.010g/dL to 0.40g/dL in water.
- Datapack and Certificate of Analysis for each standard available on our website.

| Compound | | qty. | cat.# |
|---|---|---|---|
| 0.010g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36276 |
| 0.015g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36232 |
| 0.02g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36233 |
| 0.025g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36234 |
| 0.04g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36235 |
| 0.05g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36257 |
| 0.08g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36262 |
| 0.1g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36236 |
| 0.15g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36237 |
| 0.16g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36417 |
| 0.2g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36238 |
| 0.3g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36239 |
| 0.4g/dL forensic ethanol solution | 1 mL/ampul | 5-pk. | 36266 |

## Rxi® Guard/Retention Gap Columns (fused silica)

- Extend column lifetime.
- Excellent inertness—obtain lower detection limits for active compounds.
- Sharper chromatographic peaks by utilizing retention gap technology.
- Maximum temperature: 360 °C.

| Nominal ID | Nominal OD | 5-Meter | 5-Meter/6-pk. | 10-Meter | 10-Meter/6-pk. |
|---|---|---|---|---|---|
| 0.25 mm | 0.37 ± 0.04 mm | 10029 | 10029-600 | 10059 | 10059-600 |
| 0.32 mm | 0.45 ± 0.04 mm | 10039 | 10039-600 | 10064 | 10064-600 |
| 0.53 mm | 0.69 ± 0.05 mm | 10054 | 10054-600 | 10073 | 10073-600 |

Rxi® GC Columns

## Sky™ 1.0 mm ID Straight Inlet Liners

Exceptionally inert, Sky™ inlet liners with state-of-the-art deactivation improve trace-level analysis.

- Increase accuracy and precision by preventing loss of sensitive analytes—even when using wool.
- Achieve lower detection limits for a wide range of active compounds.
- Ensure liner-to-liner reproducibility through consistent manufacturing and extensive testing.


### Sky™ 1.0mm ID Straight Inlet Liner

for Agilent GCs equipped with split/splitless inlets

| ID x OD x Length | qty. | cat.# |
|---|---|---|
| Straight, Sky Technology, 1.0mm x 6.3mm x 78.5mm | ea. | 23333.1 |
| 1.0mm x 6.3mm x 78.5mm | 5-pk. | 23333.5 |
| 1.0mm x 6.3mm x 78.5mm | 25-pk. | 23333.25 |

Visit **www.restek.com/liners** for our full selection.

## Restek Electronic Leak Detector

Don't let a small leak turn into a costly repair—protect your analytical column by using a Restek leak detector.

**Leak Detector Specifications**

| | |
|---|---|
| Detectable Gases: | Helium, nitrogen, argon, carbon dioxide, hydrogen |
| Battery: | Rechargeable lithium ion internal battery pack (12 hours normal operation) |
| Operating Temperature Range: | 32–120 °F (0–48 °C) |
| Humidity Range: | 0–97% |
| Warranty: | One year |
| Certifications: | CE, Ex, Japan |
| Compliance: | WEEE, RoHS |

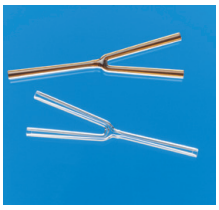
**Limits of Detection**

These gases can be detected with the Restek electronic leak detector at the following leak rates:

| Minimum Detectable Gas Limits and Indicating LED Color: |
|---|
| Helium, 1.0 x $10^{-5}$, red LED |
| Hydrogen*, 1.0 x $10^{-5}$, red LED |
| Nitrogen, 1.4 x $10^{-3}$, yellow LED |
| Argon, 1.0 x $10^{-4}$, yellow LED |
| Carbon dioxide, 1.0 x $10^{-4}$, yellow LED |

Gas detection limits measured in atm cc/sec.

## Universal "Y" Press-Tight® Connectors



An alternative method of performing dual-column confirmational analyses!

- Split sample flow onto two columns—perform confirmation analysis with a single injection.
- Split a single column flow to two detectors.

| Description | ea. | 3-pk. |
|---|---|---|
| Universal "Y" Press-Tight Connector | 20405 | 20406 |
| Universal "Y" Press-Tight Connector, Deactivated | 20405-261 | 20406-261 |
| Universal "Y" Press-Tight Connector, Siltek Deactivated | 20485 | 20486 |


| Description | qty. | cat.# |
|---|---|---|
| Leak Detector With Hard-Sided Carrying Case and Universal Charger Set (U.S., UK, European, Australian) | ea. | 22655 |
| Small Probe Adaptor for Leak Detector | ea. | 22658 |
| Dynamic Duo Combo Pack (Restek Leak Detector and ProFLOW 6000 Flowmeter) | kit | 22654 |
| Soft-Sided Storage Case for Leak Detector or ProFLOW 6000 Flowmeter | ea. | 22657 |

Avoid using liquid leak detectors on a GC! Liquids can be drawn into the system and/or into the leak detector.

*Caution: The Restek electronic leak detector is designed to detect trace amounts of hydrogen in a noncombustible environment. It is NOT designed for determining leaks in a combustible environment. A combustible gas detector should be used for determining combustible gas leaks under any condition. When using it to detect hydrogen, the Restek electronic leak detector may only be used for determining trace amounts in a GC environment.

## Restek carries a full line of headspace essentials

including screw-thread headspace vials & magnetic screw-thread caps!

# Restek Has Added ISO Guide 34 and 17025 Accreditations

## We Now Offer a Full Line of Certified Reference Materials!

Restek is proud to announce that our reference standard manufacturing and QA testing labs in Bellefonte, PA, have earned ISO Guide 34 and 17025 accreditations through A2LA. More than ever, you can rely on Restek for all of your reference standards, and now, you can also experience the advantages of our ISO accreditations:

- **Satisfy regulatory requirements** by sourcing CRMs from an accredited supplier.
- Benefit from the **exceptional product quality and customer service** needed to meet strict ISO 9001, Guide 34, and 17025 guidelines.
- Get the **same reliability and documentation with custom-formulated solutions** as you do with stock standards—both fall under Restek's accreditation.
- **Consolidate orders** by ordering primary- and secondary-source reference standards, GC and LC columns, sample prep supplies, and accessories from one vendor.

We invite you to visit **www.restek.com/iso** to learn more about our ISO quality credentials and view our certificates (including scopes of accreditation).

If you have any questions or would like more information, feel free to contact customer service at **814-353-1300, ext. 3**, or **csreps@restek.com**

***Note:*** *If your lab must use certified reference materials (CRMs), please be sure to tell your Restek representative when ordering so we can help you meet your regulatory requirements as we transition our inventory.*

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# Thermal Desorption: A Practical Applications Guide

## III. Defence and Forensic

## Introduction to Markes International Ltd.

Formed in 1997, Markes International Ltd. is one of the world's leading suppliers of thermal desorption (TD) equipment for monitoring trace toxic and odorous chemicals in air, gas and materials. Serving fast growing markets from environmental health and safety to materials testing and from food / flavour / fragrance to defence / forensic, Markes' global customer base includes major industry, government agencies, academia and the service laboratory sector.

Markes has introduced several highly successful brands of TD instruments to the market including: UNITY™ – a universal TD platform for single tubes, the 100-tube ULTRA™ TD autosampler, the Air Server™ interface for canisters and on-line sampling, the µ-CTE™ Micro-Chamber / Thermal Extractor for materials testing, the TT24-7™ for continuous on-line monitoring and the TC-20™ multi-tube conditioner.

Markes also supplies a wide range of sampling accessories and consumables for all TD application areas.

## What is TD?

Since the early 1980s, thermal desorption has provided the ultimate versatile sample introduction technology for GC / GC-MS. It combines selective concentration enhancement with direct extraction into the carrier gas and efficient transfer / injection all in one fully automated and labour-saving package.

Markes International Ltd. UK headquarters

## Overview

Thermal desorption is now recognised as the technique of choice for environmental air monitoring and occupational health & safety. Relevant standard methods include: ISO/EN 16017, EN 14662 (parts 1 & 4), ASTM D6196, US EPA TO-17 and NIOSH 2549. Related applications include monitoring chemical warfare agents (CWA) in demilitarisation / destruction facilities & civilian locations (counter-terrorism).

TD is also routinely used for monitoring volatile and semi-volatile organic compounds (S)VOCs in products and materials. Examples include residual solvents in packaging & pharmaceuticals, materials emissions testing and food / flavour / fragrance profiling.

This publication presents several real world applications for thermal desorption in forensic science and monitoring chemical warfare agents (CWA). Accompanying publications cover the application areas of:

- Food, flavour, fragrance & odour profiling
- Emissions from materials
- Environmental monitoring and occupational health & safety

## Forensic Applications for TD

Thermal desorption is extensively used for forensic science. Key applications include:

- Detection of drugs of abuse
- Arson residue analysis for accelerants
- Forensic analysis of drugs
- Detection of trace explosive vapours
- Shotgun propellant
- Forensic analysis of inks

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## Chemical Warfare Agents (CWA)

Markes thermal desorption technology provides the ultimate pre-concentration and analytical solution for CWA applications. Military & civilian security agencies and government scientists from across the world are working with Markes TD systems for monitoring:

- CW stockpile sites
- Personal exposure of military personnel
- Agent destruction facilities
- Key civilian locations (counter-terrorism)

Relevant TD options from Markes International include both automated processing of off-line sorbent tube samples (UltrA-UNITY) and continuous near-real time (NRT) detection (TT24-7). Systems can be installed at-line in destruction facilities, in off-line laboratories or in mobile labs for deployment to incident sites. Both on- and off-line TD systems offer rugged operation, quantitative recovery, optimum resolution / speciation (to minimise false positives) and lowest possible detection limits (ng/m$^3$ and below).

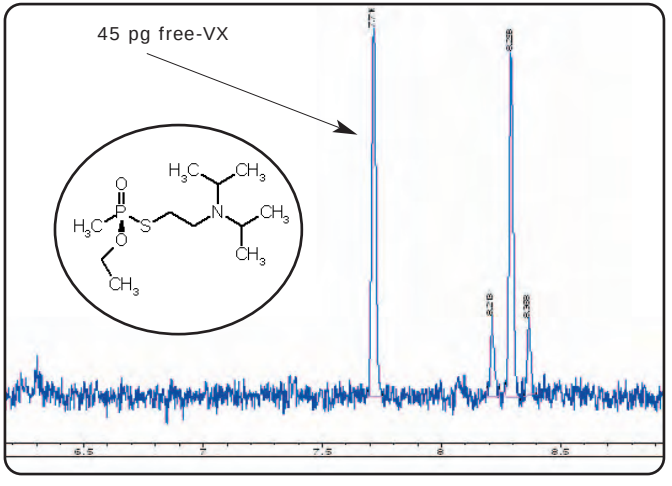
## TT24-7

The transportable, near-real time TT24-7 TD system enables continuous on-line and at-line sampling and pre-concentration of airborne chemical agents. The enriched vapour sample is subsequently analysed by GC-(MS) or direct MS technology.

Continuous monitoring is essential to protect security agency personnel and the general public from potential exposure to highly toxic chemical agents.

The TT24-7 incorporates two, electrically-cooled (Peltier) large-capacity traps which are sampled sequentially at high flow rates (~ 500 ml/min), allowing efficient pre-concentration of trace-level agent in the shortest possible time (*i.e.* providing near real-time analysis).

# Chemical warfare agents: Free-VX




Analysis of 45 pg of free-VX using sorbent tube sampling and off-line TD-GC analysis

Concentration: Sub-ppt vapour concentrations and pg levels on tube

## Background:

The nerve agent VX and the Russian equivalent (RVX) (ethyl S-2-diisopropyl aminoethylmethyl phosphonothioate) are some of the most toxic CW compounds in existence today. The analysis of free (*i.e.* underivitised) VX/RVX at sub ppt levels is very challenging because of the low volatility, "stickiness" and high reactivity of these compounds. VX and RVX are typically monitored using off-line sorbent tubes sampling large (>500 L) air volumes with subsequent analysis by thermal desorption GC/FPD or GC/MS. However, the TD system used must be highly inert and uniformly heated. Markes UltrA-UNITY is ideal for the analysis of underivitised VX/RVX at trace levels.

## Typical TD-GC conditions:

Sampling: Silcosteel™ CW tubes

TD system: UltrA-UNITY

Primary desorption: 8 mins at 300°C

Trap: Chemical weapons trap

Split: Splitless Analysis: GC-FPD

Reference: TDTS44 The analysis of free-VX from sorbent tubes at low and sub-nanogram levels



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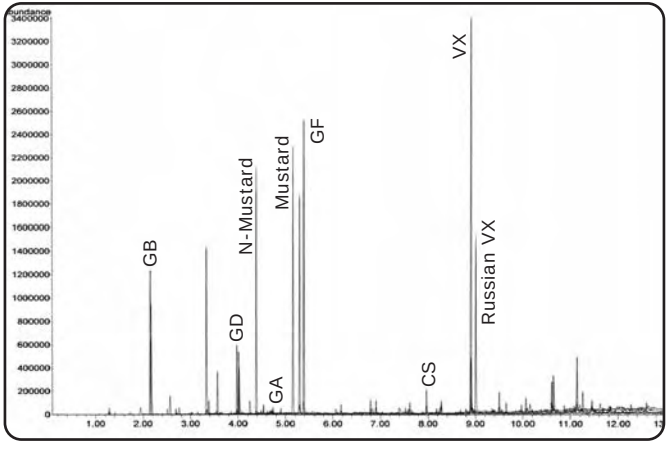
# Chemical warfare agents: DAAMS tubes




Analysis of mixed CW standard (5 ng level) by UNITY TD with GC-MS

## Typical analytes:

GB, GD, GA, GF, CS, VX, RVX, mustard, N-mustard

## Concentration:

ppt to ppb levels (sub/low ng levels on tube)

## Background:

The ability to screen for multiple CW agents, using a single TD-GC-MS method is extremely useful having applications in both the military and civil defense arenas. Where mixed CW material is stockpiled and/or destroyed there is a need to monitor the operational environment both as a check on the occupational health of plant operatives and for confirmatory analysis (DAAMS - Depot Air Analysis Monitoring Systems) of on-line systems. Similarly, for civil defence, monitoring the location of any known or suspected chemical incident is essential to identify the specific agent released, so that correct remedial and decontamination procedures can be actioned.

## Typical TD-GC conditions:

Sampling: Silcosteel CW tubes - manual pumps or automated sequential tube sampling (MTS-32™ - picture inset)

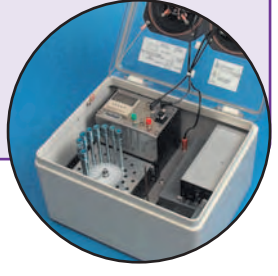
TD system: ULTRA-UNITY

Primary desorption: 8 mins at 300°C

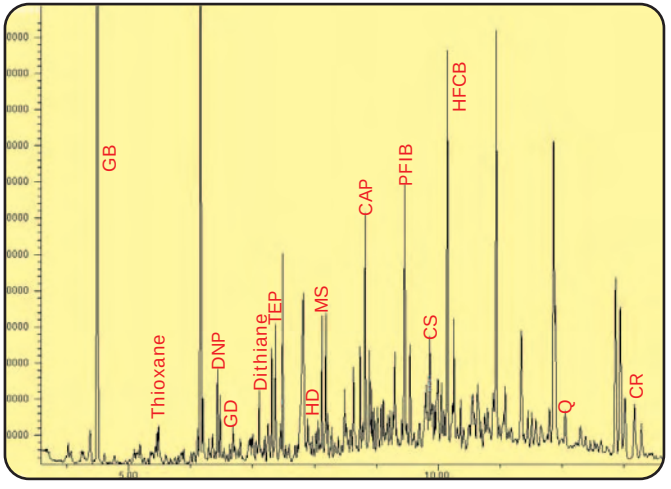
Trap: Chemical weapons trap

Split: 15 ml/min (secondary desorption)

Analysis: GC-MS (SCAN)



# Chemical warfare agents: Trace target analytes in a complex background




CW test mix diluted to 2.5 ppm with diesel fuel and analysed with spectral deconvolution software

## Background:

Analysing complex real air samples for the presence of trace CWA material can be very demanding as the background levels of other organic vapours (hydrocarbons, solvents, *etc.*) can be high relative to that of the target compunds. The risk is that this can cause high incidences of "false-positive" results which is problematical and potentially dangerous; for example, if it triggers large scale evacuations of public buildings. MS detection reduces this risk as it provides 3D data, including spectral information, for each compound.

In a recent development, retention time and spectral data can be further processed by a mathematical procedure (deconvolution) using software such as AMDIS which is provided by NIST in the USA. This identifies individual components in co-eluting / overlapping peaks and provides much greater confidence in the correct identification of trace target compounds thus eliminating false positives.

## Typical TD-GC conditions:

As the previous page

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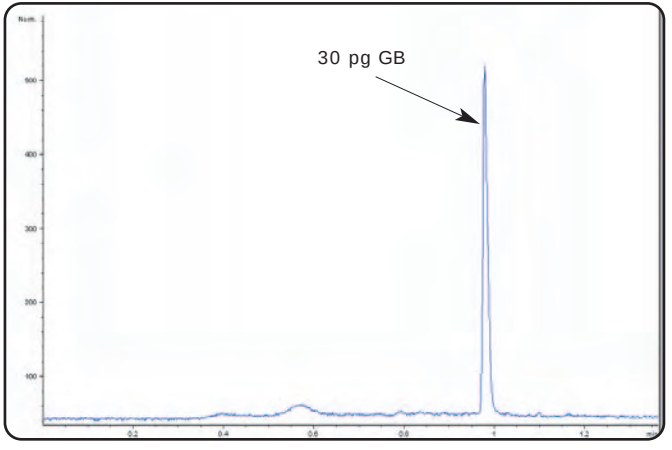
# Chemical warfare agents: NRT monitoring




Monitoring CW Agents using the TT24-7 NRT System with GC-FPD – An illustration with GB

## Background:

Near Real Time (NRT) monitoring of extremely toxic compounds such as the G type nerve agents, requires continuous sampling with no time 'blind' spots and rapid on-line analysis. At trace levels, TT24-7 focusing traps desorb super-efficiently producing sharp peaks (opposite) for optimum sensitivity.

The NRT mode of operation is an absolute requirement for CW monitoring at military-stockpile sites or demilitarisation / destruction facilities and can also be used for continuous monitoring of civilian locations in case of terrorist attack.

## Typical TD-GC conditions:

Sampling: Continuous monitoring

Sampling time / flow: 10 mins at 600 ml/min

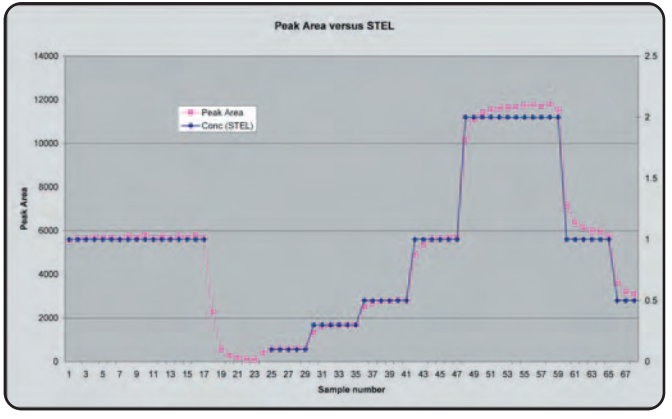
TD system: TT24-7

Traps: Chemical weapons traps

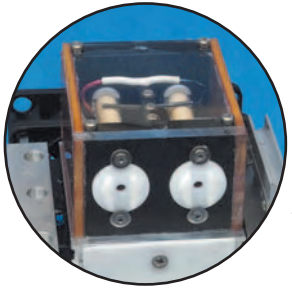
Analysis: GC-FPD

Reference: TDTS63 Using the TT24-7 with twin electrically-cooled focusing traps for continous monitoring of trace level toxic chemicals (*e.g.* CW agents) in air

## TT24-7 performance




TT24-7 data tracks atmospheric concentration exactly - Nominal set point (blue), actual measured (pink)



TT24-7 dual trap

### Background:

NRT monitoring of chemical warfare agents and other toxic chemicals offers early alert and compound identification in the event of a chemical incident. Organisations, such as the Centre for Disease Control (CDC) in the USA, have defined parameters for 'NRT' monitoring including completion of the entire sampling and analytical process within 15 mins plus continuous sampling of air with no 'blind' spots. This requires dual reciprocating sampling traps such that sampling can continue on one channel, while the other is desorbed and analysed. Air is drawn into the system using either positive sample pressure or by vacuum pump at electronic mass flow controlled rates of ~500 ml/min for up to 10 mins. Sampling of each channel is followed by rapid desorption and fast GC, MS or GCMS analysis (*i.e.* < 5 minutes). The TT24-7 TD tracks actual atmospheric concentrations closely with negligible time lag (see opposite).

Reference: TDTS63 Using the TT24-7 with twin electrically-cooled focusing traps for continous monitoring of trace level toxic chemicals (*e.g.* CW agents) in air

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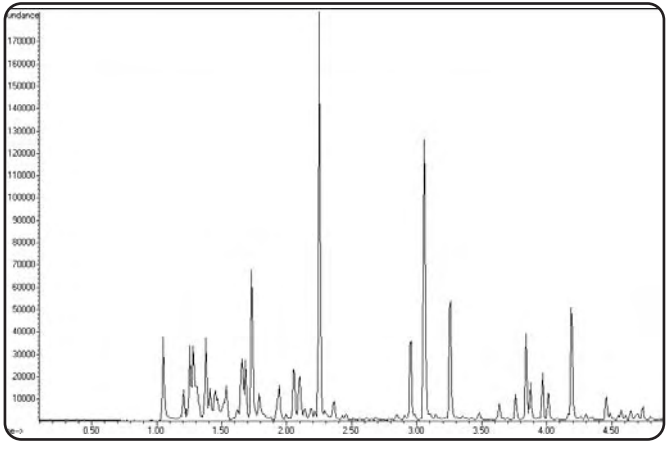
# Arson residue analysis: Gasoline vapours



Sample of headspace from cloth soaked in petrol.

Typical analytes:
Hydrocarbons

Concentration: ppb to % levels

## Background:

In suspected arson cases, it is often necessary to identify the fuel accelerant that was used to start the fire. A representative sample of debris is typically collected from the scene using a nylon bag, and then returned to the laboratory. The bag and contents are first heated (gently) to help release fuel vapours into the headspace of the bag. A small hole is then made in the bag allowing a measured volume of headspace to be withdrawn using a gas syringe and transferred into a thermal desorption tube for pre-concentration. The tube is then analysed by TD-GC-(MS) allowing the VOC profile of the fire debris headspace to be analysed for fuels / accelerants.

## Typical TD-GC conditions:

Sampling: ~100 ml of headspace transferred to Tenax tube.

TD system: ULTRA-UNITY

Primary desorption: 5 mins at 280°C

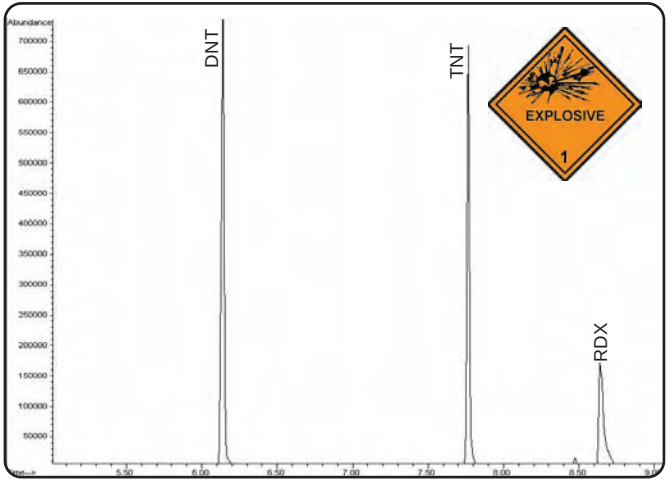
Trap: General purpose hydrophobic

Split: 30 ml/min

Analysis: GC-MS

Reference: TDTS58 The application of TD-GC(-MS) as a tool in forensic investigations

# Explosive vapours




Detection of explosives at low ng levels illustrates the inertness of the UNITY flow-path

## Typical analytes:

DNT, TNT, RDX

## Concentration: Typically ppt to ppb levels of vapours in air

## Background:

Both military and civilian security agencies need to monitor for explosives. Thermal desorption is used for monitoring trace explosive vapours in air at crime scenes, from possible arms storage locations and from vehicles suspected of being used to transport bombs or other weapons.

The high boiling point and reactive nature of explosives necessitates the use of inert sample tubes and sorbent materials.

## Typical TD-GC conditions:

Sampling: Silcosteel tubes packed with quartz wool and Tenax TA™

TD system: ULTRA-UNITY

Primary desorption: 3 mins at 180°C followed by 2 mins at 210°C

Trap: CW trap

Split: 18 ml/min

Analysis: GC-MS

Reference: TDTS58 The application of TD-GC(-MS) as a tool in forensic investigations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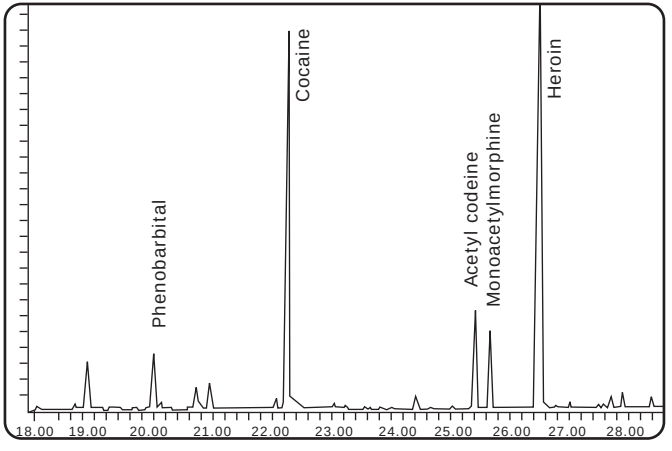
# Direct desorption for detecting drugs of abuse in house dust




Direct desorption of house dust, indicating presence of drugs of abuse such as heroin & cocaine

## Typical analytes:

- Phenobarbital
- Cocaine
- Acetyl codeine
- Monoacetylmorphine
- Heroin

Concentration: ppb to %

## Background:

Many real-world samples can be tested for proscribed drugs using direct thermal desorption / extraction with GC-MS. In this example, gentle direct desorption was used to detect drugs of abuse in house dust collected from a UK crime scene. High levels of heroin and cocaine plus traces of other drugs were identified. Direct TD eliminates sample preparation so reducing the risk of contamination. This technique is suitable for detection and identification rather than absolute quantification of the drugs. Direct desorption of a pure drug sample would also facilitate detailed analysis of impurities allowing the source of the drug to be traced.

## Typical TD-GC conditions:

Sampling: Small amount of dust placed inside glass tube, secured between 2 plugs of quartz wool

Primary desorption: 10 mins at 150°C

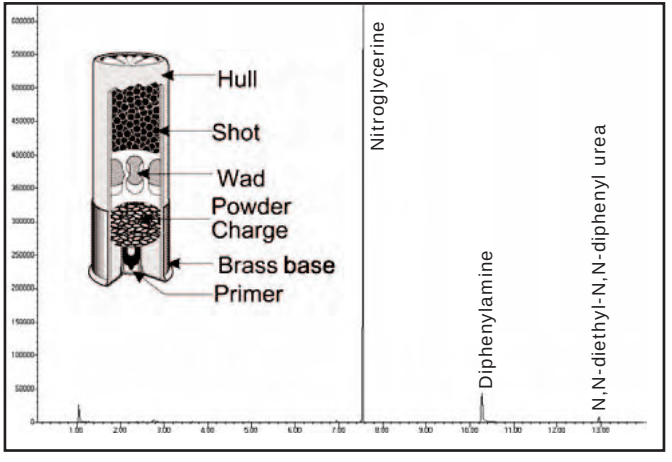
Trap: High boilers trap

Split: 10 ml/min

Analysis: GC-MS

Reference: TDTS58 The application of TD-GC(-MS) as a tool in forensic investigations

# Shotgun propellant




Direct desorption of fine particles of firearm propellant

## Analytes:

N,N-diethyl-N,N-diphenyl urea, diphenylamine, nitroglycerine

## Concentration: ppm to % in particles

## Background:

The composition of small particles suspected to be firearm propellant can be analysed by gentle, direct desorption inside a Silcosteel TD tube. Volatile / semi-volatile components of interest are released into the carrier gas stream during the desorption process while solid residues remain behind in the sample tube. This allows clean analysis of the propellant, without matrix interference, thus optimising detection limits.

## Typical TD-GC conditions:

Sampling: Small pellet of shotgun propellant placed inside an empty thermal desorption tube

Primary desorption: Direct desorption at 60°C

Trap: CW trap

Split: 20 ml/min

Analysis: GC-MS

Reference: TDTS58 The application of TD-GC-(MS) as a tool in forensic investigations

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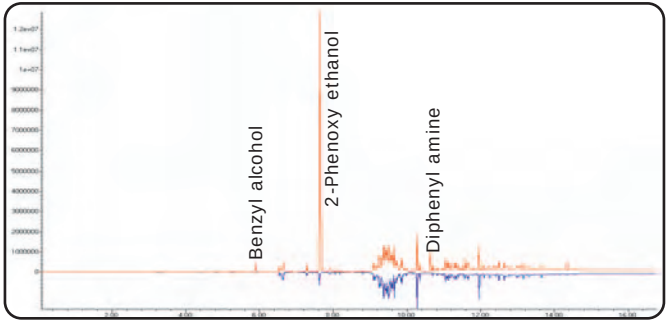
# Forensic analysis of ballpoint pen ink




Black ink: fresh (top) and aged (bottom). The blue chromatograms are from blank paper

Analytes:
2-Phenoxy ethanol, benzyl alcohol, diphenylamine

## Background:

In general, inks are composed of dyes in solvents and other materials that impart selected characteristics. Ink analysis is usually limited to comparisons of the organic dye components. However, this does not allow inks with similar formulations to be distinguished nor does it allow forensic scientists to tell how long ink has been on a particular document.

Direct desorption of paper samples, with and without writing, can be used to generate a comprehensive profile / "fingerprint" of the ink comprising both solvents and dye components. This facilitates detailed forensic analysis of the ink used - age, source, matches with other documents, *etc.*

## Typical TD-GC conditions:

Sampling: Direct desorption
TD System: UNITY
Primary desorption: 15 mins at 100°C
Trap: General purpose - graphitised carbon
Split: 20 ml/min
Analysis: GC-MS

# The Markes International advantage

- Markes is the market leader in TD
- Unparalleled reputation for product quality and reliability
- Excellence in technical and applications support
- For further information on Markes comprehensive range of instruments, sampling accessories and consumables please use one of the contact numbers / email address below or browse the web site

The Markes International team





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United Kingdom

Clinical, Forensic & Toxicology

# LC/MS/MS Analysis of Metabolites of Synthetic Cannabinoids JWH-018 and JWH-073 in Urine

By Amanda Rigdon*, Paul Kennedy**, and Ty Kahler*

*Restek Corp., **Cayman Chemical

## Abstract

A liquid chromatographic method was developed to resolve a comprehensive set of metabolites of JWH-018 and JWH-073. In addition to the chromatographic analysis method, an extraction method was developed to recover a broad range of synthetic cannabinoid metabolites, including carboxylic acid metabolites that are not traditionally recovered using a high pH liquid/liquid extraction. The extraction and analysis methods were used to identify and quantify the significant metabolites in several authentic urine samples. In addition to the identification of known metabolites, two previously undocumented metabolites were detected.

The chromatographic method detailed in this application note employed a 5 µm Ultra Biphenyl 50 mm x 2.1 mm RP-HPLC column and MS/MS detection. The quantitative range validated for all metabolites was 1 ng/mL to 500 ng/mL in urine. Linearity for all metabolites was $r \geq 0.9982$. Accuracy for QC samples ranged from 89% to 100%. The extraction procedure gave metabolite recoveries from urine that ranged from 43% to 78%.

Based on the data shown here, this method is suitable for quantification of metabolites of JWH-018 and JWH-073 to support broader research studies that positively identify clinically significant metabolites and their concentrations in urine.

## Introduction

Since 2008, synthetic cannabinoids such as JWH-018 and JWH-073 have gained popularity in the United States and Europe. These compounds are smoked as components of herbal incense mixtures that, until recently, were commercially available and legally sold. In early 2011, the US Drug Enforcement Agency (DEA) placed several of the most popular synthetic cannabinoids—including JWH-018 and JWH-073—on their Schedule 1 list, making the possession or consumption of these compounds illegal.

Because of the scheduling of JWH-018 and JWH-073, laboratories are now being tasked with developing methods to analyze urine for synthetic cannabinoids. Research has shown that the parent compounds are extensively metabolized prior to excretion and, therefore, are present at very low levels in urine samples [1]. The more abundant metabolites are better targets for screening assays; however, since the illicit use of synthetic cannabinoids is relatively recent, limited research has been completed to determine the



In previous work, several metabolites of JWH-018 and JWH-073 were identified using both in vivo and in vitro studies. The identified metabolites include mono- and di-hydroxylated metabolites, as well as carboxylated metabolites [1,2]. The hydroxylated and carboxylated metabolites are generally extracted separately due to differences in pKa values for these compounds. Both groups of metabolites present some chromatographic challenges; the hydroxylated analytes exist as multiple positional isomers that are indistinguishable by MS/MS, and the carboxylated compounds are hydrophilic, making them difficult to retain using RP-HPLC.

The purpose of this work was to develop an extraction and analysis method suitable for the identification and quantification of a wide range of known and unknown synthetic cannabinoid metabolites in urine. Our goal was to establish methodology that simplified extraction and provided reliable chromatographic resolution of clinically relevant metabolites.

## Experimental

The overall experimental design included calibrators prepared in urine at 7 levels (1, 10, 25, 50, 100, 250, and 500 ng/mL), blanks from 6 separate sources (3 male, 3 female), 2 quality control (QC) samples fortified at 40 ng/mL, and 6 legally obtained authentic samples. Quantification and partial validation was performed using this set of samples. Extraction efficiency was determined by fortifying a post-extraction blank at 10 ng/mL and comparing the area of the resulting peak to the corresponding calibrator, which was fortified prior to extraction.

*Sample Preparation*

Urine samples were fortified with synthetic cannabinoid metabolites at the calibrator and QC levels defined above. Internal standard concentration was 40 ng/mL in urine for all samples, except the double blank.

Fortified and blank samples were hydrolyzed by adding 1 mL of sample to 1 mL of beta-glucuronidase from keyhole limpet (Sigma-Aldrich cat.# G8132) and incubating at 60 °C for 3 hours. The beta-glucuronidase solution was prepared at 5,000 Fishman units/mL in 100 mM ammonium acetate buffer (pH = 5.0).

Hydrolyzed samples were then extracted using 6 mL, 500 mg C18 high-load endcapped Resprep® SPE cartridges (cat.# 24052) according to the following procedure. Note that the cartridge should not be allowed to go dry until step 6.

1. Add 1 mL 5 mM ammonium acetate in 0.1% acetic acid (pH = 4.2) to hydrolyzed sample.
2. Condition cartridge with 3 rinses of 1 mL acetonitrile.
3. Condition cartridge with 3 rinses of 1 mL 5 mM ammonium acetate in 0.1% acetic acid.
4. Apply sample to cartridge and allow it to pass through under gravity.
5. Rinse cartridge with 3 portions of 1 mL 5 mM ammonium acetate in 0.1% acetic acid.
6. Dry cartridge under vacuum for 10 minutes.
7. Elute with 3 mL acetonitrile followed by 3 mL butyl chloride. Note that the use of butyl chloride is not necessary to obtain adequate recoveries. However, if it is used, add the first mL of butyl chloride to the last mL of acetonitrile to ensure the solvents are mixed.

Sample extracts were evaporated to dryness under nitrogen at 40 °C and then reconstituted in 0.5 mL (50:50) 0.05% acetic acid in water:0.05% acetic acid in acetonitrile.

*Analysis*

The instrument used for this analysis was a Shimadzu UFLCXR liquid chromatograph coupled to an AB SCIEX API 4000 LC/MS/MS detector. Instrument conditions are listed below, and MRM transitions are provided in Table I. Note that many of the analytes included here share common transitions. During compound optimization, transitions were chosen based on abundance as well as uniqueness, when possible.

Quantification was performed using the internal standards as specified in Table I. The choice of internal standard for quantification was based on both compound identification and retention time (e.g. JWH-073 4-hydroxybutyl was quantified using JWH-018 pentanoic acid-d4 rather than JWH-073 4-hydroxyindole-d7 due to the fact that its retention time was very early compared to the JWH-073 4-hydroxyindole-d7 internal standard).

**LC Conditions**

| | |
|---|---|
| Instrument: | Shimadzu UFLCXR |
| Column: | 5 µm Ultra Biphenyl 50 mm x 2.1 mm (cat.# 9109552) |
| Column Temperature: | 25 °C |
| Mobile Phase A: | 0.05% acetic acid in water (pH approx. 3.4) |
| Mobile Phase B: | 0.05% acetic acid in acetonitrile |
| Injection Volume: | 10 µL |
| Flow: | 0.5 mL/min. |

**Gradient:**

| Time | %B |
|---|---|
| 0.00 | 45 |
| 2.00 | 45 |
| 6.00 | 85 |
| 6.10 | 95 |
| 7.00 | 95 |
| 7.10 | 45 |
| 8.50 | stop |

**MS/MS Conditions**

| | |
|---|---|
| Instrument: | API 4000 MS/MS |
| Ionization Mode: | ESI+ |
| Data Acquisition Type: | MRM (non-scheduled) |
| Ion Spray Voltage: | 3,000V |
| Source Temperature: | 600 °C |
| Curtain Gas: | 40 psi (275.8 kPa) |
| Gas 1: | 40 psi (275.8 kPa) |
| Gas 2: | 40 psi (275.8 kPa) |
| CAD Gas: | 4 psi (27.6 kPa) |

**Table I** MRM transitions and internal standards used for quantification.

| Compound | MRM1 (Quant.) | MRM2 (Qual. 1) | MRM3 (Qual. 2) | Internal Standard Used for Quantification |
|---|---|---|---|---|
| JWH-073 4-hydroxybutyl | 344.1/155.1 | 344.1/127.2 | 344.1/144.0 | JWH-018 N-pentanoic acid-d4 |
| JWH-073 N-butanoic acid | 358.1/155.1 | 358.1/127.3 | 358.1/144.1 | JWH-018 N-pentanoic acid-d4 |
| JWH-018 N-pentanoic acid-d4 (IS) | 376.1/155.2 | 376.1/230.4 | 376.1/248.3 | NA |
| JWH-018 N-pentanoic acid | 372.1/155.2 | 372.1/127.1 | 372.1/144.1 | JWH-018 N-pentanoic acid-d4 |
| JWH-018 5-hydroxypentyl | 358.1/155.2 | 358.1/127.1 | 358.1/230.3 | JWH-018 N-pentanoic acid-d4 |
| JWH-073 6-hydroxyindole | 344.1/155.2 | 344.1/127.1 | 344.1/145.1 | JWH-073 4-hydroxyindole-d7 |
| JWH-073 5-hydroxyindole | 344.1/155.2 | 344.1/127.1 | 344.1/160.0 | JWH-073 4-hydroxyindole-d7 |
| JWH-073 7-hydroxyindole | 344.2/155.1 | 344.2/127.1 | 344.2/216.3 | JWH-073 4-hydroxyindole-d7 |
| JWH-018 6-hydroxyindole | 358.1/155.1 | 358.1/127.2 | 358.1/145.2 | JWH-018 N-pentanoic acid-d4 |
| JWH-018 5-hydroxyindole | 358.1/155.1 | 358.1/127.2 | 358.1/160.2 | JWH-073 4-hydroxyindole-d7 |
| JWH-018 7-hydroxyindole | 358.1/155.1 | 358.1/127.1 | 358.1/230.3 | JWH-018 N-pentanoic acid-d4 |
| JWH-073 4-hydroxyindole-d7 (IS) | 351.3/127.0 | 351.3/223.0 | 351.3/155.0 | NA |
| JWH-073 4-hydroxyindole | 344.1/155.2 | 344.1/127.2 | 344.1/160.0 | JWH-073 4-hydroxyindole-d7 |
| JWH-018 4-hydroxyindole | 358.1/155.2 | 358.1/230.2 | 358.1/127.1 | JWH-073 4-hydroxyindole-d7 |

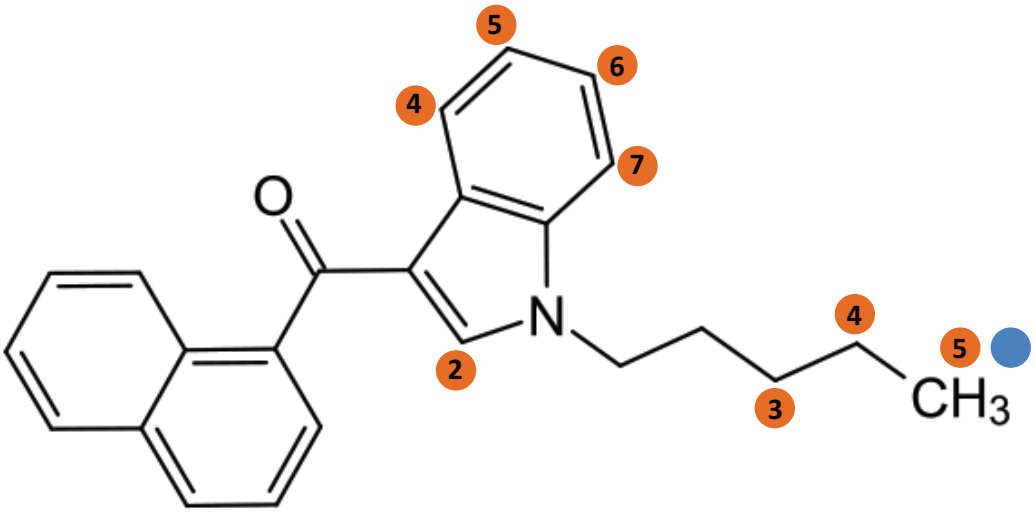
*NA=not applicable

## Results and Discussion

*Chromatography*

Chromatographic separation is essential for analyzing JWH-018 and JWH-073 metabolites due to the presence of multiple positional isomers among the mono-hydroxylated metabolites. These isomers form because each parent compound has many sites available for hydroxylation (Figure 1). Since these positional isomers have identical molecular weights and very similar fragmentation patterns, they are indistinguishable by MS/MS detectors and chromatographic resolution is required for positive identification. Representative chromatograms of low and high calibrators are shown in Figures 2 and 3. All the isomeric analytes included in this method were resolved on the Ultra Biphenyl column. By chromatographically separating these isomers, the most abundant metabolites from a given parent compound can be identified in authentic samples and methodology can be further optimized specifically for metabolites of clinical significance.

**Figure 1** Hydroxylation and carboxylation sites for JWH-018.



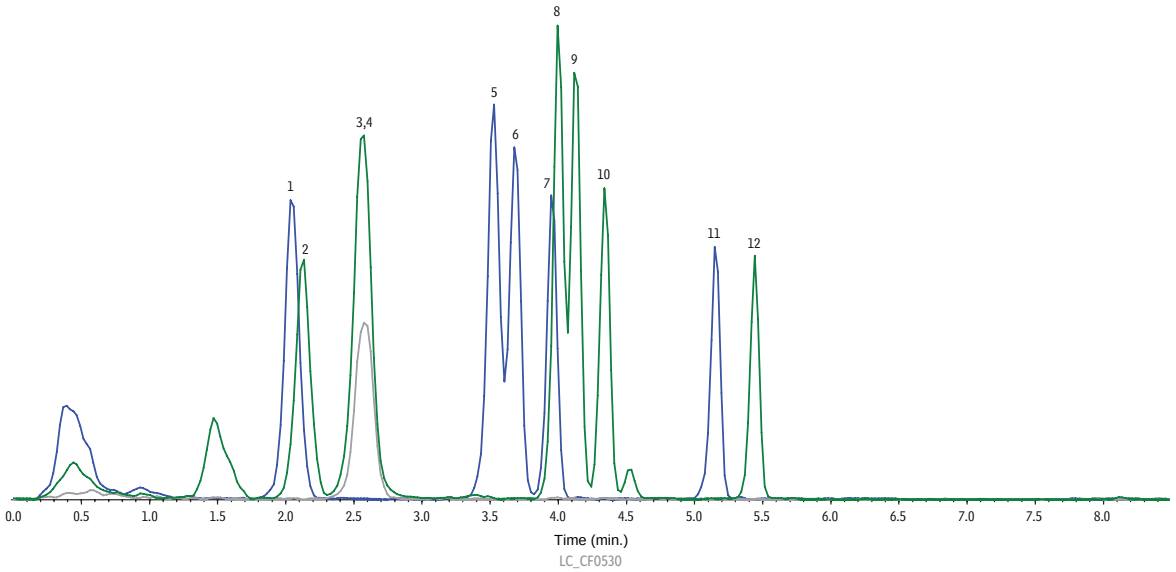

Hydroxylation site – numbers indicate position on indole ring or alkyl side chain.

Carboxylation site

Note: Possible hydroxylation and carboxylation sites are identical for JWH-073. JWH-018 2-hydroxyindole metabolite standard was not available and was not included in this method.

**Figure 2** 1 ng/mL calibration standard.

| Peaks | RT (min.) | MRM1 | MRM2 | MRM3 |
|---|---|---|---|---|
| 1. JWH-073 4-hydroxybutyl | 2.04 | 344.1/155.1 | 344.1/127.2 | 344.1/144.0 |
| 2. JWH-073 N-butanoic acid | 2.13 | 358.1/155.1 | 358.1/127.3 | 358.1/144.1 |
| 3. JWH-018 N-pentanoic acid | 2.59 | 372.1/155.2 | 372.1/127.1 | 372.1/144.1 |
| 4. JWH-018 5-hydroxypentyl | 2.57 | 358.1/155.2 | 358.1/127.1 | 358.1/230.3 |
| 5. JWH-073 6-hydroxyindole | 3.52 | 344.1/155.2 | 344.1/127.1 | 344.1/145.1 |
| 6. JWH-073 5-hydroxyindole | 3.68 | 344.1/155.2 | 344.1/127.1 | 344.1/160.0 |
| 7. JWH-073 7-hydroxyindole | 3.95 | 344.2/155.1 | 344.2/127.1 | 344.2/216.3 |
| 8. JWH-018 6-hydroxyindole | 4.00 | 358.1/155.1 | 358.1/127.2 | 358.1/145.2 |
| 9. JWH-018 5-hydroxyindole | 4.13 | 358.1/155.1 | 358.1/127.2 | 358.1/160.2 |
| 10. JWH-018 7-hydroxyindole | 4.34 | 358.1/155.1 | 358.1/127.1 | 358.1/230.3 |
| 11. JWH-073 4-hydroxyindole | 5.15 | 344.1/155.2 | 344.1/127.2 | 344.1/160.0 |
| 12. JWH-018 4-hydroxyindole | 5.44 | 358.1/155.2 | 358.1/230.2 | 358.1/127.1 |




| | |
|---|---|
| **Column** | Ultra Biphenyl (cat.# 9109552) |
| Dimensions: | 50 mm x 2.1 mm ID |
| Particle Size: | 5 µm |
| Pore Size: | 100 Å |
| Temp.: | 25 °C |
| **Sample** | |
| Diluent: | 50:50 mobile phase |
| Conc.: | 1 ng/mL extracted spiked sample |
| Inj. Vol.: | 10 µL |

| **Mobile Phase** | |
|---|---|
| A: | water + 0.05% acetic acid (pH approx. 3.4) |
| B: | acetonitrile + 0.05% acetic acid |

| Time (min.) | Flow (mL/min.) | %A | %B |
|---|---|---|---|
| 0 | 0.5 | 55 | 45 |
| 2.00 | 0.5 | 55 | 45 |
| 6.00 | 0.5 | 15 | 85 |
| 6.10 | 0.5 | 5 | 95 |
| 7.00 | 0.5 | 5 | 95 |
| 7.10 | 0.5 | 55 | 45 |
| 8.50 | stop | | |

| | |
|---|---|
| **Detector** | API 4000 |
| Model #: | API 4000 |
| Ion Source: | TurboIonSpray® |
| Ion Mode: | ESI+ |
| Ion Spray Voltage: | 3 kV |
| Curtain Gas: | 40 psi (275.8 kPa) |
| Gas 1: | 40 psi (275.8 kPa) |
| Gas 2: | 40 psi (275.8 kPa) |
| Interface Temp.: | 600 °C |
| Mode: | MRM |
| Dwell Time: | 30 ms |
| **Instrument** | API LC MS-MS |

**Notes**

Since multiple transitions are shared between analytes, only 3 transitions are shown to simplify viewing. The transitions shown are: 344.1/155.1 (blue trace), 358.1/155.1 (green trace), 372.1/155.2 (gray trace). To show analytes in full scale, internal standards are not shown.

CAD Gas was set to 4 psi.

For 50 ng/mL calibration level, see chromatogram LC_CF0531.
For 500 ng/mL calibration level, see chromatogram LC_CF0532.

**Sample was prepared according to the following method:**
1) Spike 1 mL blank urine sample with analytes and internal standards.

2) *Hydrolyze sample*:
- Add 1 mL solution of beta-glucuronidase from keyhole limpet (Sigma-Aldrich cat.# G8132). Solution is prepared at a concentration of 5,000 Fishman units/mL in 100 mM ammonium acetate buffer (pH = 5.0).
- Incubate at 60 °C for 3 hours.

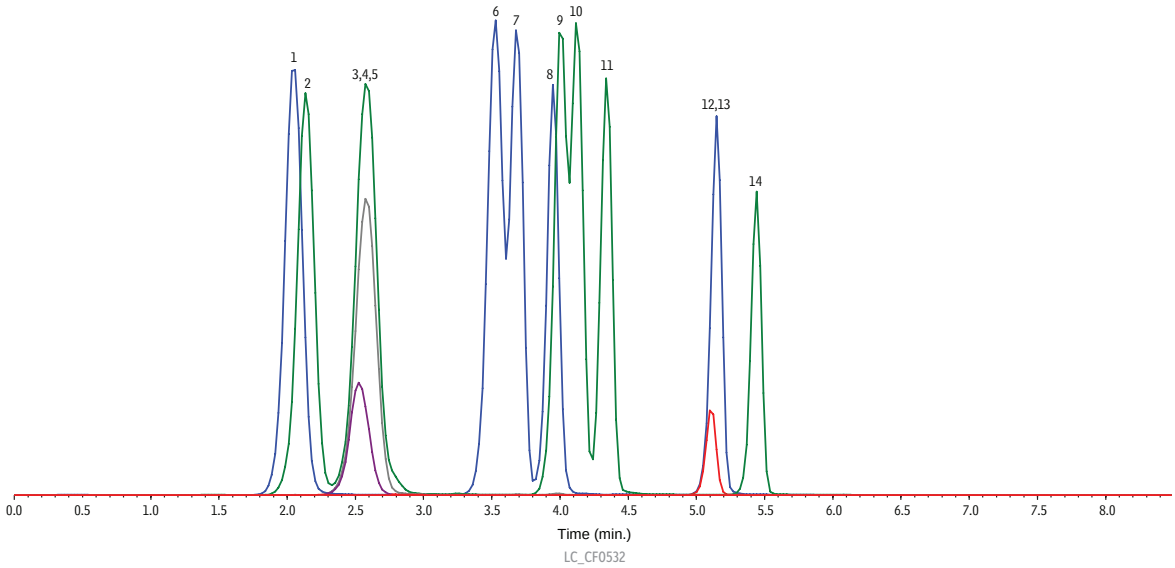
3) *Extract sample on 6 mL, 500 mg C18 high-load endcapped Resprep® SPE cartridge (cat.# 24052)*:
- Add 1 mL 5 mM ammonium acetate + 0.1% acetic acid (pH = 4.2) to sample.
- Condition cartridge with 3x 1 mL acetonitrile.
- Condition cartridge with 3x 1 mL 5 mM ammonium acetate + 0.1% acetic acid.
- Apply sample and allow to pass through under gravity.
- Rinse with 3x 1 mL 5 mM ammonium acetate + 0.1% acetic acid.
- Dry cartridge with vacuum for 10 minutes.
- Elute with 3 mL acetonitrile followed by 3 mL butyl chloride.

4) *Concentrate sample*:
- Evaporate sample to dryness under nitrogen at 40 °C.
- Reconstitute in 0.5 mL water + 0.05% acetic acid:acetonitrile + 0.05% acetic acid (50:50).

**Acknowledgement** Special thanks to Cayman Chemical for reference standards

**Figure 3** 500 ng/mL calibration standard.

| Peaks | RT (min.) | MRM1 | MRM2 | MRM3 |
|---|---|---|---|---|
| 1. JWH-073 4-hydroxybutyl | 2.05 | 344.1/155.1 | 344.1/127.2 | 344.1/144.0 |
| 2. JWH-073 N-butanoic acid | 2.14 | 358.1/155.1 | 358.1/127.3 | 358.1/144.1 |
| 3. JWH-018 N-pentanoic acid-d4 (IS) | 2.52 | 376.1/155.2 | 376.1/230.4 | 376.1/248.3 |
| 4. JWH-018 N-pentanoic acid | 2.58 | 372.1/155.2 | 372.1/127.1 | 372.1/144.1 |
| 5. JWH-018 5-hydroxypentyl | 2.58 | 358.1/155.2 | 358.1/127.1 | 358.1/230.3 |
| 6. JWH-073 6-hydroxyindole | 3.53 | 344.1/155.2 | 344.1/127.1 | 344.1/145.1 |
| 7. JWH-073 5-hydroxyindole | 3.69 | 344.1/155.2 | 344.1/127.1 | 344.1/160.0 |
| 8. JWH-073 7-hydroxyindole | 3.95 | 344.2/155.1 | 344.2/127.1 | 344.2/216.3 |
| 9. JWH-018 6-hydroxyindole | 4.00 | 358.1/155.1 | 358.1/127.2 | 358.1/145.2 |
| 10. JWH-018 5-hydroxyindole | 4.13 | 358.1/155.1 | 358.1/127.2 | 358.1/160.2 |
| 11. JWH-018 7-hydroxyindole | 4.34 | 358.1/155.1 | 358.1/127.1 | 358.1/230.3 |
| 12. JWH-073 4-hydroxyindole-d7 (IS) | 5.11 | 351.3/127.0 | 351.3/223.0 | 351.3/155.0 |
| 13. JWH-073 4-hydroxyindole | 5.15 | 344.1/155.2 | 344.1/127.2 | 344.1/160.0 |
| 14. JWH-018 4-hydroxyindole | 5.44 | 358.1/155.2 | 358.1/230.2 | 358.1/127.1 |




**Column**
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 5 µm
Pore Size: 100 Å
Temp.: 25 °C

**Sample**
Diluent: 50:50 mobile phase
Conc.: 500 ng/mL extracted spiked sample
Inj. Vol.: 10 µL

**Mobile Phase**
A: water + 0.05% acetic acid (pH approx. 3.4)
B: acetonitrile + 0.05% acetic acid

| Time (min.) | Flow (mL/min.) | %A | %B |
|---|---|---|---|
| 0 | 0.5 | 55 | 45 |
| 2.00 | 0.5 | 55 | 45 |
| 6.00 | 0.5 | 15 | 85 |
| 6.10 | 0.5 | 5 | 95 |
| 7.00 | 0.5 | 5 | 95 |
| 7.10 | 0.5 | 55 | 45 |
| 8.50 | stop | | |

**Detector** API 4000
Model #: API 4000
Ion Source: TurboIonSpray®
Ion Mode: ESI+
Ion Spray Voltage: 3 kV
Curtain Gas: 40 psi (275.8 kPa)
Gas 1: 40 psi (275.8 kPa)
Gas 2: 40 psi (275.8 kPa)
Interface Temp.: 600 °C
Mode: MRM
Dwell Time: 30 ms
**Instrument** API LC MS-MS

**Notes**

Since multiple transitions are shared between analytes, only 5 transitions are shown to simplify viewing. The transitions shown are: 344.1/155.1 (blue trace), 358.1/155.1 (green trace), 372.1/155.2 (grey trace), 376.1/155.2 (purple trace - internal standard), 351.3/127.0 (red trace - internal standard)

CAD Gas was set to 4 psi.

For 1 ng/mL calibration level, see chromatogram LC_CF0530.
For 50 ng/mL calibration level, see chromatogram LC_CF0531.

**Sample was prepared according to the following method:**
1) Spike 1 mL blank urine sample with analytes and internal standards.

2) *Hydrolyze sample*:
- Add 1 mL solution of beta-glucuronidase from keyhole limpet (Sigma-Aldrich cat.# G8132). Solution is prepared at a concentration of 5,000 Fishman units/ mL in 100 mM ammonium acetate buffer (pH = 5.0).
- Incubate at 60 °C for 3 hours.

3) *Extract sample on 6 mL, 500 mg C18 high-load endcapped Resprep® SPE cartridge (cat.# 24052)*:
- Add 1 mL 5 mM ammonium acetate + 0.1% acetic acid (pH = 4.2) to sample.
- Condition cartridge with 3x 1 mL acetonitrile.
- Condition cartridge with 3x 1 mL 5 mM ammonium acetate + 0.1% acetic acid.
- Apply sample and allow to pass through under gravity.
- Rinse with 3x 1 mL 5 mM ammonium acetate + 0.1% acetic acid.
- Dry cartridge with vacuum for 10 minutes.
- Elute with 3 mL acetonitrile followed by 3 mL butyl chloride.

4) *Concentrate sample*:
- Evaporate sample to dryness under nitrogen at 40 °C.
- Reconstitute in 0.5 mL water + 0.05% acetic acid:acetonitrile + 0.05% acetic acid (50:50).

**Acknowledgement** Special thanks to Cayman Chemical for reference standards

*Partial Validation Data*

The performance of the chromatographic method was evaluated based on signal-to-noise, linearity, back-calculated accuracy for calibrators, and ion suppression measurement (Table II). Adequate low level responses were obtained and good linearity ($r \geq 0.9982$) was observed for all metabolites over a quantitative range of 1 ng/mL to 500 ng/mL in urine. Comparison of back-calculated calibrator concentrations to prepared values showed accuracy was achieved at both ends of the linear range for all compounds.

Ion suppression was determined by peak area comparison of a blank sample spiked after extraction to a solvent standard. Based on the results gathered in this project, no suppression was observed, but significant enhancement occurred for several analytes.

Interferences and carryover were also evaluated in this study. A double blank sample was injected immediately after the high calibrator and no carryover or interference peaks were observed with areas greater than 5% of the low calibrator. In addition, 5 other blank samples from independent sources were analyzed and no interferences were detected in any of these samples.

**Table II** Chromatographic performance results.

| Compound | S/N (Quant. Ion) 1 ng/mL* | Linearity (r) (Quant. Ion) | Back-Calculated Accuracy 1 ng/mL** | Back-Calculated Accuracy 500 ng/mL ** | Ion Suppression (Quant. Ion) 10 ng/mL |
|---|---|---|---|---|---|
| JWH-018 N-pentanoic acid | 307 | 0.9993 | 1.08 | 0.99 | 1.04 |
| JWH-018 5-hydroxypentyl | 176 | 0.9990 | 1.06 | 0.98 | 1.16 |
| JWH-073 4-hydroxybutyl | 755 | 0.9982 | 1.10 | 0.97 | 1.13 |
| JWH-073 N-butanoic acid | 181 | 0.9986 | 1.10 | 0.97 | 1.07 |
| JWH-018 4-hydroxyindole | 221 | 0.9997 | 1.03 | 0.99 | 1.40 |
| JWH-018 5-hydroxyindole | 370 | 0.9988 | 0.78 | 1.03 | 1.38 |
| JWH-018 6-hydroxyindole | 458 | 0.9987 | 0.88 | 0.81 | 1.17 |
| JWH-018 7-hydroxyindole | 259 | 0.9997 | 0.92 | 1.00 | 1.17 |
| JWH-073 4-hydroxyindole | 490 | 0.9996 | 0.97 | 0.99 | 1.33 |
| JWH-073 5-hydroxyindole | 1,590 | 0.9995 | 0.90 | 1.00 | 1.20 |
| JWH-073 6-hydroxyindole | 1,410 | 0.9999 | 0.97 | 1.00 | 1.09 |
| JWH-073 7-hydroxyindole | 489 | 0.9995 | 0.85 | 1.02 | 1.14 |

* Signal-to-noise ratio for all MRM transitions at the limit of quantitation was greater than 10:1.

** Reported accuracy, based on the quantification ion, was 77.9%–119% for all calibrators and all transitions.

Extraction recovery was assessed by peak area comparison of a post-extraction blank fortified at 10 ng/mL with the corresponding calibrator, which was fortified prior to extraction. QC samples were also analyzed to verify method performance. As shown in Table III, the extraction procedure recovered all the metabolites with results ranging from 43% to 78%. Although some of the recoveries were low, results were acceptable for the first 4 compounds, which were later identified in the authentic samples as being clinically significant metabolites. Subsequent experimentation determined that the low recoveries were due to incomplete elution of the compounds from the SPE cartridge, rather than to poor retention on the analytical column. The elution with butyl chloride, in addition to acetonitrile, increased recoveries by only a small amount and may be eliminated if desired.

Although recoveries for some compounds were relatively low, the use of a mid-pH extraction (pH = 4.2) as opposed to a high pH extraction allowed for the recovery of the carboxylic acid metabolites as well as the mono-hydroxy metabolites. Recoveries for clinically significant metabolites ranged from 70% to 78%. Previously published methods describe the use of a high pH liquid/liquid extraction for the analysis of synthetic cannabinoid metabolites [1]. While the extraction method used by Sobolevsky et al. was suitable for hydroxylated metabolites, the recoveries of carboxylated metabolites are very low at high pH. While a second liquid/liquid extraction at low pH is required to adequately recover carboxylated metabolites, the SPE extraction method developed for this analysis is suitable for both mono-hydroxylated and carboxylated metabolites of JWH-018 and JWH-073.

**Table III** Extraction performance results.

| Compound | Avg. Accuracy 40 ng/mL QCs (n = 2)* | Extraction Recovery (Quant Ion) 10ng/mL |
|---|---|---|
| JWH-018 N-pentanoic acid | 98% | 78% |
| JWH-018 5-hydroxypentyl | 97% | 70% |
| JWH-073 4-hydroxybutyl | 99% | 72% |
| JWH-073 N-butanoic acid | 97% | 78% |
| JWH-018 4-hydroxyindole | 98% | 68% |
| JWH-018 5-hydroxyindole | 93% | 60% |
| JWH-018 6-hydroxyindole | 91% | 65% |
| JWH-018 7-hydroxyindole | 89% | 43% |
| JWH-073 4-hydroxyindole | 100% | 58% |
| JWH-073 5-hydroxyindole | 94% | 66% |
| JWH-073 6-hydroxyindole | 97% | 69% |
| JWH-073 7-hydroxyindole | 91% | 47% |

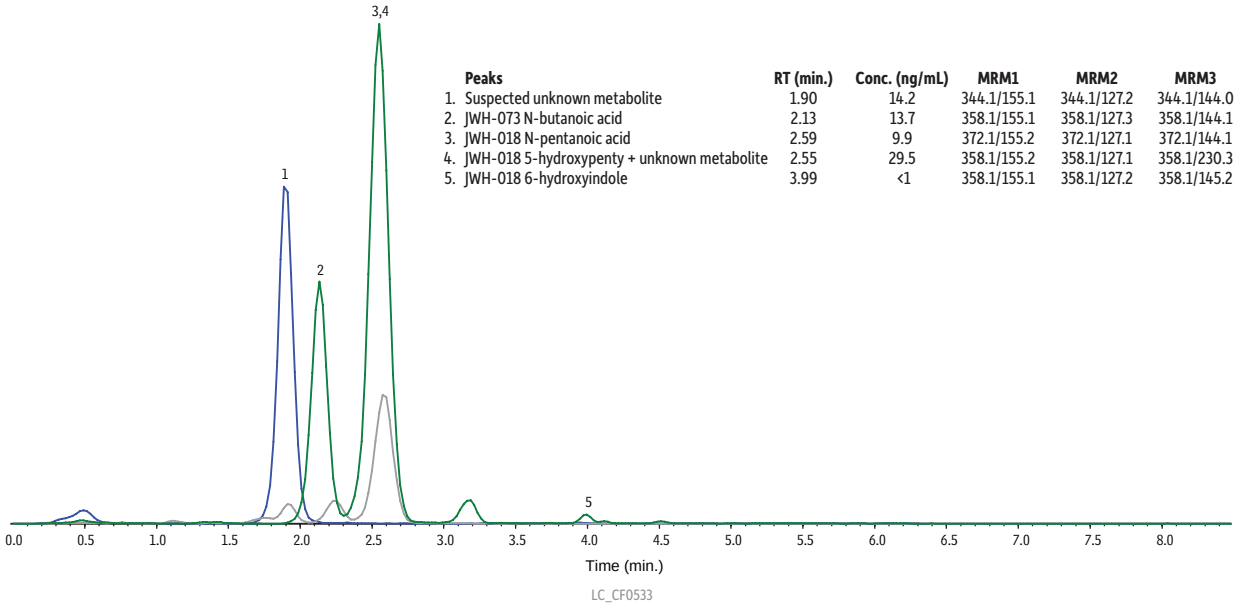
* Reported accuracy for quantification ion.

*Authentic Sample Analysis*

After validation, 6 authentic samples were prepared and analyzed according to the method established here (Table IV). All reported values met ion ratio criteria for the first qualifier MRM transition; however, most results for JWH-018 5-hydroxypentyl did not meet the ion ratio criteria for the second qualifier. As shown in Figure 4, the peak for this analyte was slightly broader than expected. To determine if these results were due to co-eluting interferences, samples and calibrators were re-analyzed using a longer column and a 60 minute isocratic method. The results from the isocratic analyses revealed a co-eluting peak with the same transitions as JWH-018 5-hydroxypentyl. This peak was not present in any of the blank samples, and based on this work, is most likely an undocumented metabolite of JWH-018. Based on recent work by NMS Labs, this unknown is most probably JWH-018 4-hydroxypentyl [3]. Chromatography for the isocratic method was not suitable for quantitation, so no quantitative results can be reported for this compound. Results shown for JWH-018 5-hydroxypentyl are the sum of JWH-018 5-hydroxypentyl and the unknown metabolite.

Although JWH-073 n-butanoic acid is present in several samples, no JWH-073 4-hydroxybutyl was detected. A large peak with the same transitions as JWH-073 4-hydroxybutyl was present in the authentic samples at a slightly earlier retention time than JWH-073 4-hydroxybutyl. Post-extraction spiking of sample #1 with 2 ng of JWH-073 4-hydroxybutyl confirmed that the observed peak was not due to JWH-073 4-hydroxybutyl. The unknown peak was not observed in any blank samples, suggesting that it is also an unknown metabolite of either JWH-018 or JWH-073. Based on recent work by NMS labs, this unknown is probably JWH-073 3-hydroxybutyl [3]. The chromatographic method used here was sufficient to partially resolve the unknown compound from the standard, and the unknown peak was quantitated using JWH-073 4-hydroxybutyl. The results for this unknown metabolite should be considered semi-quantitative.

**Figure 4** Authentic sample #1.




| Peaks | RT (min.) | Conc. (ng/mL) | MRM1 | MRM2 | MRM3 |
|---|---|---|---|---|---|
| 1. Suspected unknown metabolite | 1.90 | 14.2 | 344.1/155.1 | 344.1/127.2 | 344.1/144.0 |
| 2. JWH-073 N-butanoic acid | 2.13 | 13.7 | 358.1/155.1 | 358.1/127.3 | 358.1/144.1 |
| 3. JWH-018 N-pentanoic acid | 2.59 | 9.9 | 372.1/155.2 | 372.1/127.1 | 372.1/144.1 |
| 4. JWH-018 5-hydroxypenty + unknown metabolite | 2.55 | 29.5 | 358.1/155.2 | 358.1/127.1 | 358.1/230.3 |
| 5. JWH-018 6-hydroxyindole | 3.99 | <1 | 358.1/155.1 | 358.1/127.2 | 358.1/145.2 |

| | |
|---|---|
| **Column** | Ultra Biphenyl (cat.# 9109552) |
| Dimensions: | 50 mm x 2.1 mm ID |
| Particle Size: | 5 µm |
| Pore Size: | 100 Å |
| Temp.: | 25 °C |
| **Sample** | |
| Diluent: | 50:50 mobile phase |
| Conc.: | extracted authentic sample |
| Inj. Vol.: | 10 µL |
| **Mobile Phase** | |
| A: | water + 0.05% acetic acid (pH approx. 3.4) |
| B: | acetonitrile + 0.05% acetic acid |

| Time (min.) | Flow (mL/min.) | %A | %B |
|---|---|---|---|
| 0 | 0.5 | 55 | 45 |
| 2.00 | 0.5 | 55 | 45 |
| 6.00 | 0.5 | 15 | 85 |
| 6.10 | 0.5 | 5 | 95 |
| 7.00 | 0.5 | 5 | 95 |
| 7.10 | 0.5 | 55 | 45 |
| 8.50 | stop | | |

| | |
|---|---|
| **Detector** | API 4000 |
| Model #: | API 4000 |
| Ion Source: | TurboIonSpray® |
| Ion Mode: | ESI+ |
| Ion Spray Voltage: | 3 kV |
| Curtain Gas: | 40 psi (275.8 kPa) |
| Gas 1: | 40 psi (275.8 kPa) |
| Gas 2: | 40 psi (275.8 kPa) |
| Interface Temp.: | 600 °C |
| Mode: | MRM |
| Dwell Time: | 30 ms |
| **Instrument** | API LC MS-MS |

**Notes**

Since multiple transitions are shared between analytes, only 3 transitions are shown to simplify viewing. The transitions shown are: 344.1/155.1 (blue trace), 358.1/155.1 (green trace), 372.1/155.2 (gray trace). Internal standards are not shown.

CAD Gas was set to 4 psi.

**Sample was prepared according to the following method:**
1) Spike 1 mL blank urine sample with analytes and internal standards.

2) *Hydrolyze sample*:
- Add 1 mL solution of beta-glucuronidase from keyhole limpet (Sigma-Aldrich cat.# G8132). Solution is prepared at a concentration of 5,000 Fishman units/mL in 100 mM ammonium acetate buffer (pH = 5.0).
- Incubate at 60 °C for 3 hours.

3) *Extract sample on 6 mL, 500 mg C18 high-load endcapped Resprep® SPE cartridge (cat.# 24052)*:
- Add 1 mL 5 mM ammonium acetate + 0.1% acetic acid (pH = 4.2) to sample.
- Condition cartridge with 3x 1 mL acetonitrile.
- Condition cartridge with 3x 1 mL 5 mM ammonium acetate + 0.1% acetic acid.
- Apply sample and allow to pass through under gravity.
- Rinse with 3x 1 mL 5 mM ammonium acetate + 0.1% acetic acid.
- Dry cartridge with vacuum for 10 minutes.
- Elute with 3 mL acetonitrile followed by 3 mL butyl chloride.

4) *Concentrate sample*:
- Evaporate sample to dryness under nitrogen at 40 °C.
- Reconstitute in 0.5 mL water + 0.05% acetic acid:acetonitrile + 0.05% acetic acid (50:50).

**Acknowledgement** Special thanks to Cayman Chemical for reference standards

**Table IV** Quantitative results for authentic samples.

| Compound | Sample 1 (ng/mL) | Sample 2 (ng/mL) | Sample 3 (ng/mL) | Sample 4 (ng/mL) | Sample 5 (ng/mL) | Sample 6 (ng/mL) |
|---|---|---|---|---|---|---|
| JWH-018 N-pentanoic acid | 9.9 | 11.5 | 22.7 | 1.5 | < 1 | 44.3 |
| JWH-018 5-hydroxypentyl + unknown metabolite | 29.5* | 14.7* | 84.2* | 5.4* | 1.4* | 48.9 |
| JWH-073 4-hydroxybutyl | ND | ND | ND | ND | ND | ND |
| Unknown metabolite | 14.2 | 35.2 | 21.6 | 1.70 | < 1 | 69.7 |
| JWH-073 N-butanoic acid | 13.7 | 1.2 | 9.3 | 1.3* | ND | 1.4 |
| JWH-018 4-hydroxyindole | ND | ND | ND | ND | ND | ND |
| JWH-018 5-hydroxyindole | ND | ND | < 1 | ND | ND | ND |
| JWH-018 6-hydroxyindole | < 1 | ND | 1.1 | ND | ND | ND |
| JWH-018 7-hydroxyindole | ND | ND | ND | ND | ND | ND |
| JWH-073 4-hydroxyindole | ND | ND | ND | ND | ND | ND |
| JWH-073 5-hydroxyindole | ND | ND | ND | ND | ND | ND |
| JWH-073 6-hydroxyindole | ND | ND | ND | ND | ND | ND |
| JWH-073 7-hydroxyindole | ND | ND | ND | ND | ND | ND |

*Results did not meet ion ratio criteria (+/- 20%) for the second qualifier MRM transition.

## Conclusions

Based on the results of this partial validation, the extraction and chromatographic methods developed here are suitable for the analysis of JWH-018 and JWH-073 metabolites in human urine. The mid-range pH SPE extraction allows both mono-hydroxylated and carboxylated metabolites to be recovered from a single extraction, providing a simpler alternative to separate high pH and low pH liquid-liquid extraction procedures.

The Ultra Biphenyl column used here provides enough retention for quantitative analysis of the hydrophilic carboxylated metabolites. The column also offers the selectivity needed to separate positional isomers of the mono-hydroxylated metabolites, which must be chromatographically resolved in order to report valid data. The methods established here can be applied in clinical and forensic laboratories to optimize screening procedures for synthetic cannabinoid metabolites in urine.

**References**

[1] T. Sobolevsky, I. Prasolov, G. Rodchenkov, Detection of JWH-018 Metabolites in Smoking Mixture Post-Administration Urine, Forensic Sci. Int., 200 (2010) 141.
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[3] B. Logan, S. Kacinko, M. McMullin, A. Xu, R. Middleberg, Technical Bulletin: Identification of Primary JWH-018 and JWH-073 Metabolites in Human Urine, (2011).



RESTEK®

Lit. Cat.# CFAN1383

# GC Inlet Liner Deactivations for Basic Drug Analysis

By Kristi Sellers, Clinical/Forensic Innovations Chemist, and Lydia Nolan, Innovations Chemist

- Base-deactivated inlet liners are inert to basic drugs, for greater responses.
- Inertness of Rtx®-5Amine column is enhanced for basic compounds.
- Use this liner / column combination for the lowest %RSDs for basic drugs.

Clinical and forensic toxicologists are required to detect low levels of abused drugs in body fluids and confirm their presence by GC/MS. Typical limits of detection are 1-15ng/mL, depending on the sample matrix. For basic drugs (e.g., Figure 1), selecting the proper surface treatment for the GC inlet liner is important, because this parameter can affect responses. The surface of a glass inlet liner contains active silanol groups (Si-OH) that can act as electron pair acceptors, and react with nitrogen or oxygen electron pair donors in basic drug molecules (Figure 2).[1] These reactions usually are rapid and reversible, but they are expressed chromatographically as broad, tailing peaks and/or reduced responses. To eliminate these acid-base reactions, make chromatographic peaks sharp, Gaussian, and easy to integrate, and thereby help ensure reproducible and accurate responses, the -OH groups on the glass surface must be deactivated.

Using GC/FID responses, we evaluated several alternatives for deactivating inlet liners, to determine maximum sensitivity for basic drugs. We prepared reference standards of the free base forms of alprazolam, benzphetamine, cocaine, codeine, ketamine, methadone, and phencyclidine (Figure 1) at 100, 50, 25, 10, and 5 ng/µL concentrations, then analyzed the drugs on a base-deactivated 15m, 0.25mm ID, 0.25µm Rtx®-5Amine column (5% diphenyl/95% dimethylpolysiloxane stationary phase), using a 4mm single gooseneck inlet liner that was untreated, deactivated through an intermediate polarity deactivation process (standard liner deactivation procedure), deactivated through a base deactivation process, or deactivated through the Siltek® deactivation process. We obtained three replicate analyses for each reference standard-liner treatment combination, and evaluated the response data statistically to determine which deactivation treatment maximized sensitivity and reproducibility. We used these results to generate box plots that display the range of data distribution, or variation — an indication of the reproducibility of the performance. We chose phencyclidine (PCP) and cocaine plots to represent the nitrogen-containing and nitrogen/oxygen-containing drugs, respectively (Figure 2). The line in each box indicates the mean response.

The data show that undeactivated liners and liners that received intermediate polarity treatment provided poorer responses or reproducibility, compared to base-deactivated or Siltek® treated liners, due to the acidic nature of the undeactivated glass surface or to a small but influential number of residual acidic sites remaining on the intermediate polarity deactivated surface.

Because the undeactivated liners and intermediate polarity treated liners exhibited either low mean response or high variation, we reanalyzed the data, excluding these treatments and comparing the remaining data (for base-deactivated liners and Siltek® treated liners) for responses and reproducibility. As shown by the examples in Figure 2, base-deactivated liners and Siltek® treated liners performed equally well for cocaine, but the base-deactivated liners yielded the best responses and reproducibility for PCP. Ultimately, a base-deactivated liner would give the best overall performance. Figure 3 shows the linearity plots for all analyzed drugs, obtained using a base-deactivated liner and an Rtx®-5Amine column. Low %RSD values for ketamine (3%), phencyclidine (2%), methadone (2%), cocaine (3%), codeine (5%), and alprazolam (12%) confirm the reproducibility of data obtained from this combination.

Because nitrogen- and oxygen-containing drugs react with silanol groups on glass surfaces, it is important to use properly deactivated glass inlet liners when analyzing these compounds by GC. This work demonstrates that a base-deactivated inlet liner, used in combination with a base-deactivated column, produces high and reproducible responses for basic drugs.

**Figure 1** Nitrogen- and oxygen-containing compounds can react with silanol groups on glass surfaces, causing poor chromatography.

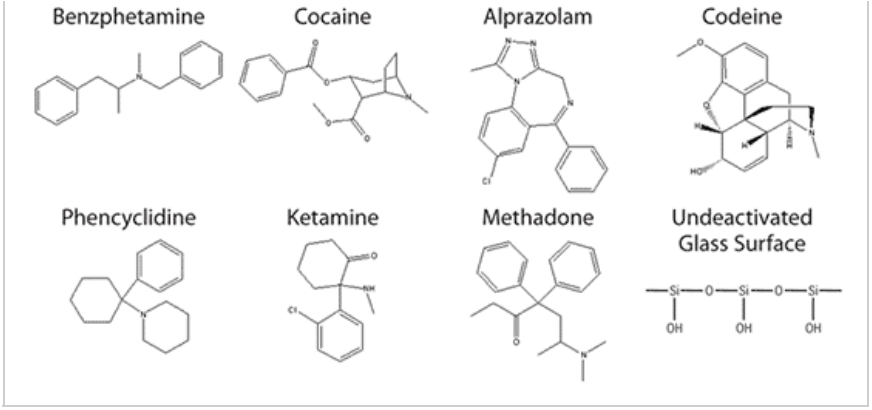



**Figure 2** A base-deactivated inlet liner provides highest mean responses for PCP.

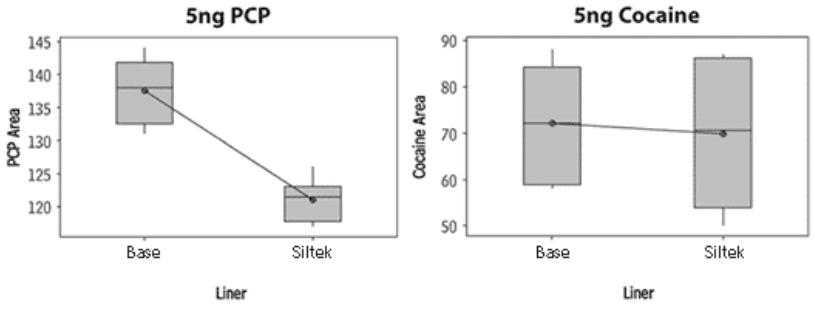
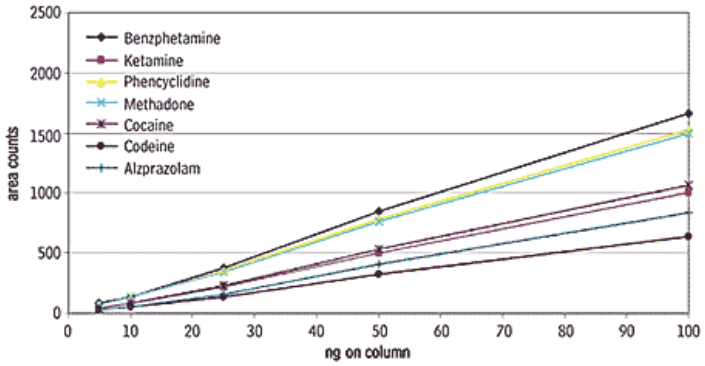



**Figure 3** Linearity plots for all drugs, analyzed using a base-deactivated inlet liner and an Rtx®-5Amine column.




**References**

1. Seyhan N. and D.C. Ege, *Organic Chemistry Health and Company*, 1984, pp.124-136.

**RELATED SEARCHES**

*Rtx-5Amine*, *ketamine*, *phencyclidine*, *methadone*, *cocaine*, *alprazolam*, *pcp*, *basic drugs*, *codeine*

RESTEK
Chromatography Products

# Fast Screening of Recalled Tylenol® for Tribromoanisole and Related Adulterants

## Using QuEChERS and GC-TOFMS

- Rapid sample preparation with QuEChERS improves turnaround time for emergency response analysis situations.
- Prepackaged QuEChERS extraction salts and snap-and-shoot standards reduce human error and save time.
- Rugged, inert, thermally stable Rxi®-5Sil MS column extends applicability to acids, bases, and higher molecular weight adulterants.


**Introduction**

The recent recall of Tylenol® pain reliever and other related products highlights the need for simple, quick sample preparation and a comprehensive analytical method for adulterants in consumer products. The rush to examine a multitude of samples in a short period of time is a common scenario for potential recalls, especially when a contaminant is found in a given product and rapid determinations need to be made to assess how widespread the problem may be.

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) sample preparation approach, originally developed to prepare fruit and vegetable samples for pesticide residue analysis, is being adopted for other applications and may be useful when rapid screening methods are required. QuEChERS employs a simple solvent shake and centrifugation step, with an optional dispersive solid phase extraction (dSPE) cleanup. In addition to being quick and easy, the use of acetonitrile in QuEChERS allows compounds containing a wide variety of chemical functionalities to be extracted, which is very important when trying to isolate an unknown adulterant. The resulting extract is appropriate for both GC/MS and LC/MS work.

The utility of the QuEChERS method is illustrated here using the aforementioned Tylenol® example, showing the applicability to this problem and, by extension, to others like it. This particular recall was due to the presence of 2,4,6-tribromoanisole (TBA) causing a musty smell in the product and, in some cases, nausea in the consumer [1]. TBA is a known breakdown product of 2,4,6-tribromophenol (TBP), which is a fumigant used on shipping pallets; TBA production occurs through a process actuated by a fungus, *Paecilomyces variotii* [2]. TBA is a common and undesirable odorant in the winemaking industry where it and similar compounds (e.g. trichloroanisole) create a situation known as cork taint [3].

This work demonstrates the potential applicability of QuEChERS sample preparation and GC-TOFMS analysis to screening methods for anisole contaminants. Advantages of methods developed based on QuEChERS and GC-TOFMS may include rapid sample screening and definitive identifications in the presence of significant amounts of matrix.

**Procedure**

TBA, TBP, 2,3,4,5-tetrachloroanisole, and pentachloroanisole were spiked into ground up Tylenol® caplets at two different concentrations and extracted using QuEChERS. Several cleanup procedures were performed for comparison and GC analysis was conducted using a sensitive, full mass-range time-of-flight MS.

*Sample Wetting and Fortification*

A bottle of recalled Tylenol® Extra Strength caplets was used for this work, although no odor of TBA was detected. Multiple caplets were ground to a fine powder using a Bamix® Mono Hand Mixer with dry grinder attachment. 1.2 g of powder, equivalent to 2 caplets (500 mg acetaminophen each) was wetted with 9 mL organic-free water for each sample for extraction. After shaking to mix well, wetted powders were fortified as follows; note that spike levels are expressed relative to approximated amount of active ingredient, not formulated product.

- Unspiked Tylenol® 100 µL of QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267)containing PCBs 18, 28, and 52 (50 µg/mL each); triphenylphosphate (20 µg/mL); tris-(1,3-dichloroisopropyl)phosphate (50 µg/mL); and triphenylmethane (10 µg/mL).
- ~1,000 ng/g spiked Tylenol® (2 samples) 5 µL of Custom Anisoles Standard #1 (cat.# 564667) containing 2,4,6-tribromoanisole, 2,3,4,5-tetrachloroanisole, and pentachloroanisole at 200 µg/mL each in methanol. 5 µL of Acid Surrogate Mix (cat.# 31025) containing 2,4,6-tribromophenol, 2-fluorophenol, and phenol-d6, diluted to 200 µg/mL in methanol. 100 µL of QuEChERS Internal Standard Mix for GC/MS Analysis.
- ~100 ng/g spiked Tylenol® 5 µL of Custom Anisoles Standard #1; 5 µL of Acid Surrogate Mix diluted to 20 µg/mL; 100 µL of QuEChERS Internal Standard Mix for GC/MS Analysis.

After fortification, each sample was allowed to soak for 1 hour prior to QuEChERS extraction. Originally the QuEChERS method was developed for high aqueous content fruits and vegetables. Here we used a reduced amount of material and sample wetting in order to increase extraction efficiency for a dry powder.

### *QuEChERS Extraction*

The EN 15662 QuEChERS method was used for sample extraction [4]. 10 mL of acetonitrile was added to a wet sample. After a 1 minute shake, Q-sep™ Q110 buffering extraction salts (cat.# 26213, 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogen citrate sesquihydrate) were added. Following another 1 minute shake, the sample was centrifuged for 5 minutes at 3,000 U/min. with a Q-sep™ 3000 centrifuge (cat.# 26230).

### *Extract Cleanup*

Four dispersive solid phase extraction methods (dSPE) were compared. For each, 1 mL portions of QuEChERS extracts were added to tubes containing drying agent and different sorbents such as primary secondary amine (PSA), C18, and graphitized carbon black (GCB) as shown below. The tubes were shaken for 2 minutes and then centrifuged for 5 minutes in the Q-sep™ 3000 centrifuge. The resulting final extracts were then analyzed with GC-TOFMS.

- Q210 (cat.# 26215): 150 mg $MgSO_4$, 25 mg PSA
- Q251 (cat.# 26125): 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18
- Q252 (cat.# 26219): 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, 50 mg GCB
- Custom dSPE tube: 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, 7.5 mg GCB

### *GC-TOFMS*

A LECO Pegasus® III GC-TOFMS instrument was used and all data were processed with LECO ChromaTOF® software. Gas chromatography was performed using an Rxi®-5Sil MS column (30 m x 0.25 mm x 0.25 µm, cat.# 13623) with a constant flow of helium at 1.2 mL/min. (40 cm/sec. at 90°C). 1 µL fast autosampler splitless injections were made into a 4mm single gooseneck liner with wool (cat.# 22405) at 250°C. The purge valve time was 60 seconds.

The GC oven program was 90 °C (1 minute), 4 °C/min. to 310 °C (2 minutes). Total run time was 58 minutes.

Electron ionization at 70 eV was used with a source temperature of 225°C. Data acquisition was from 45 to 550 amu at a rate of 5 spectra/sec.

### *Calibration and Quantification with Matrix-Matched Standards*

Matrix-matched standards were prepared at 100 pg/µL and 10 pg/µL, as these are the expected final concentrations in extracts for Tylenol® spikes (assuming 100% recoveries for the 1,000 and 100 ng/g spikes, respectively). Matrix-matched standards were prepared by adding standard solution to the final extract from a control sample, which had no measurable amounts of the compounds of interest. Actual recoveries were calculated after quantification from one-point calibration in ChromaTOF®. The internal standard method of quantification was employed using PCB 52.

## Results

The concentrations used for spikes in this case were 1,000 and 100 ng/g relative to active ingredient in the starting caplet material (estimated using labeled value). Using QuEChERS combined with GC-TOFMS, modest recoveries of all compounds were realized as can be seen in Table I. In addition, results for duplicate extracts and cleanups for 1,000 ng/g spikes, using either Q210 dSPE tubes or the custom dSPE tubes, were relatively close for each analyte. Although the spiked concentrations are higher than the odor threshold expected for an end product such as Tylenol® (TBA's odor threshold is extremely low, 0.008-0.03 ppt in water and 2-6 ppt in wine [5]), the QuEChERS approach with GC-TOFMS provides a useful technique for screening of contamination at potential levels of health concern, moderate adulteration, and for analyzing source materials such as wood pallets, for contaminants. QuEChERS can produce extracts for up to 24 samples, ready for GC or LC analysis, in less than 60 minutes, a speed conducive to the pressure of responding to a consumer product adulteration issue. In addition, the multi-compound extraction capability of the QuEChERS acetonitrile solvent offers a better chance of isolating potential adulterants from any matrix.

**Table I** Percent recoveries of potential adulterants from QuEChERS extractions: comparison of various dSPE cleanup procedures. (All samples are 1,000 ng/g, unless otherwise noted.)

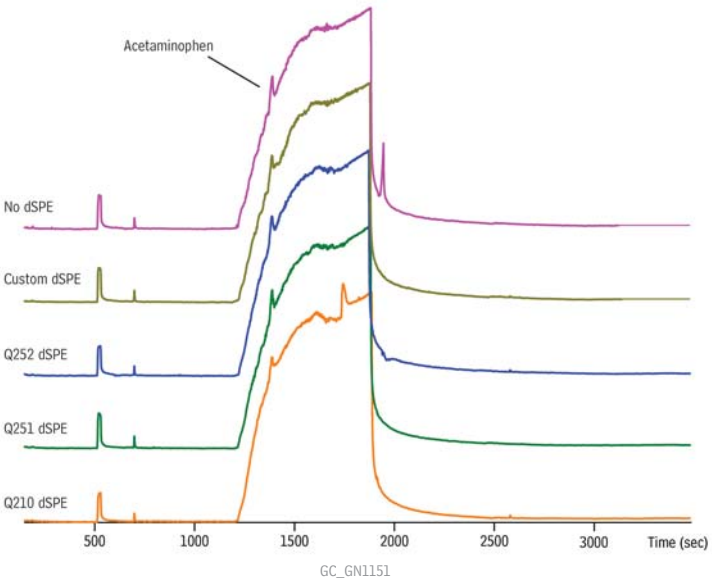
| | | Q210 | | Q251 | | Q252 | | CustomdSPE | Extract |
|---|---|---|---|---|---|---|---|---|---|
| Compound | RT (sec.) | Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 | 1,000 ng/g | 100 ng/g |
| 2,4,6-Tribromoanisole | 1097.82 | 82 | 56 | 62 | 68 | 73 | 68 | 59 | 51 |
| 2,4,6-Tribromophenol | 1133.62 | 55 | 60 | 40 | 49 | 66 | 53 | 63 | 110 |
| 2,3,4,5-Tetrachloroanisole | 1162.22 | 71 | 63 | 64 | 64 | 75 | 63 | 67 | 70 |
| Pentachloroanisole | 1256.82 | 70 | 67 | 64 | 70 | 71 | 61 | 65 | 60 |
| PCB 52 (IS) | 1611.02 | | | | | | | | |

Q210 = 150 mg $MgSO_4$, 25 mg PSA
Q251 = 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18
Q252 = 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, 50 mg GCB
Custom = 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, 7.5 mg GCB
Extract = extraction only, no clean up step was performed

The original QuEChERS approach for fruits and vegetables was developed with a novel dSPE cleanup procedure where an extract is shaken with loose sorbent material (e.g. primary secondary amine, C18, graphitized carbon black) to remove matrix coextractives like fatty acids, lipids, and pigments, that might interfere with targeted residues during instrumental analysis. Although we tried dSPE here, it is less appropriate in this application for two reasons: (1) In a true unknown adulterant situation, sorbents, especially PSA and GCB, might actually remove the adulterant from the extract, in addition to matrix interferences, leaving the adulterant undetected during instrumental analysis. (2) The gross amount of acetaminophen in the extract greatly exceeds the capacity of the dSPE sorbent, which is typically on the order of the 25-50 mg per mL extract.

Due to the overwhelming concentration of acetaminophen in the caplet powder extracts, dSPE cleanup was largely ineffective (Figure 1), but as the acetaminophen was volatile enough to chromatograph, it was not critical to remove it to prevent deposition in the injector and column. Elimination of the dSPE step did not noticeably improve, or degrade, the recovery results for TBA and TBP, or other components (Table I).

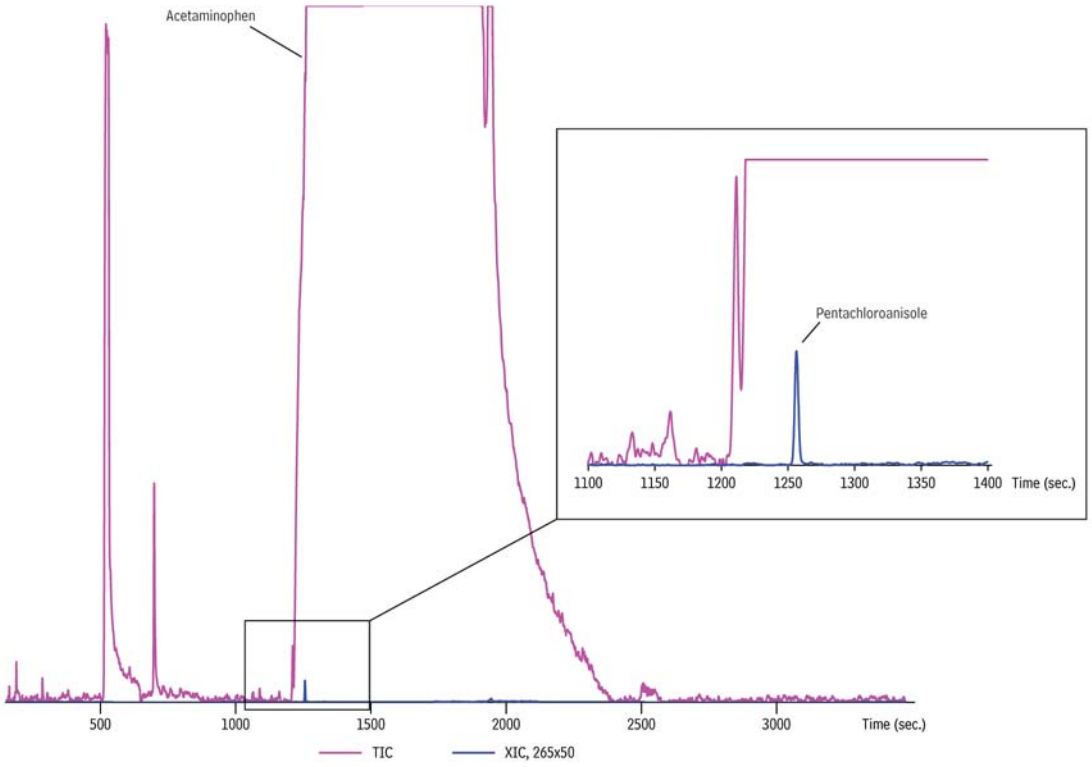
**Figure 1** Chromatograms of QuEChERS extracts of Tylenol® with different dSPE cleanups.




| | |
|---|---|
| **Column** | Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623) |
| **Sample** | QuEChERS extracts of Tylenol® spiked with TBA and anisoles |
| Diluent: | Acetonitrile |
| **Injection** | |
| Inj. Vol.: | 1 µL splitless (hold 1 min.) |
| Liner: | Gooseneck Splitless (4mm) with Wool (cat.# 22405) |
| Inj. Temp.: | 250 °C |
| Purge Flow: | 40 mL/min. |
| **Oven** | |
| Oven Temp: | 90 °C (hold 1 min.) to 310 °C at 4 °C/min. (hold 2 min.) |
| **Carrier Gas** | He, constant flow |
| Flow Rate: | 1.2 mL/min. |
| Linear Velocity: | 40 cm/sec. |
| **Detector** | TOFMS |
| Transfer Line Temp.: | 290 °C |
| Analyzer Type: | TOF |
| Source Temp.: | 225 °C |
| Electron Energy: | 70 eV |
| Mass Defect: | -20 mu/100 u |
| Solvent Delay Time: | 2 min. |
| Tune Type: | PFTBA |
| Ionization Mode: | EI |
| Acquisition Range: | 45 to 550 amu |
| Spectral Acquisition Rate: | 5 spectra/sec |
| **Instrument Notes** | LECO Pegasus 4D GCxGC-TOFMS<br>Q210 = PSA<br>Q251 = PSA and C18<br>Q252 = PSA, C18, and GCB<br>Custom = PSA, C18, and less GCB |

One reason to employ dSPE, or another cleanup step, is to remove matrix interferences that can prevent detection of potential adulterants. However, we relied on automated peak find and spectral deconvolution to detect analytes of interest among the overwhelming acetaminophen response. This is particularly evident for pentachloroanisole in the 1,000 ppb spike extract, which eluted well underneath the large acetaminophen peak (Figure 2). The disparity in concentrations is so large that the 265 m/z ion was only visible by magnifying it by 50, yet ChromaTOF® automatically located the peak and produced a deconvoluted spectrum that matched very well with the pentachloroanisole reference spectrum (Figure 3). Although this part of the application was a targeted analysis of TBA, TBP, and other anisoles, to help evaluate QuEChERS extract recoveries for these compounds in a difficult matrix, the peak find and spectral deconvolution algorithms employed here are very useful when looking for unknown contaminants. Pure sample mass spectra lead to better library searching and identification of components.

**Figure 2** Pentachloroanisole was located under the large acetaminophen peak using an automated peak find routine. (1,000 ng/g; QuEChERS extraction only, no dSPE).




| | |
|---|---|
| **Column** | Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623) |
| **Sample** | QuEChERS extracts of Tylenol® spiked with TBA and anisoles |
| Diluent: | Acetonitrile |
| **Injection** | |
| Inj. Vol.: | 1 µL splitless (hold 1 min.) |
| Liner: | Gooseneck Splitless (4mm) with Wool (cat.# 22405) |
| Inj. Temp.: | 250 °C |
| Purge Flow: | 40 mL/min. |
| **Oven** | |
| Oven Temp: | 90 °C (hold 1 min.) to 310 °C at 4 °C/min. (hold 2 min.) |
| **Carrier Gas** | He, constant flow |
| Flow Rate: | 1.2 mL/min. |
| Linear Velocity: | 40 cm/sec. |

| | |
|---|---|
| **Detector** | TOFMS |
| Transfer Line Temp.: | 290 °C |
| Analyzer Type: | TOF |
| Source Temp.: | 225 °C |
| Electron Energy: | 70 eV |
| Mass Defect: | -20 mu/100 u |
| Solvent Delay Time: | 2 min. |
| Tune Type: | PFTBA |
| Ionization Mode: | EI |
| Acquisition Range: | 45 to 550 amu |
| Spectral Acquisition Rate: | 5 spectra/sec |
| **Instrument** | LECO Pegasus 4D GCxGC-TOFMS |

## Conclusions

Shown here is a QuEChERS multi-compound extraction method that rapidly produces samples for GC or LC analysis in consumer product adulteration cases. QuEChERS is simple, efficient, and uses little solvent compared to other extraction methods. QuEChERS and GC with a sensitive, full mass-range TOFMS is a powerful approach to identifying potential adulterants in consumer products.

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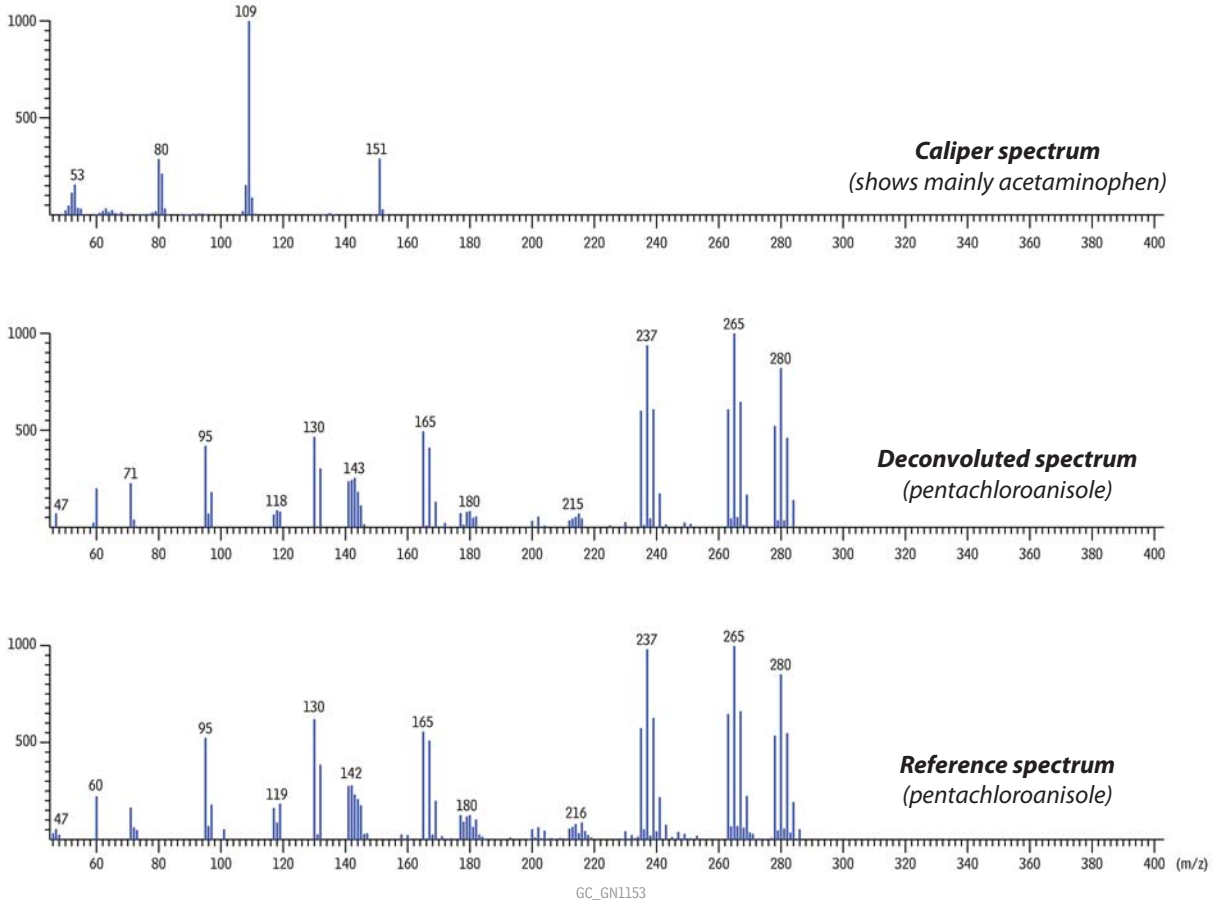
3. F.B. Whitfield, J.L. Hill, K.J. Shaw, J. Agric. Food Chem. 45 (1997) 889.
4. Foods of Plant Origin—Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Clean-up by Dispersive SPE (QuEChERS-method). (EN 15662 Version 2008.).
5. P. Chatonnet, S. Bonnet, S. Boutou, J. Agric. Food Chem. 52 (2004) 1255.


**Figure 3** The caliper spectrum taken at the peak apex of pentachloroanisole is representative of the overwhelming acetaminophen peak, but TOFMS allows spectral deconvolution to produce a sample spectrum that matches well with the reference spectrum.




| | |
|---|---|
| **Column** | Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623) |
| **Sample** | QuEChERS extracts of Tylenol® spiked with TBA and anisoles |
| Diluent: | Acetonitrile |
| **Injection** | |
| Inj. Vol.: | 1 µL splitless (hold 1 min.) |
| Liner: | Gooseneck Splitless (4mm) with Wool (cat.# 22405) |
| Inj. Temp.: | 250 °C |
| Purge Flow: | 40 mL/min. |
| **Oven** | |
| Oven Temp: | 90 °C (hold 1 min.) to 310 °C at 4 °C/min. (hold 2 min.) |
| **Carrier Gas** | He, constant flow |
| Flow Rate: | 1.2 mL/min. |
| Linear Velocity: | 40 cm/sec. |

| | |
|---|---|
| **Detector** | TOFMS |
| Transfer Line Temp.: | 290 °C |
| Analyzer Type: | TOF |
| Source Temp.: | 225 °C |
| Electron Energy: | 70 eV |
| Mass Defect: | -20 mu/100 u |
| Solvent Delay Time: | 2 min. |
| Tune Type: | PFTBA |
| Ionization Mode: | EI |
| Acquisition Range: | 45 to 550 amu |
| Spectral Acquisition Rate: | 5 |
| **Instrument** | LECO Pegasus 4D GCxGC-TOFMS |

# Capillary Columns

**Make the Switch to Rxi® columns!**

Experience what Rxi® did for many others

- Lower detection limits
- Better peak shape
- Accurate results

### Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

| ID | df (µm) | temp. limits | length | qty. | cat. # |
|---|---|---|---|---|---|
| 0.10mm | 0.10µm | -60 to 330/350°C | 10m | ea. | 43601 |
| 0.18mm | 0.18µm | -60 to 330/350°C | 20m | ea. | 43602 |
| 0.18mm | 0.36µm | -60 to 330/350°C | 20m | ea. | 43604 |
| 0.25mm | 0.10µm | -60 to 330/350°C | 15m | ea. | 13605 |
| 0.25mm | 0.10µm | -60 to 330/350°C | 30m | ea. | 13608 |
| 0.25mm | 0.25µm | -60 to 330/350°C | 15m | ea. | 13620 |
| 0.25mm | 0.25µm | -60 to 330/350°C | 30m | ea. | 13623 |
| 0.25mm | 0.25µm | -60 to 330/350°C | 30m | 6-pk. | 13623-600 |
| 0.25mm | 0.25µm | -60 to 330/350°C | 60m | ea. | 13626 |
| 0.25mm | 0.50µm | -60 to 330/350°C | 15m | ea. | 13635 |
| 0.25mm | 0.50µm | -60 to 330/350°C | 30m | ea. | 13638 |
| 0.25mm | 1.00µm | -60 to 325/350°C | 15m | ea. | 13650 |
| 0.25mm | 1.00µm | -60 to 325/350°C | 30m | ea. | 13653 |
| 0.25mm | 1.00µm | -60 to 330/350°C | 60m | ea. | 13697 |
| 0.32mm | 0.25µm | -60 to 330/350°C | 15m | ea. | 13621 |
| 0.32mm | 0.25µm | -60 to 330/350°C | 30m | ea. | 13624 |
| 0.32mm | 0.50µm | -60 to 330/350°C | 30m | ea. | 13639 |
| 0.32mm | 1.00µm | -60 to 325/350°C | 30m | ea. | 13654 |
| 0.53mm | 1.50µm | -60 to 310/330°C | 30m | ea. | 13670 |

### Rxi®-5Sil MS with Integra-Guard®

- Extend column lifetime.
- Eliminate leaks with a built-in retention gap.
- Inertness verified by isothermal testing.

| Description | qty. | cat.# |
|---|---|---|
| 15m, 0.25mm ID, 0.25µmµm Rxi-5Sil MS | ea. | 13620-127 |
| 30m, 0.25mm ID, 0.25µmµm Rxi-5Sil MS | ea. | 13623-124 |
| 30m, 0.25mm ID, 0.25µmµm Rxi-5Sil MS | ea. | 13623-127 |
| 15m, 0.25mm ID, 0.50µmµm Rxi-5Sil MS | ea. | 13635-124 |
| 30m, 0.25mm ID, 0.50µmµm Rxi-5Sil MS | ea. | 13638-124 |
| 30m, 0.25mm ID, 0.50µmµm Rxi-5Sil MS | ea. | 13638-127 |
| 30m, 0.32mm ID, 0.50µmµm Rxi-5Sil MS | ea. | 13639-125 |
| 30m, 0.32mm ID, 1.00µmµm Rxi-5Sil MS | ea. | 13654-125 |

Rxi GCColumns | 3-IN-1 TECHNOLOGY

Highest Inertness • Lowest Bleed • Exceptional Reproducibility

# Q-sep™ QuEChERS Products


### Q-sep™ 3000 Centrifuge

- Meets requirements of AOAC and European QuEChERS methodology.
- Supports 50 mL, 15 mL, and 2 mL centrifuge tubes.
- Small footprint requires less bench space.
- Safe and reliable—UL, CSA, and CE approved, 1-year warranty.

Priced to fit your laboratory's budget, the Q-sep™ 3000 Centrifuge is the first centrifuge specifically designed for QuEChERS methodology. This compact, quiet, yet powerful, unit spins at the 3,000 g-force required by the European method.

Centrifuge includes 50 mL tube carriers (6), 50 mL conical tube inserts (6), 4-place 15 mL tube carriers (6), and 2 mL tube adaptors (24).

| Description | qty. | cat.# |
|---|---|---|
| Q-sep 3000 Centrifuge, 110V | ea. | 26230 |
| Q-sep 3000 Centrifuge, 220V | ea. | 26231 |
| **Replacement Accessories** | | |
| 50mL Tube Carrier for Q-sep 3000 Centrifuge | 2-pk. | 26232 |
| 50mL Conical Tube Insert for Q-sep 3000 Centrifuge | 6-pk. | 26249 |
| 4-Place Tube Carrier for Q-sep 3000 Centrifuge | 2-pk. | 26233 |
| 2mL Tube Adaptors for Q-sep 3000 Centrifuge | 4-pk. | 26234 |

# Q-sep™ QuEChERS Products (cont.)

## Q-sep™ QuEChERS Tubes

for Extraction and Cleanup of Pesticide Residue Samples from Food Products

- Fast, simple sample extraction and cleanup using dSPE.
- Fourfold increases in sample throughput.
- Fourfold decreases in material cost.
- Convenient, ready to use centrifuge tubes with ultra pure, preweighed adsorbent mixes.

Quick, Easy, Cheap, Effective, Rugged, and Safe, the QuEChERS ("catchers") method, developed by the USDA Eastern Regional Research Center[1], has become very popular for extraction and cleanup of pesticide residue samples. Our products are available in three centrifuge tube sizes to meet the needs of both extraction and cleanup of a wide variety of sample matrices following various methods.

The researchers developed a simple two-step procedure. First, the homogenized samples are extracted and partitioned, using an organic solvent and salt solution. Then, the supernatant is further extracted and cleaned, using a dispersive SPE technique. Multiple adsorbents are placed in a centrifuge tube, along with the 1 mL of organic solvent and the extracted residues partitioned from step 1. The contents are thoroughly mixed, then centrifuged, producing a clean extract ready for a variety of GC or HPLC analytical techniques.[2] Validation and proficiency data for the QuEChERS method are available for a wide variety of pesticides in several common food matrices at www.quechers.com

| Description | Material | Methods | qty. | cat# |
|---|---|---|---|---|
| Q110 Kit | 4g $MgSO_4$, 1g NaCl, 1g TSCD, 0.5g DHS with 50mL Centrifuge Tube | European EN 15662 | 50 packets & 50 tubes | 26235 |
| Q110 Packets | 4g $MgSO_4$, 1g NaCl, 1g TSCD, 0.5g DHS | European EN 15662 | 50 packets | 26236 |
| Q150 Kit | 6g $MgSO_4$, 1.5g NaOAc with 50mL Centrifuge Tube | AOAC 2007.01 | 50 packets & 50 tubes | 26237 |
| Q150 Packets | 6g $MgSO_4$, 1.5g NaOAc | AOAC 2007.01 | 50 packets | 26238 |
| Empty 50mL Centrifuge Tube | | | 50-pk. | 26239 |
| **2mL Micro-Centrifuge Tubes for dSPE (clean-up of 1mL extract)** | | | | |
| Q210 | 150mg $MgSO_4$, 25mg PSA | European EN 15662 | 100-pk. | 26215 |
| Q211 | 150mg $MgSO_4$, 25mg PSA, 25mg C18 | | 100-pk. | 26216 |
| Q212 | 150mg $MgSO_4$, 25mg PSA, 2.5mg GCB | European EN 15662 | 100-pk. | 26217 |
| Q213 | 150mg $MgSO_4$, 25mg PSA, 7.5mg GCB | European EN 15662 | 100-pk. | 26218 |
| Q250 | 150mg $MgSO_4$, 50mg PSA | AOAC 2007.1 | 100-pk. | 26124 |
| Q251 | 150mg $MgSO_4$, 50mg PSA, 50mg C18 | AOAC 2007.1 | 100-pk. | 26125 |
| Q253 | 150mg $MgSO_4$, 50mg PSA, 50mg GCB | | 100-pk. | 26123 |
| Q252 | 150mg $MgSO_4$, 50mg PSA, 50mg C18, 50mg GCB | AOAC 2007.1 | 100-pk. | 26219 |
| **15mL Centrifuge Tubes for dSPE (clean-up of 6mL extract)** | | | | |
| Q350 | 1200mg $MgSO_4$, 400mg PSA | AOAC 2007.1 | 50-pk. | 26220 |
| Q351 | 1200mg $MgSO_4$, 400mg PSA, 400mg C18 | AOAC 2007.1 | 50-pk. | 26221 |
| Q352 | 1200mg $MgSO_4$, 400mg PSA, 400mg C18, 400mg GCB | AOAC 2007.1 | 50-pk. | 26222 |
| Q370 | 900mg $MgSO_4$, 150mg PSA | European EN 15662 | 50-pk. | 26223 |
| Q371 | 900mg $MgSO_4$, 150mg PSA, 15mg GCB | European EN 15662 | 50-pk. | 26224 |
| Q372 | 900mg $MgSO_4$, 150mg PSA, 45mg GCB | European EN 15662 | 50-pk. | 26225 |
| Q373 | 900mg $MgSO_4$, 150mg PSA, 150mg C18 | | 50-pk. | 26226 |
| Q374 | 900mg $MgSO_4$, 300mg PSA, 150mg GCB | | 50-pk. | 26126 |

PSA—primary and secondary amine exchange material
GCB—graphitized carbon black

### Sorbent Guide

| Sorbent | Removes |
|---|---|
| PSA* | sugars, fatty acids, organic acids, anthocyanine pigments |
| C18 | lipids, nonpolar interferences |
| GCB** | pigments, sterols, nonpolar interferences |

*PSA—primary and secondary amine exchange material
**GCB—graphitized carbon black

FREE Sample Packs Available!

Free sample packs of SPE cartridges are available by adding -248 to the catalog number when you call to place your order.

References (not available from Restek)
1. Anastassiades, M., S.J. Lehotay, D. Stajnbaher, F.J. Schenck, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, J AOAC International, 2003, vol 86 no 22, pp 412-431.
2. Schenck, F.J., SPE Cleanup and the Analysis of PPB Levels of Pesticides in Fruits and Vegetables. Florida Pesticide Residue Workshop, 2002.

# Reference Standards

## QuEChERS Standards

- Ready to use for QuEChERS extractions—no dilutions necessary.
- Support for GC and HPLC with MS, MS/MS, and selective detectors.

Pesticide analysis is fast and simple using QuEChERS methods. Use these cost-effective QuEChERS standards for even greater lab efficiency. Standards are compatible with all major methods, including mini-multiresidue, AOAC, and European procedures. Save time with convenient mixes or make your own blend using our full line of single component solutions.

## QuEChERS Quality Control Standards for GC/MS Analysis

| Cat.# 33268: | Cat.# 33264: |
|---|---|
| PCB 138 | anthracene |
| PCB 153 | |

50µg/mL each in acetonitrile, 5mL/ampul
cat. # 33268 (ea.)

100µg/mL in acetonitrile, 5mL/ampul
cat. # 33264 (ea.)

## QuEChERS Internal Standard Mix for GC/MS Analysis (6 components)

| | |
|---|---|
| PCB 18 | 50µg/mL |
| PCB 28 | 50 |
| PCB 52 | 50 |
| triphenyl phosphate | 20 |
| tris-(1,3-dichloroisopropyl)phosphate | 50 |
| triphenylmethane | 10 |

In acetonitrile, 5mL/ampul
cat. # 33267 (ea.)

## QuEChERS Single-Component Reference Standards

Concentration is µg/mL. ACN=acetonitrile

| Compound | Solvent | Conc. | cat.# (ea.) |
|---|---|---|---|
| PCB 18 (5mL) | ACN | 50 | 33255 |
| PCB 28 (5mL) | ACN | 50 | 33256 |
| PCB 52 (5mL) | ACN | 50 | 33257 |
| PCB 138 (5mL) | ACN | 50 | 33262 |
| PCB 153 (5mL) | ACN | 50 | 33263 |
| triphenylmethane (5mL) | ACN | 10 | 33260 |
| triphenylphosphate (5mL) | ACN | 20 | 33258 |
| tris(1,3-dichloroisopropyl) phosphate (5mL) | ACN | 50 | 33259 |

## Acid Surrogate Mix (4/89 SOW) (3 components)

- Highest concentrations commercially available.
- Convenient 1mL, 5mL, and 10mL package sizes.
- Reduces laboratory cost per sample extract.

2-fluorophenol
phenol-d6
2,4,6-tribromophenol

| Each | 15-pk. | 25-pk. |
|---|---|---|
| 2,000µg/mL each in methanol, 1mL/ampul | | |
| 31025 | 31025.15 | 31025.25 |
| 10,000µg/mL each in methanol, 1mL/ampul | | |
| 31063 | 31063.15 | 31063.25 |
| 10,000µg/mL each in methanol, 5mL/ampul | | |
| 31087 | 31087.15 | 31087.25 |
| 10,000µg/mL each in methanol, 10mL/ampul | | |
| 33029 | 33029.15 | 33029.25 |

# Fast Screening and Confirmation of Gamma-Hydroxybutyrate (GHB) in Urine

By Amanda Rigdon, Pharmaceutical Innovations Chemist and Kristi Sellers, Clinical/Forensic Innovations Chemist

*Maximize your analytical options with this versatile GHB extraction method. No derivatization means faster sample preparation. Extracts are amenable to both liquid injection GC/FID and headspace GC/MS methods.*

Gamma-hydroxybutyrate (GHB) and its precursor, gamma-butyrolactone (GBL), are controlled substances associated with drug-facilitated sexual assault. Criminal cases often hinge on lab results, which can include screening urine samples and then quantifying GHB using GC/MS. In its native state, GHB is extremely difficult to chromatograph and must be analyzed as a trimethylsilyl derivative or converted to GBL. The headspace (HS) procedure described here (adapted from an FBI Chemistry Unit method) eliminates time-consuming derivatization.[1] This procedure reduces sample preparation time and minimizes both column contamination from derivatization reagents and contamination from sample matrix caused by liquid injections.

**Improve lab efficiency and reduce contamination and matrix effects by eliminating derivatization and moving to a headspace technique.**

**Eliminate Derivatization and Reduce System Contamination**

Samples were spiked in urine and extracted according the procedure in Table I, using alpha-methylene-gamma-butyrolactone (AMGB) as an internal standard. GHB is converted to GBL with sulfuric acid, eliminating the need for derivatization (Figure 1). Note the unconverted sample shows comparable levels of GBL and AMGB, whereas GBL levels in the converted sample are significantly higher, due to the conversion of GHB to GBL.

**Table I** Extraction procedure for GHB and GBL.

1. Label two screw top test tubes per specimen. One for total GHB, the other for GBL only.
2. Add 1mL of sample (urine) to each tube.
3. Add 50µL of AMGB (internal standard) to each tube.
4. Add 150µL concentrated sulfuric acid only to tubes used for analysis of total GHB.
5. Vortex all tubes and allow them to sit 5 minutes.
6. Add 5mL methylene chloride to each tube. Shake 10 minutes to extract.
7. Centrifuge samples at 3,000 rpm for 5 minutes.
8. Transfer bottom (methylene chloride) layer to a clean test tube for drying.
9. Concentrate samples to ~100µL at 30°C under nitrogen.
10. For headspace analysis, inject 15µL of sample into a capped headspace vial. Or, for liquid injection, transfer extract to a limited volume insert.

**Reliably Screen Samples Using Existing Blood Alcohol Testing Set-Up**

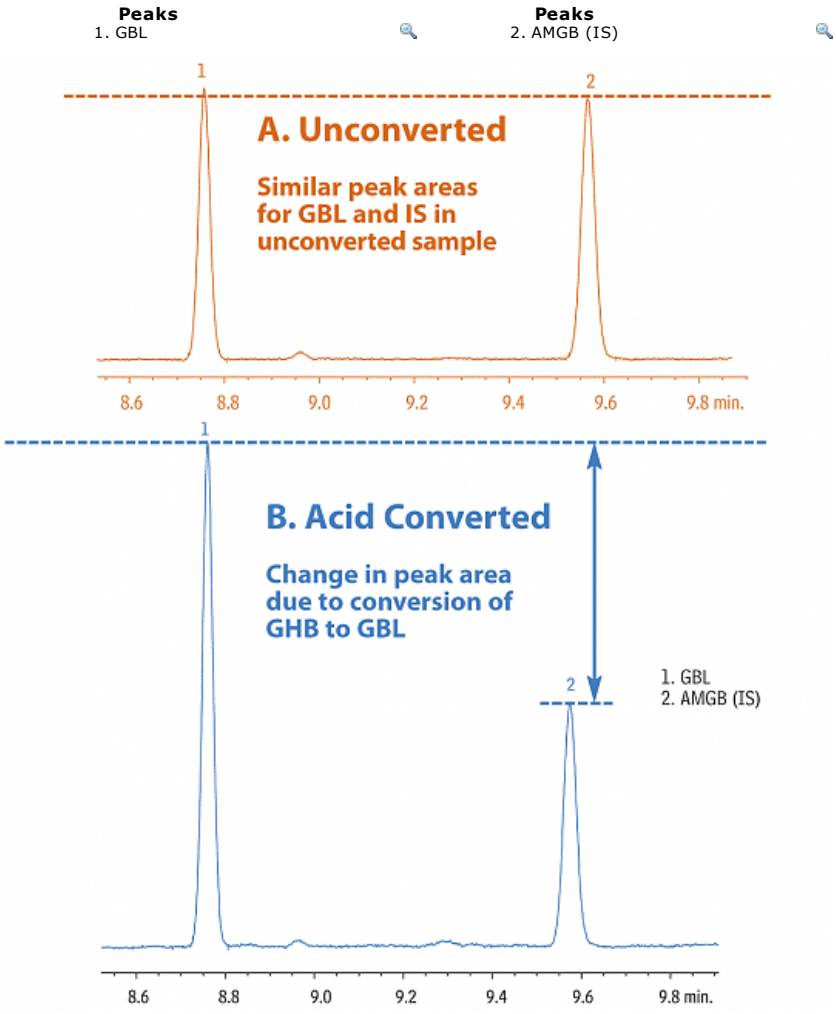
Headspace injections (using the total vaporization technique) of the final urine extracts were screened by GC/FID using an Rtx®-BAC1 column in a blood alcohol headspace GC system. This system is commonly used in clinical/forensic labs, eliminating the need for additional equipment. Excellent linear response was obtained from both unconverted ($r^2$ = 0.9992, 10-100µg/mL 4-point curve) and converted GHB in matrix ($r^2$ = 0.9910, 20-200µg/mL 4-point curve) with AMBG at 50µg/mL.

**Fast, Definitive Confirmation Analysis by Headspace GC/MS**

Positive screening results were quickly confirmed on an Rtx®-5MS column by headspace GC/MS; several quantification and qualifier ions were identified for each compound (GBL: 42, 56, 86; AMBG: 40, 68, 98). Again, excellent linearity was achieved (Figure 2) and analysis time was less than 7 minutes (Figure 3).

In summary, the versatile extraction and headspace method shown here saves lab time and minimizes contamination by eliminating the need for derivatization and by reducing matrix effects. Rapid screening is accomplished on commonly used blood alcohol GC columns, allowing labs to reduce costs by using existing equipment. Confirmation testing using the Rtx®-5MS column, provides the definitive results needed in court with a fast analysis time of less than 7 minutes.

derivatization.




GC_CF01039

| | |
|---|---|
| **Column** | Rtx®-BAC1, 30 m, 0.32 mm ID, 1.80 µm (cat.# 18003) |
| **Sample** | |
| Conc.: | 50 µg/mL GHB, GBL, and AMGB (IS) in urine<br>A: unconverted<br>B: converted with sulfuric acid |
| **Injection** | |
| Inj. Vol.: | 1000 µL headspace-syringe split (split ratio 10:1) |
| Liner: | 1mm Split (cat.# 20972) |
| Inj. Temp.: | 200 °C |
| **Headspace-Syringe** | |
| Sample Temp.: | 100 °C |
| Sample Equil. Time: | 10 min |
| **Oven** | |
| Oven Temp.: | 50 °C (hold 3 min) to 150 °C at 20 °C/min (hold 7 min) |
| **Carrier Gas** | He, constant flow |
| Flow Rate: | 1.0 mL/min |
| **Detector** | FID @ 240 °C |
| Make-up Gas Flow Rate: | 40 mL/min |

**Figure 2** GHB (analyzed as GBL) confirmation method calibration curve for headspace GC/MS analysis (10-300µg/mL in urine).

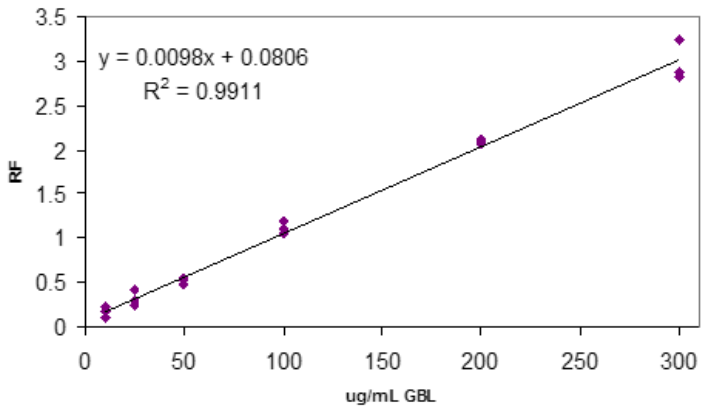
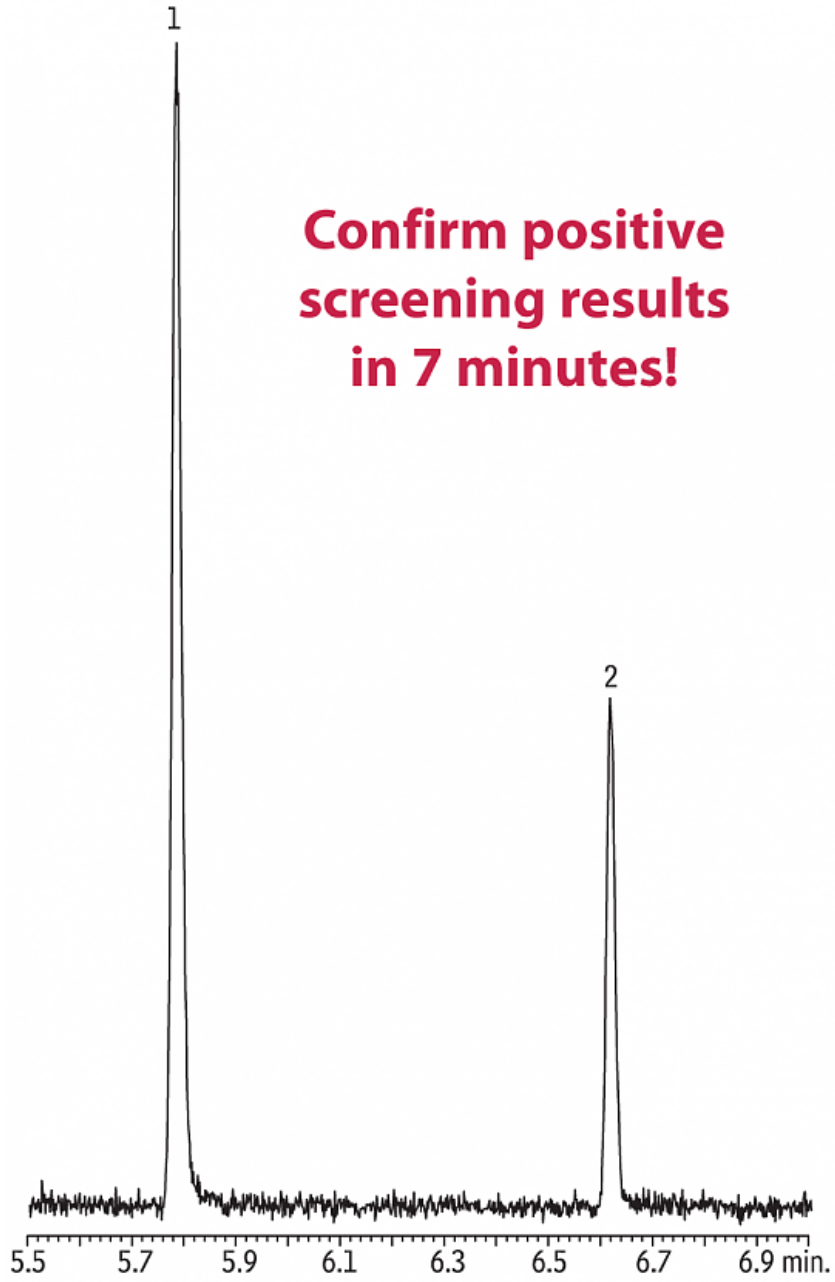



**Figure 3** Confirmation headspace GC/MS analysis of 300µg/mL converted GHB (analyzed as GBL) standard in urine.

**Peaks**
1. GBL

**Peaks**
2. AMGB (IS)






GC_CF01038

| | |
|---|---|
| **Column** | Rtx®-5MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 12623) |
| **Sample** | 300 µg/mL GHB, 50 µg/mL AMGB in urine, converted with sulfuric acid |
| **Injection** | |
| Inj. Vol.: | 1000 µL headspace-syringe split (split ratio 10:1) |
| Liner: | 1mm Split (cat.# 20972) |
| Inj. Temp.: | 200 °C |
| **Headspace-Syringe** | |
| Sample Temp.: | 100 °C |
| Sample Equil. Time: | 10 min |
| **Oven** | |
| Oven Temp.: | 50 °C (hold 3 min) to 150 °C at 20 °C/min (hold 7 min) |
| **Carrier Gas** | He, constant flow |
| Flow Rate: | 1.0 mL/min |
| **Detector** | MS |
| Mode: | Scan |
| Transfer Line Temp.: | 280 °C |
| Ionization Mode: | EI |
| Scan Range: | 20-150 amu |

**REFERENCES**

1. M.A. LeBeau, M.A. Montgomery, M.L Miller, S. G. Burmeister, J. Anal. Toxicol. 24 (2000) 421.

**RELATED SEARCHES**

*GHB*, *Gamma-Hydroxybutyrate*, *gamma-butyrolactone*, *gbl*, *date rape*, *date rape drug*, *AMGB*, *sexual assault*, *drug-facilitated*, *urine*, *headspace*

# Fast, Robust LC-MS/MS Method for Quantification of Multiple Therapeutic Drug Classes Using an Ultra Biphenyl Column

By Amanda Rigdon

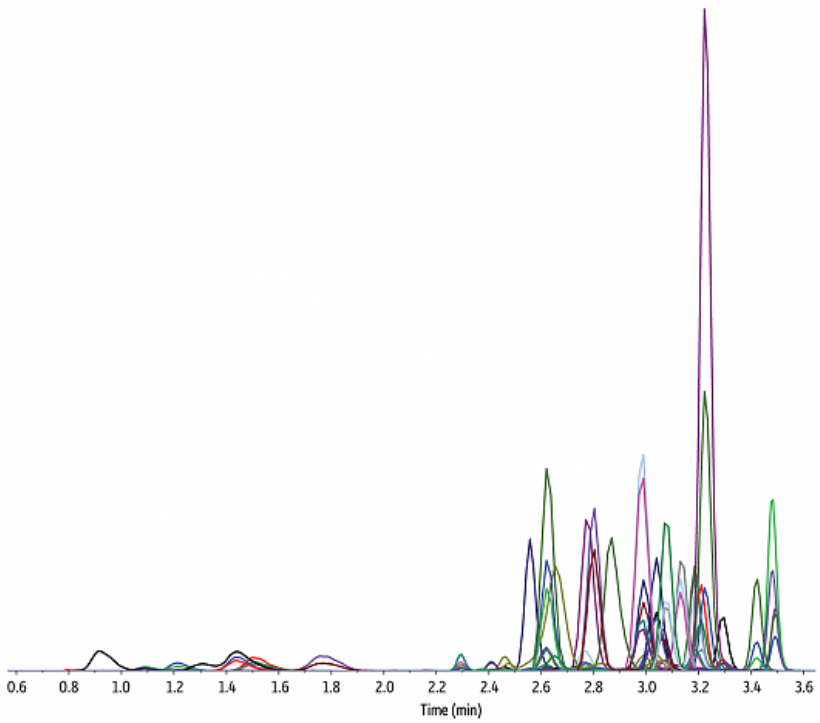
- Quantify 29 drug compounds from four drug classes in a fast, 5.5-minute analysis.
- Ultra Biphenyl column separates isobaric compounds for more definitive results.
- Highly reproducible retention times reduce downtime and reanalysis.

As demand for therapeutic drug monitoring rises, laboratories are under increased pressure to implement streamlined, cost-effective testing procedures. As with any high-volume application, the methods developed for therapeutic drug monitoring must be fast, robust, and easy to implement. Methods that can be used to quantify a wide variety of drug chemistries from a single analysis are particularly beneficial, as they reduce costs and save time. The objective of this work was to develop a fast, robust LC-MS/MS method for the quantification of 29 therapeutic drugs and metabolites in urine from several drug classes including opiates, benzodiazepines, tricyclic antidepressants, and anticonvulsants. Results from this partial validation indicate that the method used here produces good linearity, accuracy, and precision for most of the drugs tested in a fast, 5.5-minute analysis.

The method employed here uses a Shimadzu UFLCXR HPLC coupled to an AB SCIEX API 4000 MS/MS and a 5 µm Ultra Biphenyl (100 mm x 2.1 mm, cat.# 9109512) analytical column with a matching guard column (cat.# 910950212). The Biphenyl column was chosen for this work because of its versatility; it combines the performance of a traditional alkyl (e.g., C18) column with that of a phenyl column, and it offers excellent retention of both polar and nonpolar compounds. The adaptability of the Biphenyl phase makes it particularly useful for methods developed to analyze drugs from multiple classes. Matrix standards and samples were prepared using dilute-and-shoot methodology as described in Figure 1.

**Figure 1:** Analysis of 29 drug compounds and metabolites at 100 ng/mL in urine on an Ultra Biphenyl column.

| Peaks | $t_R$ (min) | MRM 1 | MRM 2 | MRM 3 |
|---|---|---|---|---|
| 1. Morphine | 0.95 | 286.1/229.1 | 286.1/201.3 | 286.1/181.1 |
| 2. Oxymorphone | 1.08 | 302.1/227.2 | 302.1/198.1 | 302.1/115.1 |
| 3. Pregabalin | 1.29 | 160.2/97.1 | 160.2/124.1 | -- |
| 4. Hydromorphone | 1.34 | 286.2/185.1 | 286.2/157.1 | 286.2/128.0 |
| 5. Gabapentin | 1.56 | 172.2/137.0 | 172.2/95.1 | 172.2/55.1 |
| 6. Codeine | 2.16 | 300.2/165.2 | 300.2/152.2 | 300.2/115.2 |
| 7. Codeine-d3 (IS) | 2.16 | 303.2/181.0 | 303.2/199.1 | -- |
| 8. Oxycodone | 2.29 | 316.2/241.2 | 316.2/256.1 | -- |
| 9. Hydrocodone | 2.33 | 300.2/199.2 | 300.2/171.2 | -- |
| 10. 7-Aminoclonazepam | 2.49 | 285.9/121.1 | 285.9/222.2 | 285.9/195.2 |
| 11. Tapentadol | 2.52 | 222.2/107.1 | 222.2/121.1 | 222.2/77.1 |
| 12. Zopiclone | 2.52 | 389.1/245.2 | 389.1/345.2 | 389.1/216.9 |
| 13. Norbuprenorphine | 2.62 | 414.2/165.4 | 414.2/223.2 | 414.2/83.1 |
| 14. 7-Aminoflunitrazepam | 2.65 | 284.1/227.2 | 284.1/135.1 | 284.1/256.1 |
| 15. Zolpidem | 2.69 | 308.2/235.0 | 308.2/263.1 | 308.2/236.2 |
| 16. Citalopram | 2.87 | 325.2/109.2 | 325.2/262.2 | 325.2/116.1 |
| 17. Fentanyl | 2.87 | 337.3/188.2 | 337.3/105.1 | 337.1/79.0 |
| 18. Buprenorphine | 2.89 | 468.3/55.2 | 468.3/396.1 | 468.3/83.2 |
| 19. Doxepin | 2.92 | 280.1/107.2 | 280.1/235.1 | -- |
| 20. Doxepin-d3 (IS) | 2.92 | 283.2/115.1 | 283.2/91.0 | -- |
| 21. Paroxetine | 2.95 | 330.2/192.1 | 330.2/70.1 | 330.2/123.1 |
| 22. Promethazine | 2.97 | 285.2/86.2 | 285.2/71.1 | 285.2/198.1 |
| 23. Nortriptyline | 3.02 | 264.2/105.1 | 264.2/233.1 | 264.2/117.1 |
| 24. Amitriptyline | 3.07 | 278.2/105.1 | 278.2/91.1 | 278.1/117.0 |
| 25. EDDP | 3.08 | 279.1/235.1 | 279.1/250.2 | 279.1/187.2 |
| 26. Lorazepam | 3.08 | 321.1/229.2 | 321.1/275.0 | 321.1/302.7 |
| 27. Sertraline | 3.09 | 307.1/276.0 | 307.1/160.1 | 307.1/158.8 |
| 28. Methadone | 3.11 | 310.3/265.3 | 310.3/105.0 | 310.3/223.1 |
| 29. Clonazepam | 3.17 | 316.1/270.1 | 316.1/240.9 | 316.1/214.2 |
| 30. Flunitrazepam | 3.31 | 314.1/268.1 | 314.1/239.1 | 314.1/183.2 |
| 31. Diazepam | 3.37 | 285.1/193.1 | 285.1/154.0 | 285.1/228.2 |
| 32. Diazepam-d5 (IS) | 3.37 | 290.1/198.2 | 290.1/233.2 | -- |




LC_CF0535

| | |
|---|---|
| **Column** | Ultra Biphenyl (cat.# 9109512) |
| Dimensions: | 100 mm x 2.1 mm ID |
| Particle Size: | 5 µm |
| Pore Size: | 100 Å |
| Temp.: | 30 °C |
| **Sample** | |
| Diluent: | Water:acetonitrile (90:10) + 0.1% formic acid |
| Conc.: | 100 ng/mL (final dilution = 20x) |
| Inj. Vol.: | 30 µL |
| **Mobile Phase** | |
| A: | Water + 0.1% formic acid |
| B: | Acetonitrile + 0.1% formic acid |

| Time (min) | Flow (mL/min) | %A | %B |
|---|---|---|---|
| 0.00 | 0.6 | 90 | 10 |
| 1.00 | 0.6 | 90 | 10 |
| 3.5 | 0.6 | 0 | 100 |
| 4.0 | 0.6 | 0 | 100 |
| 4.1 | 0.6 | 90 | 10 |
| 5.5 | 0.6 | 90 | 10 |

| | |
|---|---|
| **Detector** | AB SCIEX API 4000 MS/MS |
| Model #: | API 4000 |
| Ion Source: | TurboIonSpray® |
| Ion Mode: | ESI+ |
| Ion Spray Voltage: | 3,000 kV |
| Curtain Gas: | 40 psi (275.8 kPa) |
| Gas 1: | 60 psi (413.7 kPa) |
| Gas 2: | 60 psi (413.7 kPa) |
| Interface Temp.: | 600 °C |
| Mode: | MRM |
| **Instrument** | API LC-MS/MS |
| **Notes** | A 5 µm, 10 mm x 2.1 mm Ultra Biphenyl guard column (cat.# 910950212) was used in conjunction with this analysis. |

Sample was prepared as follows:
- Fortify urine at 100 ng/mL.
- To 1 mL of urine, add 1 mL of 100 mM ammonium acetate (pH = 5.6) containing 2,000 units of β-glucuronidase from *E. coli* (Sigma-Aldrich cat# G7396).
- Incubate for 90 minutes at 37 °C.
- Centrifuge at 3,000 rpm for 15 minutes.
- Dilute 100 µL of sample with 900 µL of water:acetonitrile (90:10) + 0.1% formic acid containing 4 ng/mL internal standard. (Total dilution factor = 20x)

**Linear Range and Sensitivity**

To evaluate linearity and sensitivity, an 11-point calibration curve covering a concentration range of 1-1,000 ng/mL was prepared in matrix. Calibration curves for each compound were built from triplicate injections using either a linear or quadratic equation, depending on the response of the individual compound. All calibration curves employed 1/x weighting. As shown in Table I, good linearity was achieved with correlation coefficient values exceeding 0.999 for most compounds.

LOQs were determined by evaluating signal-to-noise ratios for the three transitions used for each compound, and values ranged from 1 ng/mL to 5 ng/mL for most compounds. Several analytes had LOQs of 10 ng/mL; only norbuprenorphine had an LOQ of 25 ng/mL, which was expected since it is a poor responder and usually requires further sample preparation. With the exception of methadone, the quantification ion for each compound had a signal-to-noise ratio of ≥10 at the LOQ, and each qualifier ion had a signal-to-noise ratio of ≥3. Because methadone was a very high responder, the first two transitions for this drug overloaded the detector at higher concentrations, so only the third transition was used for quantification. The first two transitions may be used, but detuning these transitions is recommended to reduce response and improve linearity.

**Accuracy and Reproducibility**

Accuracy and precision at the LOQ were assessed for each compound; acceptable ranges were considered to be 90-110% recovery and ≤15% coefficient of variation (CV). Accuracy ranged from 88% to 113% for all analytes except norbuprenorphine, which typically is not determined using a dilute-and-shoot method. Precision results ranged from 1% to 23%, and all compounds except for codeine, norbuprenorphine, and sertraline had passing results of ≤15% CV for precision (Table I).

**Table I:** Partial validation results for 29 therapeutic drugs and drug metabolites.

| Compound Name | LOQ (ng/mL) | Linearity (r) | % Accuracy at LOQ | %CV at LOQ | S/N at LOQ |
|---|---|---|---|---|---|
| Morphine | 5.0 | 0.9995 | 95 | 5 | 20 |
| Oxymorphone | 5.0 | 0.9994 | 101 | 2 | 30 |
| Pregabalin | 5.0 | 0.9994 | 95 | 5 | 40 |
| Hydromorphone | 2.5 | 0.9993 | 91 | 1 | 40 |
| Gabapentin | 10.0 | 0.9994 | 98 | 5 | 10 |
| Codeine | 10.0 | 0.9990 | 109 | 18 | 50 |
| Oxycodone | 5.0 | 0.9989 | 112 | 10 | 40 |
| Hydrocodone | 5.0 | 0.9997 | 106 | 2 | 30 |
| 7-Aminoclonazepam | 2.5 | 0.9978 | 85 | 14 | 50 |
| Tapentadol | 2.5 | 0.9993 | 95 | 7 | 30 |
| Zopiclone | 10.0 | 0.9911 | 102 | 12 | 20 |
| Norbuprenorphine | 25.0 | 0.9955 | 124 | 19 | 30 |
| 7-Aminoflunitrazepam | 5.0 | 0.9993 | 91 | 12 | 40 |
| Zolpidem | 1.0 | 0.9994 | 96 | 11 | 200 |
| Citalopram | 2.5 | 0.9996 | 101 | 7 | 50 |
| Fentanyl | 1.0 | 0.9996 | 97 | 14 | 70 |
| Buprenorphine | 5.0 | 0.9996 | 99 | 2 | 40 |
| Doxepin | 5.0 | 0.9996 | 100 | 9 | 90 |
| Paroxetine | 5.0 | 0.9994 | 88 | 2 | 100 |
| Promethazine | 1.0 | 0.9997 | 94 | 12 | 30 |
| Nortriptyline | 1.0 | 0.9990 | 101 | 8 | 50 |
| Amitriptyline | 5.0 | 0.9995 | 92 | 7 | 100 |
| EDDP | 5.0 | 0.9997 | 91 | 4 | 200 |
| Lorazepam | 5.0 | 0.9994 | 99 | 13 | 20 |
| Sertraline | 10.0 | 0.9946 | 113 | 23 | 40 |
| Methadone | 1.0 | 0.9998 | 101 | 5 | 3 |
| Clonazepam | 2.5 | 0.9997 | 104 | 6 | 20 |
| Flunitrazepam | 1.0 | 0.9996 | 90 | 9 | 10 |
| Diazepam | 2.5 | 0.9994 | 84 | 6 | 40 |

Since retention time shifts can be a source of downtime and sample reanalysis, retention time reproducibility across multiple column lots was also evaluated. Replicate injections of a solvent standard were analyzed on three different lots of Ultra Biphenyl columns under the same conditions used for the samples. Retention times for each compound were determined and the maximum retention time variation across all three lots of analytical columns was just 0.13 minutes. This indicates retention times are stable and predictable, which minimizes the need to reset retention time windows when columns are changed.

**Conclusion**

Partial validation results indicate this method is suitable for the quantification of a broad range of therapeutic drugs and metabolites in urine at levels ranging from 1-1,000 ng/mL. By using a highly reproducible 5 µm Ultra Biphenyl column and the multi-drug method conditions established here, labs can reduce downtime and improve productivity.

# The Dawn of an Era

Superficially porous particles (commonly referred to as SPP or "core-shell" particles) have been proven to provide fast separations without the need for expensive Ultra High Performance Liquid Chromatography (UHPLC) instruments, thereby increasing sample throughput without capital investment. These particles feature a solid, impermeable core enveloped by a thin, porous layer of silica that decreases the diffusion path and reduces peak dispersion. As a result, they offer significantly higher efficiency than traditional fully porous particles of similar dimensions—often rivaling the efficiency of smaller particles. Core-shell particles changed LC, but they were only the beginning...

## A New Species Has Evolved

Restek is proud to announce that SPP core-shell technology has evolved with the introduction of Raptor™ LC columns. Although column efficiency, which is boosted with superficially porous particles, considerably accelerates analysis time, it has little effect on resolution (i.e., peak separation). Selectivity, on the other hand, has a substantial impact on resolution, but shows minimal improvement in analysis times. New Raptor™ LC columns bond rugged 2.7 and 5 µm superficially porous particles with Restek's unique Ultra Selective Liquid Chromatography™ (USLC®) phases to offer chromatographers the best of both worlds.

By being the first to combine the speed of SPP with the resolution of highly selective USLC® technology, Raptor™ LC columns provide the practicing analyst with the most powerful tools available for fast and efficient method development. And because they are from Restek, Raptor™ LC columns are backed by the manufacturing and quality systems you've come to trust along with the best Plus 1 service in the industry. Choose them for all of your valued assays to experience *Selectivity Accelerated.*

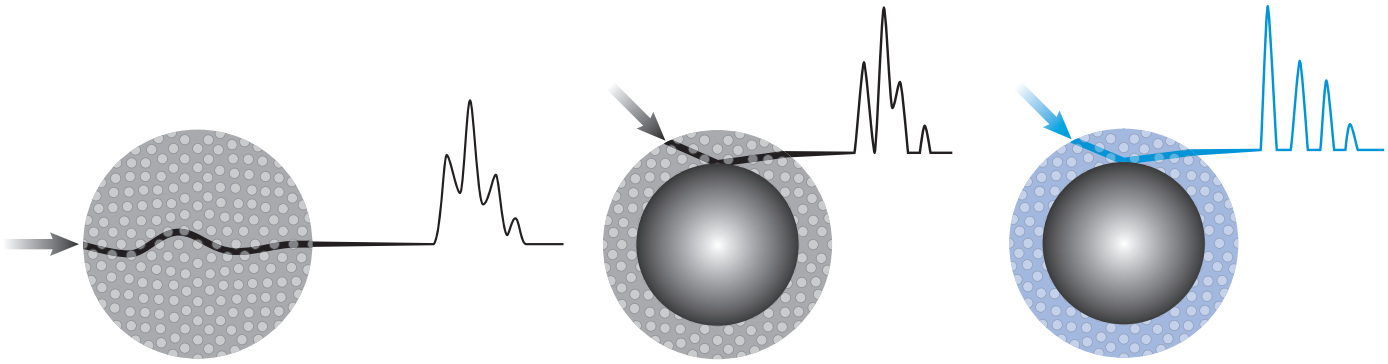
### The History of USLC® Technology

Restek extended the hydrophobic-subtraction model to describe orthogonal selectivity and then applied it to create our unique USLC® stationary phases.

Learn more at **www.restek.com/uslc**

RESTEK USLC®
Ultra Selective Liquid Chromatography

**Figure 1:** Only Raptor™ LC columns offer the higher efficiency of a superficially porous particle *plus* the improved resolution of USLC® phases.



Fully porous particles with typical stationary phases show longer retention times and less resolution.

Ordinary superficially porous particles (SPP) with typical stationary phases show shorter retention times than fully porous particles, but still exhibit less resolution.

Only Raptor™ LC columns with highly selective USLC® stationary phases show shorter retention times and greater resolution.

**Experience *Selectivity Accelerated.* Put Raptor™ LC columns and guards to the test today on your most challenging workflows.**



RESTEK

# Evolutionary Chromatography

It is only possible to fully utilize the efficiency of superficially porous particle technology when it is united with the power of USLC® selectivity. With Raptor™ LC columns, you can speed up method development and enhance sample throughput—without investing in costly UHPLC equipment—to create faster, more reliable, and more sensitive analyses.

- Run faster and avoid lengthy gradient adjustments.
- Separate isobaric and hard-to-resolve compounds with ease.
- Avoid eluting compounds near the void volume and limit ion suppression.
- Skip the complex mobile phases and multiple method modifications.

# Dissecting Raptor™ LC Columns

*A closer look at a new species*

## Adaptive Traits: Raptor™ LC Column

Restek's dedicated R&D group studied every aspect of superficially porous particles (commonly referred to as SPP or "core-shell" particles) to develop the bonding chemistries that are best suited to both the SPP construction and our highly selective USLC® phases. But we didn't stop there. In addition to implementing a new, proprietary column-packing technique, we upgraded our LC column hardware. By looking at not only the particles, but also the packing and hardware, we have made sure that you will get repeatable, rugged performance from each and every Raptor™ LC column.

### Larger 2 µm Frit

Prevents clogging better than commonly used 0.5 µm frits; boosts column lifetime and helps maintain optimal pressures.

### Rugged Label

Clearly identifies both flow direction and column; resists solvents and tearing to last as long as your column does.


### Proprietary Column-Packing Technique

Provides greater pressure stability (600 bar for 2.7 µm; 400 bar for 5 µm); achieves higher linear velocities without sacrificing efficiency or lifetime.

### Raptor™ SPP Particles

**Robust 2.7 and 5 µm Particles**
Let you run high-speed analyses without UHPLC.

**Narrow Silica Distribution**
Ensures high efficiency and consistent flows.

**Updated Bonding and QC**
Guarantee retention time stability, run to run and column to column.

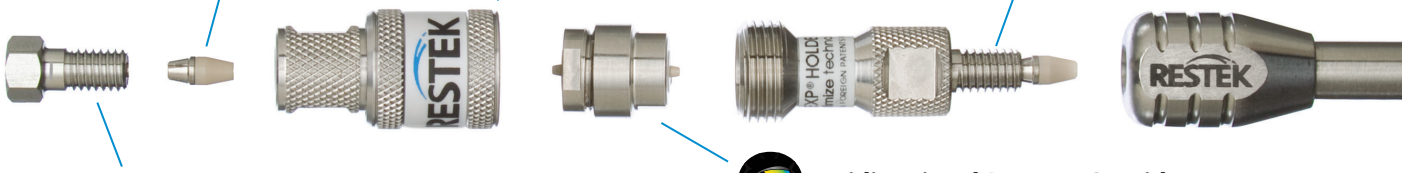
# Natural Protection: Raptor™ EXP® Guard Column

Regardless of its performance, lifespan, or frit size, we know the LC column is the most expensive consumable used for your chromatographic assay. To help protect your investment and further extend the life of our already-rugged Raptor™ LC columns, we have mated our new superficially porous particles with patent-pending guard column hardware developed by Optimize Technologies. A Raptor™ LC guard column cartridge in an EXP® direct connect holder is the ultimate in column protection.

**Patented Titanium Hybrid Ferrules**
Can be installed repeatedly without compromising high-pressure seal.

**Free-Turn® Architecture**
Allows you to change cartridges without breaking inlet/outlet fluid connections—and without tools.

**Auto-Adjusting Connection**
Provides ZDV (zero dead volume) connection to any 10-32 female port.




**Flexible Design**
Replace nut with longer or even tool-free options (below) to best suit your needs.

**Unidirectional Raptor™ Cartridge**

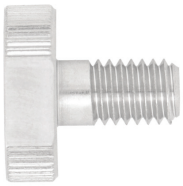
**In-Tandem Development**
Made to pair perfectly with Raptor™ LC columns.

**Superior Packing Technique**
Withstands 600 bar (2.7 µm) / 400 bar (5 µm) operating pressures.

**Restek® Quality**
Backed by the manufacturing and QC systems you trust.

View our full selection of Raptor™ EXP® guard column cartridges at **www.restek.com/raptor**

## Restek also recommends:



**Hand-Tight Nut** (cat.# 25937–25939)
Upgrade the supplied nut to install your Raptor™ EXP® guard column by hand—no tools needed.

**Long Hex-Head Nut** (cat.# 25934)
Extend the nut on your Raptor™ EXP® guard column for easier access in tight spaces—no more bumped knuckles.

**EXP® Hand-Tight Coupler** (cat.# 25940)
Achieve tool-free 8,700+ psi (600+ bar) seals anywhere in your LC system with EXP® hand-tight couplers and connectors.

Visit **www.restek.com/exp** for more EXP® hex-head fittings, couplers, replacement parts, and more!



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Pure Chromatography

**Questions about this or any other Restek® product?**
**Contact us or your local Restek® representative (www.restek.com/contact-us).**



**www.restek.com**

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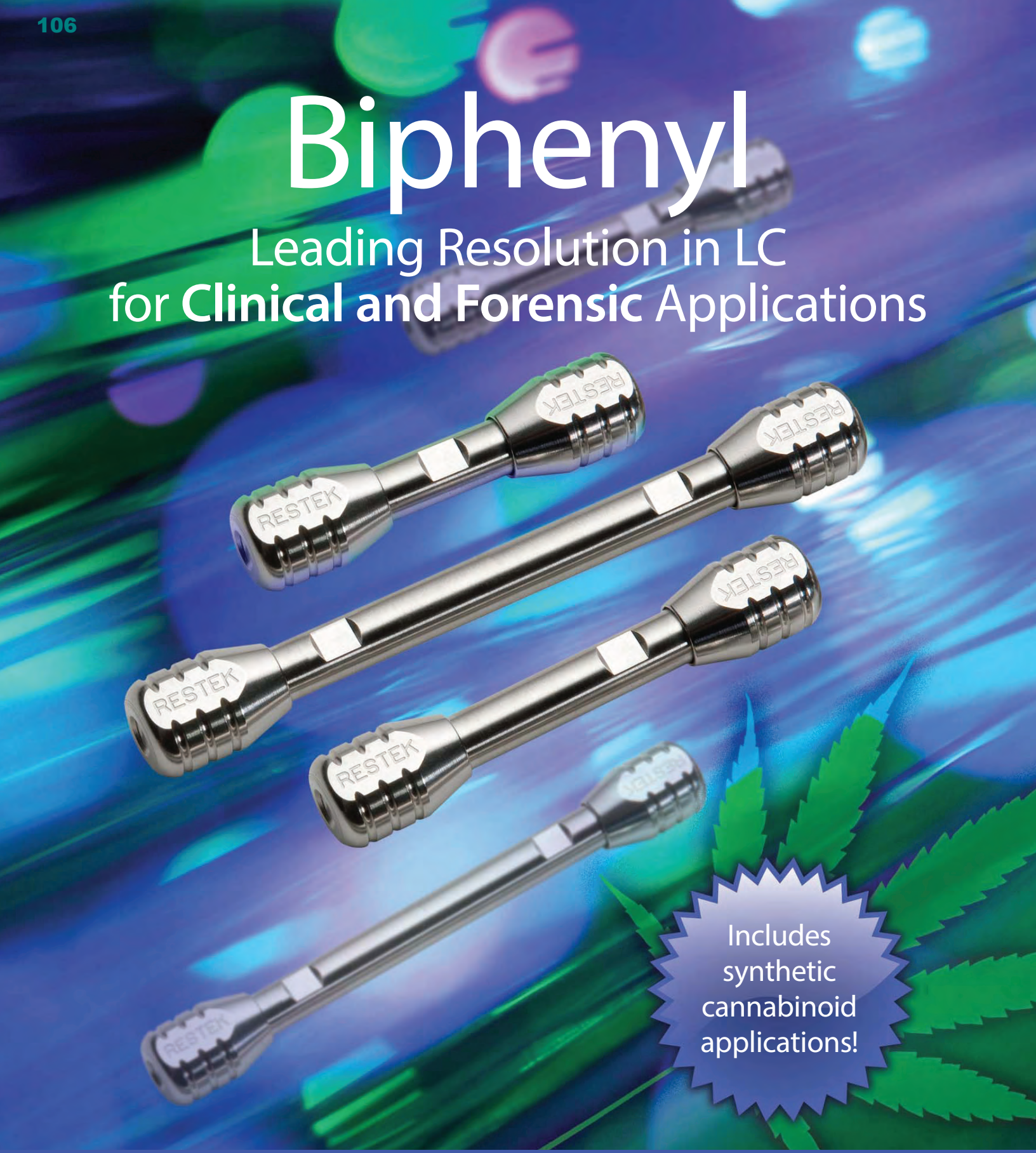
Lit. Cat.# GNTS1895C-UNV

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# Biphenyl Columns
## Greater Versatility—Increased Method Performance


BIPHENYL

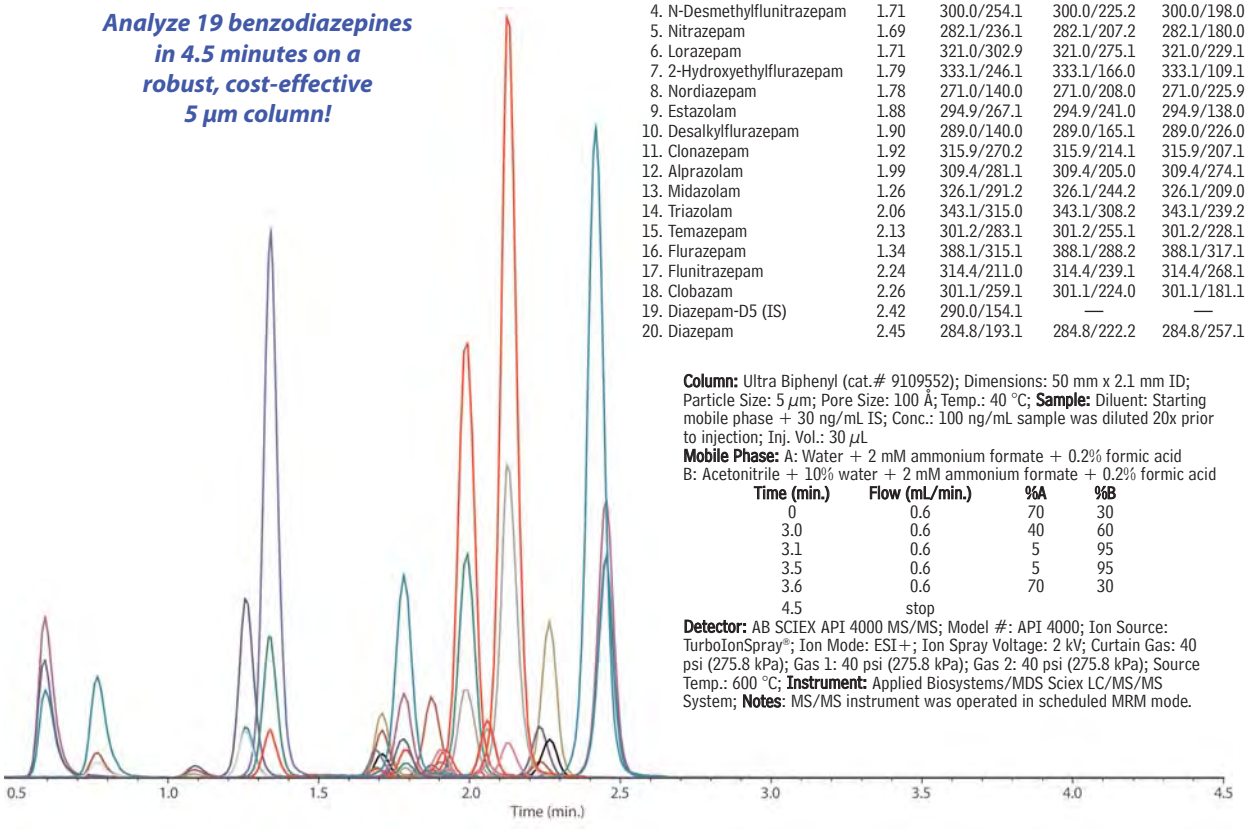
- Optimized for a wider range of compound chemistries compared to a C18 or phenyl column.
- Perfect for drugs of abuse, pharmaceuticals, metabolites, and more.
- Ultra Biphenyl columns are lot tested with a comprehensive drug panel to ensure confidence in method reproducibility.

Biphenyl columns are unique as they combine the performance of a traditional alkyl phase (C8 or C18) with that of a phenyl phase, providing unmatched versatility for clinical and forensic applications. This new phase offers excellent retention for both polar and nonpolar compounds because of the innovative end-to-end bonding of phenyl groups using an aryl linker instead of a straight chain hexyl linker. The overall result is a phase that offers the highest degree of aromatic selectivity and hydrophobic retention of any phenyl phase. To ensure reproducibility for demanding LC/MS/MS methods, each bonded lot of Ultra Biphenyl phase is tested by LC/MS with a multiclass drug panel as part of our rigorous QA process. Get optimal performance for clinical and forensic applications with versatile Biphenyl columns—**no other phase can provide both C18- and phenyl-like performance in a single column.**

## Benzodiazepines in Hydrolyzed Urine on Ultra Biphenyl

***Analyze 19 benzodiazepines in 4.5 minutes on a robust, cost-effective 5 µm column!***

| Peaks | RT (min.) | MRM1 | MRM2 | MRM3 |
|---|---|---|---|---|
| 1. 7-Aminoclonazepam | 0.59 | 286.0/250.3 | 286.0/222.2 | 286.0/121.0 |
| 2. 7-Aminoflunitrazepam | 0.77 | 284.2/135.2 | 284.2/240.2 | 284.2/226.1 |
| 3. Bromazepam | 1.10 | 315.9/182.0 | 315.9/209.1 | 315.9/260.9 |
| 4. N-Desmethylflunitrazepam | 1.71 | 300.0/254.1 | 300.0/225.2 | 300.0/198.0 |
| 5. Nitrazepam | 1.69 | 282.1/236.1 | 282.1/207.2 | 282.1/180.0 |
| 6. Lorazepam | 1.71 | 321.0/302.9 | 321.0/275.1 | 321.0/229.1 |
| 7. 2-Hydroxyethylflurazepam | 1.79 | 333.1/246.1 | 333.1/166.0 | 333.1/109.1 |
| 8. Nordiazepam | 1.78 | 271.0/140.0 | 271.0/208.0 | 271.0/225.9 |
| 9. Estazolam | 1.88 | 294.9/267.1 | 294.9/241.0 | 294.9/138.0 |
| 10. Desalkylflurazepam | 1.90 | 289.0/140.0 | 289.0/165.1 | 289.0/226.0 |
| 11. Clonazepam | 1.92 | 315.9/270.2 | 315.9/214.1 | 315.9/207.1 |
| 12. Alprazolam | 1.99 | 309.4/281.1 | 309.4/205.0 | 309.4/274.1 |
| 13. Midazolam | 1.26 | 326.1/291.2 | 326.1/244.2 | 326.1/209.0 |
| 14. Triazolam | 2.06 | 343.1/315.0 | 343.1/308.2 | 343.1/239.2 |
| 15. Temazepam | 2.13 | 301.2/283.1 | 301.2/255.1 | 301.2/228.1 |
| 16. Flurazepam | 1.34 | 388.1/315.1 | 388.1/288.2 | 388.1/317.1 |
| 17. Flunitrazepam | 2.24 | 314.4/211.0 | 314.4/239.1 | 314.4/268.1 |
| 18. Clobazam | 2.26 | 301.1/259.1 | 301.1/224.0 | 301.1/181.1 |
| 19. Diazepam-D5 (IS) | 2.42 | 290.0/154.1 | — | — |
| 20. Diazepam | 2.45 | 284.8/193.1 | 284.8/222.2 | 284.8/257.1 |




**Column:** Ultra Biphenyl (cat.# 9109552); Dimensions: 50 mm x 2.1 mm ID; Particle Size: 5 µm; Pore Size: 100 Å; Temp.: 40 °C; **Sample:** Diluent: Starting mobile phase + 30 ng/mL IS; Conc.: 100 ng/mL sample was diluted 20x prior to injection; Inj. Vol.: 30 µL

**Mobile Phase:** A: Water + 2 mM ammonium formate + 0.2% formic acid
B: Acetonitrile + 10% water + 2 mM ammonium formate + 0.2% formic acid

| Time (min.) | Flow (mL/min.) | %A | %B |
|---|---|---|---|
| 0 | 0.6 | 70 | 30 |
| 3.0 | 0.6 | 40 | 60 |
| 3.1 | 0.6 | 5 | 95 |
| 3.5 | 0.6 | 5 | 95 |
| 3.6 | 0.6 | 70 | 30 |
| 4.5 | stop | | |

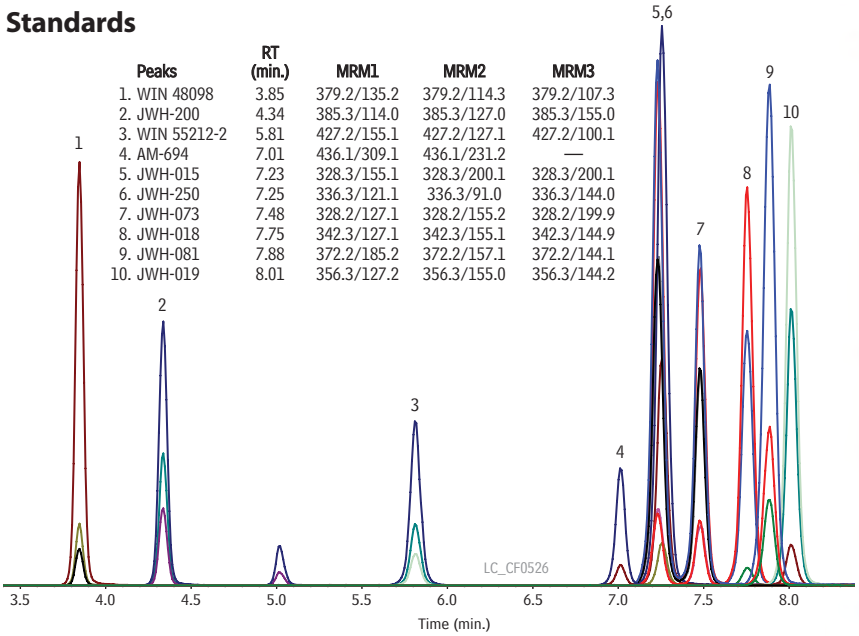
**Detector:** AB SCIEX API 4000 MS/MS; Model #: API 4000; Ion Source: TurboIonSpray®; Ion Mode: ESI+; Ion Spray Voltage: 2 kV; Curtain Gas: 40 psi (275.8 kPa); Gas 1: 40 psi (275.8 kPa); Gas 2: 40 psi (275.8 kPa); Source Temp.: 600 °C; **Instrument:** Applied Biosystems/MDS Sciex LC/MS/MS System; **Notes:** MS/MS instrument was operated in scheduled MRM mode.

LC_CF0525

## Synthetic Cannabinoids on Ultra Biphenyl

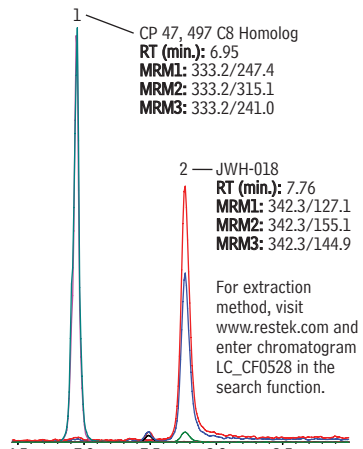
### Standards

| Peaks | RT (min.) | MRM1 | MRM2 | MRM3 |
|---|---|---|---|---|
| 1. WIN 48098 | 3.85 | 379.2/135.2 | 379.2/114.3 | 379.2/107.3 |
| 2. JWH-200 | 4.34 | 385.3/114.0 | 385.3/127.0 | 385.3/155.0 |
| 3. WIN 55212-2 | 5.81 | 427.2/155.1 | 427.2/127.1 | 427.2/100.1 |
| 4. AM-694 | 7.01 | 436.1/309.1 | 436.1/231.2 | — |
| 5. JWH-015 | 7.23 | 328.3/155.1 | 328.3/200.1 | 328.3/200.1 |
| 6. JWH-250 | 7.25 | 336.3/121.1 | 336.3/91.0 | 336.3/144.0 |
| 7. JWH-073 | 7.48 | 328.2/127.1 | 328.2/155.2 | 328.2/199.9 |
| 8. JWH-018 | 7.75 | 342.3/127.1 | 342.3/155.1 | 342.3/144.9 |
| 9. JWH-081 | 7.88 | 372.2/185.2 | 372.2/157.1 | 372.2/144.1 |
| 10. JWH-019 | 8.01 | 356.3/127.2 | 356.3/155.0 | 356.3/144.2 |




### Spice Gold Sample

(Methanol Extract)

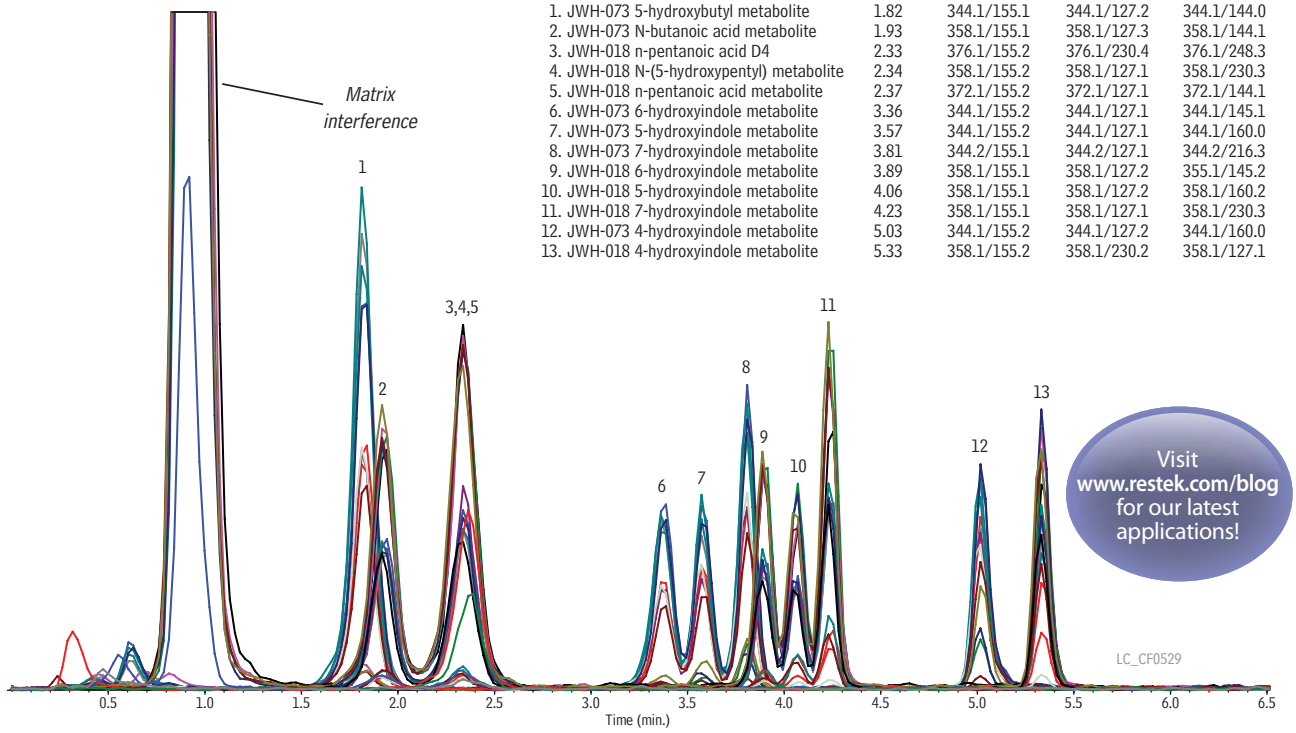



For extraction method, visit www.restek.com and enter chromatogram LC_CF0528 in the search function.

**Column**: Ultra Biphenyl (cat.# 9109552); Dimensions: 50 mm x 2.1 mm ID; Particle Size: 5 µm; Pore Size: 100 Å; Temp.: 40 °C; **Sample:** Diluent: Methanol; Conc.: 50 ng/mL; Inj. Vol.: 5 µL; **Detector**: AB SCIEX API 4000 MS/MS; Model #: API 4000; Ion Source: TurboIonSpray®; Ion Mode: ESI+; Ion Spray Voltage: 3000 kV; Curtain Gas: 40 psi (275.8 kPa); Gas 1: 40 psi (275.8 kPa); Gas 2: 40 psi (275.8 kPa); Interface Temp.: 600 °C; Mode: MRM; Dwell Time: 10 ms; **Instrument:** Applied Biosystems/MDS Sciex LC/MS/MS System

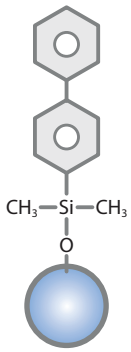
## Synthetic Cannabinoid Metabolites in Hydrolyzed Urine on Pinnacle® DB Biphenyl

| Peaks | RT (min.) | MRM1 | MRM2 | MRM3 |
|---|---|---|---|---|
| 1. JWH-073 5-hydroxybutyl metabolite | 1.82 | 344.1/155.1 | 344.1/127.2 | 344.1/144.0 |
| 2. JWH-073 N-butanoic acid metabolite | 1.93 | 358.1/155.1 | 358.1/127.3 | 358.1/144.1 |
| 3. JWH-018 n-pentanoic acid D4 | 2.33 | 376.1/155.2 | 376.1/230.4 | 376.1/248.3 |
| 4. JWH-018 N-(5-hydroxypentyl) metabolite | 2.34 | 358.1/155.2 | 358.1/127.1 | 358.1/230.3 |
| 5. JWH-018 n-pentanoic acid metabolite | 2.37 | 372.1/155.2 | 372.1/127.1 | 372.1/144.1 |
| 6. JWH-073 6-hydroxyindole metabolite | 3.36 | 344.1/155.2 | 344.1/127.1 | 344.1/145.1 |
| 7. JWH-073 5-hydroxyindole metabolite | 3.57 | 344.1/155.2 | 344.1/127.1 | 344.1/160.0 |
| 8. JWH-073 7-hydroxyindole metabolite | 3.81 | 344.2/155.1 | 344.2/127.1 | 344.2/216.3 |
| 9. JWH-018 6-hydroxyindole metabolite | 3.89 | 358.1/155.1 | 358.1/127.2 | 355.1/145.2 |
| 10. JWH-018 5-hydroxyindole metabolite | 4.06 | 358.1/155.1 | 358.1/127.2 | 358.1/160.2 |
| 11. JWH-018 7-hydroxyindole metabolite | 4.23 | 358.1/155.1 | 358.1/127.1 | 358.1/230.3 |
| 12. JWH-073 4-hydroxyindole metabolite | 5.03 | 344.1/155.2 | 344.1/127.2 | 344.1/160.0 |
| 13. JWH-018 4-hydroxyindole metabolite | 5.33 | 358.1/155.2 | 358.1/230.2 | 358.1/127.1 |




Visit www.restek.com/blog for our latest applications!

**Column:** Pinnacle® DB Biphenyl (cat.# 9409552); Dimensions: 50 mm x 2.1 mm ID; Particle Size: 5 µm; Pore Size: 140 Å; Temp.: 25 °C
For complete conditions, visit www.restek.com and enter chromatogram LC_CF0529 in the search function.




Biphenyl

## Ultra Biphenyl Columns (USP L11)

**Physical Characteristics:**

particle size: 3µm or 5µm, spherical
pore size: 100Å
carbon load: 15%
endcap: fully endcapped
pH range: 2.5 to 8
temperature limit: 80°C

| Length | 1.0mm ID cat.# | 2.1mm ID cat.# | 3.2mm ID cat.# | 4.6mm ID cat.# |
|---|---|---|---|---|
| **3µm Columns** | | | | |
| 30mm | 9109331 | 9109332 | 9109333 | 9109335 |
| 50mm | 9109351 | 9109352 | 9109353 | 9109355 |
| 100mm | 9109311 | 9109312 | 9109313 | 9109315 |
| 150mm | 9109361 | 9109362 | 9109363 | 9109365 |
| **5µm Columns** | | | | |
| 30mm | 9109531 | 9109532 | 9109533 | 9109535 |
| 50mm | 9109551 | 9109552 | 9109553 | 9109555 |
| 100mm | 9109511 | 9109512 | 9109513 | 9109515 |
| 150mm | 9109561 | 9109562 | 9109563 | 9109565 |
| 200mm | 9109521 | 9109522 | 9109523 | 9109525 |
| 250mm | 9109571 | 9109572 | 9109573 | 9109575 |

## Ultra Biphenyl Guard Cartridges

| Guard Cartridges | 3-pk. (10 x 2.1mm) | 3-pk. (10 x 4.0mm) | 2-pk. (20 x 2.1mm) | 2-pk. (20 x 4.0mm) |
|---|---|---|---|---|
| Ultra Biphenyl Guard Cartridge | 910950212 | 910950210 | 910950222 | 910950220 |

## Pinnacle® DB Biphenyl Columns (USP L11)

**Physical Characteristics:**

particle size: 1.9µm, 3µm, or 5µm, spherical
pore size: 140Å
carbon load: 8%
endcap: yes
pH range: 2.5 to 8
temperature limit: 80°C

| Length | 1.0mm ID cat.# | 2.1mm ID cat.# | 3.2mm ID cat.# | 4.6mm ID cat.# |
|---|---|---|---|---|
| **1.9µm Columns** | | | | |
| 30mm | | 9409232 | | |
| 50mm | | 9409252 | | |
| 100mm | | 9409212 | | |
| **3µm Columns** | | | | |
| 30mm | 9409331 | 9409332 | 9409333 | 9409335 |
| 50mm | 9409351 | 9409352 | 9409353 | 9409355 |
| 100mm | 9409311 | 9409312 | 9409313 | 9409315 |
| 150mm | 9409361 | 9409362 | 9409363 | 9409365 |
| **5µm Columns** | | | | |
| 30mm | 9409531 | 9409532 | 9409533 | 9409535 |
| 50mm | 9409551 | 9409552 | 9409553 | 9409555 |
| 100mm | 9409511 | 9409512 | 9409513 | 9409515 |
| 150mm | 9409561 | 9409562 | 9409563 | 9409565 |
| 200mm | 9409521 | 9409522 | 9409523 | 9409525 |
| 250mm | 9409571 | 9409572 | 9409573 | 9409575 |

## Pinnacle® DB Biphenyl Guard Cartridges

| Guard Cartridges | 3-pk. (10 x 2.1mm) | 3-pk. (10 x 4.0mm) | 2-pk. (20 x 2.1mm) | 2-pk. (20 x 4.0mm) |
|---|---|---|---|---|
| Pinnacle DB Biphenyl Guard Cartridge | 940950212 | 940950210 | 940950222 | 940950220 |

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# “The Big Pain”: Development of Pain-Free Methods for Analyzing 231 Multiclass Drugs and Metabolites by LC-MS/MS

By Sharon Lupo

As the use of prescription and nonprescription drugs grows, the need for fast, accurate, and comprehensive methods is also rapidly increasing. Historically, drug testing has focused on forensic applications such as cause of death determinations or the detection of drug use in specific populations (military, workplace, probation/parole, sports doping). However, modern drug testing has expanded well into the clinical arena with a growing list of target analytes and testing purposes. Clinicians often request the analysis of large panels of drugs and metabolites that can be used to ensure compliance with prescribed pain medication regimens and to detect abuse or diversion of medications. With prescription drug abuse reaching epidemic levels [1], demand is growing for analytical methods that can ensure accurate results for comprehensive drug lists with reasonable analysis times. LC-MS/MS is an excellent technique for this work because it offers greater sensitivity and specificity than immunoassay and—with a highly selective and retentive Raptor™ Biphenyl column—can provide definitive results for a wide range of compounds.

Typically, forensic and pain management drug testing consists of an initial screening analysis, which is qualitative, quick, and requires only minimal sample preparation. Samples that test positive during screening are then subjected to a quantitative confirmatory analysis. Whereas screening assays may cover a broad list of compounds and are generally less sensitive and specific, confirmation testing provides fast, targeted analysis using chromatographic conditions that are optimized for specific panels. C18 columns are commonly used and generally work well for hydrophobic compounds that separate using dispersive retention. However, not all compounds exhibit this type of interaction and many of today’s complex mixtures and difficult matrices require more advanced retention mechanisms. Raptor™ Biphenyl columns exploit the pi-pi (π -π) interactions of fused ring compounds with substituted electron withdrawing groups that are typical of many medications, which results in improved retention for a wider range of structurally diverse drugs and metabolites.

The Raptor™ Biphenyl column was used to develop LC-MS/MS methodology for 231 drugs and drug metabolites because it has the retention required for the wide range of compound classes tested here. It also provides improved selectivity for over 40 structurally similar drugs and metabolites, even those not normally resolved by a C18 column. The comprehensive analysis of 231 compounds shown in this article demonstrates the power and utility of the Raptor™ Biphenyl column for developing multiclass screening assays. Further, the panel-specific confirmation methods discussed later in the article can be paired either with an LC-MS/MS screening method or with traditional immunoassay screening tests. Note that stability experiments and validation in matrix should be performed to demonstrate effectiveness and reproducibility prior to implementing any method for actual sample analysis.

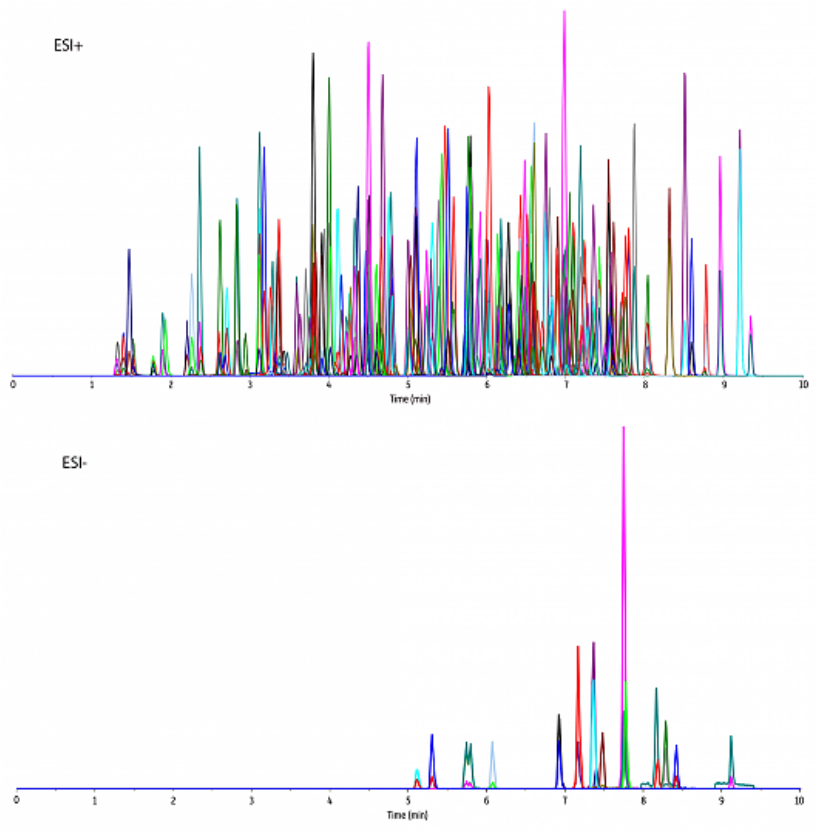
**Comprehensive Analysis of 231 Drugs and Metabolites in 10 Minutes**

Screening tests often are done by immunoassay, but this approach has several known issues. Immunoassays are less specific, which makes false positives more likely; in addition, they also are less sensitive, which increases the risk of false negatives. The emergence of definitive identification through LC-MS/MS as an alternative to paired screening and confirmational analyses speaks to the strength of LC-MS/MS as a technique [2]. However, even when using LC-MS/MS, separate screening and confirmational analyses are currently still more common than single definitive identification tests. Whether developing a screening assay or advancing to a single definitive analytical method, the power of LC-MS/MS can be maximized by using a Raptor™ Biphenyl column due to its unique retention and selectivity.

Accurately determining long lists of target analytes is a daunting task; in this case, the challenge included compounds from 10 drug classes and over 40 isomers and structurally similar compounds. Development of methodology required careful consideration of many variables, including chemical properties of the target analytes, mobile phase composition and gradient, and detector polarity. The Raptor™ Biphenyl column provided simultaneous analysis of all 231 drugs and metabolites in a fast 10-minute analysis time with an additional 2 minutes for re-equilibration (Figure 1). Good chromatographic separations were achieved and target analytes were positively identified by retention time and optimized precursor and product ions. Polarity switching was used to improve detection with most compounds (209) analyzed using positive electrospray ionization (ESI+) and the remainder (22) using negative electrospray ionization (ESI-). As noted previously, over 40 compounds that shared their precursor ion with at least one other compound were included in this assay and most were chromatographically separated allowing positive identification. Exceptions included noroxycodone and dihydrocodeine, which are distinguished in the confirmatory opioids panel; citalopram and escitalopram, which are R/S enantiomers; and levorphanol (an opioid) and dextrorphan (a hallucinogen), which are enantiomers and can be determined separately in their respective panels.

**Figure 1:** Fast, 10-minute analysis of 231 drugs and metabolites on a Raptor™ Biphenyl column by LC-MS/MS.

View peak list and conditions




LC_CF0621

**Optimized Analysis of Separate Panels for Confirmation and Quantification**

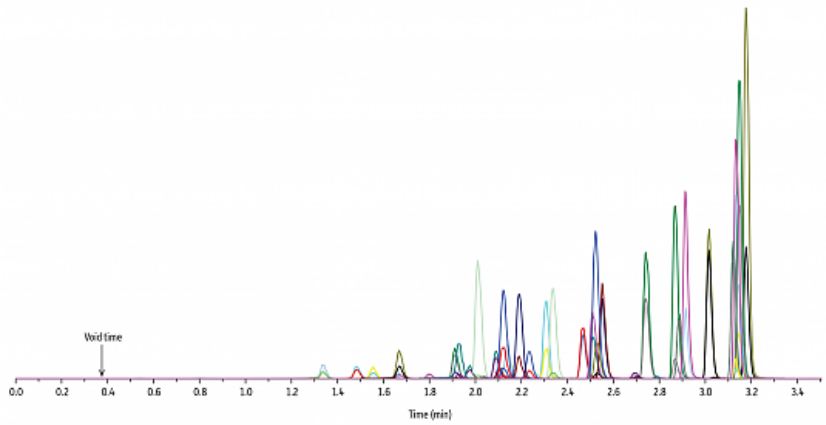
Confirmation methods were developed systematically by first grouping compounds by drug class. Compounds were separated and prepared in water as mixtures of 15-20 analytes; special care was taken to separate isobaric compounds in solutions. Two transitions were identified for each analyte and scouting gradients were run for each mixture using electrospray ionization in both positive and negative ion mode. The scouting gradients were linear (10-100% organic mobile phase), and three separate modifiers were evaluated: 1) 5 mM ammonium acetate, 2) 0.1% formic acid with 5 mM ammonium formate, and 3) 0.1% formic acid. Based on these results, optimal mobile phases, gradient conditions, and polarity were determined for each panel, and analytes were scheduled by retention time using multiple reaction monitoring (MRM).

Final optimized chromatographic conditions and results for each panel are shown in Figure 2 (opioids/metabolites), Figures 4–6 (antianxiety drugs/metabolites and barbiturates), Figure 7 (nonsteroidal anti-inflammatory drugs [NSAIDs]), Figure 8 (stimulants), Figure 9 (antiepileptic), Figure 10 (antipsychotics), Figure 11 (antidepressants), and Figure 12 (hallucinogens). While the determination of some common pain panel components is relatively straightforward, the analyses of several groups (opioids/metabolites, antianxiety drugs/metabolites and barbiturates, and NSAIDs) warrant further discussion.

Opioids are used for pain management and are among the most commonly prescribed—and abused—drugs in the world. As clinicians frequently test patients to monitor compliance, these analytes are key components in drug testing methods. These compounds present several analytical difficulties: the presence of structural isomers; poor sensitivity for buprenorphine and norbuprenorphine; and poor retention for noroxymorphone, morphine, and hydromorphone. With regard to these challenges, the chromatographic performance of the Raptor™ Biphenyl column is exceptional (Figure 2). The use of this column under gradient elution with acetonitrile and water mobile phases modified with 0.1% formic acid allowed the separation of all structural isomers. Use of LC-MS/MS provided additional specificity, which is important because 15 analytes share five precursor ions (M+H). Regarding the sensitivity issue that is common with buprenorphine and norbuprenorphine, the 2.7 µm superficially porous particle (SPP) silica in the Raptor™ Biphenyl column produced narrow peaks that improved response. The choice of acidic mobile phase for this panel also increased sensitivity compared to the use of a buffered mobile phase (Figure 3). As a final note for opioids analysis, the alternate retention mechanisms of the Raptor™ Biphenyl column increased retention of noroxymorphone, morphine, and hydromorphone. The increased separation of these small, polar target analytes from hydrophilic matrix interferences allows more accurate quantification.

**Figure 2:** Opioids/metabolites panel confirmation analysis on the Raptor™ Biphenyl column.

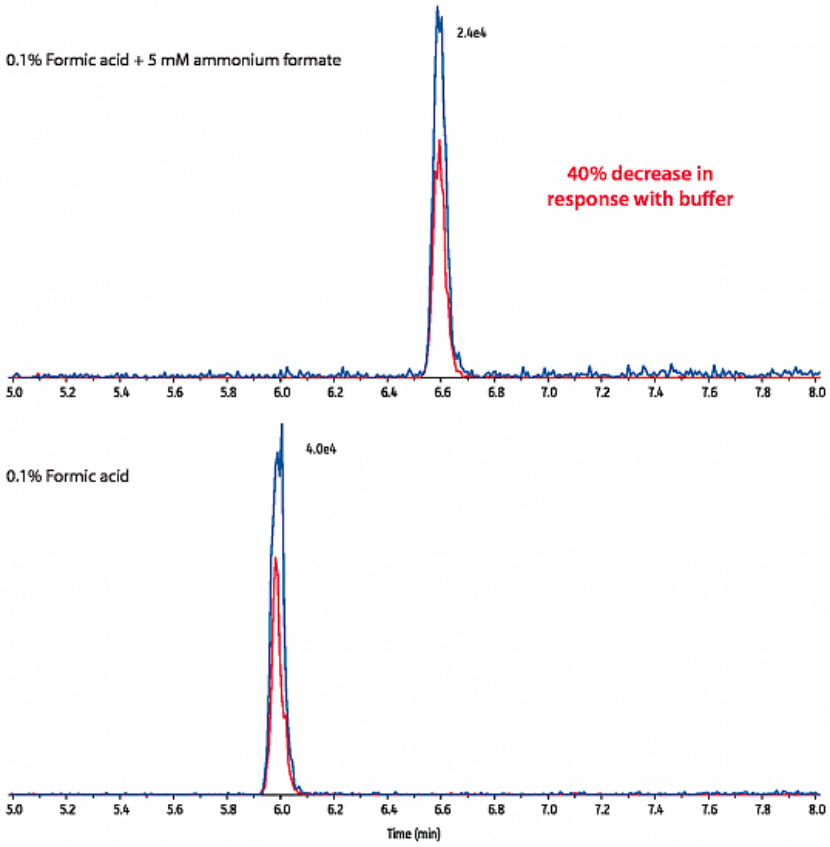
View peak list and conditions




LC_CF0610

**Figure 3:** Use of an acidic mobile phase without buffer improves the response of opioids such as buprenorphine.

View peak list and conditions

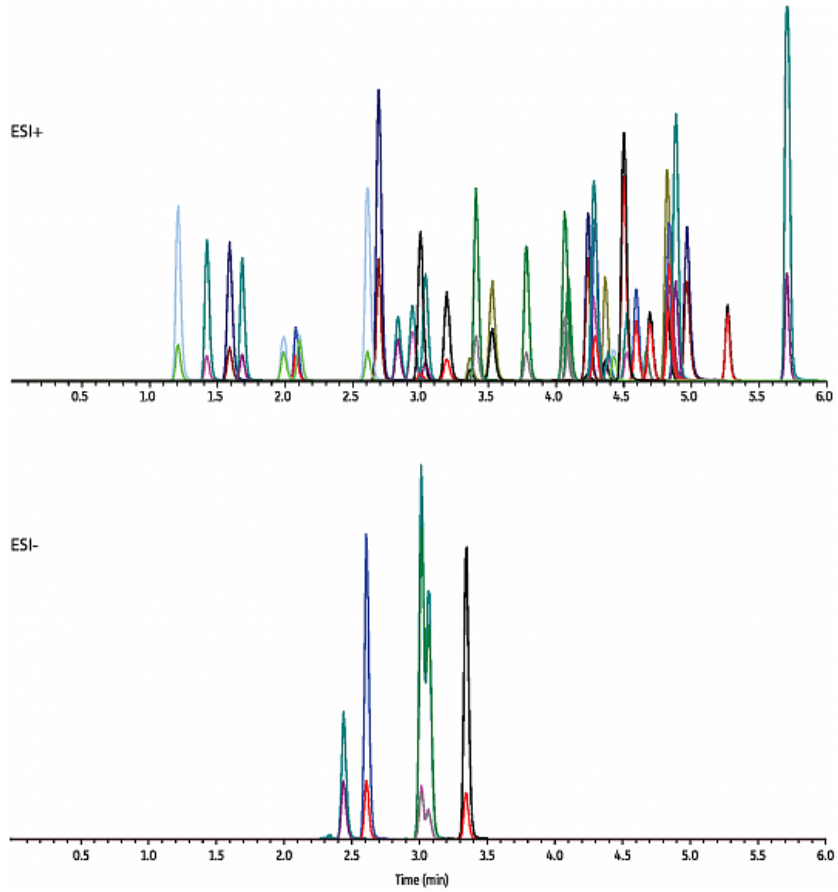



LC_CF0655

Antianxiety drugs, such as benzodiazepines, muscle relaxers, hypnotics, sedatives, and z-drugs, along with barbiturates are often abused in conjunction with other drugs, most commonly opioids [3]. Like opioids, this group also presents chromatographic challenges, namely that barbiturates are detected in negative ion mode, whereas most other drugs are detected in positive ion mode. This significantly complicates the analysis and requires polarity switching and the use of an instrument with sufficient data acquisition speed. In addition, the barbiturates amobarbital and pentobarbital are positional isomers and can be extremely difficult to resolve. If resolution between the isomers is not critical, the Raptor™ Biphenyl column allows the combined analysis of antianxiety drugs and barbiturates in just 8 minutes. The column's unique selectivity provides ~40% resolution between amobarbital and pentobarbital and efficient peak capacity, which allows optimal use of instrument data speed. Analytical conditions and results for this combined panel are shown in Figure 4. If resolution of barbiturate isomers is critical, antianxiety drugs and barbiturates can also be analyzed separately. By using the Raptor™ C18 column for the barbiturates analysis, amobarbital and pentobarbital are almost completely resolved in 6 minutes (Figure 5), while the analysis of the antianxiety drugs can be completed on the Raptor™ Biphenyl column in 5.5 minutes (Figure 6). This simpler approach improves the resolution of barbiturate isomers and is suitable for slower mass spectrometers that lack the speed required for combined analysis.

**Figure 4:** Combined antianxiety drugs/metabolites and barbiturates panel confirmation analysis on the Raptor™ Biphenyl column.

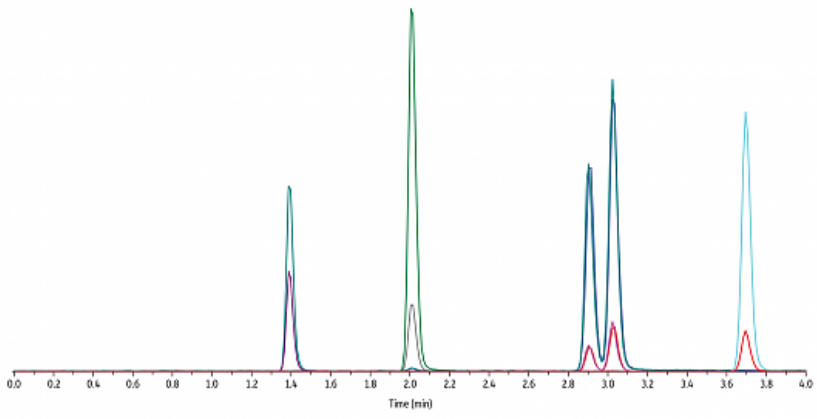
View peak list and conditions




LC_CF0657

**Figure 5:** Barbiturates analyzed separately with improved resolution in 6 minutes on the Raptor™ C18 column.

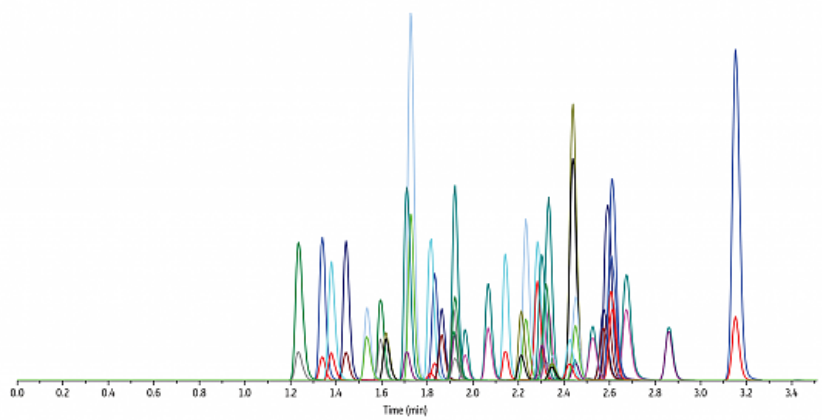
View peak list and conditions




LC_CF0619

**Figure 6:** Antianxiety drugs/metabolites analyzed separately (without barbiturates) in 5.5 minutes on the Raptor™ Biphenyl column.

View peak list and conditions

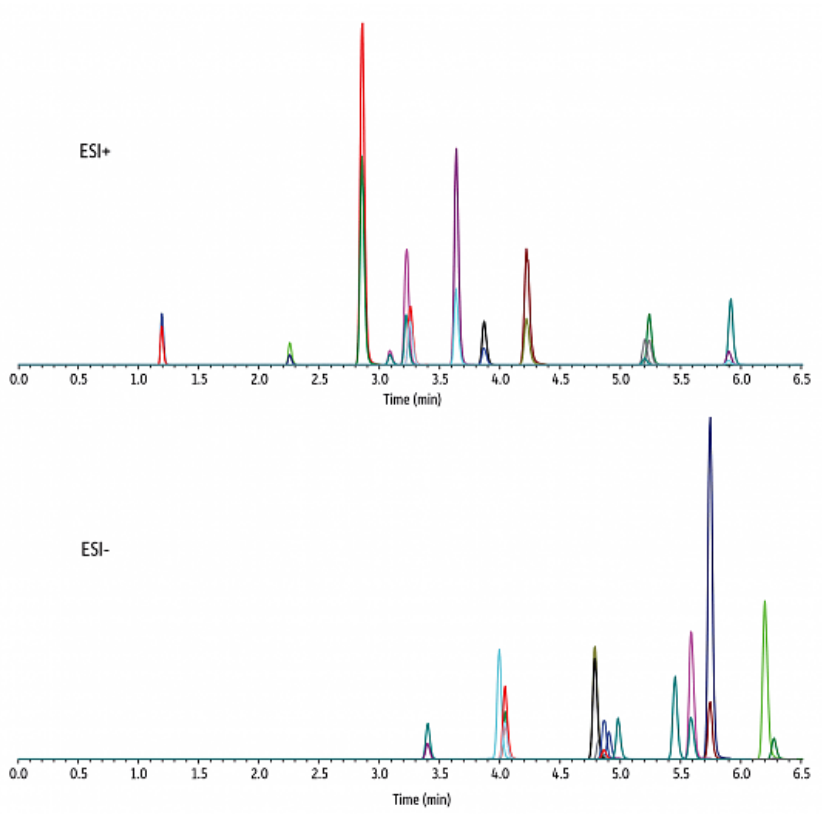



LC_CF0615

The optimized NSAIDs panel in Figure 7 is the final panel that warrants additional discussion regarding performance of the confirmational method. Although NSAIDs are generally considered safe, toxicity can occur due to their availability, widespread use, and their inclusion in combination drug formulations [4]. The primary challenge with NSAID analysis is that it is a diverse group of drugs and most detection methods are optimized for a single drug or one or more of its metabolites. For this panel, the combination of the Raptor™ Biphenyl column, use of scheduled polarity switching, and selection of mobile phases that maximized sensitivity in both positive and negative ion modes allowed the simultaneous detection of 27 NSAIDs (including acetaminophen) in one fast ~8-minute analysis.

**Figure 7:** NSAIDs panel confirmation analysis on the Raptor™ Biphenyl column.

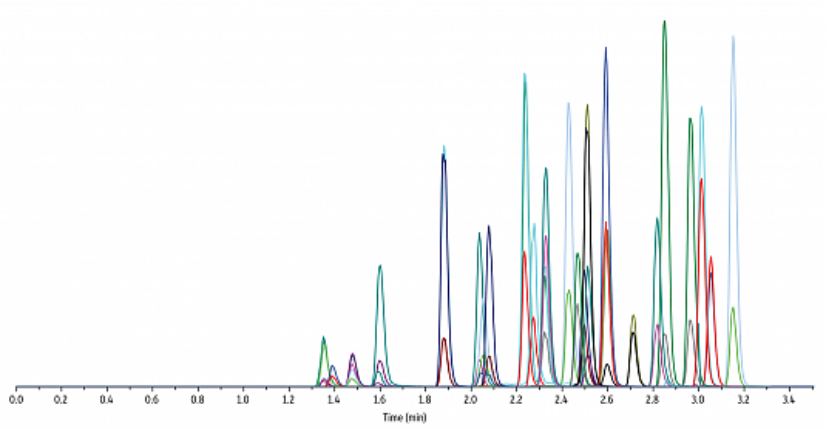
View peak list and conditions




LC_CF0620

**Figure 8:** Stimulants panel confirmation analysis on the Raptor™ Biphenyl column.

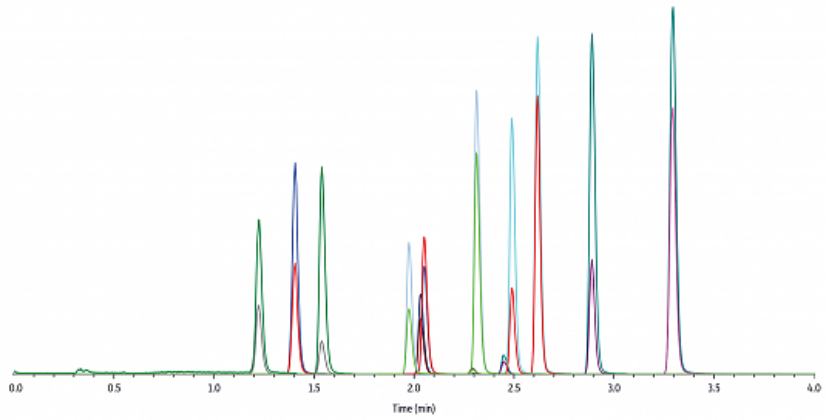
View peak list and conditions




LC_CF0617

**Figure 9:** Antiepileptic panel confirmation analysis on the Raptor™ Biphenyl column.

View peak list and conditions




LC_CF0616

**Figure 10:** Antipsychotics panel confirmation analysis on the Raptor™ Biphenyl column.

View peak list and conditions

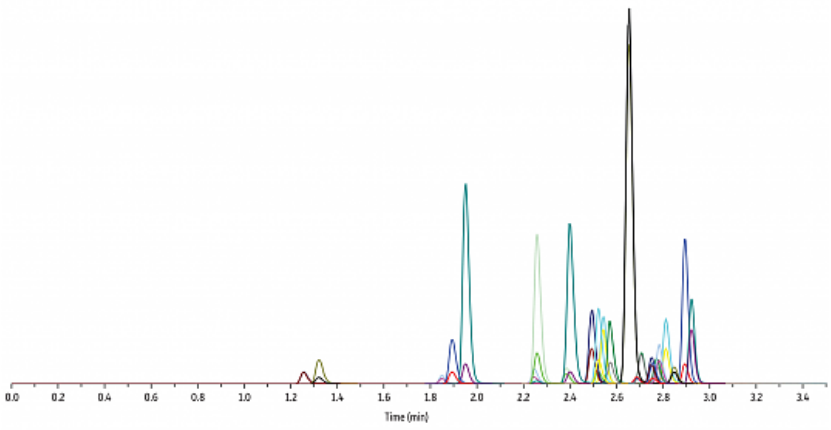
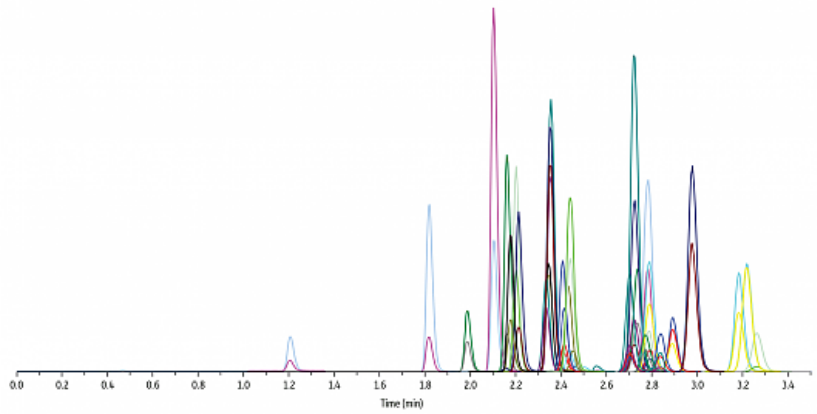


**Figure 11:** Antidepressants panel confirmation analysis on the Raptor™ Biphenyl column.

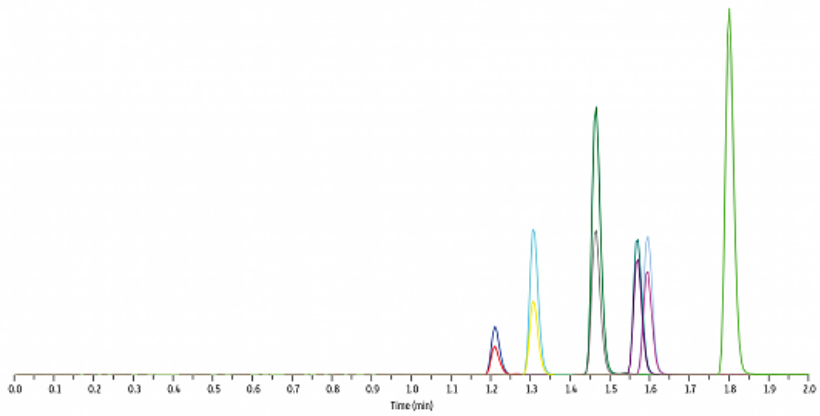
View peak list and conditions




LC_CF0609

**Figure 12:** Hallucinogens panel confirmation analysis on the Raptor™ Biphenyl column.

View peak list and conditions




LC_CF0607

**Conclusion**

As the demand for clinical and forensic drug testing increases, many drug testing facilities are turning to LC-MS/MS for its increased speed, sensitivity, and specificity. The methods shown here provide fast, accurate analysis of 231 drugs and drug metabolites. In this case, optimized method conditions and the use of a Raptor™ Biphenyl column allowed many problematic analytes—including over 40 isomers—to be identified and reported with confidence. The strong retention and unique selectivity of this column provide a powerful alternative to frequently used C18 columns.

**References**

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[3] J. Jones, S. Mogali, S. Comer, Polydrug abuse: a review of opioid and benzodiazepine combination use,

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**RELATED SEARCHES**

*pain drug analysis by lcms*

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# 5 Minute Analysis of Vitamin D in Serum by LC/MS/MS

By Amanda Rigdon, Innovations Chemist and Becky Wittrig, Global HPLC Specialist

- High sensitivity improves low level accuracy in matrix.
- 5 minute analysis time speeds up sample throughput.
- Excellent selectivity for vitamin D minimizes matrix interference.

Monitoring of vitamin D levels in patients is important for the prevention and control of disease. Vitamin D, specifically 25-hydroxy vitamin D, plays a critical role in controlling calcium and phosphate levels in the body. If these levels are not adequately controlled, bone conditions such as rickets in children or osteoporosis in adults may occur. 25-hydroxy vitamin D is a hydrophobic, fat soluble vitamin that is absorbed like a fat in the intestines. It is commonly used to diagnose conditions that interfere with fat absorption, such as Crohn's disease. Since vitamin D analysis is one of the most commonly run procedures in clinical labs, high throughput, high sensitivity analytical methods are desirable. Conventional techniques for vitamin D analysis, based on immunoassay or LC/UV, often lack adequate sensitivity, specificity, and speed; thus, interest in LC/MS/MS methods is growing. Here we establish conditions for routine vitamin D testing by LC/MS/MS which result in highly symmetric peaks that elute in just 5 minutes.

25-hydroxy vitamin D appears in several forms, but vitamin D2 and vitamin D3 are the most commonly analyzed. These forms are very similar and differ only in one methyl group and a double bond (Figure 1). Because 25-hydroxy vitamin D is a hydrophobic species, this compound is extremely amenable to reverse phase liquid chromatography (RPLC). While conventional C18 columns are commonly used in RPLC, for this analysis we selected an Ultra Aqueous C18 column instead. This phase is more retentive than a C18, which helps separate the vitamin D species from less retained matrix components. LC/MS/MS analysis using an Ultra Aqueous C18 column resulted in excellent peak shape, which contributes to enhanced sensitivity (Figure 2). To evaluate retention, human serum samples were extracted in acetonitrile and analyzed. Both vitamin D analytes were well-separated from matrix interferences (Figure 3).

Analyzing vitamin D by LC/MS/MS using an Ultra Aqueous C18 column is an ideal method for high-throughput clinical labs interested in accurate low-level detection and fast analysis times. Excellent peak shape and MS sensitivity result in faster, more accurate analysis of clinical samples.

**Figure 1** Structures of vitamins $D_2$ and $D_3$.

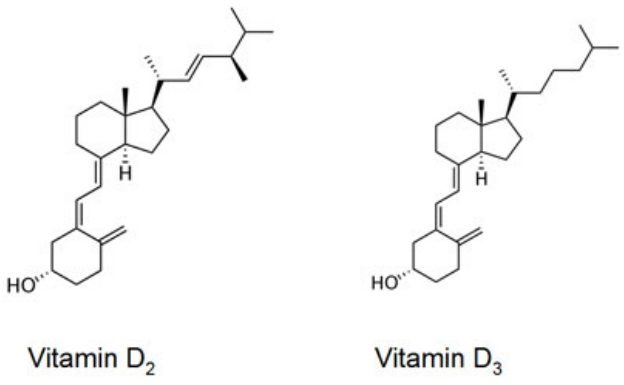
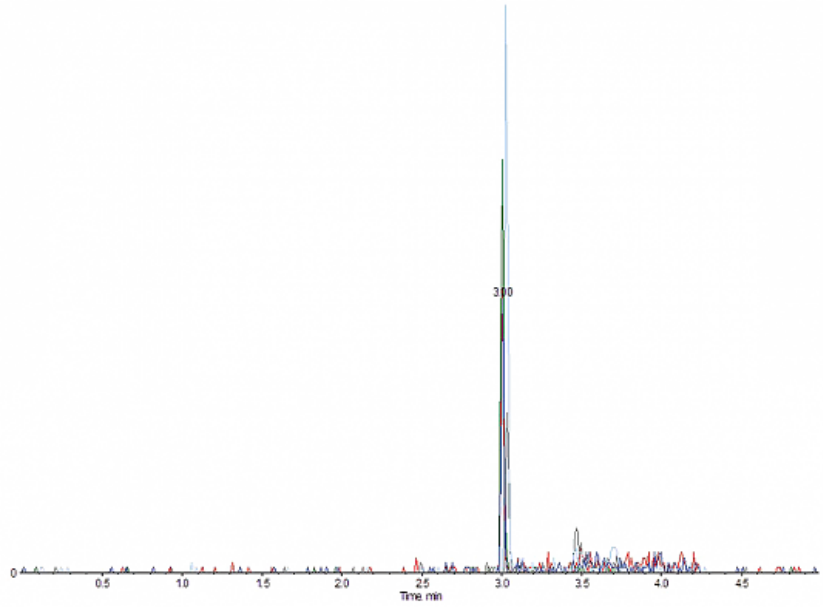



**Figure 2** Ultra Aqueous C18 columns provide outstanding peak symmetry for vitamin D, improving accuracy at low concentrations.

| Peaks | $t_R$ (min) | Q1 | Q3 | Declustering potential (V) |
|---|---|---|---|---|
| 1. d6-25-OH D3 (IS) | 3.00 | 389.3 | 211.2 | 68 |
| 2. 25-OH D3 | 3.00 | 383.3 | 211.2 | 68 |
| 3. 25-OH D2 | 3.04 | 395.3 | 229.2 | 55 |
| 4. 25-OH D3 | 3.00 | 383.3 | 229.2 | 68 |
| 5. 25-OH D2 | 3.04 | 395.3 | 269.2 | 55 |
| 6. 25-OH D2 | 3.04 | 395.3 | 119 | 55 |




LC_CF0491

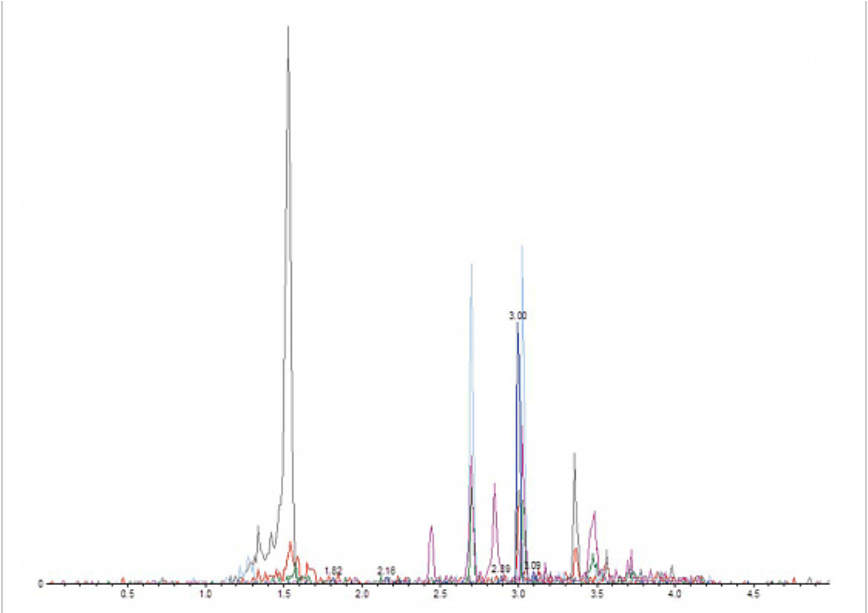
**Column**
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 3 µm
Pore Size: 100 Å
Temp.: 40 °C
**Sample** vitamin D standard
Conc.: 20 ng/mL
Inj. Vol.: 20 µL
**Mobile Phase**
A: 0.1% formic acid in water
B: 0.1% formic acid in methanol

| Time (min) | %B |
|---|---|
| 0.00 | 50 |
| 2.5 | 100 |
| 3.5 | 100 |
| 3.6 | 50 |
| 5.0 | 50 |

Flow: 0.7 mL/min
**Detector** Applied Biosystems 3200 QTRAP® LC/MS/MS System
Ion Source: TurboIonSpray®
Ion Mode: APCI+
Mode: MRM
Dwell Time: 100 ms
**Instrument** Shimadzu UFLCXR

**Figure 3** Excellent results for vitamin D in patient serum can be obtained in just 5 minutes.

| Peaks | $t_R$ (min) | Q1 | Q3 | Declustering potential (V) |
|---|---|---|---|---|
| 1. d6-25-OH $D_3$ (IS) | 3.00 | 389.3 | 211.2 | 68 |
| 2. 25-OH $D_3$ | 3.00 | 383.3 | 211.2 | 68 |
| 3. 25-OH $D_2$ | 3.04 | 395.3 | 229.2 | 55 |
| 4. 25-OH $D_3$ | 3.00 | 383.3 | 229.2 | 68 |
| 5. 25-OH $D_2$ | 3.04 | 395.3 | 269.2 | 55 |
| 6. 25-OH $D_2$ | 3.04 | 395.3 | 119.0 | 55 |



LC_CF0492

| | |
|---|---|
| **Column** | Ultra Aqueous C18 (cat.# 9178352) |
| Dimensions: | 50 mm x 2.1 mm ID |
| Particle Size: | 3 µm |
| Pore Size: | 100 Å |
| Temp.: | 40 °C |
| **Sample** | extracted serum sample |
| Inj. Vol.: | 20 µL |
| **Mobile Phase** | |
| A: | 0.1% formic acid in water |
| B: | 0.1% formic acid in methanol |

| Time (min) | %B |
|---|---|
| 0 | 50 |
| 2.5 | 100 |
| 3.5 | 100 |
| 3.6 | 50 |
| 5.0 | 50 |

| | |
|---|---|
| Flow: | 0.7 mL/min |
| **Detector** | Applied Biosystems 3200 QTRAP™ LC-MS/MS system |
| Ion Source: | TurboIonSpray® |
| Ion Source: | APCI |
| Ion Mode: | APCI+ |
| Mode: | MRM |
| Dwell Time: | 100 ms |
| **Instrument** | Shimadzu UFLCXR |

**RELATED SEARCHES**

*vitamin D*, *RPLC*, *Ultra Aqueous C18*, *vitamins*, *LC/MS/MS*

General Interest

# USLC™ Columns Put the Right Tools in Your LC Method Development Toolbox

## Understanding Selectivity in Reversed Phase Separations – A Simplified Approach to HPLC and UHPLC Column Selection

By Rick Lake and Ty Kahler

*The most significant influence on chromatographic peak separation, or resolution, is column selectivity. Unfortunately, column selectivity is also the least understood and most underutilized parameter. To improve selectivity, method developers often concentrate on manually altering mobile phases, operational parameters, and instrumentation. But because stationary phases offer more significant selectivity differences, you can drastically speed up HPLC and UHPLC method development by instead focusing on column choice. In this article, we discuss column selection for reversed phase separations and, using the hydrophobic-subtraction model (H-S model), identify a set of just 4 stationary phases—Restek's USLC™ column set—that encompasses the widest selectivity range available on the market.*

### The Role of Selectivity in Liquid Separations

When performing a liquid separation, we generally focus on choosing the right instrumentation—especially since the recent advent of UHPLC—and end up choosing columns rather hastily, either by proximity (using the column that is already on the instrument or in the closest drawer) or by habit (using a column that has offered problem-free service in the past). While never optimal, this practice should be particularly concerning for a method developer because improper column choice can lead to needlessly labor- and time-intensive method development. If we consider the impact of column selectivity on peak separation, or resolution, we can see why choosing the right column can be so advantageous.

Resolution is the result of 3 cumulative terms: efficiency (N), retention capacity (k), and selectivity (α). How well we resolve our analytes, and how quickly we do so, depends upon our ability to control these 3 factors. Of the 3, the selectivity term mathematically affects resolution to the greatest degree (Equation 1). Put another way, resolution is largely a function of selectivity.

**Equation 1:** Selectivity is the driving parameter of resolution, as it affects peak separation to the greatest degree.

$$R = \tfrac{1}{4}\sqrt{N} \times (k/(k+1)) \times (\alpha-1)$$

*Efficiency* *Retention Factor* *Selectivity*

## Changing Columns to Create Significant Changes in Selectivity

Since resolution is largely a function of selectivity, any discussion about improving resolution should focus primarily on altering selectivity. It has often been taught in HPLC method development that one can effectively alter selectivity by adjusting mobile phases to reach a desired separation. This, of course, is true. However, mobile phase adjustment can be laborious—often involving many preparation adjustments and column equilibration times—and typically creates only marginal selectivity differences. In addition, some elution profiles are not practical with certain mobile phases and detection modes, including mass spectrometry (MS) and refractive index (RI).

On the other hand, changing stationary phases (i.e., columns) can be much easier and can also result in more significant selectivity differences because stationary phases can offer alternate and even orthogonal separations. These alternate separations can also be scouted very quickly using precise scouting gradients.

With the number of columns commercially available today, choosing the right one can be difficult, even overwhelming. By quantifying stationary phase selectivity, we can create new guidelines for effectively and easily choosing columns to help reduce method development time and increase method ruggedness.

## Quantifying Column Selectivity Using the Hydrophobic-Subtraction Model (H-S Model)

Many models exist for choosing solvents and mobile phase additives, but not until recently has stationary phase characterization received much attention. Column selectivity has been largely overlooked due, in part, to its complexity, particularly for liquid separations. But now that Snyder et al. have proposed their popular hydrophobic-subtraction model (H-S model) [1], we can begin to compare and quantify stationary phase selectivity in reversed phase separations and determine (often through orthogonal separations) which stationary phases produce the greatest degree and range of selectivity differences. Only then can we identify a small set of columns that will form the contents of an efficient and effective method development toolbox.

The H-S model is a novel treatment that empirically defines reversed phase selectivity by analyzing a varied collection of solute test probes and then utilizing 5 established selectivity parameters—hydrophobicity (H), steric hindrance (S*), hydrogen bond acidity (A), hydrogen bond basicity (B), and cation exchange activity (C)—to identify the contributions of silica sorbents and stationary phases on selectivity. This model has been used by many organizations, including United States Pharmacopeia (USP), to find column equivalency.

The selectivity value (Fs) of the H-S model is normally used to find the similarity between columns, but it can conversely be used to find column ***dis***similarity, even orthogonality, to highlight selectivity differences and simplify column selection. Table I compares a variety of stationary phases and reveals which phases offer increased selectivity. (Because the H-S model evaluates the contributions of both stationary phase and silica support on selectivity, we intentionally kept the silica support constant throughout our experiments to isolate the effect of stationary phases on selectivity.) Each value was calculated relative to a C18 benchmark. The columns showing high Fs values—like the 4 Restek USLC™ phases shown in **blue**—exhibit the greatest dissimilarity in selectivity relative to the C18, so they are excellent choices when a C18 does not provide the selectivity needed.

**Table I:** The Fs term of the hydrophobic-subtraction model (H-S model) can numerically determine which stationary phases are most dissimilar to a C18, illustrating the phases needed to extend the selectivity range in reversed phase chromatography. The 4 Restek USLC™ phases are shown in **blue**.

| Terms Calculated from the Hydrophobic-Subtraction Model (H-S Model) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Stationary Phase Type | Hydrophobicity | Steric Hindrance | Hydrogen Bond Acidity | Hydrogen Bond Basicity | Cation Exchange Activity | Selectivity Function | Rank Dissimilarity |
| | H | S* | A | B | C | Fs | |
| Ultra C18 (control) | 1.051 | 0.033 | -0.032 | -0.023 | 0.057 | 0.0 | — |
| Ultra C8 | 0.0871 | 0.013 | -0.0199 | 0.019 | -0.032 | 11.2 | 8 |
| Ultra C4 | 0.0738 | -0.010 | -0.276 | 0.019 | 0.032 | 11.3 | 7 |
| Ultra C1 | 0.613 | -0.054 | -0.408 | 0.016 | -0.032 | 17.9 | 6 |
| Ultra Aqueous C18 | 0.808 | -0.128 | 0.378 | 0.013 | 0.0229 | 25.4 | 5 |
| Ultra Biphenyl | 0.661 | -0.189 | -0.283 | 0.042 | 0.204 | 28.4 | 4 |
| Ultra Cyano† | 0.409 | -0.041 | -0.801 | -0.011 | -0.110 | 29.1 | 3 |
| Ultra PFP Propyl | 0.671 | -0.092 | -0.213 | -0.007 | 0.658 | 52.0 | 2 |
| Ultra IBD | 0.672 | -0.035 | -0.052 | 0.233 | -0.564 | 63.7 | 1 |

All columns were tested using the same silica support.
† NOTE: The cyano phase also ranks high in terms of dissimilarity, but the more rugged PFP Propyl phase was ultimately chosen for the USLC™ column set because it better withstands the low pH levels required for mass spectrometry while offering equally heightened retention of basic compounds.

### Characterizing Selectivity at the Molecular Level

Often during method development, after we have made our initial column choice, we still find ourselves struggling to resolve compounds as we try to find a "better" column. This difficulty is often due to an inability to find a column with ***alternate*** selectivity. Quantifying stationary phase selectivity (Table I) is a very important step in identifying a small and effective column set for method development, but we must further define selectivity at a molecular level to ensure that the columns in our method development toolbox exhibit not just ***high*** selectivity, but also ***alternate*** selectivity based on potential analyte types.

Selectivity (α) is practically determined from the difference in retention factors (k) of 2 peaks. Therefore, to produce alternate selectivity, we must alter the retention of one peak relative to the other. (Increasing the retention of both peaks equally results in higher retention capacity, but no change in selectivity because the difference between the 2 peaks does not change.) If we focus column selection on intermolecular interactions, we can see how specific phases create selectivity by altering the retention profile of specific solutes in relation to others—true selectivity.

So before we can confirm alternate selectivity, we first need to characterize the types of intermolecular interactions commonly encountered in reversed phase chromatography (RPC). In our experiments, we measured 4 major types of interactions—dispersion, polarizability, hydrogen bonding, and cation exchange. To further simplify things and more easily define a guideline, we can relate these measured interactions to chemical properties as noted below:

- ***Dispersion*** is the term for the van der Waals interactions that exist to some extent in all organic molecules, including polar molecules. It is the major driver for RPC and is a major retention mechanism for alkyl phases (i.e., C1 through C18). Since the retention is proportionate to the hydrophobicity of the molecule, we can call these interactions ***hydrophobic retention***.
- ***Polarizability*** is the ability of a stationary phase to change its electron distribution in the presence of an analyte and induce a dipole interaction. It is commonly seen in phenyl-based columns and is the main reason we often switch from a C18 to a phenyl to find alternate selectivity. The Restek Biphenyl column has 2 phenyl rings to enhance polarizability. These interactions are most commonly seen in dipolar, unsaturated, or conjugated compounds and fused-ring compounds with electron withdrawing groups (like nitro groups). For our purposes, we will define these interactions simply as ***dipolar retention***.
- ***Hydrogen bonding*** is used in RPC when a solute and a stationary phase form a chemical bond in which a hydrogen atom of one molecule is attracted to an electronegative atom, especially a nitrogen, oxygen, or fluorine, of another molecule. Although hydrogen bonding results in retention of other solute types, we will focus on its ability to increase retention for acidic compounds and will call it ***acidic retention***.
- ***Cation exchange*** is an electrostatic interaction between a cationic solute and an anion within the stationary phase. Cation exchange, or electrostatic interaction, is most commonly employed in RPC for the retention of protonated bases. Therefore, for simplicity, we will call it ***basic retention***.

Table II outlines the common solute retention profiles for the specific interactions we measured in our experimentation. With these intermolecular interactions defined, we can now use their retention profiles to determine which highly selective columns produce alternate selectivity for specific compound types, thereby radically simplifying column selection.

**Table II:** Common retention profiles measured for modern reversed phase columns as they relate to molecular interactions.

| Solute Interaction | Type of Solute Retained | Common Phase Category | H-S Model Term | Probes Measured |
|---|---|---|---|---|
| **Dispersion** | Hydrophobic | C18 | H | Toluene, Ethylbenzene |
| **Polarizability** | Dipolar | Biphenyl | n/a* | Anisole, Benzonitrile |
| **Hydrogen Bonding** | Acidic | Polar Embedded | B | 4-Butylbenzoic Acid, Mefenamic Acid |
| **Cation Exchange** | Basic | Fluorinated Phenyl | C | Berberine, Amitriptyline, Nortriptyline |

* Because polarizability is not measured by the H-S model, Restek used anisole and benzonitrile probes to mathematically determine the degree of polarizability of each stationary phase.

### Extending the H-S Model to Simplify Column Choice

To determine a simplified guideline for column selection, Restek has extended the H-S model by analyzing empirical selectivity data of our stationary phases (Table I) against the RPC molecular interactions described in Table II. Through matching stationary phases to specific solute types based on these measured intermolecular attractions, we can aid method development in 2 significant ways: First, we can find a small set of columns with a wide range of alternate selectivity for use in method development. Second, we can define a process for selecting columns based on the chemical properties of our analytes when scouting column selectivity.

Extrapolating the retention data for the solute probes in the H-S model allows us to correlate the retention characteristics of specific solutes to stationary phase types. Ultimately, this correlation has enabled us to match column type to the selective retention of our analytes' chemical properties, making column selection truly definable by the chemical composition of our analytes.

Figure 1 illustrates the retention profile of a C18 compared with the profiles of the 4 Restek Ultra Selective Liquid Chromatography™ (USLC™) columns. We can see changes in selectivity across these columns as illustrated by the circled areas showing heightened retention for particular solute types. (Selectivity is the retention of one solute relative to another.) The 4 USLC™ columns exhibit varied retention profiles based upon solute type and, therefore, will exhibit alternate selectivity relative to one another. Because we have a small, quantified column set—4 Restek USLC™ phases—that is highly selective ***and*** exhibits significantly different retention profiles based on specific solute chemical properties, we can now match columns to specific analytes and, thus, simplify method development.

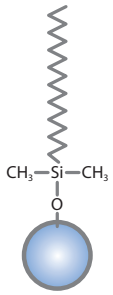
**Figure 1:** Stationary phase selectivity can be determined by looking for column types with varying retention profiles. When compared to a C18, the 4 Restek USLC™ phases offer diverse retention profiles—that is, a true range in selectivity.

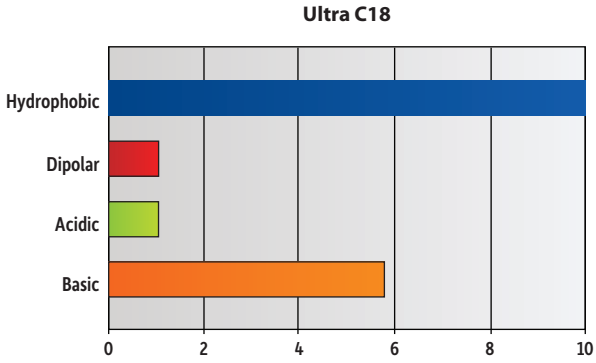
Restek Phase:
**C18**

Stationary Phase Category:
C18 (L1)

Ligand Type:
Densely bonded and fully end-capped octadecyl silane

Properties:
- General purpose.
- Strong hydrophobic retention.



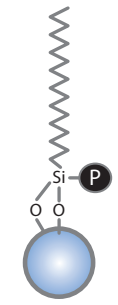

**C18 Benchmark**

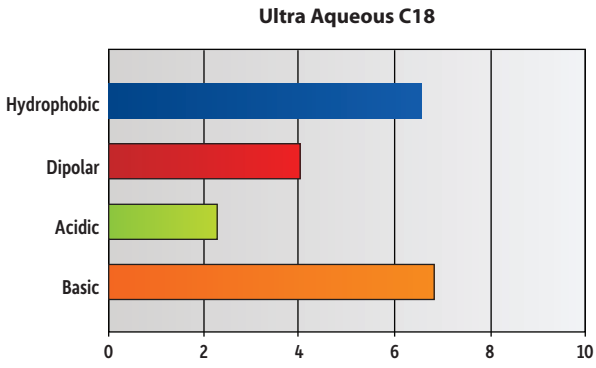
Restek USLC™ Phase:
**Aqueous C18**

Stationary Phase Category:
Modified C18 (L1)

Ligand Type:
Proprietary polar modified and functionally bonded C18

Properties:
- General purpose with a well-balanced retention profile.
- Compatible with 100% aqueous mobile phases.
- Ideal for multi-component LC-MS analyses.




**Well-balanced retention profile.**

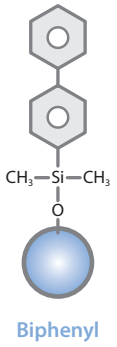
**Figure 1**, *continued*

Restek USLC™ Phase:
**Biphenyl**

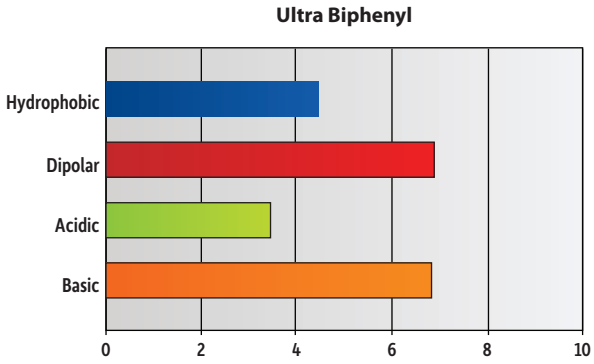
Stationary Phase Category:
Phenyl (L11)

Ligand Type:
Unique Biphenyl

Properties:
- Increased retention for dipolar, unsaturated, or conjugated solutes.
- Enhanced selectivity when used with methanolic mobile phase.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.




Biphenyl



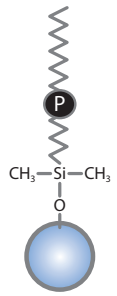

**Heightened retention for dipolar compounds.**

Restek USLC™ Phase:
**IBD**

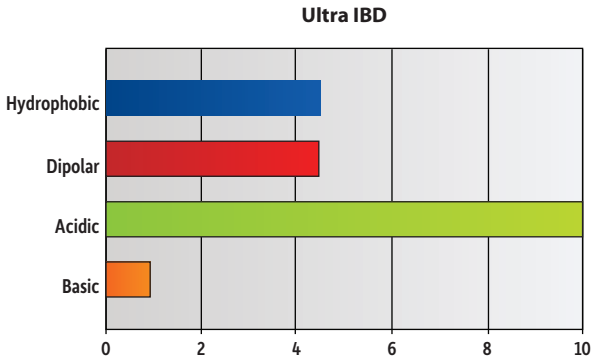
Stationary Phase Category:
Polar Embedded Alkyl (L68)

Ligand Type:
Proprietary polar functional embedded alkyl

Properties:
- Increased retention for acids and water-soluble compounds.
- Compatible with 100% aqueous mobile phases.
- Capable of reversed phase and HILIC separations.




IBD



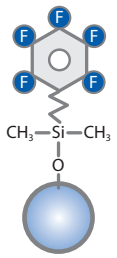

**Heightened retention for acidic compounds.**

Restek USLC™ Phase:
**PFP Propyl**

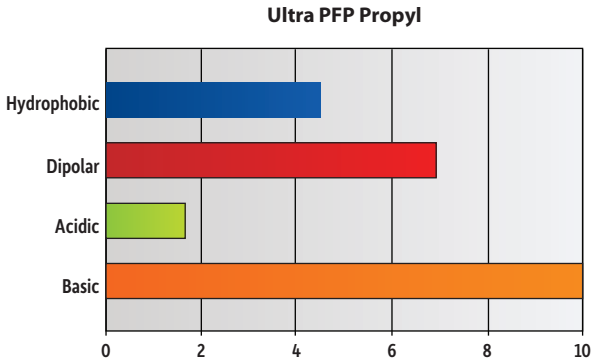
Stationary Phase Category:
Proprietary end-capped pentafluorophenyl propyl (L43)

Ligand Type:
Fluorophenyl

Properties:
- Increased retention for charged bases and electronegative compounds.
- Capable of reversed phase and HILIC separations.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.




PFP Propyl




**Heightened retention for basic compounds.**

All columns were tested using the same silica support.

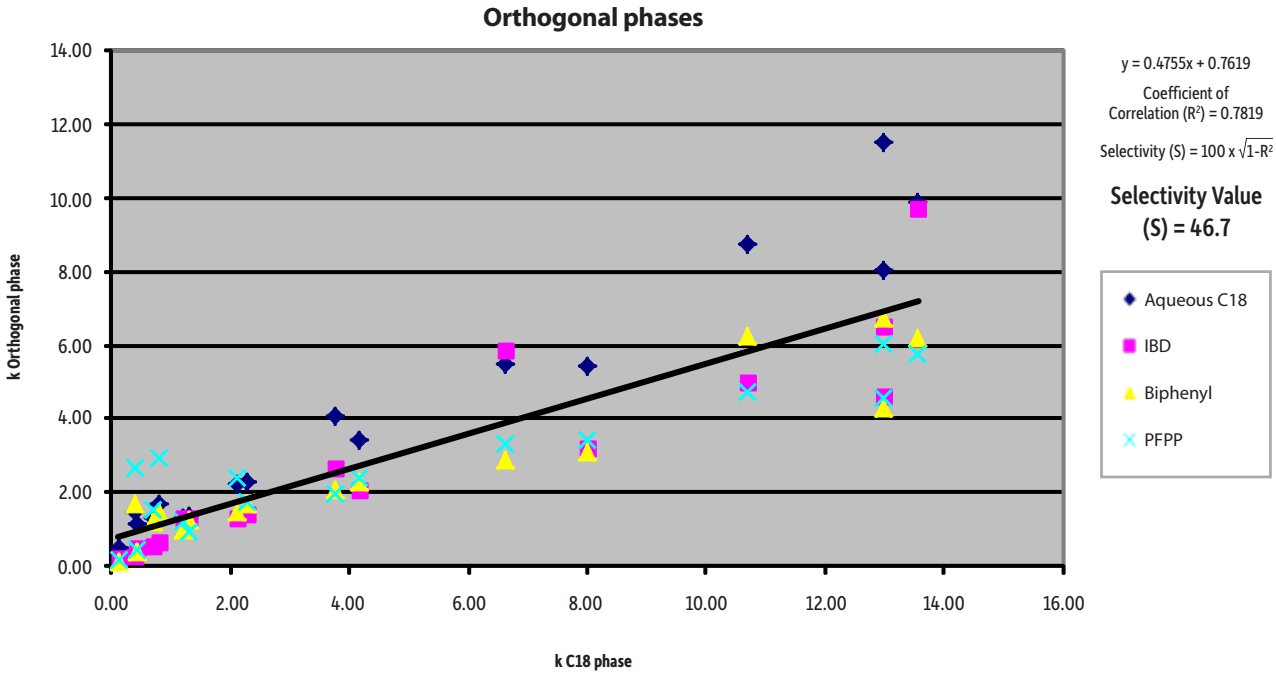
### Confirming the Alternate Selectivity of the USLC™ Column Set

To further confirm that each USLC™ column provides alternate selectivity—not only when compared to the C18 benchmark, but also when compared to the other columns in the set—we quantified the column set's range of selectivity (S) as described by Neue et al. [2]. Looking at the retention characteristics of the H-S model solute probes, we can define selectivity as the degree of scatter along the regression line when comparing stationary phases to the conventional C18 benchmark (Figure 2).

Two very similar stationary phases will produce similar retention for the solute probes and, when graphed, will show high linearity and high correlation. Two very ***dis***similar, or alternately selective, stationary phases that differ in the retention of the solute probes will show a high degree of scatter around the regression line. More scatter reveals that columns are more different, or orthogonal, from one another because it shows larger differences in selectivity. To measure this difference and use it as a means of comparing stationary phases, we can calculate a selectivity (S) value for the columns in the USLC™ column set. Note that because silica and mobile phase contributions could also alter the retention of the test probes, it is important to use identical silica supports and mobile phase compositions as to not bias the results and to allow focus only on the stationary phase contributions to selectivity.

With a selectivity value (S) of 46.7, Restek USLC™ phases produce an incredible range of alternate selectivity —using only 4 columns.

**Figure 2:** Restek has extended the selectivity range for commercially available columns and defined a column set—the 4 USLC™ phases—that is ideal for simplified column selection and ease of method development.




All columns were tested using the same silica support.

## Conclusion: The Right Tools for Maximum Selectivity

The H-S model offers the chromatographic method developer a practical approach to column selection. With a simplified model described above, we can now easily create predictable and alternate selectivity, effectively influencing the most significant factor contributing to resolution. Now that we have identified the small USLC™ column set with a wide range of quantified selectivity, we can quickly determine the best column for nearly any instrument platform and reversed phase or HILIC application by referencing predefined retention profiles. This column set can also be used to get the most out of column switching by providing a functional column set.

The Restek USLC™ column set, consisting of a balanced Aqueous C18, a Biphenyl, a fluorinated PFP Propyl, and a polar embedded IBD, has a profile that encompasses the widest range of reversed phase selectivity available today. Putting the right tools—like the USLC™ column set—in your method development toolbox means maximum alternate selectivity and peak separation with minimal effort.

## Acknowledgements

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## References

[1] L.R. Snyder, J.W. Dolan, P.W. Carr, The Hydrophobic-Subtraction Model of Reversed-Phase Column Selectivity, J. Chromatogr. A 1060 (2004) 77.

[2] U.D. Neue, J.E. O'Gara, A. Mendez, Selectivity in Reversed-Phase Separations Influence of the Stationary Phase, J. Chromatogr. A 1127 (2006) 161.

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