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In this issue, see **page 2**.

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IN THIS ISSUE

Comprehensive 2D Gas Chromatography – Making GC Separations Work Harder

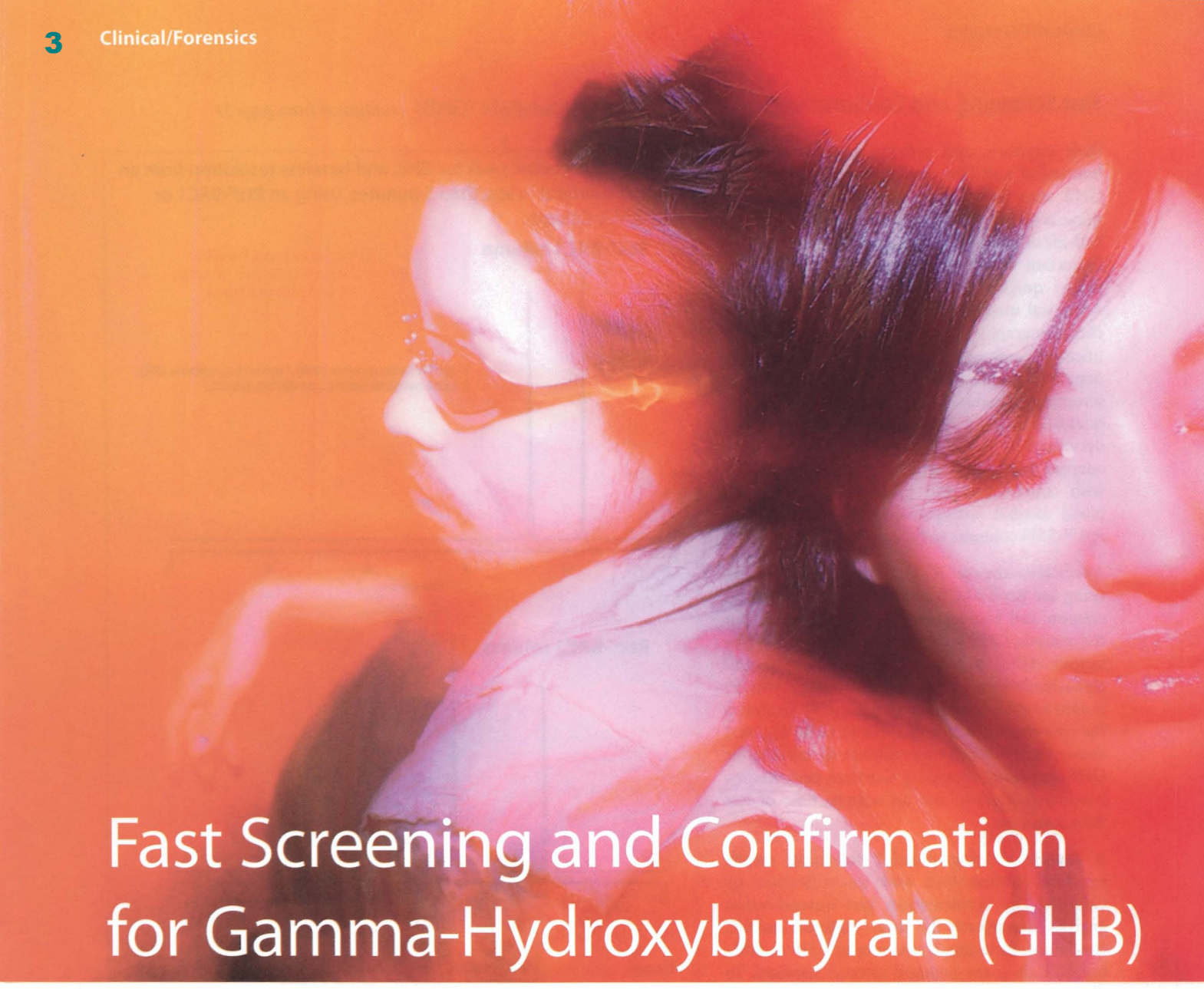
By Dr. Philip Marriott, Professor of Chemistry, RMIT University, Melbourne, Australia, philip.marriott@rmit.edu.au

We are entering a period in its development where the expectations of comprehensive two-dimensional gas chromatography (GC×GC) should – justifiably – match the rhetoric. Since its inception about 15 years ago, researchers who have made it their (life) goal to develop and promote GC×GC have waxed lyrical about the advantages of GC×GC to the GC community. If we were to list the three primary contributions that are often ascribed to GC×GC, these would be: (i) greater separation capacity; (ii) greater sensitivity; and (iii) retention structure in the 2D data presentation that permits the analyst to identify, or predict the identity of, related compounds based on the molecular properties that control retention. At this point, I should admit that I count myself guilty of being amongst those who have promulgated these advantages! Further, I also strongly support the position of GC×GC, and the benefits it holds for volatile and semi-volatile chemical analysis. And if these benefits are indeed general outcomes of GC×GC, then it is only logical that, sooner or later, this coupled column technique will supplant the single-column method that has served us so well for many years. But we might query whether single column GC has really served us so well. Admittedly, it has been just about all we had, so we have had to learn to live with its inherent limitations. Just as we might have recognised, and been frustrated by, the limited separation capacity of single column GC (i.e., as we searched for more complete understanding of the molecular composition of complex samples), analysts turned their attention to GC/MS which became routinely available. Considerable effort was devoted to implement solutions based on mass-detection to provide the necessary unique identification of individual compounds in (grossly) overlapping chromatograms. The mantra that MS can solve (all) our overlap problems probably became a crutch that somewhat numbed our realisation, according to my Research Group's philosophy, that often "the only Solution is better Resolution".

So, now that we have this new tool, what does it mean to the analyst? Well, in a simple answer – everything! With extra separation, the rationale for having to rely on MS for compound measurement (as opposed to identification) might now be negotiable. This is a considerable conceptual departure from the classical reliance on GC/MS. Extra sensitivity is a useful property to analysts, but this may be a lesser advantage of GC×GC. The ability to remove column bleed from solute elution does have benefits (when doing GC×GC/MS). The most significant advantage is separation power. To be able to resolve many more compounds immediately enables a much more complete 'picture' of the composition of a sample. Picture is used deliberately here, since the 2D GC presentation is very much akin to a picture. The comparison of 1D GC results is via a conventional GC trace – a one-dimensional time-response plot. The comparison is limited by the extent to which peaks coincide, or give multiple compound responses at one point. In GC×GC, the greater separation and picture-style GC plot means that we can simply compare two 2D pictures. Each compound now resides in its own 2D location which is determined by, or depends upon, the specific chemical-physical properties of a molecule which generate the peak position though specific interactions with the column stationary phases. The 2D plot has been called a chemical property retention map, which has axes controlled by retention mechanisms on each of the two columns. Choice of column phases is crucial to the effectiveness with which compounds are located within the available 2D space. Here, we will not consider how we generate the GC×GC experiment (i.e., the modulation methods used), however a few comments on the column selection are warranted in this text.

In GC×GC we usually couple a long 1D column directly to a short 2D column (or a regular elution column to a fast elution column). The second column has to work hard! We ask it to resolve peaks that are overlapping on the 1D column. Being about 1 m in length, with a need to complete continual, on-the-fly analyses of effluent from the 1D column within about 4-5 s, performance is everything. We use high carrier flow and narrow bore columns, but actual conditions are flexible. We now commonly find some regions of 1D GC analyses where up to 5 – 10 or more compounds co-elute. This is clearly beyond the scope of MS

Continued on page 31.



Fast Screening and Confirmation for Gamma-Hydroxybutyrate (GHB)

Using Restek Columns in Headspace GC or GC/MS Systems

By Kristi Sellers, Clinical/Forensic Innovations Chemist

- Use an Rtx®-BAC1 column or Rtx®-BAC2 column for GHB screening.
- Confirm and quantify positive GHB screens by using an Rxi™-5ms column.
- Fast, reliable screening; accurate confirmation and quantification.

In the last ten 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. The rise in use of GHB and GHB-type products as recreational drugs is primarily due to

Continued on page 4.

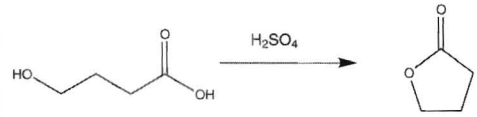
Fast Screening and Confirmation for Gamma-Hydroxybutyrate (GHB) *(continued from page 3)*

their euphoric and sedative properties. 1,4-butanediol and GBL are quickly metabolized to GHB after ingestion and are analyzed as such. Because GHB is endogenous in humans, and has a half-life of one hour or less after injection, it is very important to collect biofluids (typically blood or urine) quickly for toxicological investigation. Analytical methods for GHB usually employ gas chromatography and mass spectrometry for quantification and confirmation. The methodology described here establishes a headspace GC-FID screening procedure followed by confirmation and quantification by headspace GC/MS, and was developed by the FBI Chemistry Unit.[1] We have adapted Rtx®-BAC1 and Rtx®-BAC2 columns—with court-tested and proven performance in blood alcohol analyses—and new, highly inert Rxi™-5MS columns to the methods.

A typical headspace GC-FID blood alcohol system, using an Rtx®-BAC1 column or an Rtx®-BAC2 column, can be adapted for GHB screening. For the analysis, GHB is converted to gamma-butyrolactone (GBL) to improve chromatography, and alpha-methylene-gamma-butyrolactone (AMGB) is used as the internal standard. Figure 1 illustrates the conversion reaction of GHB to GBL. Figure 2 shows that either Restek column is suitable for GHB screening, providing Gaussian peak shapes, baseline resolution, and an analysis time of less than 5 minutes.

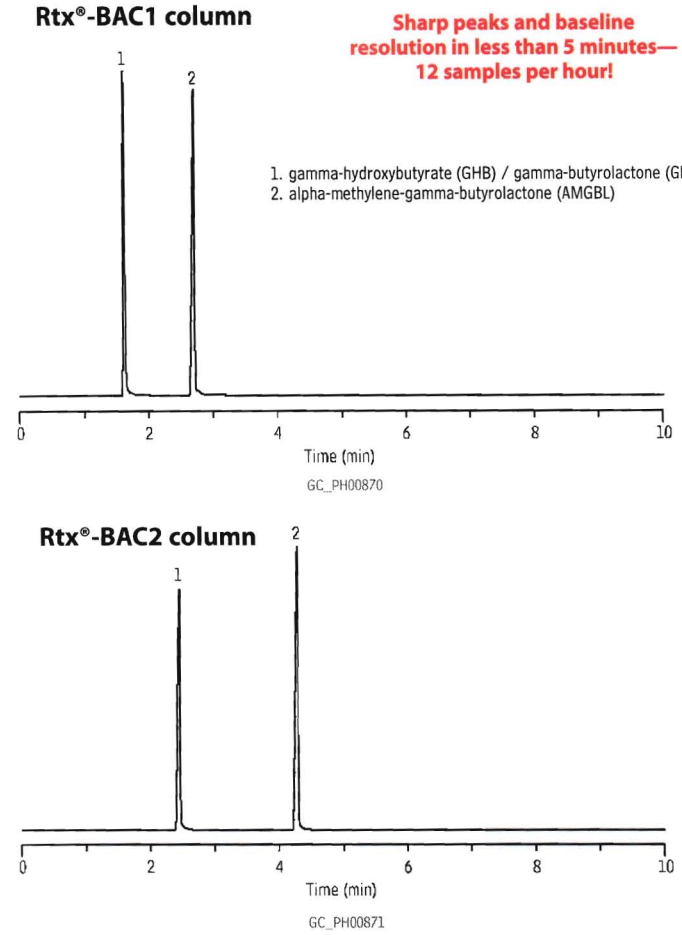
A sample yielding positive screening results requires confirmation and quantification by GC/MS. The confirmation and quantification analysis incorporates the same headspace and GC conditions, including conversion of GHB to GBL, but GBL-d6 is the required internal standard. To illustrate GBL and GBL-d6 separation and peak shape on an Rxi™-5ms column we analyzed 1µL of a standard, using GC/MS. (Figure 3). This typical liquid injection shows the two compounds are partially resolved on the Rxi™-5ms column, and positively identified using full scan. Then, extracted ion data (EI) were obtained(Figure 4). After positive identification, GHB is quantified by comparing the areas of the deuterated and undeuterated GBL extracted ions.

Figure 1 Conversion of GHB to GBL.



gamma-hydroxybutyrate (GHB) — gamma-butyrolactone (GBL)

Figure 2 Symmetric peak for GHB, and baseline resolution from an internal standard in less than 5 minutes, using an Rtx®-BAC1 or Rtx®-BAC1 Column.



Column:	Rtx®-BAC1 30m, 0.32mm ID, 1.8µm (cat.# 18003) and Rtx®-BAC2 30m, 0.32mm ID, 1.2µm (cat.# 18002), connected via universal "Y" Press-Tight® connector (cat.# 20405)
Sample:	GHB, GBL, α-methylene-γ-butyrolactone (AMGBL), 10µg/mL each in water
Inj.:	1.0mL headspace, split (split ratio 1:10), 1mm split inlet liner (cat.# 20972)
Inj. temp.:	200°C
Carrier gas:	helium, constant pressure
Linear velocity:	44cm/sec. @ 50°C
Oven temp.:	50°C (3 min.) to 150°C @ 20°C/min. (hold 7 min.)
Det:	FID @ 240°C
Headspace autosampler:	Teledyne/Tekmar HT3
Sample/platen temp.:	100°C
Sample equilibration:	15 min.
Mixing time:	5 min.
Vial pressure:	10psig
Vial pressurization time:	2 min.
Loop fill time:	2 min.
Transfer line temp.:	120°C

Figure 3 An Rxi™-5ms column provides the symmetric peaks and resolution needed for reliable confirmation of GHB.

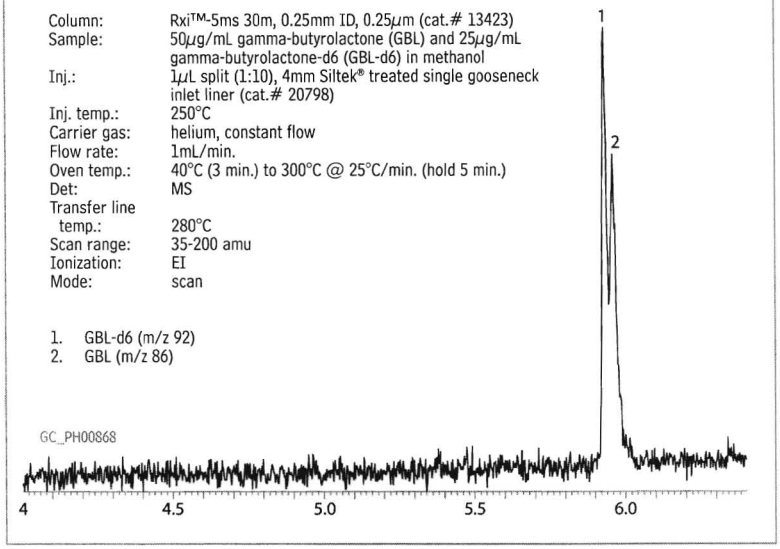
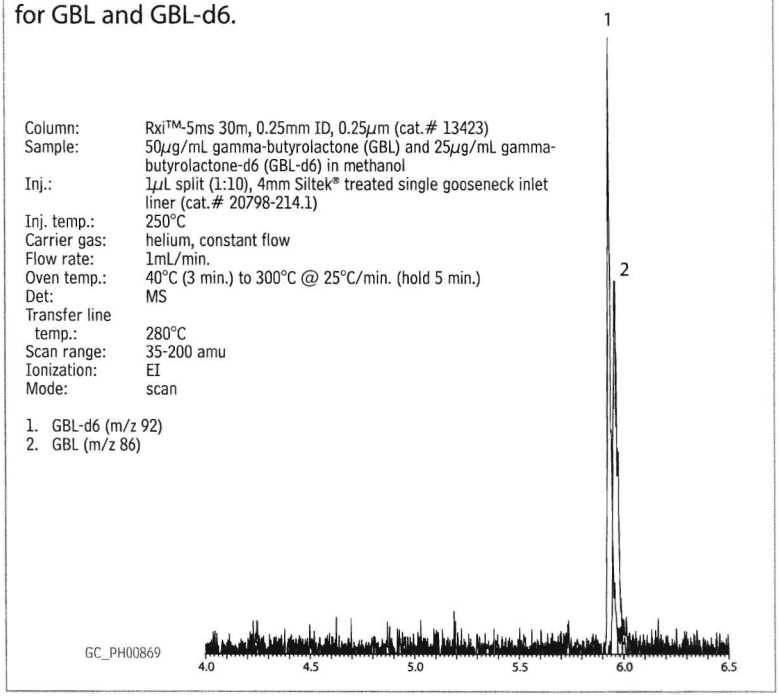


Figure 4 Overlay of extracted ion chromatograms for GBL and GBL-d6.



free literature

Clinical/Forensics Products & Applications for GC & HPLC (2006/07 Edition)
This 64-page catalog presents a wealth of information about clinical and forensic analyses by GC and HPLC. Clinical applications include analgesics, antihistamines, cardiac medications, CNS depressants, cold & sinus medications, steroids, and more; forensic applications include arson accelerants, anesthetics, barbiturates, blood alcohol, butyrolactone, cannabinoids, cocaine, opiates, and more. Includes references to many additional free Restek publications that discuss specific subjects in detail. **lit. cat.# 59989A**

Because this methodology for analyzing GHB in biofluids employs sample introduction through a headspace technique, the need for injector and column maintenance is dramatically reduced. The use of an existing headspace GC system for blood alcohol analysis eliminates the need for additional equipment and allows rapid and reliable screening, using the same Rtx®-BAC1 or Rtx®-BAC2 column. For positive results, an Rxi™-5ms column in a GC/MS system provides accurate confirmation and quantification of GHB.

Reference
1 LeBeau, M.A., M.A. Montgomery, M.L Miller, and S.G. Burmeister, J. Anal. Toxicol. 24 (6): 421-428 (Sept. 2000).

Rtx®-BAC1 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #	price
0.32mm	1.80	-20 to 240/260°C	30-Meter	18003	$485
0.53mm	3.00	-20 to 240/260°C	30-Meter	18001	$510

Rtx®-BAC2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #	price
0.32mm	1.20	-20 to 240/260°C	30-Meter	18002	$485
0.53mm	2.00	-20 to 240/260°C	30-Meter	18000	$510

Rxi™-5ms Columns (fused silica)

(Crossbond® 5% diphenyl / 95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.18mm	0.18	-60 to 330/350°C	20-Meter	13402	$370
0.18mm	0.36	-60 to 330/350°C	20-Meter	13411	$370
0.20mm	0.33	-60 to 330/350°C	12-Meter	13497	$230
0.20mm	0.33	-60 to 330/350°C	25-Meter	13498	$365
0.20mm	0.33	-60 to 330/350°C	50-Meter	13499	$630
0.25mm	0.25	-60 to 330/350°C	15-Meter	13420	$260
0.25mm	0.25	-60 to 330/350°C	30-Meter	13423	$435
0.25mm	0.25	-60 to 330/350°C	60-Meter	13426	$780
0.25mm	0.50	-60 to 330/350°C	15-Meter	13435	$260
0.25mm	0.50	-60 to 330/350°C	30-Meter	13438	$435
0.25mm	0.50	-60 to 330/350°C	60-Meter	13441	$780
0.25mm	1.00	-60 to 330/350°C	15-Meter	13450	$260
0.25mm	1.00	-60 to 330/350°C	30-Meter	13453	$435
0.25mm	1.00	-60 to 330/350°C	60-Meter	13456	$780

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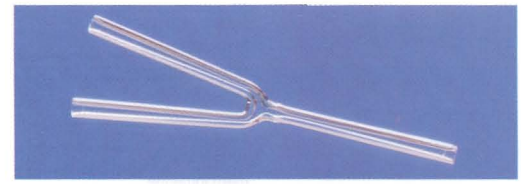

Description		ea./price
Universal "Y" Press-Tight® Connector	20405	$61
Deactivated Universal "Y" Press-Tight® Connector	20405-261	$62
Siltek® Treated Universal "Y" Press-Tight® Connector	20485	$63

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Drugs of Abuse Analytical Reference Materials

by Ken Herwehe, Analytical Reference Materials Product Manager

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Exempted Drug of Abuse Reference Materials

1000µg/mL in 1 mL purge & trap methanol

Compound	cat.#	price
Benzodiazepines		
alprazolam	34042	$23
bromazepam	34043	$23
chlordiazepoxide	34044	$23
clobazam	34045	$23
clonazepam	34046	$23
diazepam	34047	$23
flunitrazepam	34049	$23
flurazepam	34050	$23
lorazepam	34051	$23
nitrazepam	34053	$23
oxazepam	34054	$23
prazepam	34055	$23
temazepam	34056	$23
triazolam	34057	$23
Cocaine & Metabolites		
cocaine	34015	$23
benzoylecgonine	34016	$23
ecgonine	34017	$23
ecgonine methyl ester	34018	$23
Methadone & Metabolites		
methadone	34005	$23
Amphetamines & Metabolites		
d-amphetamine	34020	$23
(+)methamphetamine	34021	$23
Opiates & Metabolites		
codeine	34000	$23
hydrocodone	34002	$23
hydromorphone	34063	$23
morphine	34006	$23
oxycodone	34007	$23
oxymorphone	34065	$23
Cannibinoid & Metabolites		
cannabidiol	34011	$23
cannabinol	34010	$23
Barbituates		
amobarbital	34028	$23
aprobarbital	34029	$23
barbital	34030	$23
butabarbital	34031	$23
butalbital	34032	$23
DL-glutethimide	34058	$23
hexobarbital	34033	$23
mephobarbital	34034	$23
methohexital	34035	$23
pentobarbital	34036	$23
phenobarbital	34037	$23
secobarbital	34038	$23
talbutal	34039	$23
thiamylal	34040	$23
thiopental	34041	$23
Other		
benzphetamine	34022	$23
cocaethylene*	34066	$23
fenfluramine	34023	$23
levorphanol	34003	$23
meperidine	34004	$23
meprobamate	34059	$23
methaqualone	34064	$23
methyprylon	34060	$23
pentazocine	34062	$23
phencyclidine	34027	$23
phendimetrazine	34025	$23
phenmetrazine	34026	$23
phentermine	34024	$23
dextro-propoxyphene	34008	$23
thebaine	34009	$23

*1000µg/mL in 1mL acetonitrile.

Blood Alcohol Standards

Compound	qty.	cat.#	price
0.015g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36232	$26
1mL/ampul	10-pk.	36332	$41
5mL/ampul	ea.	36240	$26
20mL/ampul	ea.	36248	$46
0.02g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36233	$26
1mL/ampul	10-pk.	36333	$41
5mL/ampul	ea.	36241	$26
20mL/ampul	ea.	36249	$46
0.025g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36234	$26
1mL/ampul	10-pk.	36334	$41
5mL/ampul	ea.	36242	$26
20mL/ampul	ea.	36250	$46
0.04g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36235	$26
1mL/ampul	10-pk.	36335	$41
5mL/ampul	ea.	36243	$26
20mL/ampul	ea.	36251	$46
0.05g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36257	$26
1mL/ampul	10-pk.	36259	$41
5mL/ampul	ea.	36258	$26
20mL/ampul	ea.	36260	$46
0.08g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36262	$26
1mL/ampul	10-pk.	36264	$41
5mL/ampul	ea.	36263	$26
20mL/ampul	ea.	36265	$46
0.1g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36236	$26
1mL/ampul	10-pk.	36336	$41
5mL/ampul	ea.	36244	$26
20mL/ampul	ea.	36252	$46
0.15g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36237	$26
1mL/ampul	10-pk.	36337	$41
5mL/ampul	ea.	36245	$26
20mL/ampul	ea.	36253	$46
0.2g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36238	$26
1mL/ampul	10-pk.	36338	$41
5mL/ampul	ea.	36246	$26
20mL/ampul	ea.	36254	$46
0.3g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36239	$26
1mL/ampul	10-pk.	36339	$41
5mL/ampul	ea.	36247	$26
20mL/ampul	ea.	36255	$46
0.4g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36266	$26
1mL/ampul	10-pk.	36268	$41
5mL/ampul	ea.	36267	$26
20mL/ampul	ea.	36269	$46

Blood Alcohol Mix Resolution Control Standard

(8 components)

acetaldehyde
acetone
acetonitrile
ethanol (NIST certified value)
ethyl acetate
isopropanol
methanol
methyl ethyl ketone

0.100g/dL each in water, 1mL/ampul

cat. # 36256 (ea.) $31

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by Ken Herwehe, Analytical Reference Materials Product Manager

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Organochlorine Pesticide Mix #2 (Rev), Method 525.2 (8 components)

chlorobenzilate
chloroneb
chlorothalonil
DCPA methyl ester (Dacthal®)
heptachlor epoxide (isomer A)
trans-nonachlor
cis-permethrin
trans-permethrin

500µg/mL each in acetone, 1mL/ampul
cat. # 33011 (ea.) $35

Organonitrogen Pesticide Mix #1 (Rev), Method 525.2 (37 components)

alachlor
ametryn
atraton
atrazine
bromacil
butachlor
butylate
chlorpropham
cyanazine (Bladex)
cycloate
diphenamid
EPTC
etridiazole (Terrazole®)
fenarimol
fluridone (Sonar®)
hexazinone (Velpar®)
metolachlor
metribuzin
MGK-264
molinate
napropamide (Devrinol®)
norflurazon
pebulate
prometon
prometryne
pronamide (propyzamide)
propachlor
propazine
simazine
simetryn
tebuthiuron
terbacil
terbutryn
triadimefon
tricyclazole (Beam)
trifluralin
vernolate

500µg/mL each in acetone, 1mL/ampul
cat. # 33012 (ea.) $195

Organophosphorus Pesticide Mix #1 (Rev), Method 525.2 (7 components)

chlorpyrifos (Dursban®)
dichlorvos (DDVP)
disulfoton sulfone
ethoprop (ethoprophos)
methyl paraoxon (Parathion methyl-O-analog)
mevinphos (Phosdrin)
stirofos (tetrachlorvinphos)

500µg/mL each in acetone, 1mL/ampul
cat. # 33013 (ea.) $45

Method 525.2 Semivolatile Mix (revised)

(28 components)

acenaphthylene
anthracene
benzo(a)anthracene
benzo(a)pyrene
benzo(b)fluoranthene
benzo(ghi)perylene
benzo(k)fluoranthene
benzylbutylphthalate
bis(2-ethylhexyl)adipate
bis(2-ethylhexyl)phthalate
chrysene
dibenzo(a,h)anthracene
diethylphthalate
dimethylphthalate
di-*n*-butylphthalate
2,4-dinitrotoluene
2,6-dinitrotoluene
di-*n*-octylphthalate
fluoranthene
fluorene
hexachlorobenzene
hexachlorocyclopentadiene
indeno(1,2,3-cd)pyrene
isophorone
naphthalene
pentachlorophenol*
phenanthrene
pyrene

1,000µg/mL each in acetone, (*pentachlorophenol at 4,000µg/mL), 1mL/ampul
cat. # 31899 (ea.) $80

Method 525.2 PCB Congener Mix (8 components)

2-chlorobiphenyl (BZ#1)
2,3-dichlorobiphenyl (BZ#5)
2,4,5-trichlorobiphenyl (BZ#29)
2,2',4,4'-tetrachlorobiphenyl (BZ#47)
2,2',3',4,6-pentachlorobiphenyl (BZ#98)
2,2',4,4',5,6'-hexachlorobiphenyl (BZ#154)
2,2',3,3',4,4',6-heptachlorobiphenyl (BZ#171)
2,2',3,3',4,5',6,6'-octachlorobiphenyl (BZ#200)

200µg/mL each in acetone, 1mL/ampul
cat. # 32420 (ea.) $56

Organochlorine Pesticide Mix AB # 3

(20 components)

aldrin
α-BHC
β-BHC
δ-BHC
γ-BHC (lindane)
α-chlordane
γ-chlordane
4,4'-DDD
4,4'-DDE
4,4'-DDT
dieldrin
endosulfan I
endosulfan II
endosulfan sulfate
endrin
endrin aldehyde
endrin ketone
heptachlor
heptachlor epoxide (B)
methoxychlor

2,000µg/mL each in hexane:toluene (1:1), 1mL/ampul
cat. # 32415 (ea.) $71

Method 525.2 Nitrogen/Phosphorus Pesticide Mix #2 (6 components)

carboxin
diazinon
disulfoton
fenamiphos
merphos
terbufos

1,000µg/mL each in acetone, 1mL/ampul
cat. # 32423 (ea.) $64

Metribuzin

1,000µg/mL in acetone, 1mL/ampul
cat. # 32436 (ea.) $23

Method 525.2 Fortification Recovery Standard

p-terphenyl-d14

1,000µg/mL in methylene chloride, 1mL/ampul
cat. # 31828 (ea.) $23

Method 525.2 Internal Standard Mix

acenaphthene-d10
chrysene-d12
phenanthrene-d10

1,000µg/mL each in acetone, 1mL/ampul
cat. # 31825 (ea.) $27

Method 525.2 Surrogate Standard Mix

2-nitro-*m*-xylene
perylene-d12
pyrene-d10
triphenylphosphate

1,000µg/mL each in acetone, 1mL/ampul
cat. # 31826 (ea.) $27

Method 525.2 GC/MS Performance Check Mix

4,4'-DDT
DFTPP (decafluorotriphenylphosphine)
endrin

1,000µg/mL each in acetone, 1mL/ampul
cat. # 31827 (ea.) $27

Rapid Analysis of Steroid Hormones by GC/MS

Using the New Rxi™-1ms Column

By Kristi Sellers, Clinical/Forensic Innovations Chemist

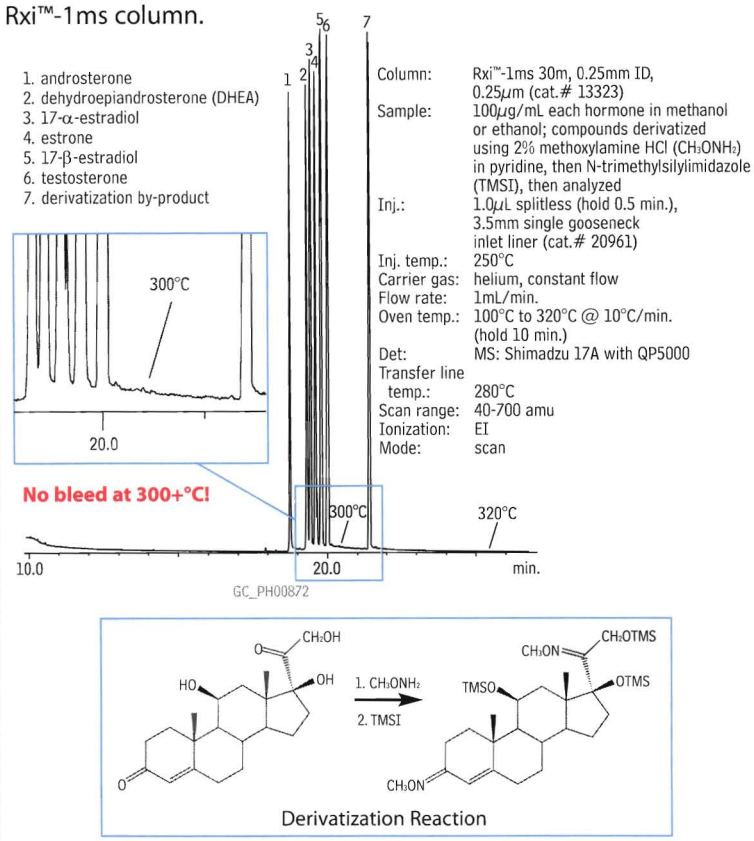
- Resolve 6 common steroid hormones in less than 25 minutes.
- Ultra-low bleed column greatly reduces background interferences.
- Stable performance at 300°C or above.

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 and Gaussian peak shape 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 (Figure1) 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.



Reference

1. http://www.meridianvalleylab.com/steroid_dept.html

Rxi™-1ms Column (fused silica)

(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	13323	$435

For other dimensions, please vist our website.

Enhanced Resolution of Endocrine Disrupting Hormones

Using an Allure® Biphenyl Column and LC-TOFMS

By Robert Freeman, Environmental Innovations Chemist, Rick Lake, Pharmaceutical Innovations Chemist, and Lydia Nolan, Innovations Chemist

- Enhanced selectivity for closely related hormones.
- Complete resolution of 7 common sex hormones in less than 8 minutes.
- Increased confidence in identifications, using a LECO TOFMS system.

Endocrine disrupting chemicals in the environment are a topic of growing concern. Evidence suggests that the developmental and reproductive systems of both fish and wildlife have been affected.[1] A variety of commonly used chemicals have endocrine disrupting properties, but the sex hormones (estrogens, progestogens and androgens) carry the most estrogenic potency.[2] The primary sources are believed to be human excretion and agriculture runoff. Since these compounds generally are not affected by standard wastewater treatment practices, it is believed they are routinely discharged into receiving streams. For this reason, we sought to develop a procedure to detect endocrine disrupting hormones in aqueous matrices.

Chemically, the sex hormones are steroids. Steroids are a unique class of compounds, in that all structural variation is centered on a common conjugated ring system (Figure 1), from which double bonding and various functional groups produce chemical diversity. Estrogens possess a hydroxyl group at position 3, while progestogens and androgens possess a carbonyl group (Figure 2). Typically a complex functional group at position 17 denotes a synthetically produced steroid.

Because steroids are neutral compounds, we evaluated both alkyl (i.e., C18) and phenyl stationary phases to determine the optimum phase for resolving steroid hormones. Alkyl stationary phases separate analytes on the basis of overall hydrophobicity. Phenyl phases offer a different separation mechanism: interactions among π-π electrons, between the phenyl ligand and the analytes. often, these π-π interactions can produce alternate and enhanced selectivity.[3]

A downside to phenyl phases is that they typically show only moderate retention, compared to octadecylsilyl (ODS) alkyl phases. In contrast, the Allure® Biphenyl phase – a surface chemistry consisting of two phenyl groups bonded end-to-end – provides a greater concentration of phenyl groups, in sterically favorable positioning, and thereby increases π-π interactions. An Allure® Biphenyl column exhibits an overall increase in retention capacity and analyte interaction, and provides highly effective separations of compounds exhibiting differences in π-π interactions (Figure 3).

Figure 1 Separations of steroids are especially challenging because all steroid molecules are based on a common structure.

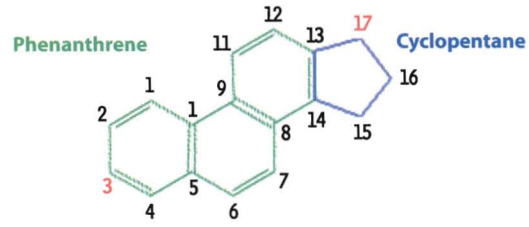
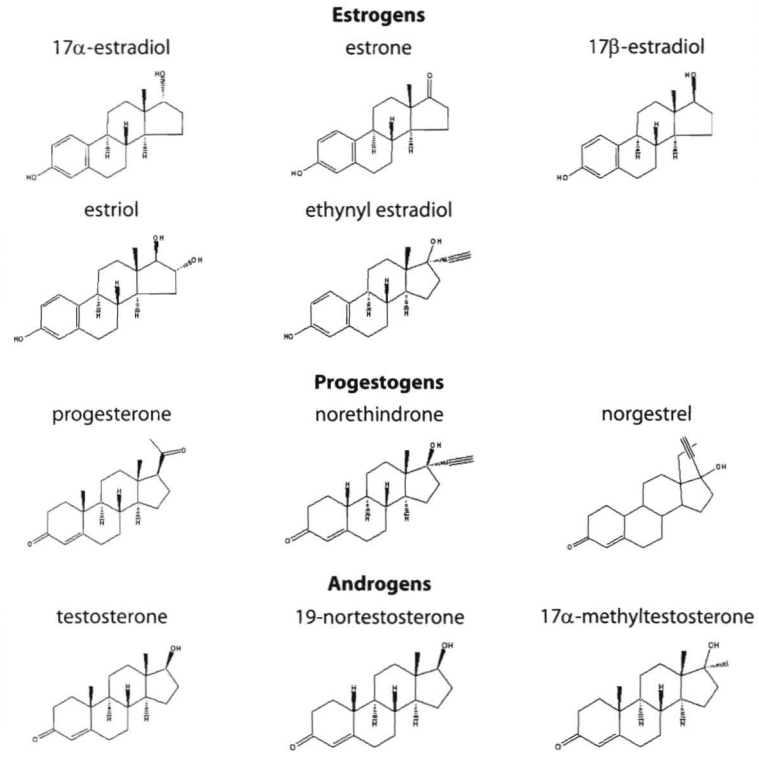


Figure 2 Estrogens include a hydroxyl group at position 3, progestogens and androgens include a carbonyl group.



Acknowledgement
We are grateful to Paul Kennedy, Ph.D, LECO Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396, for his assistance with this analysis.

Figure 3 An Allure® Biphenyl column provides superior selectivity and retention for steroids (UV detection).

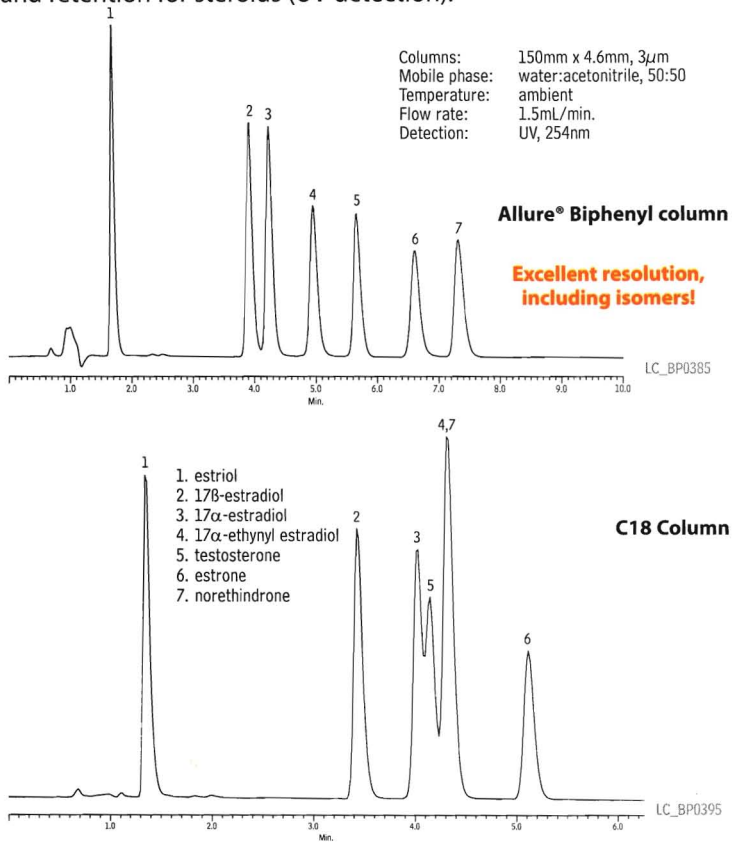
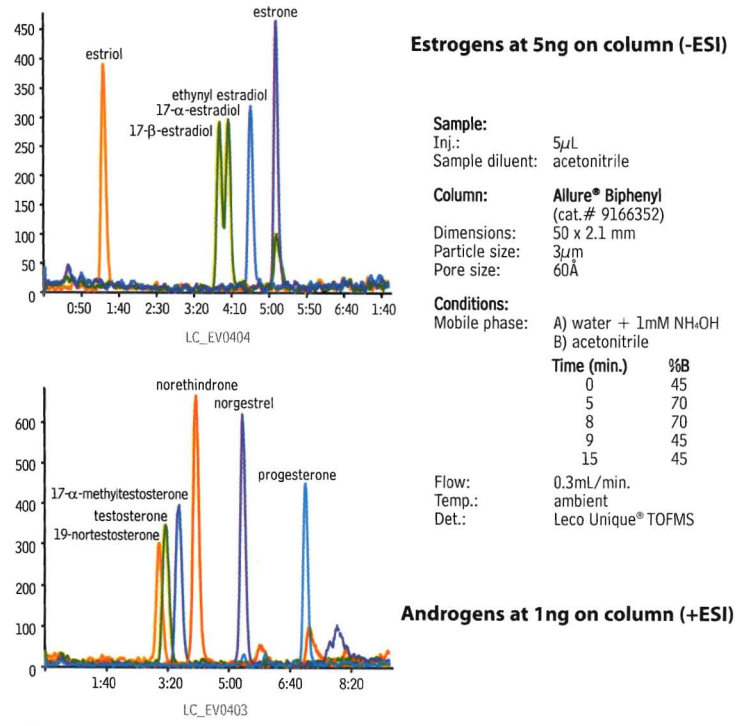


Figure 4 Sensitive analysis of steroids, using an Allure® Biphenyl column, and LECO Unique® TOFMS.



To monitor steroid sex hormones in water, we first developed an extraction procedure, using styrene-divinylbenzene solid phase extraction disks and methyl *tert*-butyl ether (MTBE) as the extraction solvent. We conditioned the extraction disks with acetonitrile and MTBE to remove any potential interferences. After rinsing the disk with distilled water and loading the disks with one liter of sample we used 10mL of MTBE to elute the sample. Prior to analysis the final 10mL extract was concentrated to 2mL and exchanged to acetonitrile.

We recognized that the complexity of environmental sample mixtures and matrices often would make difficult a complete chromatographic separation of the steroid sex hormones by HPLC, and qualitative detection with a non-selective detector (UV-Vis). Mass spectrometry, with secondary separation based on m/z, increased our confidence in the qualitative identifications. We selected LECO Corporation's Unique® LC-TOFMS system for its high data acquisition rate – 100 spectra/sec. The ChromaTOF® software Peak Find algorithm can deconvolute closely eluting peaks, and mass can be determined accurately, to within 5ppm, to calculate possible molecular formula. Because the ionization potential differs among the groups of steroid sex hormones, both negative and positive ESI was used. The estrogens were amenable to negative ESI, while the androgens and progestogens showed much greater sensitivity when we used positive ESI (Figure 4). We believe this difference is because of the differing functional groups at position 3.

These analyses demonstrate that the Allure® Biphenyl stationary phase, through π-π interactions, offers excellent selectivity for compounds with unsaturation differences in their hydrocarbon ring structures. Additionally, the secondary separation power of the Unique® TOFMS system and ChromaTOF® software allows overall analysis time to be reduced, through optimized column dimensions and run conditions, while qualitative identification is maintained.

References

1 http://www.epa.gov/scipoly/oscpendo/
2 Kuster, M., M.J. Lopez, and D. Barcelo, Estrogens and Progesterons in Wastewater, Sludge, Sediments, and Soil, pp. 3 Handbook of Environmental Chemistry
3 http://www.restek.com/fantasia/pdfCache/580020.pdf

Allure® Biphenyl Columns

3µm Column, 2.1mm	cat. #	price
30mm	9166332	$364
50mm	9166352	$364
100mm	9166312	$390
3µm Column, 4.6mm	**cat. #**	**price**
30mm	9166335	$364
50mm	9166355	$364
100mm	9166315	$390

For other column dimensions, and columns with 5µm packing, please visit our website.

New Rxi™-1ms Capillary GC Column

For Low Level GC/MS Analyses

By Robert Freeman, Environmental Innovations Chemist

- Inert, low-bleed column for reliable results.
- Save time – analyze acidic and basic compounds under the same conditions.
- Guaranteed reproducible performance, column to column.

The second column in our new Rxi™ GC column line – the Rxi™-1ms column – will provide the same outstanding performance as the Rxi™-5ms column, with equally superior inertness, ultra-low bleed, and excellent batch to batch reproducibility.

Our first test for this 100% dimethylpolysiloxane phase column was an analysis of a complex mixture of semivolatile organic compounds. The extensive target list was comprised of many classes of compounds including chloroacetanilides, chlorotriazines, triazinones, uracils, polcyclic aromatic hydrocarbons, and phthalates. Figure 1 shows peak shape and selectivity are equally good for all of these diverse compounds, and all are eluted in an acceptable analysis time.

Excellent Inertness

In addition to analyzing these compounds, we analyzed an acidic compound (2,4-dinitrophenol) and a basic compound (pyridine), each at 0.5ng on column, to assess column inertness. Column activity reveals itself through poor response and peak tailing for such active compounds, and these two compounds present both varying difficulties in a GC/MS analysis and differing modes of degradation. Figure 2 shows the excellent peak shapes and responses for these compounds on the 30m x 0.25mm ID, 0.25µm film column.

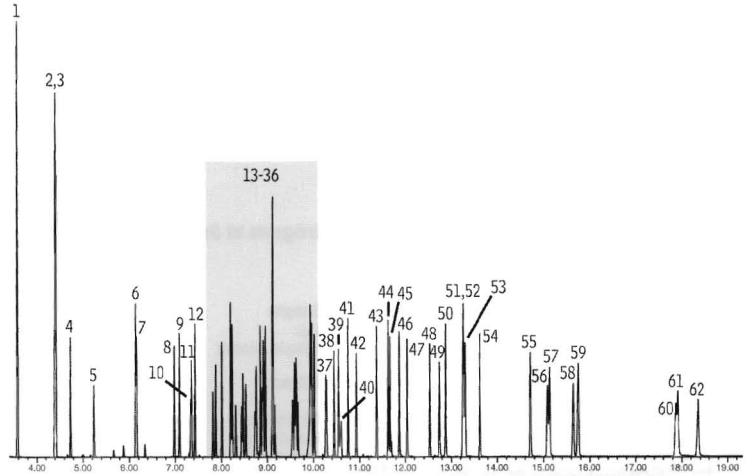
Phenols are notorious for breakdown and peak tailing, caused by interaction with the surface of an active inlet liner or an active column. Nitrophenols and pentachlorphenol, for example, very often exhibit poor peak shape and/or poor response. Figure 3 shows the 30m x 0.25mm ID, 0.25µm Rxi™-1ms column provides very good peak shapes for phenols. Peak responses are well above method requirements.

Ultra-Low Bleed

In addition to excellent inertness, Rxi™-1ms columns exhibit very low bleed. Figure 4 is focused on the end of the chromatogram for semivolatiles. At 330°C, bleed is much lower than the signals for 0.5ng of target analytes. This exceptional signal-to-noise differential for late eluting compounds assures better detection limits.

Figure 1 Excellent selectivity and peak shapes for common drinking water semivolatiles at 10ng, using an Rxi™-1ms column.

1. 2-fluorophenol (surr.)
2. bis(2-chloroethyl)ether
3. phenol-d6 (surr.)
4. 1,4-dichlorobenzene-d4 (int. std.) •
5. nitrobenzene-d5 (surr.)
6. naphthalene-d8 (int. std.) •
7. naphthalene
8. 1-methylnaphthalene
9. 2-methylnaphthalene
10. hexachlorocyclopentadiene
11. EPTC
12. 2-fluorobiphenyl (surr.)
13. 2,6-dinitrotoluene
14. dimethylphthalate
15. acenaphthylene
16. acenaphthene-d10 (int. std.) •
17. acenaphthene
18. 2,4-dinitrotoluene
19. 1-naphthalenamine
20. molinate
21. 2-naphthalenamine
22. 5-nitro-o-toluidine
23. diethylphthalate
24. fluorene
25. propachlor
26. diphenylamine
27. 2,4,6-tribromophenol (surr.)
28. simazine
29. prometon
30. atrazine
31. hexachlorobenzene
32. 4-aminobiphenyl
33. terbacil
34. phenanthrene-d10 (int. std.) •
35. phenanthrene
36. anthracene
37. metribuzin
38. acetochlor
39. alachlor
40. bromacil
41. di-*n*-butylphthalate
42. metolachlor
43. fluoranthene
44. pyrene
45. butachlor
46. *p*-terphenyl-d14 (surr.)
47. *p*-dimethylaminoazobenzene
48. benzyl butyl phthalate
49. 2-acetylaminofluorene
50. bis(2-ethylhexyl)adipate
51. benzo(a)anthracene
52. chrysene-d12 (int. std.) •
53. chrysene
54. bis(2-ethylhexyl)phthalate
55. di-*n*-octylphthalate
56. benzo(b)fluoranthene
57. benzo(k)fluoranthene
58. benzo(a)pyrene
59. perylene-d12 (int. std.) •
60. indeno(1,2,3-cd)pyrene
61. dibenzo(a,h)anthracene
62. benzo(ghi)perylene



GC_EV00833

Column:	Rxi™-1ms, 30m, 0.25mm ID, 0.25µm (cat.# 13323)
Sample:	US EPA Method 525.2 mix: custom 525.2 calibration mix, SV Internal Standard Mix (cat.# 31206), B/N Surrogate Mix (4/89 SOW) (cat.# 31024), Acid Surrogate Mix (4/89 SOW) (cat.# 31025)
Inj.:	1.0µL, 10µg/mL each analyte (internal standards 100µg/mL), split (10:1) 4mm Drilled Uniliner® inlet liner (hole at bottom) (cat.# 20756)
Instrument:	Agilent 6890
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1.2mL/min.
Oven temp.:	50°C (hold 1 min.) to 265°C @ 20°C/min., to 330°C @ 6°Cmin. (hold 1 min.)
Det.:	Agilent 5973 MSD
Transfer line temp.:	280°C
Scan range:	35-550 amu
Solvent delay:	3.20 min.
Tune:	DFTPP
Ionization:	EI

• Internal standards at 100ng on-column.

Figure 2 An Rxi™-1ms column has excellent selectivity for basic or acidic compounds, under the same conditions. (0.5ng each; extracted ion chromatograms).

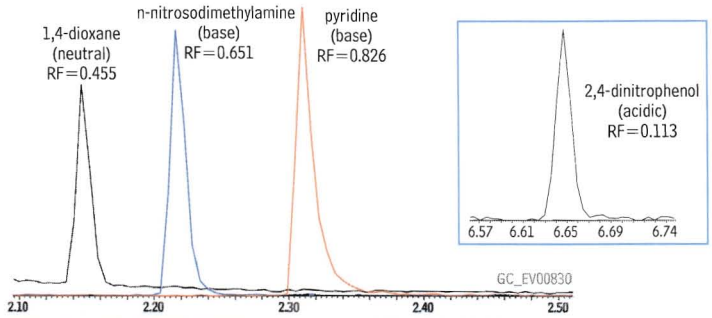
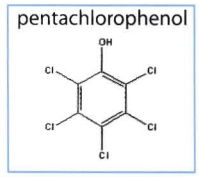


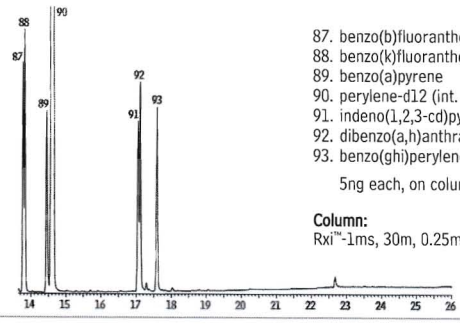
Figure 3 Acidic analytes at 5.0ng on an Rxi™-1ms column (extracted ion chromatogram).

1. phenol
2. 2-chlorophenol-d4 (surr.)
3. 2-chlorophenol
4. 2-methylphenol (*o*-cresol)
5. 2-nitrophenol
6. 2,4-dimethylphenol3,5,6-d3 (surr.)
7. 2,4-dimethylphenol
8. 2,4-dichlorophenol
9. 3-nitro-*o*-xylene (int. std.)
10. 4-chloro-3-methylphenol
11. 2,4,6-trichlorophenol
12. 2,4-dinitrophenol
13. 4-nitrophenol
14. 2,3,4,5-tetrachlorophenol (int. std.)
15. 2-methyl-4,6-dinitrophenol
16. 2,4,6-tribromophenol (surr.)
17. pentachlorophenol



Column: Rxi™-1ms, 30m, 0.25mm ID, 0.25µm (cat.# 13323)
Sample: US EPA Method 528 Mix: Phenols Fortification Mix, EPA 528 (cat.# 31695), Internal Standard Mix, EPA 528 (cat.# 31696), Surrogate Standard Mix, EPA 528 (cat.# 31697)
Inj.: 1.0µL, 5µg/mL each analyte (internal standards 25µg/mL), split (10:1) 4mm Drilled Uniliner® inlet liner (hole at bottom) (cat.# 20756)
Instrument: Agilent 6890
Inj. temp.: 250°C
Carrier gas: helium, constant flow
Flow rate: 1.2mL/min.
Oven temp.: 70°C (hold 0.5 min.) to 130°C @ 8°C/min., to 300°C @ 50°C/min. (hold 1 min.)
Det.: Agilent 5973 MSD

Figure 4 Exceptionally low bleed for Rxi™-1ms columns at 330°C.



87. benzo(b)fluoranthene
88. benzo(k)fluoranthene
89. benzo(a)pyrene
90. perylene-d12 (int. std.)
91. indeno(1,2,3-cd)pyrene
92. dibenzo(a,h)anthracene
93. benzo(ghi)perylene

5ng each, on column

Column:
Rxi™-1ms, 30m, 0.25mm ID, 0.25µm (cat.# 13323)

Based on these results, we highly recommend the new Rxi™-1ms column for low-level analyses that require a 100% dimethylpolysiloxane phase.

Rxi™-1ms Columns (fused silica)

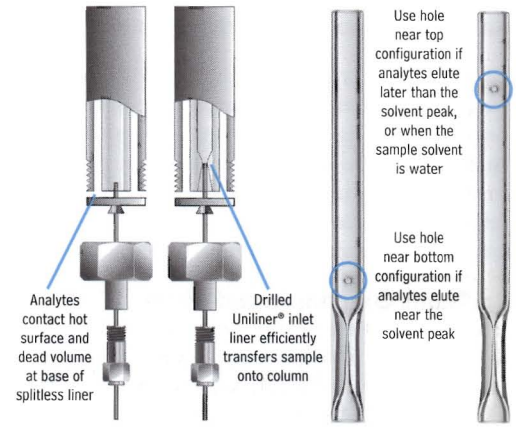
(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.18mm	0.18	-60 to 330/350°C	20-Meter	13302	$370
0.20mm	0.33	-60 to 330/350°C	12-Meter	13397	$230
0.20mm	0.33	-60 to 330/350°C	25-Meter	13398	$365
0.20mm	0.33	-60 to 330/350°C	50-Meter	13399	$630
0.25mm	0.25	-60 to 330/350°C	15-Meter	13320	$260
0.25mm	0.25	-60 to 330/350°C	30-Meter	13323	$435
0.25mm	0.25	-60 to 330/350°C	60-Meter	13326	$780
0.25mm	0.50	-60 to 330/350°C	15-Meter	13335	$260
0.25mm	0.50	-60 to 330/350°C	30-Meter	13338	$435
0.25mm	0.50	-60 to 330/350°C	60-Meter	13341	$780
0.25mm	1.00	-60 to 330/350°C	15-Meter	13350	$260
0.25mm	1.00	-60 to 330/350°C	30-Meter	13353	$435
0.25mm	1.00	-60 to 330/350°C	60-Meter	13356	$780
0.32mm	0.25	-60 to 330/350°C	15-Meter	13321	$280
0.32mm	0.25	-60 to 330/350°C	30-Meter	13324	$460
0.32mm	0.25	-60 to 330/350°C	60-Meter	13327	$820
0.32mm	0.50	-60 to 330/350°C	15-Meter	13336	$280
0.32mm	0.50	-60 to 330/350°C	30-Meter	13339	$460
0.32mm	0.50	-60 to 330/350°C	60-Meter	13342	$820
0.32mm	1.00	-60 to 330/350°C	15-Meter	13351	$280
0.32mm	1.00	-60 to 330/350°C	30-Meter	13354	$460
0.32mm	1.00	-60 to 330/350°C	60-Meter	13357	$820
0.53mm	0.50	-60 to 330/350°C	15-Meter	13337	$310
0.53mm	0.50	-60 to 330/350°C	30-Meter	13340	$515
0.53mm	1.00	-60 to 330/350°C	15-Meter	13352	$310
0.53mm	1.00	-60 to 330/350°C	30-Meter	13355	$515
0.53mm	1.50	-60 to 330/350°C	15-Meter	13367	$310
0.53mm	1.50	-60 to 330/350°C	30-Meter	13370	$515
0.53mm	1.50	-60 to 330/350°C	60-Meter	13373	$880

restek innovation!

The Drilled Uniliner®

To reduce the effects of surface activity in the injection port liner, and focus on the effects of the column on active analytes, we used a Drilled Uniliner® inlet liner. This liner eliminates contact between the active compounds and active metal surfaces in the injector, ensuring an inactive sample pathway for analyte transfer from the injection port to the column. For more information, request **lit. cat.# 59877**.



GC/MS Low-Level for Semivolatiles in Drinking Water

Excellent Responses at 10ng On Column, Using an Rxi™-5ms Column

By Robert Freeman, Environmental Innovations Chemist

- Inert, ultra- low bleed column improves low level analyses.
- Excellent peak shapes and responses for active analytes.
- Drilled Uniliner® inlet liner minimizes sample breakdown in the injector.

Semivolatile organic chemical contaminants in drinking water are target compounds in many analytical methods, worldwide. US EPA Method 525.2, for example, is a general purpose solid-phase extraction/GC/MS procedure for identifying and quantifying a wide range of semivolatile compounds. Analytes, and introduced internal standards and surrogates, are extracted from a 1-liter water sample by passing the sample through a solid phase extraction disk containing a bonded C18 phase (e.g., Resprep™-C18, cat. #24004). Target compounds are trapped on the disk, then eluted in a small amount of solvent. The extract is concentrated by evaporating the solvent, and the sample components are separated, identified, and quantified by GC/MS.

As is true for many other semivolatiles methods, the extensive target compound list for Method 525.2 encompasses numerous classes of analytes. These diverse compounds present varying difficulties in the analysis, including differing modes of degradation. Coupled with the continual need for lower levels of detection, these challenges make extreme demands on the chromatography column, and the analysis requires an inert, thermally stable, low-bleed stationary phase. To meet these needs, we recommend a 30 meter, 0.25mm ID, 0.25µm Rxi™-5ms column. Enhanced surface deactivation provides Rxi™-5ms columns with exceptional inertness and ultra-low bleed, ensuring resolution and symmetric peaks for these difficult analytes.

Figure 1 shows the total ion chromatogram for 88 semivolatiles commonly analyzed in drinking water, and listed in US EPA Method 525.2, at 10ng each on an Rxi™-5ms column. Resolution and peak shapes are exceptionally good.

To minimize analyte degradation in the injection port, and discrimination among analytes by molecular weight, we recommend installing a Drilled Uniliner® inlet liner in the injection port. This liner forms a Press-Tight® seal with the inlet end of the column, eliminating contact between the sample and the hot metal surfaces in the injection port and assuring near-complete sample transfer. The small hole in the wall of the liner allows the liner to be used with split/splitless injections. As an additional precaution to minimize analyte breakdown, we use a pulsed splitless injection (50psi / 0.3 min.; 1µL sample) to reduce the time the analytes spend in the injection port.

Exceptional inertness and ultra-low bleed enable an Rxi™-5ms column to perform exceptionally well in analyses of complex mixtures of semivolatile compounds. We recommend pairing an Rxi™-5ms column with our recently revised analytical reference mixes for semivolatile pollutants in water, listed on page 16 of this *Advantage*. Restek can provide all the materials needed for a semivolatiles analysis: extraction disks, analytical reference materials, and a column capable of excellent responses for all target analytes at low on-column concentrations.

Rxi™-5ms Columns (fused silica)

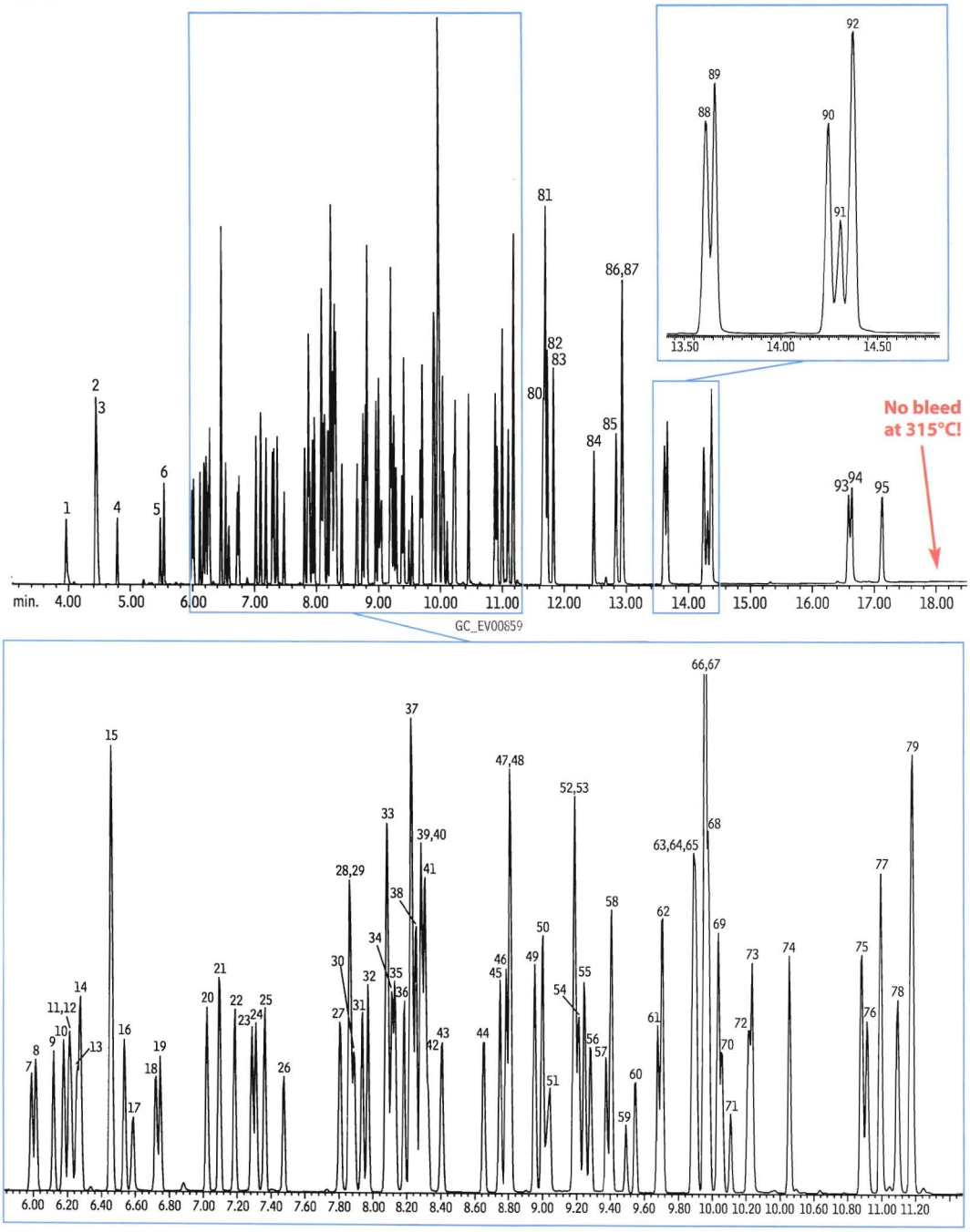
(Crossbond® 5% diphenyl / 95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	13423	$435

for more info

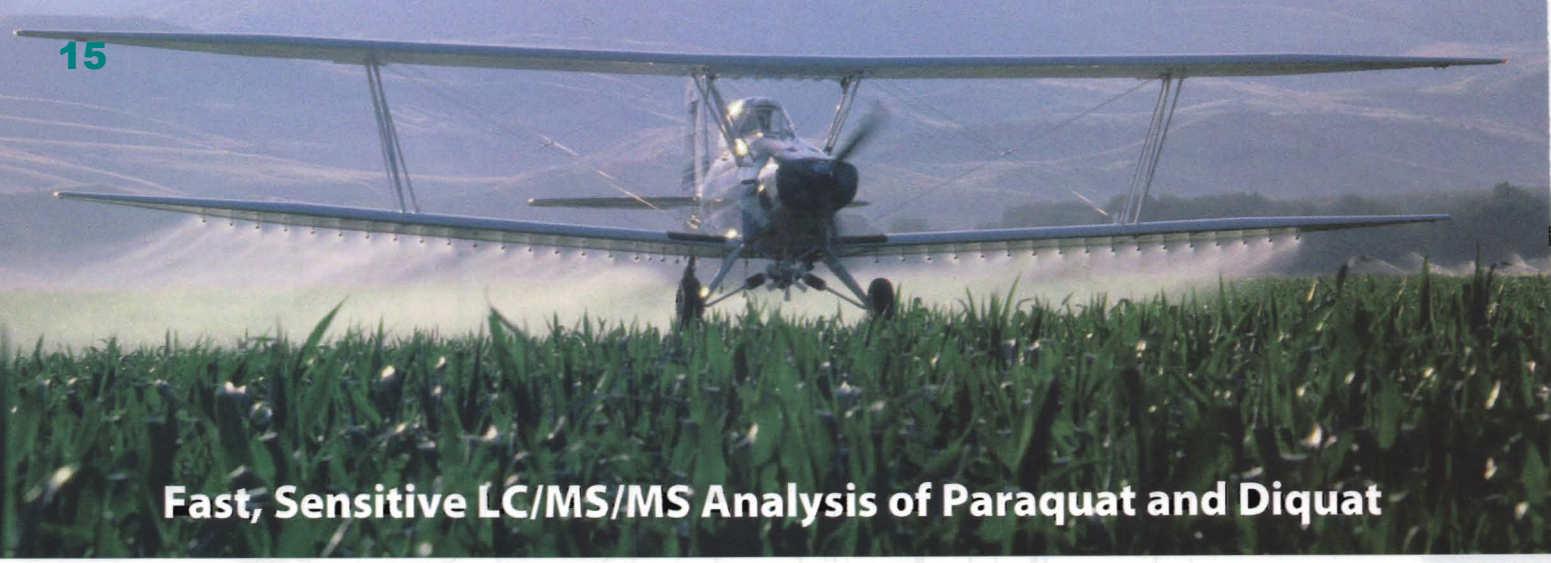
For more information about Drilled Uniliner® inlet liners see page 11 and request **lit. cat.# 59877**, or visit our website: www.restek.com

Figure 1 Excellent resolution and symmetric peaks for commonly analyzed drinking water semivolatiles, at 10ng each on an Rxi™-5ms column.



1. isophorone
2. 2-nitro-*m*-xylene (SS)
3. naphthalene
4. dichlorvos (DDVP)
5. hexachlorocyclopentadiene
6. EPTC
7. mevinphos
8. butylate
9. vernolate
10. dimethyl phthalate
11. pebulate
12. etridiazole (Terrazole®)
13. 2,6-dinitrotoluene
14. acenaphthylene
15. acenaphthene-d10 (IS)
16. chlorneb
17. tebuthiuron
18. 2,4-dinitrotoluene
19. molinate
20. diethyl phthalate
21. fluorene
22. propachlor
23. ethoprop (ethoprophos)
24. cycloate
25. chlorpropham
26. trifluralin
27. atraton
28. hexachlorobenzene
29. prometon
30. simazine
31. atrazine
32. propazine
33. pentachlorophenol
34. terbufos
35. pronamide (propyzamide)
36. diazinon
37. phenanthrene-d10 (IS)
38. phenanthrene
39. disulfoton
40. methyl paraoxon
41. anthracene
42. terbacil
43. chlorothalonil
44. metribuzin
45. simetryn
46. ametryn
47. alachlor
48. prometryn
49. terbutryn
50. di-*n*-butyl phthalate
51. bromacil
52. cyanazine (Bladex)
53. metalochlor
54. chlorpyrifos (Dursban®)
55. triademefon
56. Dacthal® (DCPA)
57. MGK-264 (isomer A)
58. diphenamid
59. MGK-264 (isomer B)
60. merphos
61. heptachlor epoxide
62. fluoranthene
63. stirofos
64. disulfoton sulfone
65. butachlor
66. pyrene-d10 (SS)
67. fenamiphos
68. pyrene
69. napropamide (Devrinol®)
70. *trans*-nonachlor
71. merphos oxide
72. tricyclazole (Beam)
73. carboxin
74. chlorobenzilate
75. butyl benzyl phthalate
76. norflurazon
77. bis(2-ethylhexyl) adipate
78. hexazinone (Velpar®)
79. triphenylphosphate (SS)
80. benzo(a)anthracene
81. chrysene-d12 (IS)
82. chrysene
83. bis(2-ethylhexyl) phthalate
84. fenarimol
85. *cis*-permethrin
86. trans-permethrin
87. di-*n*-octyl phthalate
88. benzo(b)fluoranthene
89. benzo(k)fluoranthene
90. benzo(a)pyrene
91. fluridone (Sonar®)
92. perylene-d12 (SS)
93. indeno(1,2,3-cd)pyrene
94. dibenzo(a,h)anthracene
95. benzo(ghi)perylene

Column:	Rxi™-5ms, 30m, 0.25mm ID, 0.25µm (cat.# 13423)
Sample:	US EPA Method 525.2 mix, 10µg/mL each analyte, 25µg/mL each internal standard and surrogate: Method 525.2 Semivolatile Mix (cat.# 31899), Organonitrogen Pesticide Mix #1 (cat.# 33012), Organonitrogen Pesticide Mix #2 (cat.# 33011), Organophosphate Pesticide Mix #1 (cat.# 33013), Nitrogen/Phosphorous Pesticide Mix #2 (cat.# 32423), Method 525.2 Internal Standard Mix (cat.# 31825), Method 525.2 Surrogate Standard Mix (cat.# 31826)
Instrument:	Agilent 6890
Inj.:	1.0µL, pulsed splitless injection: 50psi (0.3 min.), 80mL/min. (0.15 min.), gas saver 15mL/min. (1 min.), 4mm Drilled Uniliner® inlet liner, hole near bottom (cat#20771)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1.2mL/min.
Oven temp.:	90°C (1 min.) to 270°C @ 20°C/min., to 315°C @ 6°C/min.
Det.:	Agilent 5973 MSD
Interface line temp.:	280°C
Scan range:	35-550 amu
Solvent delay:	3.00 min.
Tune:	DFTPP
Ionization:	EI



Fast, Sensitive LC/MS/MS Analysis of Paraquat and Diquat

Using an API 3200™ Mass Spectrometer and an Ultra Quat HPLC Column

Houssain El Aribi, Ph.D., LC/MS Product and Application Specialist, MDS SCIEX*, Becky Wittrig, Ph.D., HPLC Product Manager, C. Vernon Bartlett, HPLC R&D Scientist, and Julie Kowalski, Innovations Team Chemist, Restek Corporation

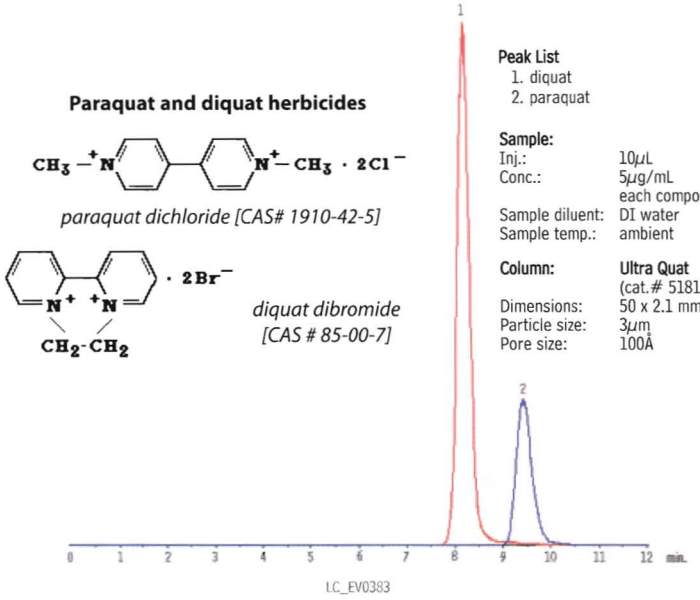
- Complete resolution of paraquat & diquat – with a simple, isocratic mobile phase!
- Superior sensitivity—5ppb paraquat or 0.1ppb diquat—without preconcentration.
- Significantly faster than conventional methodologies.

Restek chemists designed the Ultra Quat HPLC column specifically for analyses of quaternary amine compounds. This unique column makes possible a simple HPLC/UV analysis for paraquat and diquat[1] – a significant improvement over alternative methodologies. Now, in collaboration with scientists at MDS Sciex, we have developed a fast, highly sensitive LC/MS method for analyzing these challenging target compounds.

Charged quaternary amines, such as paraquat and diquat, exhibit little or no retention on C18 or other alkyl stationary phases. In our HPLC/UV procedure, our Ultra Quat mobile phase modifier (Ultra Quat Reagent Solution, cat.# 32441) increases the interactions between paraquat and diquat and the Ultra Quat stationary phase, providing the necessary retention and resolution. For compatibility with MS detection, however, we needed a volatile mobile phase additive. Low concentrations of heptafluorobutyric acid (HFBA) effectively shield the positive charges of paraquat and diquat, increasing interactions between the quaternary amines and the Ultra Quat stationary phase.

Figure 1 shows the excellent separation of paraquat and diquat, at a concentration of 5µg/mL each in water, achieved by using an API 3200™ mass spectrometer. We used multiple reaction monitoring (MRM) – a standard technique for quantitative LC/MS/MS – for this application. In MRM, pairs of target precursor ions and unique fragment ions are used for quick and accurate identification of target species. Collision induced

Figure 1 Fast, sensitive LC/MS/MS analysis of paraquat and diquat, using an API 3200™ mass spectrometer and an Ultra Quat HPLC column.



Conditions:

Mobile phase:	10mM heptafluorobutyric acid:acetonitrile, 95:5 (v/v)
Flow:	0.3mL/min.
Temp.:	ambient
Det.:	Applied Biosystems API 3200™
Interface:	electrospray
Ion Mode:	positive
Temp.:	600°C
Ion Spray™ voltage:	5500V
Collision exit potential:	3V
Curtain Gas™:	15psi
Gas supply 1:	70psi
Gas supply 2:	60psi
Quantitation:	(MRM)
Q1/Q3:	unit resolution
Dwell time:	200 ms

Precursor Ion (amu)	Fragment Ion (amu)	DP (V)	Collision Energy (eV)
diquat, 183+	157+	30	30
paraquat, 93 (2+)	171+	20	20

*Data courtesy of Houssain El Aribi, Ph.D., LC/MS Product and Application Specialist, MDS SCIEX, 71 Four Valley Drive, Concord, Ontario, Canada, L4K 4V8

Table 1 MRM transitions and MS conditions used to generate CID spectra for paraquat and diquat.

Precursor Ion (m/z)	Fragment Ions (m/z)	DP (V)	Collision Energy (eV)
Paraquat [M^{2+} - H^+] 185	170a 169b	40	30
Paraquat-d8 [M^{2+} - D^+] 193 (int. std.)	178a	40	30
Diquat [M^{2+} - H^+] 183	157a 168b	35	30
Diquat-d4 [M^{2+} - D^+] 186 (int. std.)	158a	35	30

Figure 2 CID spectra for paraquat$^+$ at CE = 25eV.

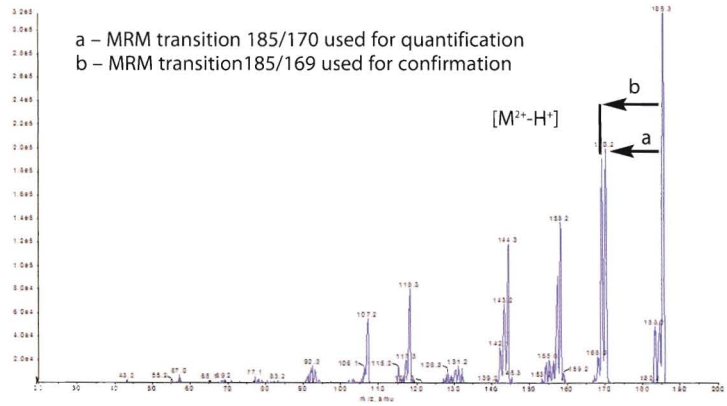
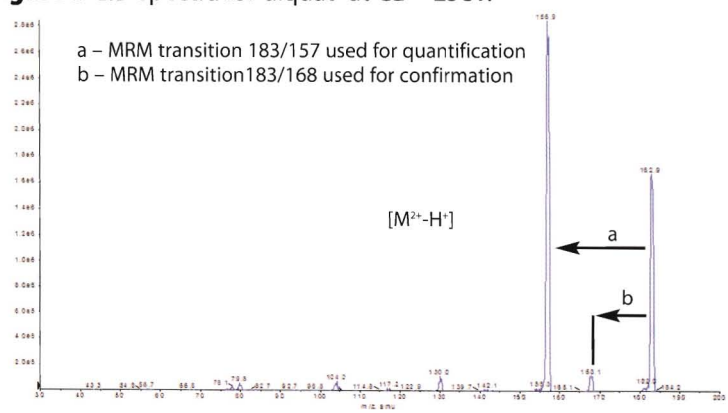


Figure 3 CID spectra for diquat$^+$ at CE = 25eV.



free literature

Simple, Sensitive HPLC/UV Analysis for Paraquat and Diquat Using High-Recovery Solid Phase Extraction and an Ultra Quat HPLC Column

These highly charged quaternary amines are poorly retained on alkyl stationary phases. Using only acetonitrile, water, and a solvation-blocking reagent, our separation system alters the interactions among analyte, mobile phase, and stationary phase, and promotes solubility of the analytes in the stationary phase. In our system, the detection limit is 6ppb for either herbicide, and the analysis is completed in less than 10 minutes. An optimized solid phase extraction cartridge concentrates the herbicides for the analysis. **lit. cat.# 580006**

Environmental HPLC: Applications-Columns-Reference Materials

Restek HPLC columns support environmental HPLC applications with rapid analysis times and effective analyte resolution. Sample turn-around can be 50% faster, or more, than with alternative columns. In addition, we prepare analytical reference materials and sample clean-up products for these methods. Applications in this publication include polyaromatic hydrocarbons, carbamates, phenoxyacid herbicides, explosives, carbonyls, and paraquat/diquat. **lit. cat.# 59741A**

dissociation (CID) is used to generate the fragment ions. CID spectra for paraquat and diquat are shown in Figures 2 and 3. This approach has been used in many pharmaceutical and environmental applications, to generate unmatched limits of detection or quantification, precision, and accuracy. For accurate quantification, we used paraquat-d8 and diquat-d4 as internal standards (Table 1), to compensate for matrix effects and to correct for random and systematic errors in separation and detection.

For triplicate injections of 8 concentrations of analytes in deionized water and in lake water, from 5µg/100mL to 100µg/100mL for paraquat and from 0.1µg/100mL to 100µg/100mL for diquat, correlation coefficients for calibration curves were >0.995, using a linear fit and 1/x weighting factor. These results indicate that quantification can be performed with good linearity and sensitivity. Minimum detection limits (MDL) for the method, for paraquat and diquat in deionized water, were 5µg/L and 0.1µg/L, respectively.

LC/MS is a powerful tool for analyses of challenging environmental contaminants. In LC/MS analyses of paraquat and diquat, the combination of an Applied Biosystems API 3200™ mass spectrometer and an Ultra Quat HPLC column ensures fast, sensitive, and accurate results.

Reference

1. *Simple, Sensitive HPLC/UV Analysis for Paraquat and Diquat, Using High-Recovery Solid Phase Extraction and an Ultra Quat HPLC Column* Applications Note 580006, Restek Corporation, Feb. 2006.
Reference available from Restek on request.

Ultra Quat Columns & Guard Cartridges

5µm Column, 4.6mm	cat. #	price
150mm	9181565	$399
150mm (with Trident™ Inlet Fitting)	9181565-700	$414
Ultra Quat Guard Cartridges		
10 x 2.1mm	918150212	$131
10 x 4.0mm	918150210	$131
20 x 2.1mm	918150222	$131
20 x 4.0mm	918150220	$131

Paraquat & Diquat Calibration Mix

diquat dibromide paraquat dichloride
1,000µg/mL each in water, 1mL/ampul
cat. # 32437 (ea.) $26

free literature

HPLC Essentials

Genuine Restek Replacement Parts will keep your Agilent, Beckman, Hitachi, PerkinElmer, Shimadzu, Thermo Separation Products, or Waters system running smoothly and chromatography sharp. Restek parts equal or exceed the performance of original components. **lit. cat.# 59012A**

Assaying Local Anesthetics by GC/FID

Optimizing System Suitability, Using an Rxi™-5ms Column

By Rick Lake, Pharmaceutical Innovations Chemist

- Rxi™-5ms column assures excellent peak shapes for basic compounds.
- Stable, reproducible retention times.
- Easy conformance to stringent system suitability criteria.

Local anesthetics are biologically active compounds that reversibly inhibit the propagation, or broadcasting, of signals along nerve cell pathways. Because of this action, they are widely used as drug compounds to produce temporary analgesia (loss of pain) and paralysis (loss of muscle movement). Anesthetic compounds are formulated into a large number and wide variety of drug products, ranging from over-the-counter topical ointments to clinical injectables, and they often are formulated in combination with other active ingredients. Therefore, many analyses of local anesthetics involve manufacturing assays, like potency and stability assays, which require high-throughput and reproducible results. These assays require the fulfillment of system suitability criteria and, for this reason, we investigated assaying local anesthetics by GC/FID, using common system suitability parameters as evaluation criteria.

By GC standards, a local anesthetic is a high molecular weight, weakly basic, active compound. We took these characteristics into account when we chose the column and inlet liner for this application. Considering that these analytes are basic and active, the deactivation of the inlet liner and capillary column is very important. For superior inertness, we chose to use an Rxi™-5ms column.

When analyzing high molecular weight compounds – the normal case in pharmaceutical assays – discrimination and irreproducible injections sometimes occur, primarily due to incomplete vaporization of the analytes. This can be especially problematic for analysts who must meet stringent system suitability criteria. Some liners, like the laminar cup and cup splitter, were designed specifically for samples containing high molecular weight compounds. These liner designs aid in sample vaporization, but at a cost of reduced internal volumes and intricate flow paths that can cause poor reproducibility when such liners are used with a solvent that has a large expansion volume, like methanol. In this application, we used our conventional, intermediate polarity deactivated, split liners packed with intermediate polarity deactivated wool. Wool in the liner provides a large surface area, for rapid vaporization, but the liner still delivers a uniform vapor cloud to the split point.

Under these conditions, chromatography from a six-replicate system suitability analysis (Figure 1) was well within normal acceptance criteria (Table 1). USP tailing, approximately 1.00 for all analytes, shows the exceptional inertness of the Rxi™-5ms column. In addition, retention times and area responses were extremely stable. The Rxi™-5ms column, coupled with an appropriate inlet liner, provides the stability and deactivation necessary to afford easier conformance to system suitability criteria. The 10-minute analysis time for these compounds ensures high sample throughput.

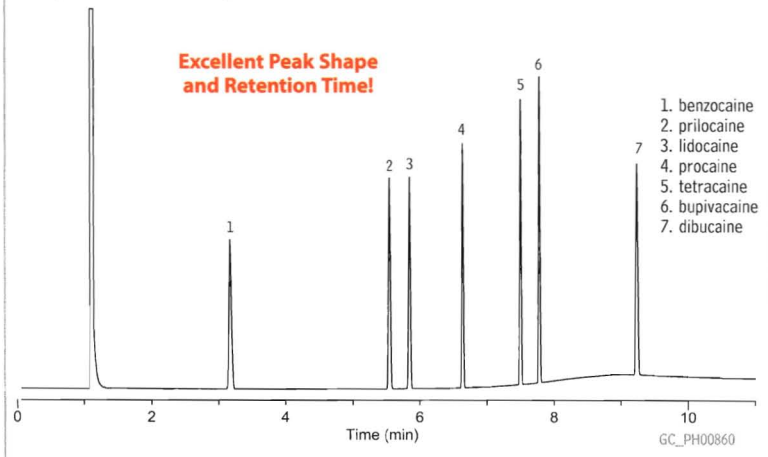
Rxi™-5ms Column (fused silica)

(Crossbond® 5% diphenyl / 95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.53mm	1.00	-60 to 330/350°C	30-Meter	13455	$515

For other dimensions, see page 5.

Figure 1 An Rxi™-5ms column provides excellent peak shape and stable retention times for basic compounds, for easier conformance to system suitability criteria.



Column:	Rxi™-5ms 30m, 0.53mm ID, 1.00µm (cat.# 13455)
Sample:	50µg/mL each component in methanol
Inj.:	1.0µL split (10:1), 4mm split inlet liner with wool (cat # 20781)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	5.0mL/min.
Oven temp.:	200°C (4 min.) to 320°C @ 30°C/min. (hold 3 min.)
Det.:	FID @ 300°C

Table 1 An Rxi™-5ms column provides exceptionally stable retention times and area responses.

Compound	Peak Area (%RSD)	Retention Time (%RSD)	USP Tailing	Efficiency
benzocaine	0.85	0.03	1.00	55858
prilocaine	1.36	0.02	1.00	(isothermal)
lidocaine	1.01	0.02	1.00	
procaine	1.83	0.03	1.00	
tetracaine	1.78	0.01	1.00	
bupivacaine	1.64	0.02	1.02	
dibucaine	1.17	0.06	1.00	
Mean	1.38	0.03	1.00	

six-replicate system suitability analysis

Optimized RP-HPLC Method for Hydroxybenzoic Acids

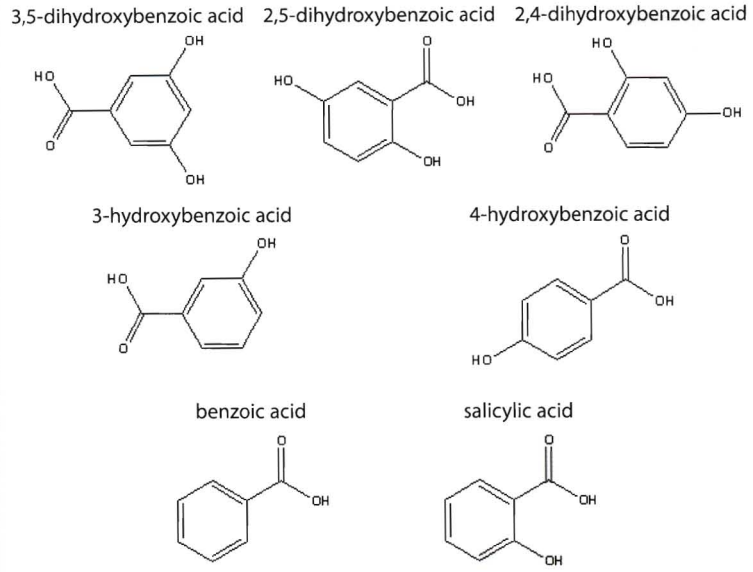
Balanced Retention for a Range of Polarities, Using an Ultra Aqueous C18 Column

By Rick Lake, Pharmaceutical Innovations Chemist

- Useful retention of more polar and less polar analytes.
- Ultra Aqueous C18 column is compatible with 100% aqueous mobile phases.
- Ideal for samples that encompass a broad range of analyte polarity.

Hydroxybenzoic acids are important pharmacological compounds. They serve as active drug substances (aspirin, for example), as well as preservatives in drug products. In some cases, they represent impurities in drug products. Their analysis sometimes can be difficult, not only because they represent a wide range of applications, but primarily because they encompass a wide range of polarity. Chemically, benzoic acid, the basic structure for these analytes, consists of a benzene ring with a carboxyl group (Figure 1). Hydroxybenzoic acids share the same basic structure, but contain additional hydroxyl groups on the benzene ring (Figure 1). The additional hydroxyl groups' varied positions and numbers create differences among the analytes' overall polarity and solubility. Because these compounds represent such varying chemistry and polarity, finding an alkyl (C18) HPLC column that can effectively assay them all could be very difficult, but such a column could be of value for resolving these compounds from active drugs or from chemically similar impurities.

Figure 1 Hydroxybenzoic acids share the same basic structure, but have varying polarity.

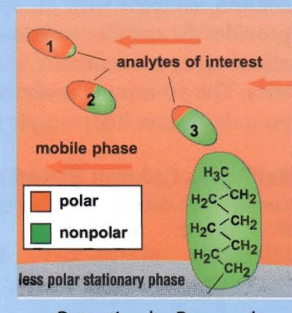


Using identical conditions, we analyzed a group of hydroxybenzoic acids on a conventional C18 stationary phase column, on a C18 column with a polar group within (intrinsic to) the alkyl bonded phase (an IBD phase*), and on an Ultra Aqueous C18 column. Our objective was to find the optimum stationary phase for resolving analytes with a varying number of polar functional groups.

Overall, the Ultra Aqueous C18 column provided the best balance of retention for more polar and less polar analytes (Figure 2A), completely resolving our test mix when used with a simple gradient mobile phase. The conventional C18 column exhibited retention very similar to that of the Ultra Aqueous C18 column for the less polar analytes, benzoic acid and salicylic acid, but it showed less retention and resolution for the more polar compounds (Figure 2B). The intrinsically base deactivated column, on the other hand, exhibited opposite characteristics – retention similar to the Ultra Aqueous C18 column for the more polar compounds, but little retention of the less polar compounds (Figure 2C).

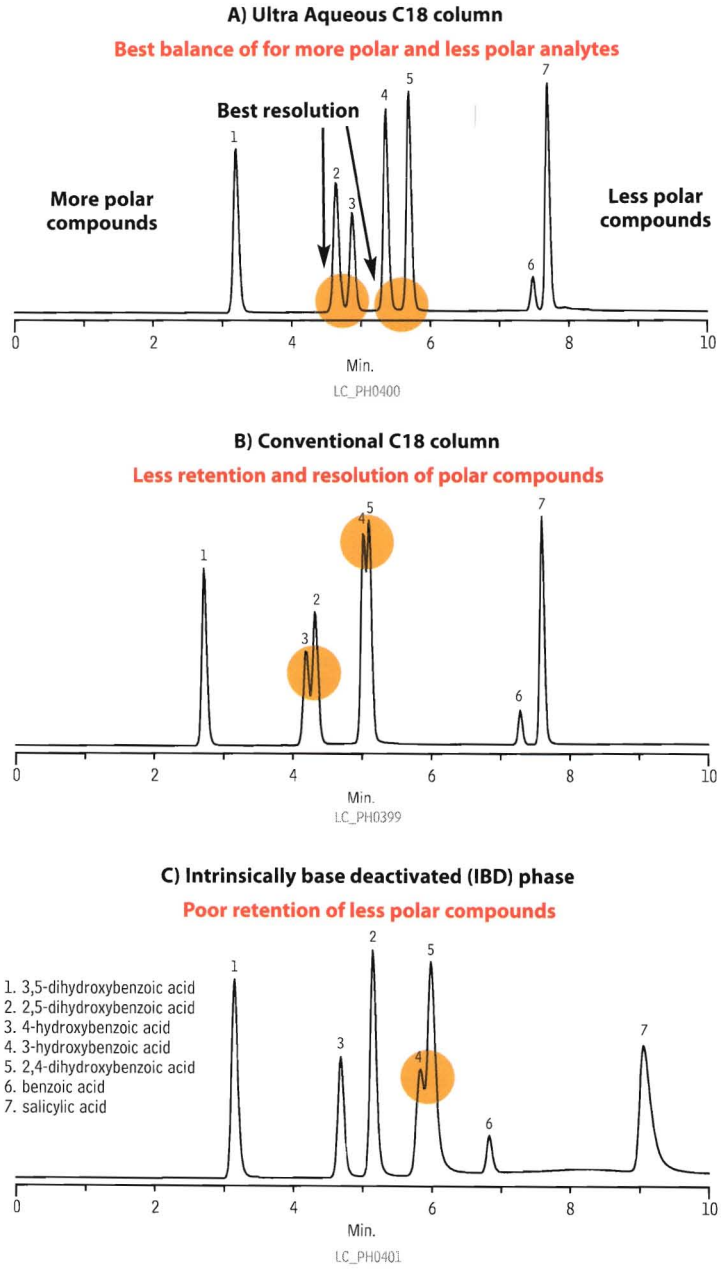
Options for Analyzing Polar Compounds

Many types of alkyl phases currently are available to the analyst, making column selection difficult. Although all alkyl phases possess the same basic structure – a specific length of alkyl chain bonded to a silica surface (typically C1-C30, with C18 being the most common) – various attached polar groups create selectivity and retention differences among columns. For example, a conventional C18 phase is comprised of a monomerically bonded straight 18 carbon alkyl chain, meaning every alkyl chain has a single, direct attachment to the silica surface. These phases are excellent for retaining nonpolar compounds, but they show very limited retention for polar compounds. One common bonding technique for increasing retention of polar compounds on an alkyl phase is to attach a polar group within, or intrinsic to, the alkyl phase. These phases, known as intrinsically base deactivated (IBD) phases, show increased retention for polar compounds because the embedded polar groups are capable of interaction with polar portions of analyte molecules. (These phases also have a deactivating effect on basic compounds, by creating an electrostatic barrier.) Polarity also can be added to an alkyl phase by adding polar end caps to active sites on the silica surface, or by adding polar side chains to the alkyl attachment. Interactions with polar compounds also can be increased through the use of a polymeric bonding chemistry.



Retention by Reversed

Figure 2 An Ultra Aqueous C18 column shows suitable retention for polar or nonpolar compounds, providing enhanced selectivity for a broad range of polarity.



Sample:
Inj.: 5µL
Conc.: 50µg/mL each component
Sample diluent: mobile phase

Column: Ultra Aqueous C18 (cat.# 9178568)
Dimensions: 150 x 4.6 mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: A: 20mM potassium phosphate (pH 2.5); B: acetonitrile

Time (min.)	%B
0.00	20
5.00	50
10.00	50

Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 210nm

It is well documented that Ultra Aqueous C18 columns are compatible with 100% aqueous mobile phases, because the stationary phase has sufficient polar character to prevent dewetting or hydrophobic collapse.[1] Our current analyses reveal yet another advantage to the slight polar character of this column: by providing the best resolution of analytes exhibiting a wide range of polarity, the Ultra Aqueous C18 column demonstrates that it also can be used to retain, and separate, more polar or less polar compounds – or mixtures of both.

Reference

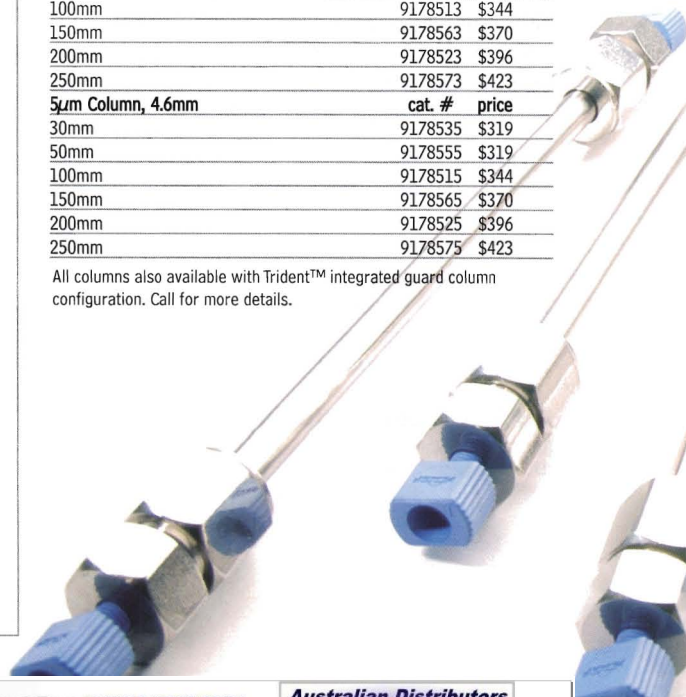
1 *Ultra Aqueous C18 HPLC Columns: Achieve Stable Retention in 100% Aqueous Mobile Phase* Restek Corporation, 2002 (lit. cat.# 59371). Reference available on request.

*The intrinsically base deactivated (IBD) phase shows increased retention for polar compounds, because the embedded polar groups are capable of interaction with polar portions of analyte molecules.

Ultra Aqueous C18 Columns

3µm Column, 2.1mm	cat. #	price
30mm	9178332	$344
50mm	9178352	$344
100mm	9178312	$370
3µm Column, 3.2mm	**cat. #**	**price**
30mm	9178333	$344
50mm	9178353	$344
100mm	9178313	$370
3µm Column, 4.6mm	**cat. #**	**price**
30mm	9178335	$344
50mm	9178355	$344
100mm	9178315	$370
5µm Column, 2.1mm	**cat. #**	**price**
30mm	9178532	$319
50mm	9178552	$319
100mm	9178512	$344
150mm	9178562	$370
200mm	9178522	$396
250mm	9178572	$423
5µm Column, 3.2mm	**cat. #**	**price**
30mm	9178533	$319
50mm	9178553	$319
100mm	9178513	$344
150mm	9178563	$370
200mm	9178523	$396
250mm	9178573	$423
5µm Column, 4.6mm	**cat. #**	**price**
30mm	9178535	$319
50mm	9178555	$319
100mm	9178515	$344
150mm	9178565	$370
200mm	9178525	$396
250mm	9178575	$423

All columns also available with Trident™ integrated guard column configuration. Call for more details.



GC Analysis of Total Reduced Sulfurs at ppbv Levels

Using an Rxi™-1ms Column and Sulfur Chemiluminescence Detection

by Silvia Martinez, Innovations Chemist

- Reliable results for ppbv concentrations of highly active sulfur compounds.
- Inert, low bleed column resolves all analytes.
- Column compatible with SCD and other sulfur-specific detectors.

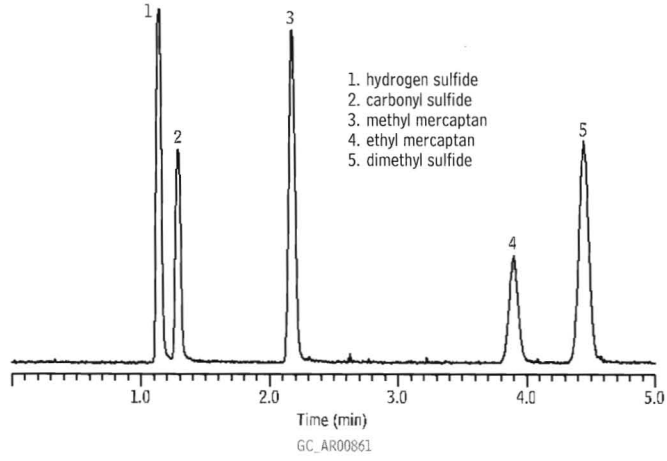
Through the Clean Air Act, the United States Environmental Protection Agency (US EPA) regulates and limits the emission of toxic air pollutants. The determination of total reduced sulfurs, as required by CFR Title 40, requires the use of methods and equipment capable of providing full resolution as well as high sensitivity. Methods TO-15, TO-16 and TO-16A describe GC procedures that apply to the determination of reduced sulfurs from stationary sources, such as recovery furnaces, lime kilns, smelt dissolving tanks, fuel gas combustion devices, tail gas control units, and others. Method TO-16 specifies detectable concentrations of ppbv levels for dimethyl disulfide, dimethyl sulfide, hydrogen sulfide, and methyl mercaptan. While these methods do not specify the analytical GC column to use, they do state that the column must resolve the sulfur compounds.

Our new 100% dimethylpolysiloxane column, the Rxi™-1ms column, provides the ultra-low bleed required for low level detection and quantification of sulfur compounds. Its exceptional inertness allows complete separation of these very reactive compounds, with excellent peak shape, at ppbv levels. When this column is coupled with a sulfur chemiluminescence detector (SCD), the analysis is fast and simple.

For our example analysis, we collected a 20mL sample of a gaseous mixture of hydrogen sulfide, carbonyl sulfide, methyl mercaptan, ethyl mercaptan, and dimethyl sulfide in helium, using a Sulfinert®-treated stainless steel sample loop. We transferred the sample to a SilcoCan™ air monitoring canister and pressurized the can to 30psig with dry nitrogen. The Sulfinert® passivation treatment on both the sample loop and canister prevented adsorption losses of the highly active sulfur compounds. We introduced a 1mL aliquot of the diluted gaseous mixture into the Rxi™-1ms column via a second Sulfinert®-treated stainless-steel sample loop, using helium as a carrier, and analyzed the sample isothermally at 30°C.

Figure 1 shows the chromatography for the reduced sulfur compounds, demonstrating full resolution in less than 5 minutes. For collecting, storing, and analyzing active sulfur compounds at levels as low as single parts per billion, the performances of Sulfinert® passivated containers and transfer systems, and inert Rxi™-1ms columns, simply can't be equaled.

Figure 1 Total resolution of reduced sulfur compounds, in less than 5 minutes, using an Rxi™-1ms column.



Column:	Rxi™-1ms, 30m, 0.32mm ID, 4.00µm (cat.# 13396)
Sample:	hydrogen sulfide, carbonyl sulfide, methyl mercaptan, ethyl mercaptan, dimethyl sulfide, 100 ppbv each in helium
Inj.:	1.mL splitless, direct
Sample loop temp.:	30°C
Carrier gas:	helium, constant pressure
Linear velocity:	48cm/sec. @ 30°C
Oven temp.:	30°C
Det.:	sulfur chemiluminescence detector
Det. temp.:	800°C

Sample storage & transfer:
SilcoCan™ air monitoring canister with Siltek® treated 1/4" valve (cat.# 24182-650); Sulfinert® treated gas sample loop, 1cc (cat.# 22848); Sulfinert® treated gas sample loop, 10cc (custom order)

Rxi™-1ms Columns (fused silica)

(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.32mm	4.00	-60 to 330/350°C	30-Meter	13396	$460

for more info

SilcoCan™ Canisters

The best alternative for ambient air monitoring

Recovery data for low ppb levels of active sulfur-containing compounds show why SilcoCan™ canisters are the best choice for monitoring TO-14, TO-15, or reactive sulfur compounds. lit. cat.# 59011A

Sulfinert®-Treated Sample Cylinders

Store Active Sulfur Compounds at ppb Levels

by Neil Mosesman, Air Sampling Products Manager

- Stable storage of sulfur compounds at ppb levels.
- D.O.T. rated to 1800psi at room temperature.
- High quality cylinders manufactured by Swagelok®.

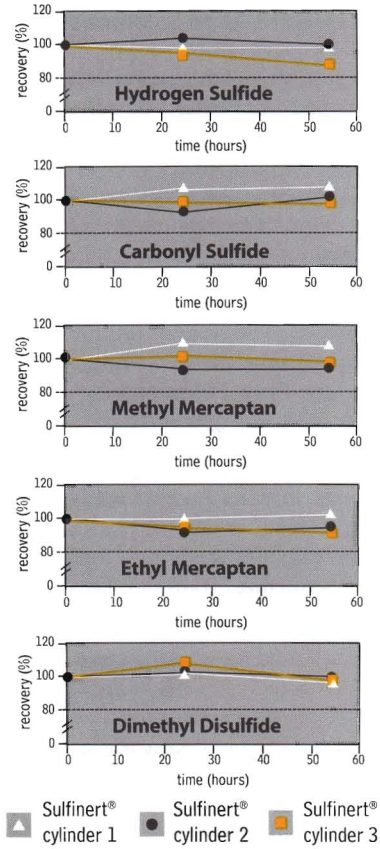
Refinery and natural gas samples often contain trace amounts of sulfur-containing compounds which can interfere with reactions or poison catalysts in petrochemical processes. Because sulfur compounds quickly react with stainless steel surfaces, accurate determination of these compounds is impossible when samples are collected and stored in untreated sample cylinders. Restek's Sulfinert® passivation technique bonds an inert silica layer into the surface of stainless steel, preventing active compounds from reacting with or adsorbing to the steel.

To characterize Sulfinert® surfaces, we tested the stability of 17ppbv standards of sulfur compounds in three Sulfinert® sample cylinders over a 54-hour period. Dimethyl sulfide, which is not adsorbed by stainless steel, was used as an internal standard.

The Sulfinert®-treated cylinders were inert to the reactive sulfur compounds over the 54-hour test period (Figure 1). Hydrogen sulfide exhibited greater than 85% recovery; methyl mercaptan, ethyl mercaptan, carbonyl sulfide, and dimethyl disulfide exhibited greater than 90% recovery.

Sulfinert®-treated gas sampling equipment is ideal for collecting and storing samples containing ppb levels of sulfur compounds, such as natural gas or beverage-grade carbon dioxide. Sulfinert® treatment ensures that sulfur compounds or other highly active compounds remain stable during transport from the field to the laboratory.

Figure 1 Stability of sulfur compounds is remarkable in Sulfinert®-treated cylinders.



Sulfinert® Treated Swagelok® Sample Cylinders

These cylinders are made from 304 grade stainless steel with 1/4" female NPT threads on both ends.

Size	qty.	cat.#	price
75cc	ea.	24130	$202
150cc	ea.	24131	$228
300cc	ea.	24132	$233
500cc	ea.	24133	$258
1000cc	ea.	24134	$430
2250cc	ea.	21394	$829

Sulfinert® Treated Alta-Robbins Sample Cylinder Valves

- All wetted parts Sulfinert® treated for inertness.
- Compatible with Sulfinert® treated Swagelok® sample cylinders.
- Large, durable, Kel-F® seat ensures leak-free operation.

Description	qty.	cat.#	price
1/4" NPT Exit	ea.	21400	$177
1/4" Compression Exit	ea.	21401	$177
1/4" NPT with Dip Tube*	ea.	21402	$253
1/4" NPT with 2850psi Rupture Disk	ea.	21403	$354
1/4" NPT Male Inlet x 1/4" Female Outlet with 2850psi Rupture Disk	ea.	21404	$354

*Specify dip tube length or % outage when ordering (maximum length = 5.25"/ 13.3cm)

restek innovation!

Sulfinert® treated sampling apparatus.

Sample Cylinder (cat. # 24133)

Cylinder Valve (cat. # 21400)

Rupture Disc Tee (cat. # 21396)

For Sulfinert® treated fittings, tubing, and sample loops, refer to our catalog or visit our website.

for more info

For information about Restek surface coatings, please visit **www.restekcoatings.com**

How Good is Your PONA Column?

Data-Based Decisions Help Simplify the Choice

By Barry L. Burger, Innovations Chemist

- When tested, most PONA columns do not meet ASTM D-6730 method specifications.
- Restek 100-meter PONA column meets all ASTM specifications.
- Restek PONA column is compatible with hydrogen carrier gas.

So, you're ready to purchase a PONA column. But, with all the options available today, which manufacturer do you purchase the column from, and what criteria do you consider in making your selection? Do you select the most expensive column, thinking higher price means quality, and therefore higher performance? Or, do you take the advice of the guy in the laboratory down the hall when he tells you it doesn't matter whose column you buy – they are all the same? That statement cannot be further from reality. Many variables affect how well a column will perform in the demanding ASTM D-6730 method: column length and ID, polymer deposition, and column deactivation, to name a few. These all vary among manufacturers, and the effects of these variations are substantiated by data.

To assist you in making a data-based decision when selecting a PONA column for use in the ASTM D-6730 method, Restek purchased designated versions of the 100 meter x 0.25mm ID x 0.5df PONA column from four vendors. We evaluated these columns, and our own Rtx™-1PONA column, using the proposed D-6730 method that calls for hydrogen carrier gas, which reduces tridecane retention time from 140 minutes to approximately 70 minutes. (For more advantages of using hydrogen as the carrier gas, see *Advantage* 2006.02, pages 18-19.)

We performed the comparisons using an Agilent 6890 GC equipped with a flame ionization detector and ChemStation data collection software. In all analyses we used hydrogen carrier gas in the constant flow mode, adjusted the dead time to 3.50 ±0.05 minutes at 35°C, and set a split ratio of 150:1. Data presented here were generated at 35°C, as specified by the ASTM method, to determine if a column is suitable for adding a tuning column and performing the PONA analysis. We used Transition Labs' (Golden, Colorado) DHA Oxy-Setup mix (Transition Labs part number 94100) for this determination. We evaluated all five columns under the same conditions, and measured each against the specifications for ASTM D-6730, as follows:

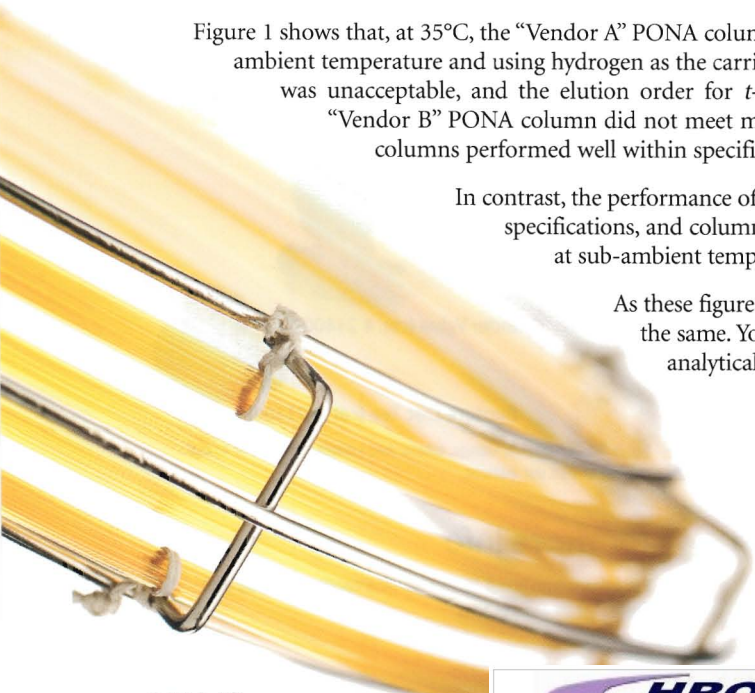
Parameter	**ASTM D-6730 Specification**
theoretical plates for C5:	450,000 - 550,000
K′ for C5:	0.45 - 0.50
peak asymmetry for *t*-butanol:	>1.00 - <5.00
resolution of *t*-butanol/2-methylbutene-2:	3.25 - 5.25

On opening the competitor PONA column containers we discovered that only one of the four manufacturers provided QA data pertinent to the ASTM 6730 method – each of the other three provided a chromatogram of a sample unrelated to the method. Further, one column did not meet the ASTM D-6730 minimum efficiency specification of 450,000 theoretical plates.

Figure 1 shows that, at 35°C, the "Vendor A" PONA column did not meet ASTM D-6730 method specifications. Further, at sub-ambient temperature and using hydrogen as the carrier gas, per ASTM D-6730 method, peak asymmetry for the oxygenates was unacceptable, and the elution order for *t*-butanol and 2-methylbutene-2 was reversed. Similarly, at 35°C, the "Vendor B" PONA column did not meet method specifications. At 35°C, the "Vendor C" and "Vendor D" PONA columns performed well within specifications, but column efficiency was less than ideal.

In contrast, the performance of the Restek PONA column at 35°C was well within ASTM 6730 method specifications, and column efficiency exceeded the specification. The column also performed well at sub-ambient temperature and using hydrogen as the carrier gas.

As these figures show, all PONA columns – or any columns, for that matter – are *NOT* the same. You the customer, have the final say about which vendor to select for your analytical column needs. If you make data based decisions, you can choose wisely.



Rtx®-1PONA Column (fused silica)

(Crossbond® 100% dimethyl polysiloxane)*

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.50	-60 to 300/340°C	100-Meter	10195	$810

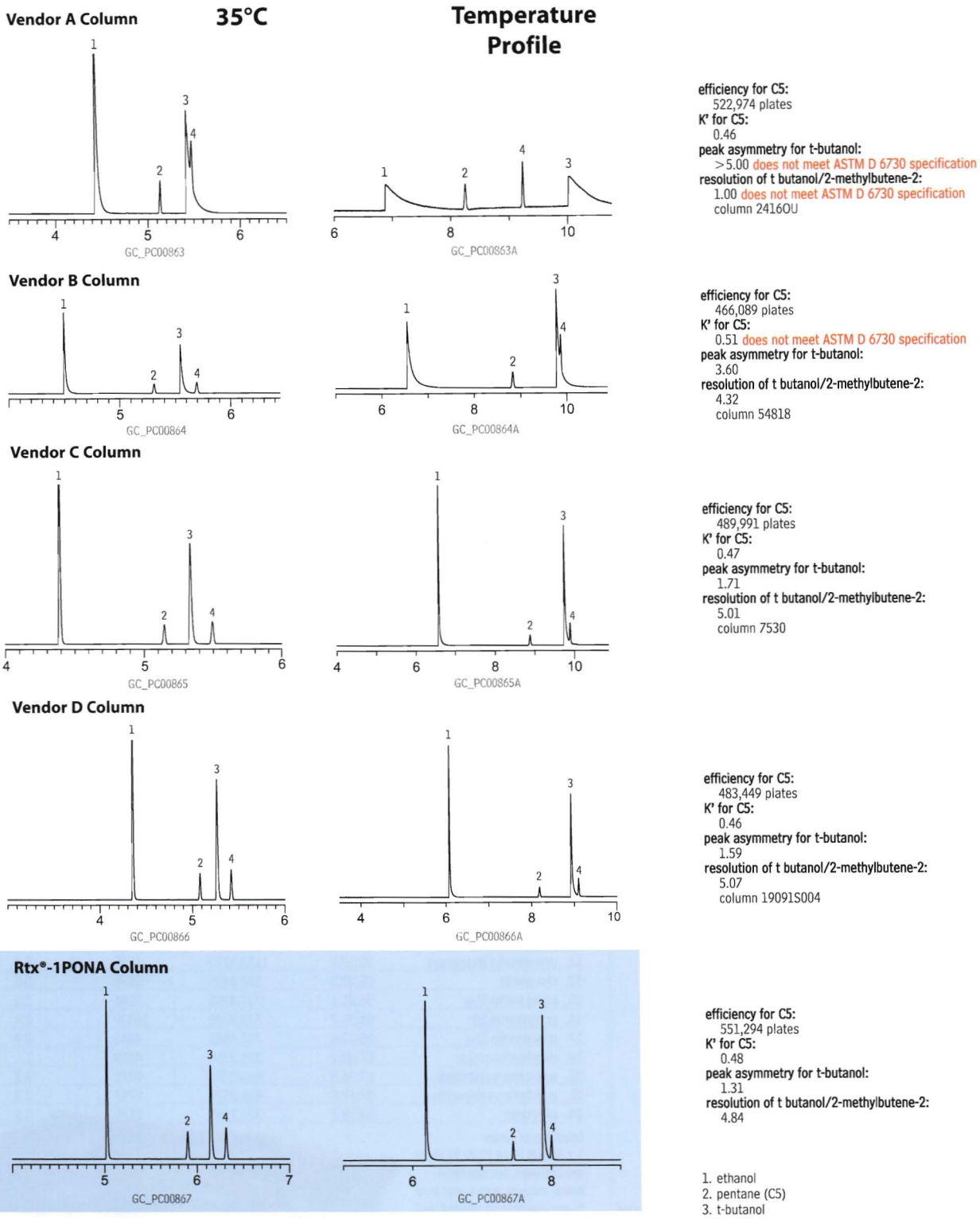
*Optimized phase for hydrocarbon analysis

Rtx®-5PONA Tuning Column (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	1.0	-60 to 325°C	5-Meter	10196	$75

Figure 1 An Rtx-1PONA column offers superior performance for ASTM D-6730 method specifications.



Column:	100m, 0.25mm ID, 0.50μm
Sample:	DHA Oxy-Setup mix (Transition Labs #94100)
Inj.:	0.01μL split (split ratio 150:1)
Inj. temp.:	275°C
Carrier gas:	hydrogen
Linear velocity:	48cm/sec.
Oven temp.:	35°C and Method D 6730 temperature profile
Det.:	FID
Det. temp.:	300°C

Temperature Profile

Column A:	5°C > 8.23 min. > 22°C/min. > 48 min.
Column B:	5°C > 8.84 min. > 22°C/min. > 48 min.
Column C:	5°C > 8.87 min. > 22°C/min. > 48 min.
Column D:	5°C > 8.19 min. > 22°C/min. > 48 min.
Rtx®-1PONA:	5°C > 8.20 min. > 22°C/min. > 48 min.

Rapid, Reproducible HPLC Analysis for Flavonoids in Cocoa

Using a LECO Unique® LC-TOFMS System and an Ultra Aqueous C18 Column

By Julie Kowalski, Restek Innovations Chemist, and Brian Shofran, LECO Corporation

- 15-minute screening for flavonoids.
- Excellent selectivity, using an Ultra Aqueous C18 column.
- Reliable identifications and reproducible results for complex samples.

Flavonoids are complex polyphenolic compounds, with diverse aromatic substitutions, that contribute to color, flavor, fragrance—and toxicity—of many foods. Interest in flavonoids has exploded because of links to antioxidant activity and, possibly, to control and prevention of disease.[1,2] Flavonoid contents of foods have been difficult to study, due to sample complexity and generally low abundances of the target compounds. Cocoa is rich in the flavan-3-ol flavonoids, including catechin, epicatechin, and procyanidin (Figure 1), and these are screened for as marker compounds. In finished chocolate and cocoa products, amounts of flavonoids depend primarily on the amounts of nonfat cocoa solids, on bean type, and on processing. Flavonoids can be destroyed by heat or other processing, like dutching, which is common in the production of cocoa and chocolate products.

We developed a rapid screening method for catechin, epicatechin, and procyanidin content, and screened commercial cocoa products for flavan-3-ol content. We prepared samples by mixing the cocoa products with liquid nitrogen, powdering the frozen mixes, and extracting samples with deionized water: methanol (1:4). Extracts were

Figure 1 Flavan-3-ol flavonoids are screened for as marker compounds.

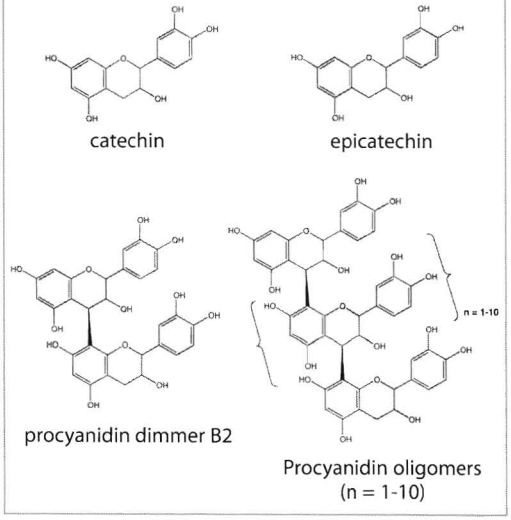
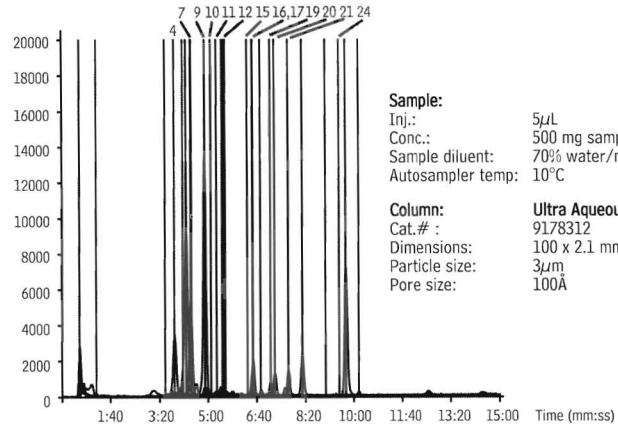


Figure 2 Extracted ion chromatogram of a cacao sample.



Sample:
Inj.: 5µL
Conc.: 500 mg sample extract
Sample diluent: 70% water/methanol
Autosampler temp: 10°C

Column: Ultra Aqueous C18
Cat.# : 9178312
Dimensions: 100 x 2.1 mm
Particle size: 3µm
Pore size: 100Å

Conditions:
Mobile phase: A: 0.1% formic acid in water; B: acetonitrile:methanol, 50:50 (v/v)

Time (min.)	%B
0	10
10	60
15	60

Flow: 400µL/min.
Temp.: 30°C
Det.: UV @ 210nm

Numbered peaks are listed in Table 1

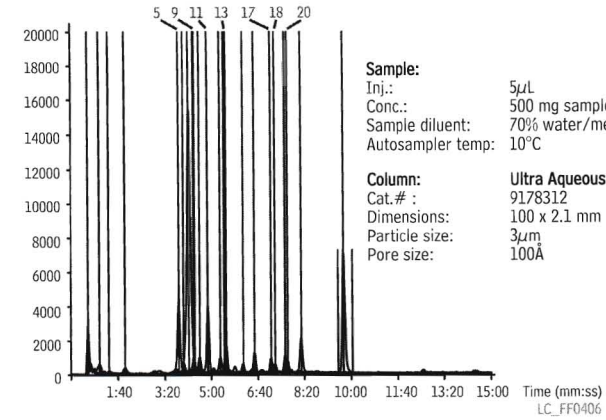
Mass Spectrometry
Instrument: Leco Unique® LC-TOFMS High Flow ESI Source
ESI voltage: (-) 3500 V
Desolv. temp.: 300°C
Nebulizer pres.: 375kPa
Desolv. gas: nitrogen, 7L/min.
Interface temp.: 100°C
Nozzle: (-) 160V
Data acq. rate: 4 spectra/sec.

Table 1 Components in the cacao sample.

Peak	RT (min:sec)	Unique Mass	Area	Area %
4. catechin (monomer)	03:50.4	289.1818	28618	6.7
7. procyanidin B2	04:24	577.3722	34559	8.0
9. epicatechin	04:53.8	289.1841	93682	21.8
10. procyanidin C1	05:06.2	865.5671	10221	2.4
11. procyanidin (tetramer)	05:17.8	1153.8179	1585	0.4
12. clovamide	05:29.3	358.2409	3528	0.8
15. procyanidin II-g	06:21.1	737.4785	5246	1.2
16. procyanidin B5	06:31.7	577.3745	10339	2.4
17. procyanidin II-a	06:32.6	707.4643	4043	0.9
19. dideoxyclovamide	07:08.2	326.2384	4839	1.1
20. quercetin-galactoside	07:16.8	463.279	9471	2.2
21. quercetin-arabinoside	07:44.6	433.2524	9797	2.3
24. quercetin	09:30.2	301.1595	2179	0.5

Identities of peaks 1,2,3,5,6,8,13,14,18,22,23,25,26 are unknown, but retention times, masses, areas, and area % are available on request, and will be listed in our next Buzz electronic newsletter.

Figure 3 The flavonoid composition of cocoa powder is readily distinguished from that of cacao, using our column and detection system.



Sample:
Inj.: 5µL
Conc.: 500 mg sample extract
Sample diluent: 70% water/methanol
Autosampler temp: 10°C

Column: Ultra Aqueous C18
Cat.# : 9178312
Dimensions: 100 x 2.1 mm
Particle size: 3µm
Pore size: 100Å

Conditions:
Mobile phase: A: 0.1% formic acid in water; B: acetonitrile:methanol, 50:50 (v/v)

Time (min.)	%B
0	10
10	60
15	60

Flow: 400µL/min.
Temp.: 30°C
Det.: UV @ 210nm

Numbered peaks are listed in Table 2

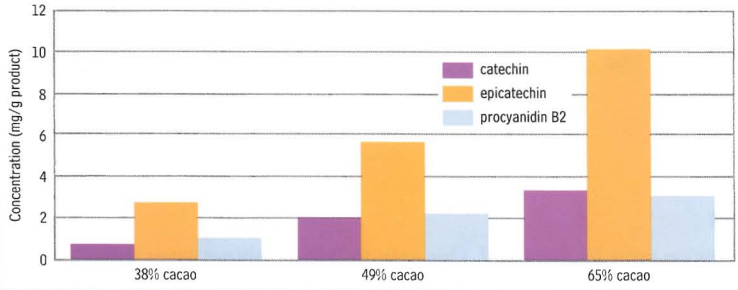
Mass Spectrometry
Instrument: Leco Unique® LC-TOFMS High Flow ESI Source
ESI voltage: (-) 3500 V
Desolv. temp.: 300°C
Nebulizer pres.: 375kPa
Desolv. gas: nitrogen, 7L/min.
Interface temp.: 100°C
Nozzle: (-) 160V
Data acq. rate: 4 spectra/sec.

Table 2 Flavonoid components in cocoa powder exhibit virtually the same retention times as in cacao.

Peak	RT (min:sec)	Unique Mass	Area	Area %
5. catechin (monomer)	03:50.4	289.1806	35151	8.7
9. procyanidin B2	04:25.0	577.3661	3928	1.0
11. epicatechin	04:52.8	289.1802	28030	6.9
13. procyanidin C1	05:28.3	358.2432	3287	0.8
17. procyanidin (tetramer)	07:08.2	326.2279	7088	1.8
18. clovamide	07:16.8	463.2485	6002	1.5
20. procyanidin II-g	07:43.7	433.2532	6047	1.5

Identities of peaks 1,2,3,4,6,7,8,10,12,14,15,16,19,21,22 are unknown, but retention times, masses, areas, and area % are available on request, and will be listed in our next Buzz electronic newsletter.

Figure 4 Concentrations of flavonoids in Venezuelan cacao, determined using an Ultra Aqueous C18 column and a LECO Unique® LC-TOFMS system.



centrifuged, concentrated, and filtered.[3] For a detailed description of sample preparation, refer to the LECO website www.leco.com.

An Ultra Aqueous C18 column is an excellent choice for this analysis, because it is designed to perform reversed phase separations well and reproducibly when the mobile phase has a high aqueous content. Using a 100mm x 2.1mm Ultra Aqueous C18 column and the automated peak find LECO ChromaTOF software in the Unique® LC-TOFMS system, we separated and identified 26 flavonoid compounds in a cacao sample (Figure 2 and Table 1).*

Next, using the automated peak find software in ChromaTOF, we identified flavonoids in cocoa powder (Figure 3 and Table 2). Processing of cacao reduces the amount of catechins and procyanidins in cocoa components. If an alkalizing step is present in the process, this also leads to a remarkable decrease in the content of catechins and procyanidins. For peaks identified in the cocao and cocoa powder samples, retention time did not differ by more than 0.01 seconds (Tables 1 and 2). The analysis was completed and conditions returned to the initial mobile phase composition in 15 minutes.

Subsequently, we analyzed three samples from Venezuela, containing differing amounts of cacao. Quantitative results were determined through ChromaTOF. Analytical results for these samples are shown in Figure 4. As expected, based on data in Table 1, epicatechin was substantially higher than catechin in each sample. Also as expected, catechin, epicatechin, and procyanidin B2 content increased with increasing amounts of cacao.

A LECO Unique® LC-TOFMS system and an Ultra Aqueous C18 column assure rapid, excellent resolution, reliable identification and quantification, and highly reproducible retention times for flavonoid compounds – even in very complex mixtures.

References
1. Prior, R.L., *et al., Procyanidin and catechin content and antioxidant capacity of cocoa and chocolate products, J. Agric. Food Chem.* 54: 4057-4061 (2006).
2. Hurst, W.J., *et al., Antioxidant activity and polyphenols and procyanidin contents of selected commercially available cocoa-containing and chocolate products in the United States, J. Agric. Food Chem.* 54: 4062-4068 (2006).
3. Andreas-Lacueva, *et al., An LC method for the analysis of cocoa phenolics, LC*GC Eur.* 902-905 (2000).

LECO Corporation, 14950 Technology Court, Fort Myers, FL, 33912

*Cacao is the sum of the products derived from the cacao bean – chocolate liquor, cocoa, and cocoa butter[2].

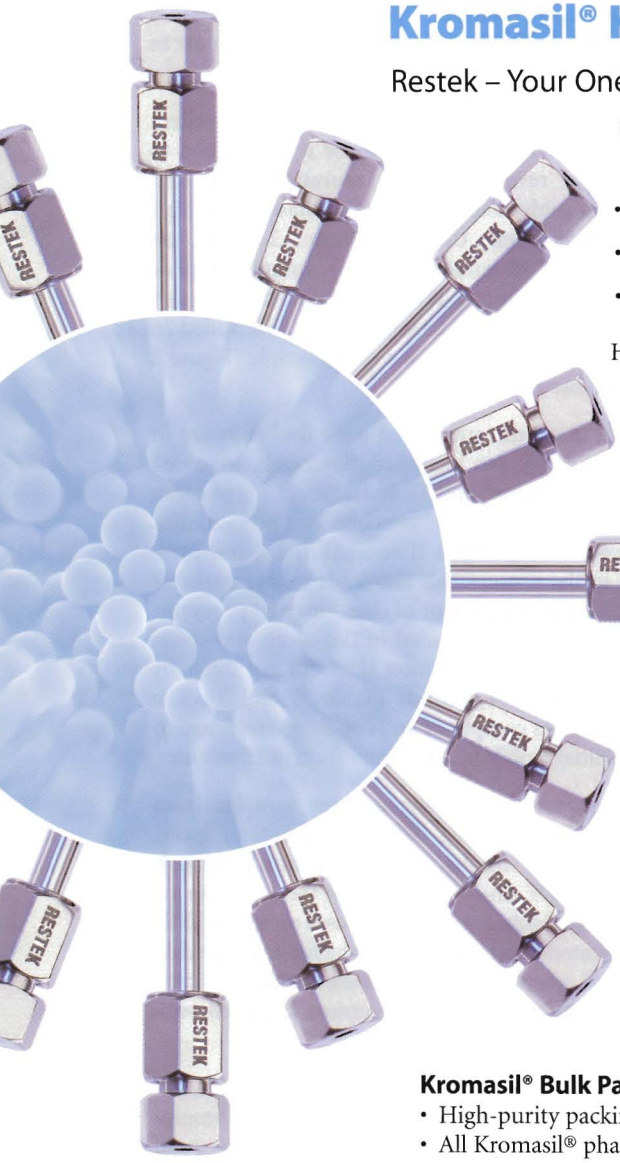
Ultra Aqueous C18, 5µm Column

3µm Column, 2.1mm	cat. #	price
100mm	9178312	$370

Kromasil® HPLC Bulk Packing Materials

Restek – Your One Source for Kromasil® Bulk Packing Materials

By Becky Wittrig, Ph.D., HPLC Product Manager



- The Kromasil® HPLC products you know and trust – available from Restek!
- Perfectly spherical, totally porous bulk silica products.
- Wide range of bonded phases and particle sizes.

HPLC grade silica materials differ greatly from one manufacturer to another. Factors that affect the selectivity of a silica substrate include the surface area and chemical purity of the substrate, the pore structure, and the pore diameter distribution. Kromasil® HPLC silica products consist of highly spherical, porous particles in sizes from 3.5µm to 16µm and larger. The surface properties of the silica have been optimized, including a narrow pore size distribution and well-defined pore structure. For chromatographic separations, this ensures higher efficiencies, smaller pressure drops, and excellent lot-to-lot reproducibility.

Kromasil® spherical silicas are produced using a sol-gel technique, which yields a mechanically strong particle with a large surface area (330m2/g for 100Å silica). Metal impurities are carefully monitored, as trace metals in the silica structure increase the surface acidity and can lead to tailing peaks for basic or chelating compounds. Typical metal content for Kromasil® silicas is shown in Table 1.

Table 1 Typical chemical purity for Kromasil® spherical silicas.

Metal	Content (ppm)
Na	<25
Al	<10
Fe	<10

Based on AAS or ICP measurements.

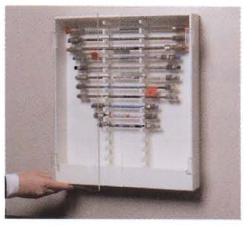
Kromasil® bulk packings are available from Restek in a wide range of particle sizes and bonded phases. For more information, please contact Restek technical service at **800-356-1688** or **814-353-1300, ext. 4.**

Kromasil® Bulk Packings

- High-purity packing materials in 10 and 16µm.
- All Kromasil® phases available.

Description	min. qty.	cat.#	200-499 grams	500-999 grams	≥1000 grams
Kromasil® 100Å Silica, 10µm	200g	92000	$7.93/gram	$6.90/gram	$6.13/gram
Kromasil® 100Å Silica, 16µm	200g	92001	$6.35/gram	$5.52/gram	$4.91/gram
Kromasil® 100Å C8, 10µm	200g	92030	$11.90/gram	$10.35/gram	$9.20/gram
Kromasil® 100Å C8, 16µm	200g	92031	$9.52/gram	$8.28/gram	$7.36/gram
Kromasil® 100Å C18, 10µm	200g	92040	$11.90/gram	$10.35/gram	$9.20/gram
Kromasil® 100Å C18, 16µm	200g	92041	$9.52/gram	$8.28/gram	$7.36/gram
Kromasil® 100Å Chiral DMB, 10µm	200g	92080	$34.88/gram	$30.33/gram	$26.96/gram
Kromasil® 100Å Chiral DMB, 16µm	200g	92081	$27.90/gram	$24.26/gram	$21.57/gram
Kromasil® 100Å Chiral TBB, 10µm	200g	91990	$34.88/gram	$30.33/gram	$26.96/gram
Kromasil® 100Å Chiral TBB, 16µm	200g	91991	$27.90/gram	$24.26/gram	$21.57/gram

Other phases and particle sizes available on request.



30-Column Storage Cabinet

Tired of stacks of HPLC columns on your lab benches? This easy-to-install cabinet saves space and protects columns; the hinged door is clear to allow quick identification of column labels or tags.

Description	dimensions	qty.	cat.#	price
30 Column Cabinet	17 3/8 x 15 x 2 7/8"	ea.	25159	$119

*Please note: Columns in photograph are not included.

Cool Tools!

For Thermo Instruments

Jet Removing Tool for Thermo GCs: Focus GC / TRACE™ GC / Ultra/TRACE™ GC x GC

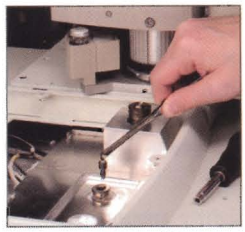
- Unique, ergonomic handle—easy to grip.
- Easily loosens the FID jet.

new for 2006

Remove FID cell assembly.

Slip tool over FID jet.

Turn counterclockwise to loosen jet.



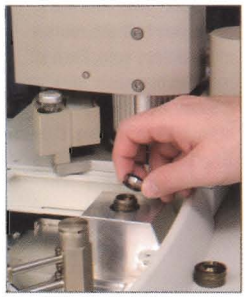
Use tweezers (cat. #20101) to remove jet.

Description	Similar to TF part #	qty.	cat.#	price
Jet Removing Tool for Thermo	205-019-00	ea.	24936	$50

Liner Cap Removing Tool for Thermo GCs: Focus GC / TRACE™ GC / Ultra/TRACE™ GC x GC

- Unique, ergonomic handle—easy to grip.
- Easily loosens the liner cap from the injector.

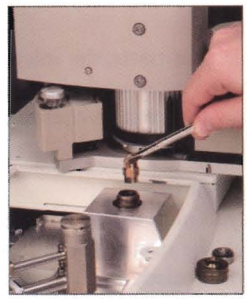
new for 2006



Remove septum cap, septum holder, septum, and septum support.

Place tool on liner cap. Align two pins on bottom of tool with two open slots on liner cap.

Turn counterclockwise to loosen liner cap.

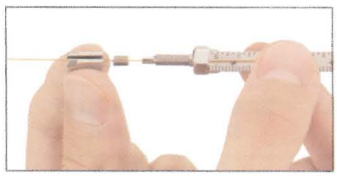


Use tweezers (cat. #20101) to remove liner cap.

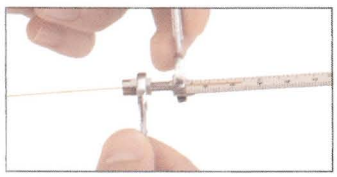
Description	Similar to TF part #	qty.	cat.#	price
Liner Cap Removing Tool for Thermo	205-070-10	ea.	24937	$50

Capillary Installation Gauge for TRACE™ and Focus SSL (M4 Ferrules)

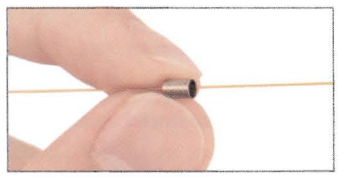
- Seats ferrule onto column for consistent installations.
- Prevents crushed column ends.
- Made from high-quality stainless steel.



Install nut and ferrule onto column. Cut column end squarely. Slide column into installation gauge to recommended insertion distance. Finger-tighten column nut.



Tighten assembly to ensure a properly seated ferrule. Loosen assembly and remove column and column nut.



The ferrule will be properly seated, and should remain in place when light force is applied. If it slides loosely on the column, repeat procedure.

Description	qty.	cat.#	price
Capillary Installation Gauge for TRACE™ & Focus SSL (M4 ferrules)	ea.	22330	$70

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Cool Tools for GC and HPLC
Restek innovation saves you time and money.

lit. cat.# 59879

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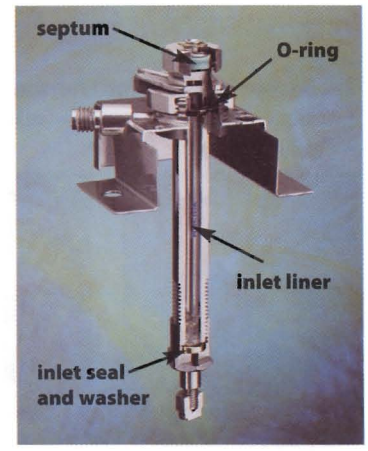
innovation

Easily seat ferrules for consistent installations!

Peak Performers

Injection Port Maintenance with FastPack™ Inlet Kits

by Donna Lidgett, GC Accessories Product Manager



What are the benefits of using a FastPack™ Inlet Kit?

FastPack™ inlet kits include all the parts needed to maintain your system. Injection port maintenance should be performed prior to installing any capillary column, and on a routine basis, based on the number of injections made and the cleanliness of the samples. Maintenance includes replacing the injection port (inlet) liner, the critical inlet seals, and the septum.

Why replace an injection port liner?

For optimum column performance, the injection port (inlet) liner must be free of septum particles, sample residue, or ferrule fragments. Peak shape degradation, poor reproducibility, sample decomposition, and ghost peaks all are associated with using a dirty (contaminated) liner.

Why replace the critical seal?

The critical seal must fit tightly around the inlet liner, to prevent carrier gas from leaking around the outside of the liner. Replace the critical seal prior to installing an inlet liner.

Why replace the septum?

The septum maintains a leak-tight seal that excludes air from the inlet. Frequent replacement prevents fragmentation and leaks. Multiple injections and continuous exposure to hot injection port surfaces will decompose the septum and can create particles, which can fall into the inlet liner. Septum particles are a potential source of ghost peaks, loss of inertness, and carrier gas flow occlusion. Allow a new septum sufficient time to condition in the injector, to reduce the incidence of ghost peaks. To avoid contamination, always use forceps when handling septa.

FastPack™ Inlet Kits are a great way to make routine maintenance easy. Each kit includes one each: inlet liner (choose from four popular styles), Viton® O-ring, 0.8mm ID gold-plated inlet seal, inlet seal washer, 11mm Thermolite® septum.

Why replace the inlet seal?

In Agilent split/splitless injection ports, the inlet seal sits at the base of the injector. Dirt, non-volatile residue, septum fragments, and other undesirable materials contaminate the inlet seal and decrease analytical linearity. The only way to maintain optimum performance is by frequently changing the inlet seal and ensuring the seal is leak-tight.

FastPack™ Inlet Kits for Agilent GCs

- Convenient: all the parts you need in one package—no hunting for individual items.
- Economical: costs less than the sum of the individual parts.
- Clean: Mylar® bag is factory sealed; no contamination of the products from weeks in the lab.

1 pack includes 5 maintenance kits

Deactivated Liner	cat.#	1 pack (5 kits)	5-19 packs	20 or more packs
4mm Splitless	21101	$193/pk.	$183/pk.	$173/pk.
4mm Splitless Gooseneck	21102	$213/pk.	$203/pk.	$193/pk.
4mm Splitless Double Gooseneck	21103	$253/pk.	$241/pk.	$228/pk.
4mm Split with Wool*	21104	$203/pk.	$193/pk.	$183/pk.

*Liner dimensions are 4mm ID, 6.3mm OD, 78.5mm long. Liners in other kits are 6.5mm OD.

Liners for Agilent/Finnigan GCs	Benefits/Uses	ID*/OD & Length (mm)	Similar to Agilent part #	cat.#/price ea.	5-pk.	25-pk.
2mm Splitless	trace samples <2µL	2.0 ID 6.5 OD x 78.5	5181-8818 (ea.) 5183-4703 (5-pk.) 5183-4704 (25-pk.)	20712 $23	20713 $77	20714 $233
4mm Splitless	trace samples >2µL	4.0 ID 6.5 OD x 78.5	210-3003 (ea.) 210-3003-5 (5-pk.)	20772 $19	20773 $58	20774 $233
Siltek® 4mm Splitless	trace samples >2µL	4.0 ID 6.5 OD x 78.5	—	20772-214.1 $24	20773-214.5 $78	20774-214.25 $322
Gooseneck Splitless (4mm) w/ Wool†	trace samples >2µL	4.0 ID 6.5 OD x 78.5	5062-3587 (ea.) 5183-4693 (5-pk.) 5183-4694 (25-pk.)	22405 $29	22406 $81	22407 $329
4mm Split w/ Wool	universal, use with Agilent 7673 autosampler	4.0 ID 6.3 OD x 78.5	19251-60540 (ea.) 5183-4691 (5-pk.) 5183-4692 (25-pk.)	20781 $23	20782 $67	20783 $274
Siltek® 4mm Split w/ Wool	universal, use with Agilent 7673 autosampler	4.0 ID 6.3 OD x 78.5	—	20781-213.1 $42	20782-213.5 $116	20783-213.25 $442

Liners for Varian 1075/1077 GCs

	Benefits/Uses:	ID*/OD & Length (mm)	Similar to Varian part #	ea.	cat.#/price 5-pk.	25-pk.
 2mm Splitless	trace samples <2µL	2.0 ID 6.3 OD x 74	01-900109-05	20721 $35	20722 $97	20723 $437
 4mm Splitless	trace samples >2µL	4.0 ID 6.3 OD x 74	01-900109-05	20904 $25	20905 $97	20906 $437
 Splitter w/ Wool	universal, use with rapid autosamplers	4.0 ID 6.3 OD x 72	01-900109-01	20792 $35	20793 $111	20794 $501

Liners for Varian 1177 GCs

	Benefits/Uses:	ID*/OD & Length (mm)	Similar to Varian part #	ea.	cat.#/price 5-pk.	25-pk.
 4mm Split w/Glass Frit	universal	4.0 ID 6.3 OD x 78.5	39-26119-36	21045 $39	21046 $171	—
 4mm Split w/ Wool	universal	4.0 ID 6.3 OD x 78.5	39-26119-34	—	21079 $67	—

Liners for Varian 1078/1079 GCs

	Benefits/Uses:	ID*/OD & Length (mm)	Similar to Varian part #	ea.	cat.#/price 5-pk.	25-pk.
 1078/1079 Split w/ Frit	dirty samples, non-active compounds	3.4 ID 5.0 OD x 54	03-918464-00	21708 $39	21709 $171	—
 1078/1079 Splitless	trace samples <2µL	2.0 ID 5.0 OD x 54	03-918466-00	21711 $29	21712 $113	—

Liners for Shimadzu GCs

	Benefits/Uses:	ID*/OD & Length (mm)	Similar to Shimadzu part #	ea.	cat.#/price 5-pk.	25-pk.
 17A & 2010 Split/Splitless w/ Wool	universal, for most common analyses	3.5 ID 5.0 OD x 95	221-41444-00	20955 $25	20956 $89	20957 $317
 Siltek® 17A & 2010 Split/Splitless w/ Wool	universal, for most common analyses	3.5 ID 5.0 OD x 95	—	20955-213.1 $44	20956-213.5 $138	20957-213.25 $485

Liners for PerkinElmer GCs

	Benefits/Uses:	ID*/OD & Length (mm)	Similar to PE part #	ea.	cat.#/price 5-pk.	25-pk.
 Splitless (2mm ID)	trace samples	2.0 ID 5.0 OD x 100	N6502007	20730 $29	20731 $113	20732 $475
 Auto SYS™ Splitless	headspace & purge & trap	1.0 ID 6.2 OD x 92.1	N6502006	21272 $29	21273 $120	21274 $483
 Baffle Splitter	universal, for most common analyses	3.5 ID 5.0 OD x 100	N6502008	20736 $22	20737 $85	—
 Cup Splitter	high & low MW compounds	3.5 ID 5.0 OD x 100	—	20739 $61	20740 $241	—

Liners for Thermo Finnigan TRACE™ and Focus SSL GCs

	Benefits/Uses:	ID*/OD & Length (mm)	Similar to TF part #	ea.	cat.#/price 5-pk.	25-pk.
 Splitless (3mm ID)	trace samples	3.0 ID 8.0 OD x 105	453 20032	20942 $34	20943 $130	20944 $570
 Siltek® Splitless (3mm ID)	trace samples	3.0 ID 8.0 OD x 105	—	20942-214.1 $39	20943-214.5 $150	20944-214.25 $660

*Nominal ID at syringe needle expulsion point.
†Use this liner for increased sensitivity.

All liners are **100% deactivated**

All liners are shipped intermediate polarity (IP) deactivated unless otherwise requested.

Commonly Asked GC Questions

Answered by the Restek Chromatography Information Services Group

How do I know which guard column would be best for my application?
Restek offers guard columns and transfer lines ranging from 0.025mm ID to 0.53mm ID, from 1 to 10 meters long, in fused silica or Silcosteel® treated stainless steel. Guard columns are available with nonpolar, intermediate polarity, or polar deactivation, and with several specialty deactivations.

- In most applications in which nonpolar to moderately polar solvents are used; we recommend an intermediate-polarity (IP) deactivated guard column.
- For most polar solvents except water, we generally suggest a polar deactivated guard column.
- For water-based samples, we recommend our water-resistant Hydroguard™ guard columns. This deactivation is designed to withstand the harsh "steam-cleaning" that occurs when water is rapidly vaporized in the column.
- For applications that require a highly inert surface to minimize analyte breakdown, such as pesticides analysis, we recommend a Siltek® deactivated guard column.
- For amines or other basic compounds, we offer base-deactivated guard columns.

Also, note that for many of our popular stationary phases, we offer Integra-Guard™ columns – an analytical column with an integral guard column. This eliminates the connection between the guard column and the analytical column. Much information about guard columns is presented in our free publication #59319.

What are all those different capillary column temperatures listed in your catalog?
The first temperature listed is the minimum operating temperature for the column. The two temperatures separated by a slash symbol (/) are the maximum isothermal operating temperature and the maximum temperature program temperature, respectively. The maximum temperature program temperature is the maximum temperature to which the column may be exposed briefly without causing damage. For most stationary phases, the maximum temperature program temperature is approximately 20°C above the maximum isothermal temperature. In addition to these temperatures, the polymer stability temperature sometimes is listed. This is the maximum temperature to which the polymer phase can be exposed before degradation.

I see ghost peaks when I inject a sample or standard, and my mass spectrometer identifies these peaks as a siloxane material. Is there a problem with my column?
Capillary columns can produce a varying amount of baseline noise (siloxane bleed), usually containing fragment ions at m/z 73, 207, and 281, but they will not produce any distinct peaks in an analytical run. The most common sources of distinct siloxane peaks are septum bleed and the chemicals used to deactivate the injection port liner and the glass wool packing material.

Sometimes I experience problems when using a 1701-type column for my pesticides analysis. Are there other column choices?
On-column breakdown of chlorinated pesticides, such as endrin, methoxychlor, and DDT, are common with cyano-containing phases, such as 1701-type phases. Fortunately, there are other column choices. These include a few standard phases, such as our Rtx®-35, Rtx®-35MS, and Rtx®-50 phases. In addition, Restek has developed several specialized columns for pesticides, including Rtx®-CLPesticides & Rtx®-CLPesticides2, and Stx®-CLPesticides & Stx®-CLPesticides2 columns. These columns eliminate on-column breakdown problems, improve separation, and reduce analysis time. Information about these columns, and example chromatography, can be found on our website: www.restek.com

Can I order a fused silica column in a column cage to fit my small GC oven?
Yes. We offer several special cage options for non-standard and portable GC ovens. Please contact our **informations services** group at **800-356-1688, ext. 4**, for specifics, or **customer service (ext. 3)** for prices Please note that we cannot cage or recage columns from other manufacturers.

the Restek Chromatography Information Services Group

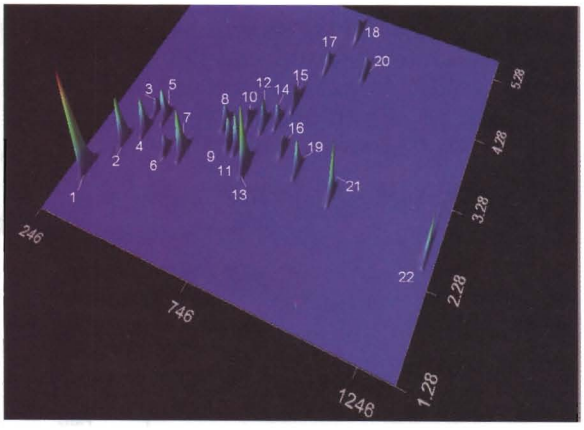
Comprehensive 2D Gas Chromatography – Making GC Separations Work Harder *Continued from page 2.*

deconvolution. GC deconvolution through real separation is a more rugged and desirable outcome – and we can still combine GC×GC with MS for further identification. It is certainly true that GC×GC demands improved performance capabilities of GC instruments, new software, and better column quality control (e.g., improved batch-to-batch column reproducibility for the 1 m lengths of 2D columns that we use). These cannot be realised without the compliance of instrument and column manufacturers. As examples of generic GC×GC applications, a low polarity (5% phenyl) 1D column coupled to a short polar (wax phase) 2D column is useful for essential oils, but recently wax-low polarity column combinations have proved equally valuable. For petrochemicals, where higher temperature operation is needed, a low polarity (5% phenyl) 1D column coupled to a short polar (50% phenyl) 2D column is often used. For environmental analysis of PCBs, a carborane phase 2D column has been reported, where selectivity towards the extent of compound planarity is sought. In this short commentary, there is no space to engage in specifics of certain GC×GC methods, but obviously there is considerable opportunity to optimise methods, and use sound principles of GC and phase selection to get the best out of GC×GC.

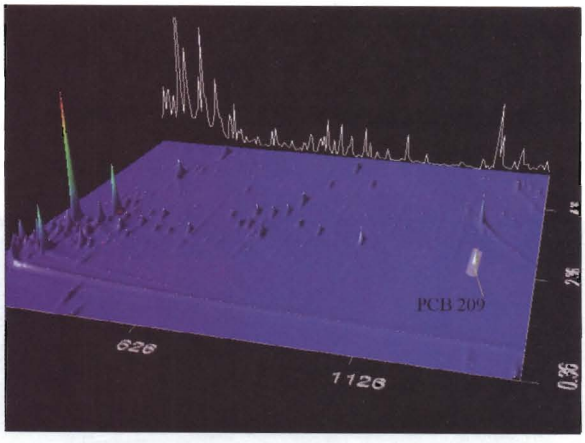
We are at the threshold of a new era in GC, and getting the best out of our GC×GC methods is a task that an increasing number of analysts will be striving for. The comments of Professor Walt Jennings (Restek Advantage, 2006.01) also ring true for GC×GC. When a method has been developed for GC×GC, and one of the columns has to be replaced, to what extent will the 2D plot faithfully reproduce our archived or master analytical result? This must be addressed in the two-dimensional experiment, to prove that the analyst can have confidence that their data interpretation protocols survive column change and routine maintenance of the system. But with the impressive capabilities of GC×GC, it is important that analytical methods and the greater information content it offers are supported by validated and reliable operation.

Editor's note: Dr. Marriott is one of the world's leading experts in 2D-GC.

An Example of 2D Gas Chromatography see *Advantage* 2005.1



GCxGC analysis of organochlorine pesticides combines primary column and confirmation column results.



Organochlorine pesticides separated from interferences in tomato extract.

Columns:	Rtx®-5 9m, 0.18mm ID, 0.20μm (10m column, cat.# 40201, with 1m removed) Rtx®-200 1m, 0.18mm ID, 0.20μm (1m of 10m column, cat.# 45001)
Inj.:	1μL, split, 250°C, split ratio 50:1
Oven:	Primary: 50°C (0.2 min.), 30°C/min. to 140° (no hold), 5°C/min. to 250°C (no hold) Secondary: 50°C offset from primary oven
Instrument:	LECO GCxGC/ECD
Modulator:	Temperature offset: 30°C Modulation time: 6 sec
Det.:	ECD, 325°C, 150mL/min. nitrogen makeup gas, 50Hz

Tradeshow Schedule

We'd be happy to talk with you at any of the following meetings or shows. We'll post our booth numbers as they become available to us.

September, 2006

Date	September 17-21
Show	120th AOAC Annual Meeting & Exposition
Location	Hyatt Regency, Minneapolis, MN

October, 2006

Date	October 3-7
Show	SOFT 2006 Annual Meeting
Location	Hilton Austin, Austin, TX
Date	October 7-10
Show	ACIL 69th Annual Meeting
Location	San Antonio Marriott River Center, San Antonio, TX
Date	October 11
Show	Chromatography Society Triad Symposia
Location	AstraZeneca, Charnwood, England
Date	October 11-12
Show	Midwestern Association of Forensic Scientists (MAFS)
Location	Hyatt downtown, Indianapolis, IN
Date	October 17-19
Show	Gulf Coast Conference
Location	Moody Gardens Convention Center & Hotel, Galveston Island, TX
Date	October 24-25
Show	Chromatography Society Triad Symposia
Location	Pfizer, Sandwich, England
Date	October 31 - November 3
Show	SEMA Show
Location	Las Vegas Convention Center, Las Vegas, NV

November, 2006

Date	November 1-2
Show	WWEM Water, Wastewater and Environmental Monitoring Conference Exhibition & Workshops
Location	Telford International Centre, Telford, Shropshire, England
Date	November 1-4
Show	32nd Annual NEAFS Meeting
Location	Tarrytown DoubleTree Hotel, Tarrytown, NY
Date	November 6-7
Show	California Association of Toxicologists
Location	Miramonte Resort and Spa, Palm Springs, CA
Date	November 6-10
Show	SWAFS/NWAFS Joint Meeting and Training Conference
Location	Doubletree Hotel, Colorado Springs, CO
Date	November 9
Show	2006 Anachem Symposium
Location	Burton Manor, 27777 Schoolcraft Rd., Livonia, MI
Date	November 13-16
Show	2006 Eastern Analytical Symposium
Location	Garden State Exhibit Center, Somerset, NJ
Date	November 21-22
Show	Chromatography Society Triad Symposia
Location	GlaxoSmithKline, Stevenage, England

For latest updates, see our Tradeshow Calendar

GET YOUR MIX

Time-Saving MegaMix™ Environmental Reference Mixes.

- Largest number of target analytes in one mix, formulated for maximum stability.
- Available for US EPA methods 8260, 8270, 502.2, 524.2, 525.2, 624, 625, SOM01.1, OLC 03.2, OLM 04.2, Skinner List volatiles, Skinner List semivolatiles.

MegaMix™ mixes simplify preparation of calibration mixes, and shorten preparation time, because they include a maximum numbers of compatible target analytes. In some applications a second calibration analysis has been required for coeluting target compounds, but the MegaMix™ formulation ensures all included analytes can be calibrated in one analysis (e.g., 3- and 4- methylphenol with other components in OLC 03.2 semivolatiles mix; *m*- & *p*- xylene with other components in OLC 03.2 volatiles mix).

Save time, save effort, minimize potential for preparation problems – use MegaMix™ reference mixes, only from Restek or authorized distributors.