Flavors & Fragrances by Gas and Liquid Chromatography

Becky Wittrig, Ph.D.
RESTEK CORPORATION
Flavors & Fragrances by Gas and Liquid Chromatography

I. Flavor Compounds by HPLC
II. Flavor Compounds by GC
III. Flavor Profiling Techniques
IV. Fragrance Analysis
Analysis of Flavors by HPLC

- Suitable for less volatile, thermally unstable compounds
- Flavor Compounds by HPLC
  - Vanillin & ethyl vanillin
  - Capsaicins (heat)
  - Spice compounds
  - Browning reaction products
Flavor Compounds by HPLC
Vanillin & Ethyl Vanillin

- Real Vanilla Extract
  - Vanillin, + many other flavor compounds
  - >35% alcohol by volume
- Vanilla Flavorings
  - Vanillin & ethyl vanillin
- HPLC Analysis
  - AOAC method 990.25
  - Reversed phase with UV detection
Flavor Compounds by HPLC
Vanillin & Ethyl Vanillin

Vanillin / Ethyl Vanillin Standard  
Vanilla Extract
Flavor Compounds by HPLC

Vanillin & Ethyl Vanillin by HPLC

Separation Conditions:

**Column:** Ultra C8, 150 x 4.6 mm, 5µm

A: 1.2% acetic acid

<table>
<thead>
<tr>
<th>Time</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>20</td>
<td>40</td>
</tr>
<tr>
<td>25</td>
<td>20</td>
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</tbody>
</table>

B: methanol

Flow Rate: 1mL/min

UV@ 254 nm
Flavor Compounds by HPLC

Heat Content in Foods

- Heat Content
  - Sensory – Scoville Heat Units
  - Traditionally Measured by Trained Panelists
    - Can be subjective
    - Involved procedure
- HPLC Procedures
  - American Spice Trade Association (ASTA)
  - AOAC International
  - Calculate Scoville Heat Units - relate to sensory
Flavor Compounds by HPLC

Heat Content in Foods

Source: http://www.mohotta.com
**Flavor Compounds by HPLC**

**Heat Content in Foods**

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**Ultra C18**

150 x 4.6 mm, 5µm

**Conditions:**

- A: 1% acetic acid
- B: acetonitrile

**Time %B**

<table>
<thead>
<tr>
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<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
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<td>12</td>
<td>80</td>
</tr>
<tr>
<td>13</td>
<td>50</td>
</tr>
</tbody>
</table>

**Flow Rate:** 1mL/min  
**UV@ 280 nm**

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Use fluorescence for greater sensitivity!
Flavor Compounds by HPLC
Heat Content of Habenero Nuggets

Ultra C18
150 x 4.6 mm, 5µm

Conditions:
Same as previous chromatogram

- capsaicin
- nordihydrocapsaicin
- dihydrocapsaicin

min
# Flavor Compounds by HPLC

Analyzing Spice Components

Monitoring the Chemical Compounds Responsible for the Distinctive Flavor/Aroma

<table>
<thead>
<tr>
<th>Spice</th>
<th>Compound(s)</th>
</tr>
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<tbody>
<tr>
<td>Anise</td>
<td><em>(E)</em>-Anethole, methyl chavicol</td>
</tr>
<tr>
<td>Caraway</td>
<td><em>d</em>-Carvone, carvone derivatives</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>Cinnamaldehyde, eugenol</td>
</tr>
<tr>
<td>Fennel</td>
<td><em>(E)</em>-Anethole, fenchone</td>
</tr>
<tr>
<td>Nutmeg</td>
<td>Sabinine, <em>α</em>-pinene, myristicin</td>
</tr>
<tr>
<td>Saffron</td>
<td>Safranal</td>
</tr>
<tr>
<td>Turmeric</td>
<td>Turmerone, zingeriberone, 1,8-cineole</td>
</tr>
</tbody>
</table>
Flavor Compounds by HPLC

Piperine

Pinnacle II Cyano
150 x 4.6 mm, 5µm

Conditions:
50:50 water:acetonitrile
Flow Rate: 1mL/min
UV@ 345 nm
Browning Reaction Products
HMF Standard by HPLC

Column:  Ultra C18 (Restek Corp.), 250 mm x 4.6 mm, 5 μm
Mobile Phase A:  90:10 water:methanol, 10 mM ammonium formate
Mobile Phase B:  10:90 water:methanol, 10 mM ammonium formate
Gradient:  0-5 min at 100% A, to 100% B at 10 min, 10 min. hold
Flow:  0.5 mL/min.
Temperature:  ambient
Detector:  UV @ 280 nm
Injection Volume:  10 μL
HMF in Grape Juice by HPLC

No SPE Treatment

HMF + interferences

C18 SPE Treatment

HMF
Sample Prep: HMF in Grape Juice

Extraction Procedure

1. Conditioning
   a. Apply 3 mL methanol
   b. Apply 3 mL deionized water
2. Sample Application
   a. Apply 4 mL sample to moist SPE tube, gravity feed
3. Wash
   a. Pull remaining sample through tube, using vacuum
   b. Apply 3 mL water
   c. Remove excess water from bed under vacuum
4. Elution
   a. Apply 2 mL elution solvent, gravity feed, dilute to volume
HMF by LC/MS
Analysis of Flavors by GC

- GC is suitable for volatile, thermally stable compounds
- Higher resolution separations generally possible
- Flavor Compounds by GC
  - Alcoholic beverages
  - Volatiles
Flavor Compounds by GC

- Determine the Goals of the Analysis
  - QC / purity determinations
  - Comparison of flavors
  - Reverse engineering

- Detection
  - FID & MSD most common
  - Specialty detectors (GCO, AED)
Flavor Compounds by GC
Sampling Techniques

- **Liquid Sampling**
  - Split/splitless injection
  - Direct or on-column injection
  - Large volume injection

- **Headspace Sampling**
  - Static
  - Dynamic (purge & trap)
  - Thermal desorption

- **Extraction Techniques**
Flavor Compounds in Scotch

Split injection

Peak List
1. Acetaldehyde
2. Methanol
3. Ethanol
4. Acetone
5. Isopropanol
6. n-Propanol
7. Ethyl acetate
8. Isobutanol
9. Acetic acid
10. Isoamyl alcohol
11. Active amyl alcohol
Flavor Compounds in Scotch by GC

- **Analytical Column**
  - Rtx®-1301, 60m x 0.25mm x 1.4µm
  - Linear velocity: H₂ @ 40 cm/sec.

- **Temperature Program**
  - 35°C (5 min. hold) to 100°C @ 1°C/min.

- **Injector**
  - 100:1 split
  - 1.0 µL neat injection using a Cyclosplitter® sleeve

- **Detector**
  - FID @ 200°C
Large Volume Injectors (LVI)

- Large volume injected at low temperature
  - Must have early solvent vent
  - Injector rapidly heated after venting
- Injection must not over-fill the pre-column
  - Injection rate must match the evaporation rate
- Used in the analysis of trace level components
Large Volume Injectors (LVI)
Malt Whiskey

Chromatogram courtesy of Kevin MacNamara, Irish Distilleries
Chromatogram Conditions

Large Volume Injectors – Malt Whiskey

Column: Stabilwax®-DA, 30m x 0.18mm, 0.18µm

Oven temp.: 60°C(2 min. hold) to 100°C at 20°C/min, to
240°C at 5°C/min, 10 min. hold

Carrier gas: Helium @ 45 cm/sec

Quad Temp: 150°C

Source Temp: 230°C

Scan range: 30-400AMU

Ionization: 70eV EI

Inj Volume: 10µL LVI (splitless) @ 10 µL/min

Injector: Gerstel CIS Injector

35°C for 2 min, then 10°C/sec to 300°C,
hold 5 min.

He vent flow: 600 mL/min with 1.8 min. vent end time
Flavor Profiling
Headspace Sampling Techniques

• **Advantages**
  - “Cleaner” samples
  - Minimal sample preparation

• **Types**
  - Static headspace
  - Dynamic headspace
  - Thermal desorption
Flavor Profiling
Static Gas Extraction

- **Advantages**
  - Excellent screening tool
  - Inexpensive
  - Minimal sample carryover
  - Easy to perform

- **Disadvantages**
  - Less sensitive
  - Involves preparing calibration in sample matrix
Flavor Profiling: Static Gas Extraction

- Constant ratio between liquid & gas phases at equilibrium

Where $K = \frac{C_{\text{gas}}}{C_{\text{liquid}}}$

- Large $K$ values favor the gas state
- $C_{\text{gas}}$ is directly proportional to peak area

Lit. #59895 — Headspace Guide
Flavor Profiling: Static Gas Extraction

Volatile from 2 different batches of chewing gum. The headspace was sampled after heating to 60°C.
Flavor Profiling: Static Gas Extraction
Residual solvents in decaffeinated lemon tea

1. Methanol
2. iso-Propanol
3. Methylene chloride
4. Methyl acetate
5. n-Butanol (IS)
Flavor Profiling: Dynamic Gas Extraction Purge & Trap Inlet System

- Concentrates volatiles
- Dynamic extraction of solids and liquids
- Adsorbent trap
- Desorb (10-80 mL/min)
- Narrow bore column (split flow)
- Part of GC system
Flavor Profiling: Dynamic Gas Extraction
Purge & Trap Sampling

1. Wet Purge

- Carrier gas bubbled through the matrix
- Volatiles in matrix diffuse into carrier gas & are carried away
- Typical flows: 40-50 mL/min. (can heat)
- Typical purge time: 10-12 min.
Flavor Profiling: **Dynamic Gas Extraction**

Purge & Trap Sequence

2. Dry Purge

- Trap is dried by purging with gas only
- Typical time: 1-4 min.
Flavor Profiling: **Dynamic Gas Extraction Purge & Trap Sequence**

3. **Desorb Preheat**

- Trap is heated without flow
- Typical temp: 5° below desorb temperature
- Minimizes retention on the trap
Flavor Profiling: Dynamic Gas Extraction
Purge & Trap Sequence

4. Desorb

- Trap is backflushed into column
- Typical time: 2 - 4 minutes
- Typical flow: 10-80 mL/min.
- Typical temp: 180° - 250°C
Flavor Profiling: **Dynamic Gas Extraction**

Purge & Trap Sequence

5. Trap Bake

- Trap is baked clean with flow
- Typical time: $8^+$ minutes
- Typical temp: higher than desorb temperature
- Avoid overheating adsorbents
Flavor Profiling: Dynamic Gas Extraction
Typical Adsorbents for Purge & Trap

- 10cm Carbopack B
- 6cm Carboxen 1000
- 1cm Carboxen 1001

Vocarb® 3000
Flavor Profiling: \textbf{Dynamic Gas Extraction}

Requirements of a Trap

- Retention of polar & non-polar compounds
- Hydrophobic characteristics
- Reproducible desorption
  - Increasing levels of adsorbency
- Able to withstand a broad temperature range
Flavor Profiling: Dynamic Gas Extraction
Connecting a Purge & Trap to the GC Column

- Via the injection port
- Directly to the column
  - 1/16" union
  - Silica transfer line from 6 port valve directly to the column
- Low volume injector
Interfacing to a GC/MS System

- Narrow bore column flows (0.5-1.3 mL/min.) permit a direct interface
- 0.53mm ID columns require:
  - Open Split Interface
  - Excess Flow
  - or
  - Jet Separator
  - Vacuum

Open Split Interface  or  Jet Separator
Purge & Trap GC/MS of Volatiles

Abundance

THF

4-Heptanone

Benzene

Styrene

Minutes
Purge & Trap GC/MS of Volatiles

**GC Parameters**
- **Column:** Rtx-5MS, 30m x 0.25mm x 1.0um
- **Injector:** 250°C, 20:1 split
- **Carrier gas:** Helium at 1 mL/min, constant flow
- **Oven:** 50°C to 92°C at 3°C/min, to 220°C/min. at 20°C/min. (1 min. hold)

**MSD Parameters**
- **Temperature:** 280°C
- **Scan Range:** 35-260, 1 min. solvent delay
- **Ionization:** EI @ 70eV

**Purge & Trap Parameters**
- **Concentrator:** Tekmar LSC-3100 with Vocarb 3000 (type K) trap
- **Purge:** 10 min. at 40 mL/min, 60°C
- **Dry purge:** 3 min. at 40 mL/min.
- **Desorb:** 2 min. at 40 mL/min, 245°C
Thermal Desorption Techniques

- Heat sample to drive volatiles into headspace
- Can trap and concentrate volatiles
  - Cryofocusing step
- Minimal sample preparation
- Commercially available units
Short-Path Thermal Desorption System

Valve 1

Carrier Gas

Valve 2

Sample tube

Septum purge vent

Split purge vent

To GC
Analysis of Fragrances by GC

• Typically Very Complex
  – 100+ Components
  – Range of compound types, volatilities

• Fragrance Compounds by GC
  – Essential Oils
  – Chiral Compounds
Essential Oil Analysis by GC

- Complex Samples
  - Hundreds of volatiles
  - Many at trace levels
- Multiple Uses / Products
  - Spices, perfumes, fragrances, medicinals
- Multiple Phases Can be Used
  - Low polarity (OV1)
  - High polarity (Waxes, trifluoropropyl, cyano phases)
Spearmint Oil Analysis by GC

60m x 0.25mm x 0.25um Stabilwax®, 0.2uL neat oil, 100:1 split.
75°C (4 min hold) to 200°C @4°C/min (10 min. hold), H2@40cm/s.
# Peak List for Spearmint Oil Analysis

<table>
<thead>
<tr>
<th></th>
<th>Peak Name</th>
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<tbody>
<tr>
<td>1</td>
<td>α-pinene</td>
</tr>
<tr>
<td>2</td>
<td>β-pinene</td>
</tr>
<tr>
<td>3</td>
<td>Sabinene</td>
</tr>
<tr>
<td>4</td>
<td>Myrcene</td>
</tr>
<tr>
<td>5</td>
<td>α-terpinene</td>
</tr>
<tr>
<td>6</td>
<td>L-limonene</td>
</tr>
<tr>
<td>7</td>
<td>1,8-cineole</td>
</tr>
<tr>
<td>8</td>
<td>cis-ocimene</td>
</tr>
<tr>
<td>9</td>
<td>γ-terpinene</td>
</tr>
<tr>
<td>10</td>
<td>p-cymene</td>
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<tr>
<td>11</td>
<td>Terpinolene</td>
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<tr>
<td>12</td>
<td>3-octyl acetate</td>
</tr>
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<td>3-octanol</td>
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<td>L-menthone</td>
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<td>Trans-dihydrocarvyl acetate</td>
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<td>cis-carvyl acetate</td>
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<td>Trans-carveol</td>
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<td>cis-jasmone</td>
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<tr>
<td>30</td>
<td>Viridiflorol</td>
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</table>
Chiral GC: Definition of Chirality

- Any compound that does not contain a plane of symmetry
- Compound can exist as enantiomers
  - Non-superimposable mirror images
- Typically a carbon center with 4 different substituents
Chiral Compound: Linalool
Beta-Cyclodextrin Phases
Derivatized with various functional groups
Limonene on Rt-βDEXse

**Column:**
30m x 0.32mm x 0.25µm
Rt-βDEXse

**Oven temperature:**
40°C (1 min.) 2°/min to 230°C

**Linear velocity:**
80 cm/sec with hydrogen carrier
Chiral compounds on Rt-βDEXse

ethyl 2-methylbutyrate

R S

styrene oxide

camphor
Rose oxides and $\beta$-citronellol
Commercial Geranium Oil

**Column:** $\text{Rt-} \beta\text{DEXsa}$
30m x .25mm x .25µm

**Carrier:** Hydrogen @ 40cm/sec

**Oven temperature program:** 60°C 1°/min to 110°C; GC-FID
Commercial Geranium Oil

Column: Rt-βDEXsa
30m x .25mm x .25µm

rose oxides
linalool
β-citronellol
Summary of Flavor & Fragrance Analysis

- Chromatography is a Powerful Tool for Flavor & Fragrance Analyses
  - Headspace GC for volatiles
  - HPLC for less thermally stable compounds

- What is the Goal of the Analysis?
  - Evaluate a flavor or fragrance material
  - Identify an off flavor