UHPLC on any HPLC system for Food Analysis

Dr. Frank Michel
Frank.Michel@sial.com
History of HPLC particle design

Irregular

Difficult to pack, clogging, not very robust

Total Porous

Current state-of-the-art in HPLC
Efficiency:
\[ N = \frac{L_{\text{col}}}{H} \Leftrightarrow H = \frac{L_{\text{col}}}{N} \]
van Deemter equation:
\[ H = A + \frac{B}{u} + Cu \]

\[ A = 2\lambda d_P \]

From: Veronika R. Meyer, Practical High-Performance Liquid Chromatography
Efficiency:
\[ N = \frac{L_{\text{col}}}{H} \iff H = \frac{L_{\text{col}}}{N} \]
van Deemter equation:
\[ H = A + \frac{B}{u} + Cu \]

\[ B = 2\gamma D_m \]
Efficiency:
\[ N = \frac{L_{\text{col}}}{H} \iff H = \frac{L_{\text{col}}}{N} \]
van Deemter equation:
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\[ C = (f_1 d_p^2 + f_2 d_f^2) \]
\[ B = 2\gamma D_m \]
Efficiency:
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van Deemter equation:
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\[ A = 2\lambda d_p \]

\[ C = (f_1d_P^2 + f_2d_f^2) \]

\[ B = 2\gamma D_m \]
van Deemter curves for different particle sizes

\[
H = A + \frac{B}{u} + C u
\]

Pressure (psi) vs. HETP (\(\mu m\))

Mobile phase velocity (mm/sec)
Particle size: Influence on efficiency and pressure

Doubling the efficiency by halving the particle size results in a pressure increase by a factor of four.

\[ N \propto \frac{1}{d_p} \]

\[ P \propto \frac{1}{d_p^2} \]

<table>
<thead>
<tr>
<th>Particle (µm)</th>
<th>psi</th>
<th>bar</th>
<th>N</th>
</tr>
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<tbody>
<tr>
<td>1.8</td>
<td>5889</td>
<td>406</td>
<td>27,500</td>
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<tr>
<td>2.5</td>
<td>3089</td>
<td>213</td>
<td>20,000</td>
</tr>
<tr>
<td>3</td>
<td>2118</td>
<td>146</td>
<td>16,500</td>
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<td>5</td>
<td>769</td>
<td>53</td>
<td>10,000</td>
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<tr>
<td>10</td>
<td>189</td>
<td>13</td>
<td>5,000</td>
</tr>
<tr>
<td>15</td>
<td>87</td>
<td>6</td>
<td>3,750</td>
</tr>
<tr>
<td>20</td>
<td>44</td>
<td>3</td>
<td>2,500</td>
</tr>
</tbody>
</table>

10 cm column, 3 mm/s linear velocity
Pellicular particles do have only a thin porous skin (low capacity)

Irregular

Difficult to pack, clogging, not very robust

Total Porous

Current state-of-the-art in HPLC

Pellicular

The NEW technology

Fused-Core™

Fused-Core is a trademark of Advanced Materials Technology, Inc.
Fused-Core technology

Innovative approach in HPLC by Dr. J. J. Kirkland in 2007
Porous silica with high capacity on solid, non-porous core
Highly pure silica

- 2.7 µm silica particle
- 1.7 µm solid core
- 0.5 µm porous SiO$_2$ layer
- 90 Å pore size
- Very narrow particle size distribution
- C18, C8, HILIC (Si and OH5), RP-Amide, Phenyl-Hexyl, Peptide ES C18, PFP (F5)
Fused-Core technology

Innovative approach in HPLC by Dr. J. J. Kirkland in 2007
Porous silica with high capacity on solid, non-porous core
Highly pure silica

- 2.7 µm silica particle
- 1.7 µm solid core
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Fused-Core provides higher efficiency

The shorter diffusion pathway facilitates the mass transfer (C term)!
Fused-Core provides higher efficiency

Narrow particle size distribution!
- Bed uniformity (better A term)
- Larger frits (column lifetime)

Fused-Core particle size distribution
Average = 2.77 µm;
standard deviation = 6% of mean

Particle size distribution of a typical commercial totally porous packing
Average = 3.78 µm;
standard deviation = 19% of mean

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Comparison of pressure and efficiency

\[ H = A + \frac{B}{u} + Cu \]

\[ \Delta P = \frac{1000F\eta L}{\pi r^2 d_p^2} \]

*50x4.6 mm columns, 55/45 ACN/water*
Fused-Core technology – higher efficiency

Agilent 1200
Ascentis Express C18, 15cm x 4.6mm, 2.7 µm
1.0mL/min, 254nm, RT, 10uL inj.

4. Toluene N = 30,738
3. Benzene N = 31,696
2. Acetophenone N = 33,786
1. Uracil (marker for void time)
Pressure = 183 bar (2690 psi)

How much efficiency is possible on a normal HPLC system?
Twice the efficiency compared to 3 µm at the same back pressure

Ascentis Express C18, 2.7 µm
65 % ACN
N = 237,700 N/m, N = 35,655 N/col.
Pressure = approx. 4,000 psi

Usual C18, 3 µm
72.5 % ACN
N = 140,600 N/m, N = 21,090 N/col.
Pressure = approx. 4,000 psi

1. p-Hydroxy ethylbenzene
2. Napthalene
3. p-Xylene
4. Biphenyl

Agilent 1100 HPLC system
Column: 150 x 4.6 mm
Mobile phase: ACN/water
Flow rate: 1.5 mL/min
Injection: 2.0 µL
Detection: 220 nm
TO11/IP6A Carbonyl DNPH Mix

Ascentis Express C18, 2.7 µm
Peak 8
N = 260,720 p/m
N = 39,108 p/col

Sensitivity gap

Ascentis C18, 3 µm
Peak 8
N = 146,587 p/m
N = 21,988 p/col

Column: 150 x 4.6 mm I.D.
Mobile phase:
Ascentis Express C18 2.7 µm: 25:75, water: acetonitrile
Ascentis C18 3 µm: 30:70, water: acetonitrile
Flow rate: 1.0 mL/min.
Temp.: 30 °C
Det.: UV at 365 nm
Injection: 1 µL
Sample: 47285-U TO11/IP6A Carbonyl-DNPH Mix as indicated below in 40:60, water: acetonitrile

Peak IDs
1. Formaldehyde-2,4-DNPH (105 µg/mL)
2. Acetaldehyde-2,4-DNPH (76.4 µg/mL)
3. Acrolein-2,4-DNPH (63.2 µg/mL)
4. Acetone-2,4-DNPH (61.5 µg/mL)
5. Propionaldehyde-2,4-DNPH (61.5 µg/mL)
6. Crotonaldehyde-2,4-DNPH (53.6 µg/mL)
7. Butyraldehyde-2,4-DNPH (52.5 µg/mL)
8. Benzaldehyde-2,4-DNPH (40.5 µg/mL)
9. Isovaleraldehyde-2,4-DNPH (46.4 µg/mL)
10. Valeraldehyde-2,4-DNPH (46.4 µg/mL)
11. o-Tolualdehyde-2,4-DNPH (37.5 µg/mL)
12. m-Tolualdehyde-2,4-DNPH (37.5 µg/mL)
13. p-Tolualdehyde-2,4-DNPH (37.5 µg/mL)
14. Hexaldehyde-2,4-DNPH (42 µg/mL)
15. 2,5-Dimethylbenzaldehyde-2,4-DNPH (35 µg/mL)
Same efficiency compared to sub 2µm particles

Ascentis Express C18

0.3 mL/min
45 % acetonitrile
2130 psi
N = 12,500

Sub-2 µm Column 2

0.3 mL/min
51 % acetonitrile.
7000 psi
N = 12,170

Mobile Phase: water : acetonitrile; isoelutropic for β-Estradiol
Columns: 100 x 2.1 mm
Flow: variable
Det: 200 nm
Inj: 1µL
Elution order:
1. Estriol
2. β-Estradiol
3. Contaminant
4. Estrone
5. Estrone degradant
Increasing speed on traditional HPLC systems*

Conventional C18
25 cm x 4.6 mm I.D., 5 µm
1.0 mL/min., N = 22147
Pressure: 128 bar (1880 psi)

Ascentis Express C18
10 cm x 4.6 mm I.D., 2.7 µm
1.0 mL/min., N = 22694
Pressure: 167 bar (2450 psi)

Ascentis Express C18
10 cm x 4.6 mm I.D., 2.7 µm
1.5 mL/min., N = 21297
Pressure: 248 bar (3645 psi)

Requirement on method: N >20,000

*Agilent 1100 HPLC System
Increasing speed on traditional HPLC systems*

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*Agilent 1100 HPLC System

**Requirement on method: N > 20,000**
Determination of melamine in milk

Issue: illegal addition of melamine to milk to artificially increase the nitrogen content
Sample preparation: extraction and purification by Discovery DSC-SCX SPE tubes
Analytical method: Ascentis Express HILIC for separation of melamine and related products from milk with mass spectrometric detection.
LC-MS-MS Analysis of Melamine Extracted from Milk Spiked at 100 ng/mL at Two Transitions

MRMs (127/85 & 127/68 m/z) – MRM 127/85 was used for quantitation

Melamine and Related Hydrolysis Products

Melamine

[M+H]+ = 127.072671 Da
Monoisotopic Mass = 126.065394 Da

Cyanuric Acid

[M-H]- = 128.010165 Da
Monoisotopic Mass = 129.017441 Da

Ammelide

[M+H]+ = 129.040702 Da
Monoisotopic Mass = 128.033425 Da

Ammeline

[M+H]+ = 128.056686 Da
Monoisotopic Mass = 127.04941 Da
Results: Melamine and Hydrolysis Products on Ascentis Express HILC with MS Detection

Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles
Det.: MS(+), blue MS(-) full scan mode

1. Cyanuric acid
2. Melamine
3. Ammelide
4. Ammeline
Determination of illegal food dyes

References
Determination of illegal food dyes

Peak ID:
1. Parafuchsin
2. Basic Fuchsin
3. Methylfuchsin
4. New Fuchsin
5. Malachite Green
6. Sudan III
7. Sudan 410
Determination of illegal food dyes

Column: Ascentis Express C8, 100 x 4.6 mm
Flow rate: 0.8 mL/min
Temp: 55 °C
Detection: UV DAD: 200 – 950 nm
MS: ESI(+), SPS target 500 m/z
Inj. volume: 3 µL
Mob. Phase: (A) water with 0.1 % formic acid
(B) acetonitrile:methanol (90:10)

<table>
<thead>
<tr>
<th>Time</th>
<th>%A</th>
<th>%B</th>
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<tbody>
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<td>25</td>
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<tr>
<td>1.5</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>15.0</td>
<td>2</td>
<td>98</td>
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<td>22.0</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>25.0</td>
<td>75</td>
<td>25</td>
</tr>
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</table>
Intra-Lab Validation of QuEChERS/HPLC

HPLC

- Application of Fused-Core-Particle technology
- Stationary Phase: RP-Amide instead of C18

Enio Belotti, Luca Meni, Marco Ruggeri, Water&Life Entratico (BG) Italy
Intra-Lab Validation of QuEChERS/HPLC

According EU Guideline SANCO/3131/2007 (Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Foods and Feeds)

- Pear: example for high sugar content
- Kiwi: example for low pH
- Salad: example for high chlorophyll content
- Corn meal: example for low water content

Standard solution in ACN extracts of pesticide free samples

Calibration with 29 pesticides from different classes such as acaricides, fungicides or insecticides, concentration range 0.006 mg/kg (<LOQ) to 1 mg/kg (MRL)

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Intra-Lab Validation of QuEChERS/HPLC

Calibration curve for Ethoprophos in extract of salad
IS: Ethyltriphasphate (acc. EN 15662)

Enio Belotti, Luca Meni, Marco Ruggeri, Water&Life Entratico (BG) Italy
Intra-Lab Validation of QuEChERS/HPLC

Column: Ascentis Express RP Amide, 10 cm x 2.1 mm ID
HPLC: Shimadzu Prominence UFLC XR
MS/MS: Applied Biosystems API 3200
Mobile Phase A: NH₄HCO₂ in H₂O (5 mmol/L, 0.1 % formic acid)
Mobile Phase B: NH₄HCO₂ in MeOH (5 mmol/L, 0.1 % formic acid)
Temp.: 40 °C
Gradient:

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<th>Time (min)</th>
<th>Mobile Phase A %</th>
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<tr>
<td>0.5</td>
<td>90</td>
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<tr>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>

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Intra-Lab Validation of QuEChERS/HPLC

New method:
- High recovery
- Reproducibility

HPLC:
- Resolution
- Robustness

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rec %</th>
<th>EN 15662 Rec %</th>
<th>RSD %</th>
<th>EN 15662 RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abamectina b1a NH-4</td>
<td>95.4</td>
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<td>Abamectina b1b NH-4</td>
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<td>Acetamiprid</td>
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<td>12.2</td>
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<tr>
<td>Aldicarb</td>
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<td>5.9</td>
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<td>Azoxystrobin</td>
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<td>Buprofezin</td>
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<td>Carbendazim</td>
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<td>Carbofuran</td>
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<td>Ethoprophos</td>
<td>96.5</td>
<td>3.0</td>
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</tbody>
</table>

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Summary

Fused-Core particle provide a „kinetic advantage“

• Higher efficiency on any HPLC system
  – Twice the efficiency compared to 3 µm particles
  – Thrice the efficiency compared to 5 µm particles
  – Half backpressure compared to sub-2 µm particles
  => Increase of the column length for increased efficiency,
     Can be used on any HPLC system

• Shorter analysis times maintaining the efficiency
  – Same efficiency like 3 µm columns at half column length
  – Same efficiency like 5 µm columns at one third of column length
  – Same speed and efficiency like sub-2 µm columns at half
    backpressure => Increase of flow rate and speed

• Ruggedness and durability as known from 5 µm columns
Dziękuję za uwagę!