More Speed, Resolution and Sensitivity in HPLC & LC-MS

Without Investing in Capital Equipment
Overview of Presentation

HPLC Innovations:
- Particle technology
- Bonded phase selectivity
- Extended pH range

LC-MS and MALDI-MS:
- Chiral LC-MS → subject of later presentation
- Reducing matrix effects and ion suppression → subject of later presentation
- Solvents and additives
- Ionic liquids
HPLC Innovations

- Particle technology - Ascentis® Express with Fused-Core™ particles
- Bonded phase selectivity
- Extended pH range

Users are.....
- HPLC and LC-MS analysts

Interested in...
- Decreasing run time
- Increasing resolution
- Increasing sensitivity
- Using existing HPLC systems
Recent HPLC Innovations & the Rationale Behind Them

HPLC particle manufacturers have been focusing their product development on ways to increase sample throughput by decreasing analysis time.

- **4 min. (conventional particles)**
- **30 sec.**
- **16 sec.**

New technologies have led to significant improvements in analysis time.
Fundamentals: Resolution Equation

Innovations related to improving resolution and sensitivity leverage one or more variables in the Resolution Equation

\[ R = \frac{\sqrt{N}}{4} \cdot \frac{k}{k+1} \cdot \frac{\alpha-1}{\alpha} \]

Challenge: How to decrease run time, but maintain your separation (k, \( \alpha \) and total N)?

Conventional approaches (5 and 3 \( \mu \)m particles):
1. Decrease column length, but this reduces plates (\( N \propto L \))
2. Increase flow rate, but this causes high pressure and reduces N
Industry Response to HPLC User Needs: Two Particle Innovations

2004: UHPLC
In order to achieve faster separations, UHPLC technology was introduced. UHPLC comprises:
- Small particles (sub-2 µm) generate higher N
  - Smaller particles allow you to maintain the same number of plates required for a given separation on a shorter column
- Instruments that provide the pressure required to drive these separations
  - Smaller particles also require pressures that go beyond the capabilities of a conventional HPLC system

2007 to today: Fused-Core
A new and proven particle innovation allows the use of columns packed with Fused-Core particles to achieve the same efficiency and speed advantages as UHPLC, but at ½ the pressure, and on conventional instruments.
Innovations in Silica Particles Have Boosted Efficiency

Why the steady trend to smaller particles?
- Smaller particles provide higher efficiencies

However, smaller particles generate higher pressure
- Unique particle morphologies with lower pressure are becoming important

<table>
<thead>
<tr>
<th>Year</th>
<th>Diameter</th>
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<tr>
<td>1970s</td>
<td>10µm</td>
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<tr>
<td>1980s</td>
<td>5µm</td>
</tr>
<tr>
<td>1990s</td>
<td>3µm</td>
</tr>
<tr>
<td>2004</td>
<td>Sub-2µm</td>
</tr>
<tr>
<td>2007</td>
<td>2.7µm Fused-Core</td>
</tr>
</tbody>
</table>
Fused-Core: A New HPLC Particle Concept

The innovation behind Ascentis Express

- High-capacity layer of colloidal silica fused to non-permeable silica core.
- Efficiency of sub-2 µm porous particles.
- Low column back-pressures, comparable to 3 µm particles.
- Ruggedness of 5 µm columns; easy to prepare and resistant to blockage (2 µm frits).
- Use either traditional or ultra-HPLC instruments.
- Extend performance of UHPLC to longer columns and get even higher N.
Fused-Core Particles: Comparison of Morphology to Porous 3 µm Particles

Short diffusion path and uniform particle distribution minimize peak broadening and result in extremely high performance, comparable to sub-2 µm particles, but at much lower pressure. Higher flows and longer columns are possible.
Fused-Core Features & Benefits

Summary

• Features:
  1. Unique particle morphology
  2. ~ 3 µm particle size
  3. Tight particle size distribution

• Benefits:
  1. Efficiency equal to sub-2 µm particles
  2. Maintains efficiency at high flow rates
  3. One-half the pressure of sub-2 µm particles
  4. Uses conventional HPLC instruments (e.g. what you currently have)
  5. Even higher efficiency possible than sub-2 µm particles by using longer columns
Evolution of Improvement: Efficiency & Pressure vs. Particle

- **10 µm particles**
  - \( N_{\text{ave}} = 6,000 \)
  - \( P = 80 \text{ bar} \)

- **5 µm particles**
  - \( N_{\text{ave}} = 12,000 \)
  - \( P = 150 \text{ bar} \)

- **3 µm particles**
  - \( N_{\text{ave}} = 18,000 \)
  - \( P = 250 \text{ bar} \)

- **2.7 µm Fused-Core sub-2 µm**
  - \( N_{\text{ave}} = 36,000 \)
  - \( P = 285 \text{ bar (Fused-Core)} \)
  - \( P = \sim 600 \text{ bar (sub 2-µm)*} \)

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11 Columns: 15 cm x 4.6 mm I.D., Flow: 1.8 mL/min., Temp.: 30 °C, o- and p-xylene

*Estimated P for the sub-2 µm since 15 cm L not supplied
Fundamentals: van Deemter Equation

Efficiency in HPLC is described by the **van Deemter Equation** (where $H$ is plate height)

$$H = A + \frac{B}{u} + Cu$$

$$N = \frac{L}{H}$$

**A Term** – Particle bed uniformity (how well packed the column is)

**B Term** – Diffusion along column (how long the zone is on the column)

**C Term** – Diffusion within particle (rate of mass transfer in/out of pores and phase)

Average Linear Velocity ($v$)

$$v = \frac{L}{t_0}$$
Benefits of Tight Particle Size Distribution

Improves the A-term of the van Deemter equation

Can use 2 µm frits:
- Extremely rugged & resistant to plugging
- Sub-2µm particles typically require 0.2 µm frits

Ascentis Express: 2.7 µm +/- 6%

Typical porous silica particle: Average +/- 19%
Ascentis Express Column Technology

Fact: Pressure rises faster than efficiency and resolution as particle size is reduced

Challenge: Achieve sub-2µm efficiency at 3µm pressures

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

\[ P \propto \frac{1}{d_p^2} \]
Adverse Effect of Pressure: Increase and Non-Uniform Pressure

van’t Hoff relationship

Pressure \( \propto \) temperature, higher pressure will lead to higher temperature

Temperature does not affect all analytes equally, so selectivity differences may occur

C18 column, 10 cm x 4.6 mm (45:55) 8.7mM ammonium phosphate, pH 6.5:methanol 1.5 mL/min. 220nm

Crossover at 45°C
Ultra-High Efficiencies at 3µm Pressures

Agilent 1200
Ascentis Express C18, 15 cm x 4.6 mm, 2.7 µm
Mobile Phase: 40/60 water/ACN
Flow: 1.0 mL/min.,
Detection: 254 nm/10 mm/13 µL
Injection: 10 µL in mobile phase
Pressure: 183 bar (2690 psi)

1. Uracil \( (T_0 \text{ marker}) \)
2. Acetophenone \( N_{\text{obs}} = 33,786 \ (225K \text{ pl/m}) \)
3. Benzene \( N_{\text{obs}} = 31,696 \ (211K \text{ pl/m}) \)
4. Toluene \( N_{\text{obs}} = 30,738 \ (205K \text{ pl/m}) \)

\( N_{\text{col}} = 36,000 \) (at optimum velocity with 1µL injection)

With high pressure UHPLC systems:

- Either increase linear velocity and go much faster with minimal or no loss of \( N \).
- Or use even longer columns, generate more plates, and achieve much higher resolution and peak capacity.
HETP measured for toluene vs. the superficial velocity of the mobile phase on a C18, 15 cm x 4.6 mm, 2.7 µm Ascentis Express column, at a temperature of +35 °C. Uracil was taken as unretained compound, to calculate the column breakthrough time. Elution: 30:70 water:acetonitrile.

An H of 4µm equals 250,000 plates per meter.

Data provided by Prof. Luigi Mondello, U. Messina, Messina, Italy.
Ascentis Express: High Efficiency and Low Pressure on Conventional HPLC Instruments

Ascentis Express C18
10 cm x 4.6 mm, 2.7 µm
Pressure: 248 bar
N = 21,300

Sub-2 µm C18
5 cm x 4.6 mm, 1.8 µm
Pressure: 310 bar
N = 12,800

More efficiency near the practical system limit on conventional instruments (e.g. Agilent 1100)

Ascentis Express permits:
• Longer columns for higher N
• Higher flow rates for faster runs

Because not at $P_{\text{max}}$:
• Even more speed possible by increasing flow rate
• Even more efficiency possible by increasing column length

Increased speed or N not possible on sub-2 µm column due to pressure

Agilent 1100 (400 bar limit)
Mobile Phase: 35:65 ACN/Water
Flow Rate: 1.5 mL/min.
Detection: 254 nm
Injection: 5 µL
Ascentis Express Columns Maintain High Sample Capacity

Reversed-Phase Sample Loading (Butyl Paraben, $k \sim 3$)

60/40 Methanol/ 20 mM sodium phosphate, pH = 7.0
40 degrees C., 1.5 ml/min, 4.6 x 50 mm C18 columns

Theoretical Plates

Linear over several orders of magnitude

Micrograms Injected (in 5uL)

Fused-Core, 2.7 µm
Porous silica, 3 µm
Ascentis Express Ultra High Efficiency
Also Improves Sensitivity

Linear range improves at trace levels

Injection: 1 μL of 0.01 mg/mL = 10 ng
All chromatograms same x- and y-scale, 220 nm

2.7 μm Ascentis Express
3 μm
5 μm
Scaling Method to Fast LC on Traditional HPLC Systems

5 µm C18, 15 cm x 3 mm ID

- Column: 5 µm C18; 15 cm x 3 mm
- Flow: 0.4 mL/min. (~1.5 mL total volume per run)
- Mobile Phase: 20:80, water : acetonitrile
- Inj: 1.5 µL
- Temp: 35°C
- Pressure: 885 psi (61 bar)
- $N_{(naphthalene)}$: ~11,000
- $k_{(naphthalene)}$: 1.78

At least 4-fold increase in throughput with 3-fold solvent-saving due to short column length.

Ascentis Express, 5 cm x 3 mm ID

- Column: Ascentis Express C18; 5 cm x 3 mm
- Flow: 0.6 mL/min. (~0.5 mL total volume per run)
- Mobile Phase: 31:69, water:acetonitrile
- Inj: 0.5 µL
- Temp: 35°C
- Pressure: 1750 psi (121 bar)
- $N_{(naphthalene)}$: ~11,000
- $k_{(naphthalene)}$: 1.75

0.5 mL total run volume
Virtually No Resolution Loss at 4X Normal Flow Rate: Benefit of Flat van Deemter

**Column:** Ascentis Express C18; 5 cm x 3 mm  
**Flow:** 1.2 mL/min.  
**Mobile Phase:** 31:69, water:acetonitrile  
**Inj:** 0.5 µL  
**Temp:** 35°C  
**Pressure:** 3700 psi (255 bar) (consider move to UHPLC)  
**Rs\(_{(phenol/acet.)}\):** 3.1

9-fold increase in throughput compared to the original method on 5µm particle.

**Column:** Ascentis Express C18; 5 cm x 3 mm  
**Flow:** 2.0 mL/min. (~ 4 times normal flow)  
**Mobile Phase:** 31:69, water:acetonitrile  
**Inj:** 0.5 µL  
**Temp:** 35°C  
**Pressure:** 6400 psi (441 bar) (requires UHPLC instrument)  
**Rs\(_{(phenol/acet.)}\):** 2.8

15-fold increase in throughput compared to the original method on 5 µm particle (not possible with 5 or 3 µm due to loss of N).
Ascentis Express Chosen for High Speed LC-MS DMPK Studies*

Express columns chosen by GSK group for ruggedness and performance

The authors conclude: “The partially porous stationary phase material has demonstrated equivalent resolving power to sub-2µm materials under the ballistic gradient chromatography conditions employed, and shown to exhibit excellent resilience and performance over the analysis of thousands of protein precipitated plasma extracts, suggesting that this type of column is a valuable tool for pharmaceutical bioanalysts.”

Ballistic Gradients Possible on Ascentis Express

column: Ascentis Express C18 2 cm x 2.1 mm ID
mobile phase A: 10mM ammonium formate pH 4.0
mobile phase B: 10mM ammonium formate (95:5 acetonitrile:water) pH 4.3
temp.: ambient
det.: ESI+, LC/MS-TOF
injection: 2 µL

<table>
<thead>
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<th>gradient:</th>
<th>time</th>
<th>%A</th>
<th>%B</th>
<th>flow</th>
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<td>2.51</td>
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<tr>
<td>3.5</td>
<td>80</td>
<td>20</td>
<td></td>
<td>0.4</td>
</tr>
</tbody>
</table>

Sample (0.2 µg/mL ea.)
1. Mesoridazine
2. Doxepin
3. Desipramine
4. Losartan
5. Clomipramine
Human Serum Albumin Tryptic Digest (5 micron vs. Fused-Core)

column: 15 cm x 4.6 mm I.D.
mobile phase A: 100% water with 0.1% TFA
mobile phase B: 50:50, ACN:water, with 0.1% TFA
flow rate: 1.0 mL/min.; temp: 35 °C
det: UV at 215 nm
injection: 20 µL
gradient: 0 to 100 %B in 80 min. (linear), hold for 5 min.
Human Serum Albumin Tryptic Digest (exploded view 25-45 min)

~ 80 peaks

Ascentis Express C18

~ 40 peaks

Conventional C18
Ascentis Express Columns Installed in Waters Acquity®
Ascentis Express Column in Agilent 1200
High Performance HPLC Fittings

Permit use of Ascentis Express on any HPLC or UHPLC system
Work with other manufacturers columns
Ascentis Express Benefits

1. The same efficiency as sub-2 μm particles, but ½ the pressure
2. The same pressure as 3 μm particles, but twice the efficiency
3. Compatible with all HPLC and UHPLC instruments

Benefits over sub-2 μm porous particles:
- Uses conventional HPLC instruments
- Lower backpressure
- Longer columns permit higher efficiency
- More rugged

Benefits over 3 and 5 μm porous particles:
- Shorter run times than 5 μm (3xN) and 3 μm (2xN) particles without sacrificing resolution
- Shorter columns have same efficiency (N) and reduce solvent consumption
- Higher resolution than 5 μm and 3 μm columns of same length
Ascentis Express Columns

Phases:
- Ascentis Express C18
- Ascentis Express C8
- Ascentis Express RP-Amide
- Ascentis Express Phenyl-Hexyl
- Ascentis Express HILIC

Dimensions (Columns):
- 2.1, 3 & 4.6 mm ID
- 2, 3, 5, 7.5, 10 & 15 cm L

Dimensions (Capillaries):
- 75, 100, 200, 300 & 500 µm ID
- 5 & 15 cm L

High performance fittings

http://www.sigma-aldrich.com/express
HPLC Innovations

• Particle technology – Ascentis Express
• Bonded phase selectivity – Ascentis phases
• Extended pH range

Users are...
• HPLC and LC-MS analysts

Interested in...
• Increasing resolution
• Increasing or decreasing retention
• Using existing HPLC systems

Users can expect...
• Different elution patterns
• Optimized retention and resolution
• “Greener” mobile phases
The Power of Stationary Phase Selectivity

Two reversed-phase columns, same MP conditions
Different retention profiles

Leverage bonded phase chemistry to alter retention and/or selectivity
Selectivity is Still the Most Powerful Term in the Resolution Equation

- Selectivity ($\alpha$) has the greatest effect on resolution ($R$)
- Its effect is not as limited as the effect of efficiency ($N$) and retention ($k$)
- Selectivity is altered by:
  - Stationary phase
  - Mobile phase
  - Temperature

\[ R = \frac{\sqrt{N}}{4} \cdot \frac{k}{k+1} \cdot \frac{\alpha-1}{\alpha} \]

Examples of Selectivity Effect on Elution Profiles in RP Mode

Different bonded phases give different selectivity under the same conditions
- Affects resolution
- Affects quantitation and sensitivity

- Column: Ascentis Express phases 10 cm x 3 mm ID
- Mobile phase A: (65:35) water: acetonitrile
  - Flow rate: 0.6 mL/min.
  - Pressure: 3000 psi total, 2360 psi column
  - Temp.: 35 °C
  - Det.: UV, 250 nm
- Injection: 2 µL

Sample (0.1 mg/mL ea.)
1. Oxazepam
2. Alprazolam
3. Clonazepam
4. N-Desmethyl diazepam
5. Diazepam
# Column Selection Guide

<table>
<thead>
<tr>
<th>Compound Class / Interactions</th>
<th>Phase</th>
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<tr>
<td>hydrophobic, dispersive</td>
<td>C18</td>
</tr>
<tr>
<td>hydrophobic, dispersive</td>
<td>C8</td>
</tr>
<tr>
<td>acids, phenols, anilines, H-Bond donors</td>
<td>RP-Amide</td>
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<tr>
<td>hetero aromatic and pi-acceptors</td>
<td>Phenyl</td>
</tr>
<tr>
<td>mild pi-acids (electron pair acceptors)</td>
<td></td>
</tr>
<tr>
<td>bases, electron- and pi-donors</td>
<td>Pentafluorophenyl</td>
</tr>
<tr>
<td>highly polar</td>
<td>Silica, Cyano</td>
</tr>
</tbody>
</table>
RP Mode Column Selectivity (Orthogonality) Plots

- Compares retention ($k$) for the same compounds on two column phases.
- Use variety of analyte types to get global estimate.
- Different phases show lower correlation (widest scatter); defines orthogonality.
- Same phases show higher correlation.
HILIC Mode Column Selectivity

Benefits of HILIC:
- Retention of highly-polar analytes (e.g. metabolites)
- Complementary selectivity to RP-HPLC (often opposite order)
- Increases MS sensitivity
- Quick transfer from final steps of sample prep

HILIC application areas:
- Amino acids
- Small, polar acids (metabolomics)
- Biogenic amines
  - neurotransmitters, food/bev. contaminants
- Organophosphates (pesticides)
- Sugars
- Drug metabolites and conjugates

Ascentis Express HILIC or C18, 10 cm x 2.1 mm ID, (10:90) 100 mM ammonium formate, pH 3.0:acetonitrile. 0.4 mL/min., 35 °C; UV at 254 nm; 1 μL inj.
HPLC Bonded Phase Choices

Ascentis® Express (2.7 µm Fused-Core™ particles)
- C18
- C8
- RP-Amide
- Phenyl-Hexyl
- HILIC (silica)

Ascentis® (3, 5 and 10 µm)
- C18
- C8
- RP-Amide
- Phenyl
- Silica

Discovery® (3, 5 and 10 µm)
- HS-F5 (pentafluorophenyl)
- Cyano

SUPELCOSIL®

http://www.sigma-aldrich.com/ascentis
HPLC Innovations

- Particle technology – Ascentis Express
- Bonded phase selectivity – Ascentis phases
- Extended pH range – apHera HPLC Columns

Users are...
- HPLC and LC-MS analysts

Interested in...
- Using pH to alter resolution or retention
- Using existing HPLC systems

Users can expect...
- Different elution patterns
- Wider pH range
- Longer column lifetime
High pH RP-HPLC Separations on apHera

apHera particles
- Vinyl alcohol copolymer
- pH 2 – 12
- Higher efficiency than polystyrene particles
- 300 Å
- C4, C8, C18, NH2 bonded phase chemistries

Tricyclic Antidepressants at High pH on apHera C18

column: apHera C18, 15 cm x 4.6 mm I.D., 5 μm
mobile phase A: 10:90, (0.1M piperidine/HCl, pH 11.1):water
mobile phase B: 10:90, (0.1M piperidine/HCl, pH 11.1):acetonitrile
mobile phase mixing ratio: A:B = 40:60
flow rate: 0.6 mL/min.
temp.: 35 °C
det.: 215 nm
injection: 5 μL
sample: 50 μg/L ea. in 50:50, mobile phase A: methanol

1. Clomipramine
2. Amitriptyline
3. Imipramine
4. Norclomipramine
5. Doxepin
6. Nortriptyline
7. Desipramine
8. Nordoxepin
Sugar Analysis on apHera NH2

Higher efficiency and lower bleed than APS-silica

Columns: apHera NH2 or aminopropyl silica, 15 cm x 4.6 mm I.D., 5 µm particles
mobile phase: 20:80, water:acetonitrile
flow rate: 1.0 mL/min.
temp.: 25 °C
det.: ELSD, 45 °C, 3.5 psi nitrogen
injection: 10 µL
sample: 500 µg/mL in 30:70, water:acetonitrile

1. D(-)-Arabinose
2. D(+)Xylose
3. D(+)Mannose
4. D(+)Galactose
5. D(+)Glucose
apHera Products

High pH stability
Rugged operation
Phases:
- NH2
- C18
- C8
- C4
Analytical to preparative dimensions:
- 2 – 28 mm ID
- 1 – 30 cm L

http://www.sigma-aldrich.com/aphera
LC-MS and MALDI-MS Innovations

• Reducing matrix effects – HybridSPE®-PPT
• Chiral LC-MS – Astec CHIROBIOTIC CSPs
• Solvents and additives
• Ionic liquids for ESI and MALDI

Users are...
• LC-MS analysts

Interested in...
• Increasing MS sensitivity
• Increasing column lifetime

Users can expect...
• Reduced phospholipid interferences
• Enhanced column lifetime

HybridSPE Particles for Selective Phospholipid Removal

The phosphate groups on the phospholipids are strong Lewis bases (electron donors) and complex with the zirconium atoms on the particle surface.

The Zr atom acts as a Lewis acid (electron acceptor) because it has empty d-orbitals.
Effective Phospholipid Removal Prior to LC-MS

Almost 100% phospholipids in dog plasma were removed by HybridSPE technique, moderate phospholipids were removed by generic polymeric SPE method, and no phospholipids were removed by traditional PPT.
LC-MS and MALDI-MS Innovations

• Reducing matrix effects
• Chiral LC-MS – Astec CHIROBIOTIC® CSPs
• Solvents and additives
• Ionic liquids for ESI and MALDI

Users are...
• HPLC and LC-MS analysts
• Performing chiral HPLC separations

Interested in...
• Analyzing enantiomers by LC-MS

Users can expect...
• MS-friendly chiral separations
• Flexible MP operations

Covered in Talk #3 – “Practicing Chiral Chromatography in Your Mobile Phase Comfort Zone”
LC-MS and MALDI-MS Innovations

- Reducing matrix effects – HybridSPE-PPT
- Chiral LC-MS – Astec CHIROBIOTIC CSPs
- Solvents and additives – LC/MS CHROMASOLV
- Ionic liquids for ESI and MALDI

Users are...
- HPLC and LC-MS analysts

Interested in...
- Increased sensitivity
- Reduced instrument down-time
- Reducing MP preparation

Users can expect...
- Reduced cluster ion formation
- Better sensitivity
- Reduce MP prep time
Cluster Ion Formation in LC-MS

Mass spectra of human gastrin dissolved in 0.2% formic acid in water at high (left) and low (right) ppm alkali metal concentrations:

- Solvent impurities interfere with LC-MS sensitivity and reliability
- Also ideal as mobile phases for low-UV & as sample prep solvents

MS spectra in HPLC-grade solvent

MS spectra in LC-MS CHROMASOLV solvent
CHROMASOLV Solvents for LC-MS

Extremely high purity
Rigorous test specifications
• Metals, inorganic ions, particles
• Over 34 quality tests
Solvents:
• Water, Acetonitrile, Methanol, 2-Propanol, Ethyl Acetate
Additives:
• TFA, acetic acid, formic acid, propionic acid, ammonium acetate, ammonium formate, TFA, sodium citrate, ammonium hydroxide, ammonium bicarbonate
Blends:
• 0.1% acetic acid, formic acid, ammonium acetate, TFA and blends
• In water, acetonitrile or methanol

http://www.sigma-aldrich.com/chromasolv
LC-MS and MALDI-MS Innovations

- Chiral LC-MS – Astec CHIROBIOTIC CSPs
- Reducing matrix effects – HybridSPE-PPT
- Solvents and additives – LC/MS CHROMASOLV
- Ionic liquids for ESI and MALDI
Ionic Liquids for (+)ESI-MS of Anions (Ion Pairing) – Dication IL

1) Formation of the adduct between dication IL and perchlorate anion.

```
1,9-Nonandiyl-bis-(3-methylimidazolium) difluoride
m/z = +145.12 Da
```

```
+ ClO₄⁻
```

```
m/z = -98.95 Da
```

```
```

2) The resulting positive, singly-charged ion can be detected by (+) ESI with very high sensitivity.

```
```

```
m/z = +389.19 Da
```

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Ionic Liquids for (+)ESI-MS of Anions (Ion Pairing) – Trication IL

1) Formation of the adduct between trication IL and sulfate anion.

1,3-imidazolium-bis-(1-hexyl-benzyl-imidazolium)-trifluoride

\[ m/z = +183.20 \text{ Da} \]

\[ m/z = -47.97 \text{ Da} \]

2) The resulting positive, singly-charged ion can be detected by (+) ESI with very high sensitivity.

\[ m/z = +647.34 \text{ Da} \]
Ionic Liquids for MALDI-MS

Ionic liquids for MALDI:
- Nearly no vapor pressure, which leads to a better vacuum stability
- Clean spectra, for high resolution

1: Bradykinin
2: Angiotensin II
3: Angiotensin I
4: Bombesin
5: N-acetyl-Renin-substrate
6: ACTH, (1-17)
7: ACTH (18-39)
8: Somatostatin 28
M: Matrix

QIT-ToF peptide mass fingerprint spectra (stainless steel target)

From “Ionic Matrices for MALDI-QIT-ToF-MSn peptide sequencing and MALDI-ToF-MS peptide mass fingerprinting” T. Deierling, et al
Ionic Liquids for MALDI-MS

Ionic liquids for MALDI also form uniform coatings on the target.

Comparison of crystallization-pattern of DHB (conventional matrix) and CHCA-DE on stainless steel target

From “Ionic Matrices for MALDI-QIT-ToF-MSn peptide sequencing and MALDI-ToF-MS peptide mass fingerprinting” T. Deierling, et al
Ionic Liquids for LC-MS

Ready to use solutions with complete instructions

ESI-MS

- 1,9-Nonandiyl-bis-(3-methylimidazolium) difluoride; 5 mM in methanol:water (1:1) (75128)
- 1,3-imidazolium-bis-(1-hexyl-benzyl-imidazolium)-trifluoride; 5 mM in H₂O (08675)

MALDI-MS

- α-Cyano-4-hydroxycinnamic acid butylamine salt (CHCA-B, 67336)
- α-Cyano-4-hydroxycinnamic acid diethylamine salt (CHCA-DE, 55341)
Summary

HPLC Innovations:
- Particle technology – Ascentis® Express with Fused-Core™ Technology
- Bonded phase selectivity – Different phase chemistries of Ascentis and Ascentis Express
- Extended pH range – apHera

LC-MS and MALDI-MS:
- Chiral LC-MS – Astec chiral phases
- Ion suppression – HybridSPE™-PPT Technology
- Solvents and additives – LC-MS CHROMASOLV
- Ionic liquids – For MALDI and ESI-MS
Acknowledgements/Collaborators

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