

Echelon™ C18 Columns

- Wide pH range stability, pH 2-11
- Ideal for basic and acidic analytes
- No secondary Si-OH interaction
- High capacity: smaller particles = higher efficiency

Why is Echelon so good?

Echelon material has been designed from start to finish to optimize analysis of the most challenging basic and acidic compounds.

Pure silica means symmetric peaks.

Echelon silica is manufactured using the purest reagents available, in a process that excludes contaminants from the silica bead structure as it forms. Conventional silicas contain large amounts of metal ion (Fe, Al, Mg, Mn, Cr, Ti, and Ni) and sulfate contaminants, which reduce silica performance. Metal ions increase the acidity of surrounding silanols, promoting increased interaction with polar contaminants, like bases. With Echelon, metals are nonexistent, particularly at the silica surface where their effects are most deleterious. This resulting silica is extremely pure.

Sprite™ Echelon™ Direct Connect C18 HPLC Columns

- 10 to 40 x 2.1mm I.D. sizes
- Fast HPLC analysis
- LC-MS applications
- PEEK™ / titanium design
- Individually tested and certified

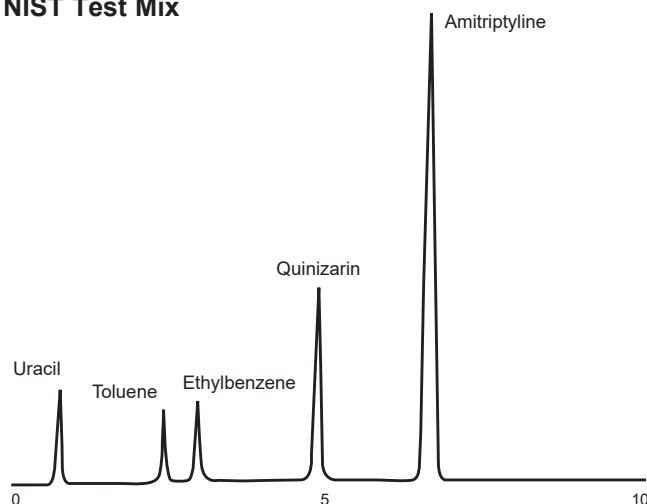


| Cat. No. | Description |
|---------------|------------------|
| Sprite AE1842 | 4.0cm x 2.1mm ID |
| Sprite AE1822 | 2.0cm x 2.1mm ID |
| Sprite AE1832 | 1.0cm x 2.1mm ID |

Advantage™ Echelon™ Specifications

Phase: C18
 Particle Size: 4µm and 5µm
 Pore Size: 100Å
 Pore Volume: 1.0mL/gm
 Surface Area: 430 m²/gm
 % Carbon: C18=18%
 End-Capping: Proprietary and extremely exhaustive
 Silica Class: Ultrapure Type B
 Hardware: PEEK™ & Stainless Steel Columns & Cartridges

NIST Test Mix



Catalog No.: ADV8002
Column: Echelon C18 150 x 4.6mm
Eluent: 80% MeOH/buffer
Flow: 1mL/min.

Quinizarin, a strong metal chelator, and amitriptyline, a very sensitive solute for residual silanols, both elute with good recovery and symmetrical peaks on Echelon.





Optimizing High-Throughput LC/MS/MS “trap-and-elute”

Brendon Kapinos¹, John Janiszewski¹, Mary Piotrowski¹, Hui Zhang¹, Carl...
¹Pfizer Global R&D, Groton, CT, ²Sound Analytics, Niantic, CT

Introduction

Rapid, robust bioanalysis is essential in modern drug discovery. Implementation of “trap-and-elute” LC enables high-throughput, high-quality analysis to drive drug discovery efforts.

The Apricot Designs Dual Arm (ADDA) autosampler was used to perform rapid “trap-and-elute” bioanalysis (10 seconds per injection).

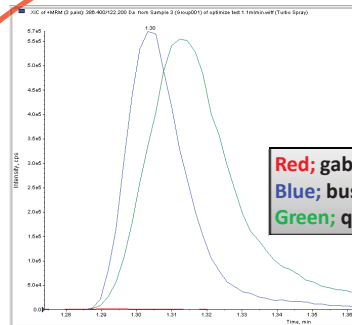
Further optimization of “trap-and-elute” parameters, including flow rate, valve timing, and columns of varying chemistries and dimensions can extend the application of this method.

Co-elution of analytes can result in ion suppression. We investigated this phenomenon using a cocktail of well-studied analytes, and pursued ways to reduce or eliminate this effect.

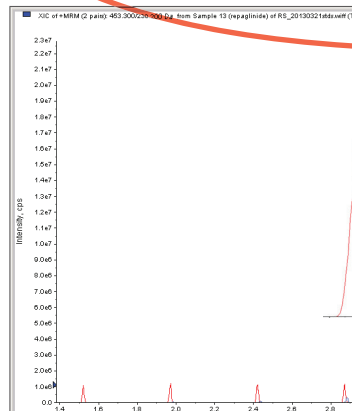
Standard vs. “Hybrid”

“Hybrid” trap-and-elute uses rapid... analytes and provide chromatog...

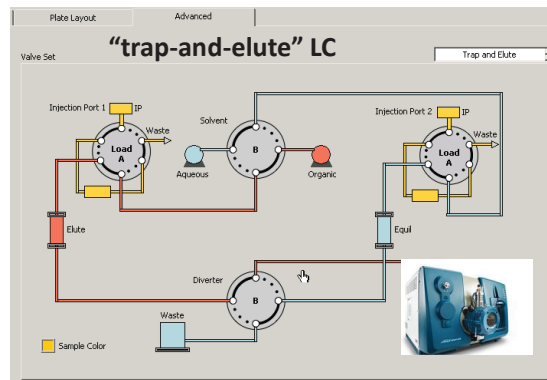
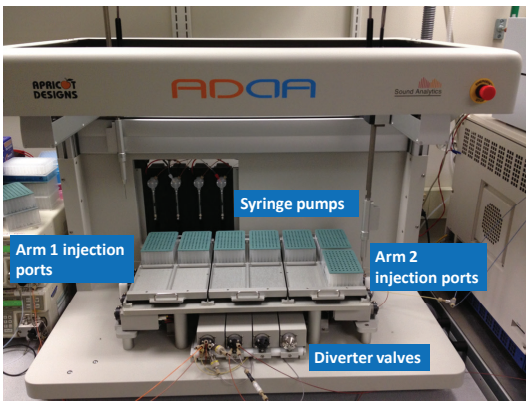
- Valve timing
- LC flow rate
- Mobile phase composition
- Column type



-Optimize Technologies 1.5x5mm trap...
 -1.2ml/min LC flow rate (standard trap...)



Materials and methods



Above: dual-arm “trap-and-elute” plumbing

- Aqueous mobile phase equilibrates one column, while organic phase elutes the other column to mass spectrometer
- Coordinated valve timing maximizes throughput (10sec/injection).

Apricot Designs Dual-Arm autosampler (ADDA) coupled to AB SCIEX API5500 was used for sample analysis.

“trap-and-elute” bioanalysis (described at right) was used to acquire all samples.

Mobile phase:

Aqueous: 95% 2mM ammonium formate, 5% 50/50 Methanol/Acetonitrile
 Organic: 45% ACN/55% 2mM ammonium formate

LC Flow rate:

1.2ml/min (standard “trap-and-elute”)
 1ml/min (“hybrid” trap-and-elute)

Columns:

-Optimize Technologies 1.5x5mm 13µ trap cartridge (standard “trap-and-elute”)
 -Analytical Sales and Services Sprite Echelon 10x2.1, 20x2.1, 30x2.1mm 4µ (“Hybrid” trap-and-elute)
 -Advanced Materials Technology HALO 5 20x2.1mm 5µ (“Hybrid” trap-and-elute)

| Valve Timing | | Detailed Valve Schedule | |
|--------------------|------|-------------------------|------------|
| Equilibrate | 8 s | Index | Time [sec] |
| DeSalt | 8 s | 1 | 0.00 |
| Sweep Time | 13 s | 2 | 8.00 |
| Dead Volume Offset | 0 s | 3 | 13.00 |
| | | 4 | 16.00 |
| | | 5 | 24.00 |
| | | 6 | 29.00 |
| | | 7 | 29.00 |
| | | 8 | 40.00 |
| | | 9 | 45.00 |
| | | 10 | 48.00 |
| | | 11 | 56.00 |
| | | 12 | 61.00 |
| | | 13 | 64.00 |
| | | 14 | 72.00 |
| | | 15 | 77.00 |
| | | 16 | 80.00 |
| | | 17 | 88.00 |
| | | 18 | 93.00 |
| | | 19 | 96.00 |

Summary of analytical

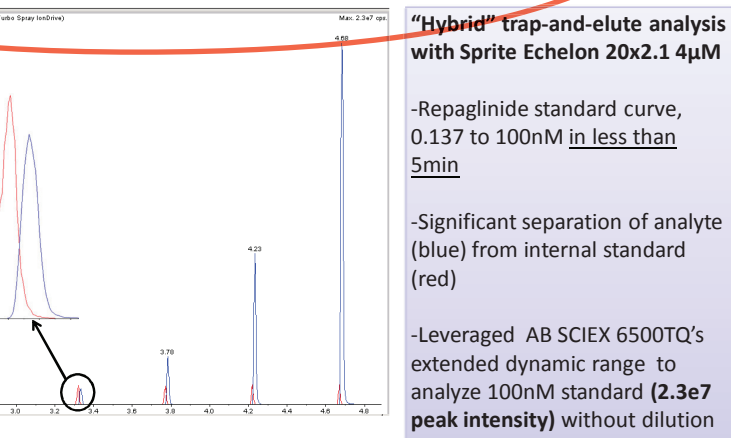
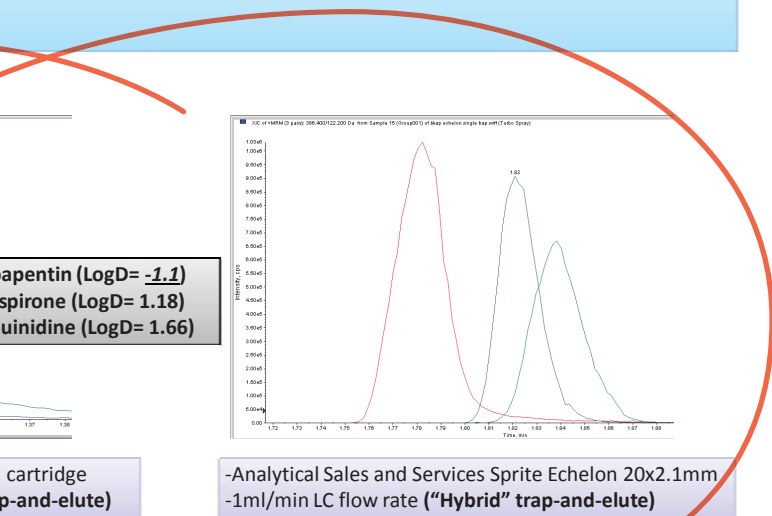
| Bioanalytical mode | Cycle time (sec/sample) |
|-------------------------|-------------------------|
| Standard trap-and-elute | 10-15 |
| “Hybrid” trap-and-elute | 30 |

“Trap-and-elute” Bioanalysis in Drug Discovery

Marie Funk¹, Wayne Lootsma², Will Schramm²

“Trap-and-elute”

Standard trap-and-elute analysis (30sec/sample) to increase retention of analyte through optimization of:



Optimal conditions

| Particle size | Column dimensions | |
|---------------|-------------------|---|
| 13µ | 1.5x5mm | Rapid “trap-and-elute” bioanalysis |
| 4-5µ | 20-40x2.1mm | <ul style="list-style-type: none"> “trap-and-elute” setup weaker eluent analytical column lower flow rate |

Alleviation of ion suppression due to co-elution

- Monitored ion suppression in standard “trap-and-elute” mode using propranolol and buspirone spiked with various concentrations of verapamil (5, 10, 20µM).
- Explored ways to reduce ion suppression, including using acidic mobile phase, increasing flow rate and Ion Spray voltage.
- Applied “Hybrid” trap-and-elute LC to attenuate ion suppression.

% Decrease in MS/MS response of propranolol and buspirone spiked with varying concentrations of verapamil (standard “trap-and-elute”)

| | [Verapamil, µM] | %Decrease in peak area compared to baseline | | |
|-------------|-----------------|---|---------------------|---------------------------|
| | | 0.01% FA mobile phase | 1.5ml/min flow rate | Ion Spray Voltage of 5500 |
| Propranolol | 5 | 71.3 | 70.2 | 58.5 |
| | 10 | 79.7 | 77.2 | 67 |
| | 20 | 84.8 | 82.4 | 75.3 |
| Buspirone | 5 | 70.3 | 73.4 | 61.5 |
| | 10 | 80.3 | 80.4 | 73.6 |
| | 20 | 86.1 | 85.9 | 81.6 |

% Decrease in MS/MS response of propranolol and buspirone spiked with varying concentrations of verapamil (Sprite Echelon 20x2.1 column, “Hybrid” trap-and-elute LC)

| | [Verapamil, µM] | % Decrease in peak area compared to baseline |
|-------------|-----------------|--|
| Propranolol | 5 | 0.7 |
| | 10 | 3.1 |
| | 20 | 15.0 |
| Buspirone | 5 | 4.1 |
| | 10 | 11.3 |
| | 20 | 25.4 |

Conclusions

- “Trap-and-elute” bioanalysis is a rugged, high-throughput method of sample analysis well-suited to drug discovery.
- Co-elution of high concentrations of analytes can produce ion suppression effects. Increasing Ion Spray voltage and, to a lesser extent LC flow rate, can reduce ion suppression.
- “Hybrid” trap-and-elute LC provides enhanced retention of polar molecules, separation of analytes and drastically reduced ion suppression while maintaining high throughput. Sprite Echelon and HALO 5 20x2.1 columns provided excellent retention and separation of analytes while retaining high throughput analysis.
- Further optimization of “Hybrid” trap-and-elute LC can improve retention, separation and increase sample throughput.

NEW! <2µm Columns

C18 100Å 1.7µm

| Cat. No. | Description |
|----------|--|
| ADV8106 | Echelon C18, 100Å, 1.7µm, 2cm x 2.1mm |
| ADV8107 | Echelon C18, 100Å, 1.7µm, 3cm x 2.1mm |
| ADV8108 | Echelon C18, 100Å, 1.7µm, 5cm x 2.1mm |
| ADV8117 | Echelon C18, 100Å, 1.7µm, 10cm x 2.1mm |

Echelon™ Citus™

5µm, 6-32 Threads for Capillary Tubing

| Cat. No. | Description |
|----------|-------------------|
| ET1637 | 3.0cm x 75µm ID |
| ET1657 | 5.0cm x 75µm ID |
| ET1617 | 10.0cm x 75µm ID |
| ET1675 | 15.0cm x 75µm ID |
| ET1627 | 25.0cm x 75µm ID |
| ET1631 | 3.0cm x 150µm ID |
| ET1651 | 5.0cm x 150µm ID |
| ET1611 | 10.0cm x 150µm ID |
| ET1615 | 15.0cm x 150µm ID |
| ET1625 | 25.0cm x 150µm ID |

Echelon™ Microbore Columns

C18 4µm

| Cat. No. | Description |
|------------|-------------------|
| ADV0303185 | 3.0cm x 0.3mm ID |
| ADV0503185 | 5.0cm x 0.3mm ID |
| ADV1003185 | 10.0cm x 0.3mm ID |
| ADV1503185 | 15.0cm x 0.3mm ID |
| ADV2503185 | 25.0cm x 0.3mm ID |
| ADV0305185 | 3.0cm x 0.5mm ID |
| ADV0505185 | 5.0cm x 0.5mm ID |
| ADV1005185 | 10.0cm x 0.5mm ID |
| ADV1505185 | 15.0cm x 0.5mm ID |
| ADV2505185 | 25.0cm x 0.5mm ID |
| ADV0310185 | 3.0cm x 1.0mm ID |
| ADV0510185 | 5.0cm x 1.0mm ID |
| ADV1010185 | 10.0cm x 1.0mm ID |
| ADV1510185 | 15.0cm x 1.0mm ID |
| ADV2510185 | 25.0cm x 1.0mm ID |

Echelon™ Analytical Columns

C18 4µm

| Cat. No. | Description |
|----------|-------------------|
| ADV8019 | 2.0cm x 2.1mm ID |
| ADV8021 | 3.0cm x 2.1mm ID |
| ADV8008 | 5.0cm x 2.1mm ID |
| ADV8009 | 10.0cm x 2.1mm ID |
| ADV8010 | 15.0cm x 2.1mm ID |
| ADV8011 | 25.0cm x 2.1mm ID |
| ADV8030 | 3.0cm x 3.0mm ID |
| ADV8004 | 5.0cm x 3.0mm ID |
| ADV8005 | 10.0cm x 3.0mm ID |
| ADV8006 | 15.0cm x 3.0mm ID |
| ADV8007 | 25.0cm x 3.0mm ID |
| ADV8029 | 3.0cm x 4.6mm ID |
| ADV8000 | 5.0cm x 4.6mm ID |
| ADV8001 | 10.0cm x 4.6mm ID |
| ADV8002 | 15.0cm x 4.6mm ID |
| ADV8003 | 25.0cm x 4.6mm ID |

NEW! 3µm Columns

C18 100Å

| Cat. No. | Description |
|----------|--------------------------------------|
| ADV8302 | Echelon C18, 100Å, 3µm, 20mm x 2.1mm |
| ADV8303 | Echelon C18, 100Å, 3µm, 30mm x 2.1mm |
| ADV8304 | Echelon C18, 100Å, 3µm, 50mm x 2.1mm |

Echelon™ Guard Cartridges & Holders

C8 & C18 Guard Cartridges: 4µm, 5/Pkg

| Cat. No. | Description |
|----------|--|
| ADV-E118 | C18 Stainless Steel Guard, 20mm x 3.2mm ID |
| ADV-E185 | C18 PEEK™ Guard Cartridge, 2cm x 2.1mm |

Fingertight Guard Holders for 20mm Columns

| Cat. No. | Description |
|-------------|----------------------|
| ADV-GuardFM | PEEK™ Guard Holder |
| ADV-GuardFF | Double Female Holder |



Echelon™ Semi-Prep & Prep Columns

C18 5µm

| Cat. No. | Description |
|-----------|------------------|
| ADV820510 | 5.0cm x 10mm ID |
| ADV821010 | 10.0cm x 10mm ID |
| ADV821510 | 15.0cm x 10mm ID |
| ADV822510 | 25.0cm x 10mm ID |
| ADV820520 | 5.0cm x 20mm ID |
| ADV821020 | 10.0cm x 20mm ID |
| ADV821520 | 15.0cm x 20mm ID |
| ADV822520 | 25.0cm x 20mm ID |



Echelon™ Prep & Semi-Prep Guard Cartridges & Holders

| Cat. No. | Description |
|--------------|--|
| ADV0220-73 | C18, 10µm, 25mm x 20mm ID, 2/Pkg |
| ADV20-HOLD25 | Prep Guard Cartridge Holder, 20mm |
| ADV0310-73 | C18, 10µm, 30mm x 10mm ID, 2/Pkg |
| ADV10-HOLD30 | Semi-Prep Guard Cartridge Holder, 10mm |

Direct Connect SS Pre-column Filter

- 5µl Dead Volume
- 0.5µm SS Frit
- Rated to 15,000 PSI



| Cat. No. | Description | Qty |
|----------|--|-----|
| 48815 | SS Direct Inline Filter Assemblies, 0.5µm Frit | 1 |
| 48001 | Replacement SS 0.5µm Frit | 5 |