

## 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

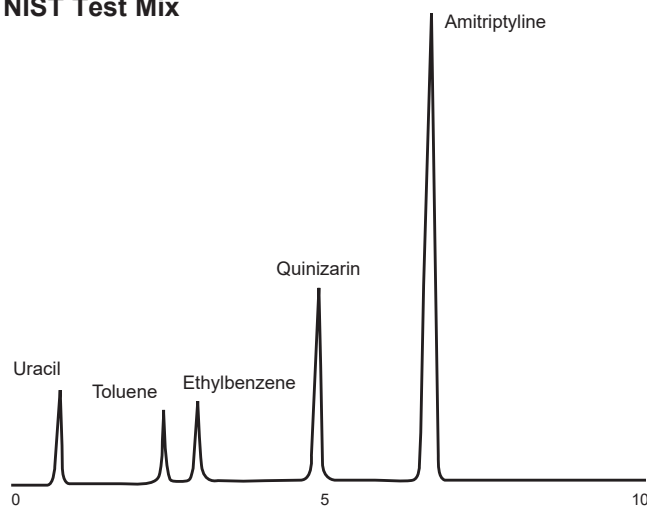


Cat. No.	Description
Sprite AE1842	4.0cm x 2.1mm ID
Sprite AE1822	2.0cm x 2.1mm ID
Sprite AE1832	1.0cm x 2.1mm ID

## Advantage™ Echelon™ Specifications

Phase: C18  
Particle Size: 4µm and 5µm  
Pore Size: 100Å  
Pore Volume: 1.0mL/gm  
Surface Area: 430 m<sup>2</sup>/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 Kapinos<sup>1</sup>, John Janiszewski<sup>1</sup>, Mary Piotrowski<sup>1</sup>, Hui Zhang<sup>1</sup>, Carl...  
<sup>1</sup>Pfizer Global R&D, Groton, CT, <sup>2</sup>Sound 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).

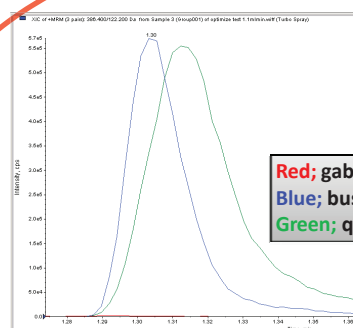
Further optimization of “trap-and-elute” parameters, including flow rate, valve timing, and columns of varying chemistries and dimensions can extend the application of this method.

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.

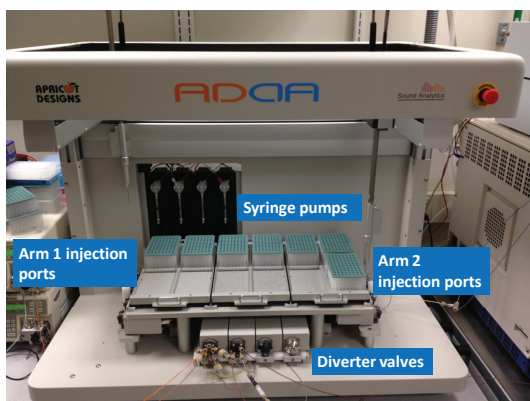
## Standard vs. “Hybrid”

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

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



## Materials and methods



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:

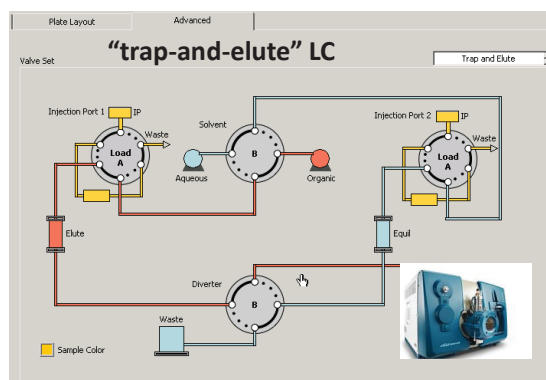
Aqueous: 95% 2mM ammonium formate, 5% 50/50 Methanol/Acetonitrile  
Organic: 45% ACN/55% 2mM ammonium formate

### LC Flow rate:

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

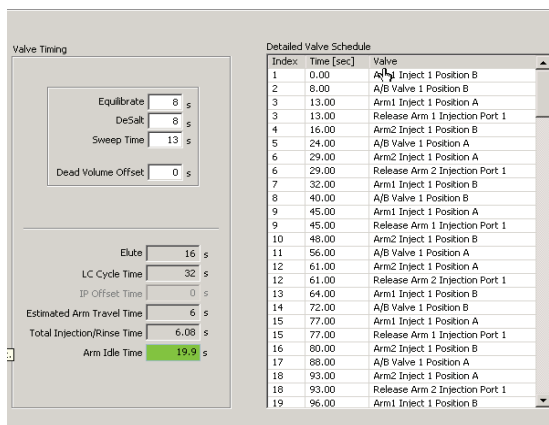
### Columns:

-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)

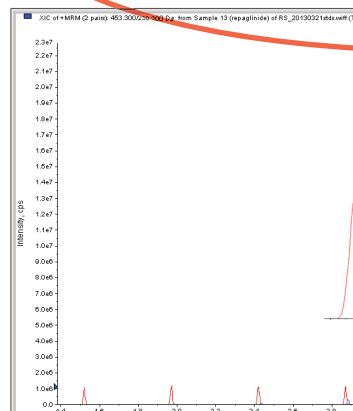


Above: dual-arm “trap-and-elute” plumbing

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



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



## 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

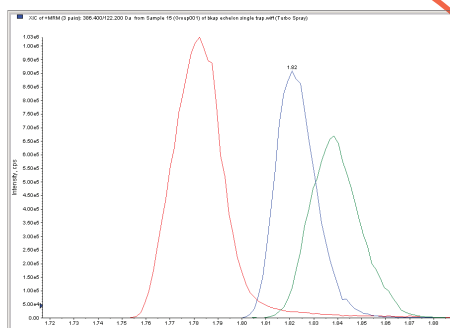
rie Funk<sup>1</sup>, Wayne Lootsma<sup>2</sup>, Will Schramm<sup>2</sup>

## “Trap-and-elute”

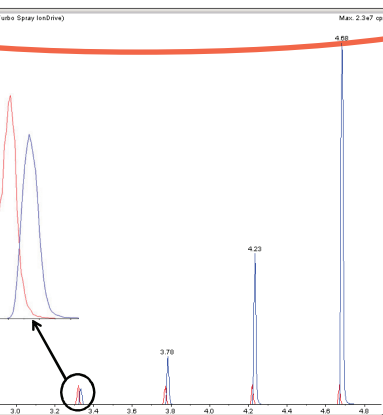
mid trap-and-elute analysis (30sec/sample) to increase retention of  
graphic separation through optimization of:

apentin (LogD= -1.1)  
spirone (LogD= 1.18)  
uinidine (LogD= 1.66)

cartridge  
p-and-elute)



-Analytical Sales and Services Sprite Echelon 20x2.1mm  
-1ml/min LC flow rate (“Hybrid” trap-and-elute)



### “Hybrid” trap-and-elute analysis with Sprite Echelon 20x2.1 4μM

-Repaglinide standard curve,  
0.137 to 100nM in less than  
5min

-Significant separation of analyte  
(blue) from internal standard  
(red)

-Leveraged AB SCIEX 6500TQ's  
extended dynamic range to  
analyze 100nM standard (**2.3e7  
peak intensity**) without dilution

## al conditions

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

## 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