



Optimizing High-Throughput LC/MS/MS “trap-and-elute” Bioanalysis in Drug Discovery

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Introduction

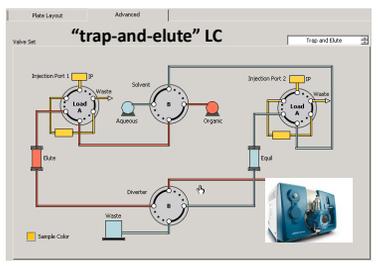
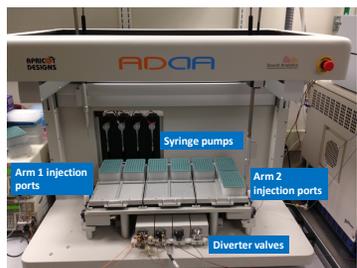
Rapid, robust bioanalysis is essential in modern drug discovery. Implementation of “trap-and-elute” LC enables high-throughput, high-quality analysis to drive drug discovery efforts.

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.

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).

Valve Timing

Equilibrate	0
DeSolv	0
Sweep Time	13
Dead Volume Offset	0

Detailed Valve Schedule

Steps	Time (sec)	Valve
1	0.00	Arm1 Inject 1 Position B
2	8.00	AB Valve 1 Position B
3	13.00	Arm2 Inject 1 Position A
4	13.00	Release Arm 1 Injection Port 1
5	24.00	AB Valve 1 Position A
6	29.00	Arm2 Inject 1 Position A
6	29.00	Release Arm 2 Injection Port 1
7	32.00	Arm1 Inject 1 Position B
8	40.00	AB Valve 1 Position B
9	45.00	Arm2 Inject 1 Position A
9	45.00	Release Arm 1 Injection Port 1
10	48.00	Arm2 Inject 1 Position B
11	56.00	AB Valve 1 Position A
12	61.00	Arm2 Inject 1 Position A
12	61.00	Release Arm 2 Injection Port 1
13	64.00	Arm1 Inject 1 Position B
14	72.00	AB Valve 1 Position B
15	77.00	Arm2 Inject 1 Position A
15	77.00	Release Arm 1 Injection Port 1
16	80.00	Arm2 Inject 1 Position B
17	88.00	AB Valve 1 Position A
18	93.00	Arm2 Inject 1 Position A
18	93.00	Release Arm 2 Injection Port 1
19	96.00	Arm1 Inject 1 Position B

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

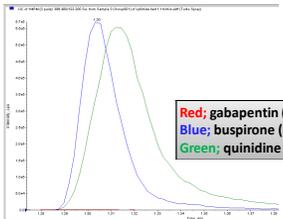
LC Flow rate:
 1.2ml/min (standard “trap-and-elute”)
 1ml/min (“hybrid” trap-and-elute)

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 S 20x2.1mm 5µ (“Hybrid” trap-and-elute)

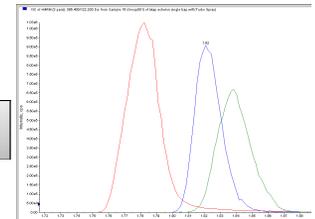
Standard vs. “Hybrid” trap-and-elute

“Hybrid” trap-and-elute uses rapid trap-and-elute analysis (30sec/sample) to increase retention of analytes and provide chromatographic separation through optimization of:

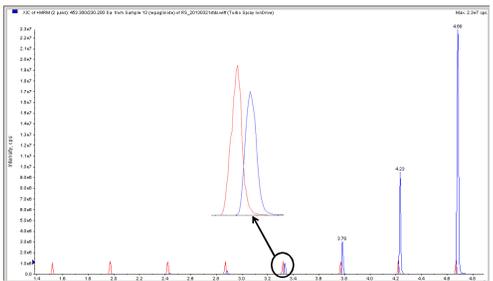
- Valve timing
- LC flow rate
- Mobile phase composition
- Column type



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

Summary of analytical conditions

Bioanalytical mode	Cycle time (sec/sample)	Particle size	Column dimensions	
Standard trap-and-elute	10-15	13µ	1.5x5mm	Rapid “trap-and-elute” bioanalysis
“Hybrid” trap-and-elute	30	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 S 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.