High-Throughput, Dual-Stream UHPLC/MS/MS Bioanalysis and Data-Deconvolution for Rapid Drug Discovery Applications

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1

High-Throughput, Dual-Stream UHPLC/MS/MS Bioanalysis and Data-Deconvolution for Rapid Drug Discovery Applications

HDO Group (Hit Discovery and Optimization)

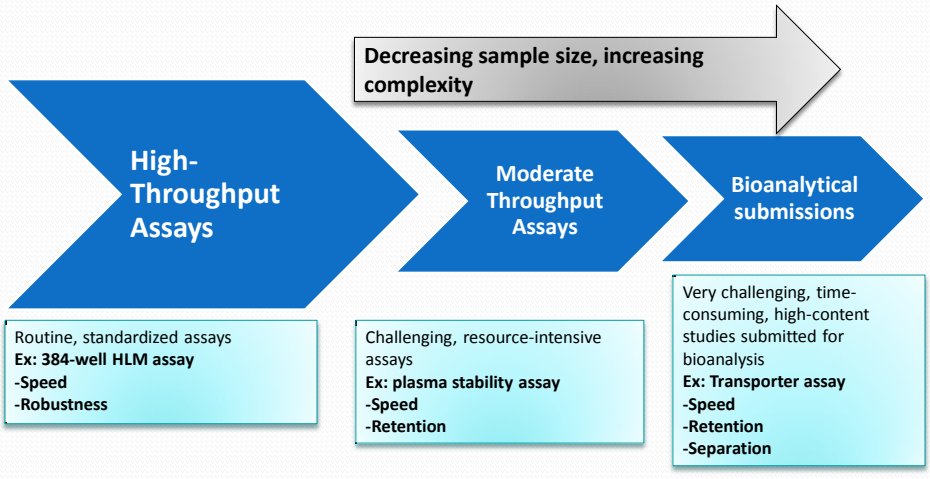
ADME Screening (96- and 384-well assays)
- -Metabolism
- -DDI & Toxicity
- -Disposition
- -Permeability & Transporters

***In Vitro* Bioanalysis**
- - Transporter Studies
- - Enzymology

Technology Development and Validation

2

Our Bioanalytical Work Spans a Broad Scope



3

Leveraging Technology to Address Portfolio Needs

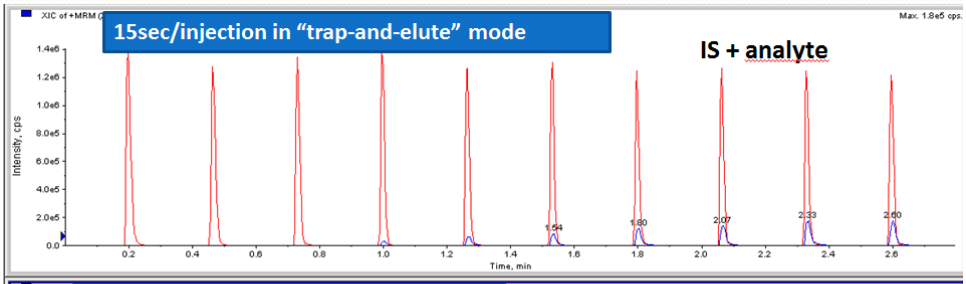
Dual-arm (dual-stream) liquid-handler-type LC/MS/MS autosampler

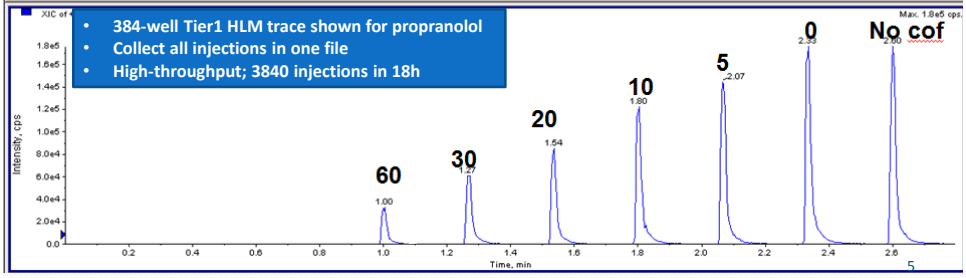
High-throughput, large deck capacity, rapid sample analysis and ***flexibility***

4 injection ports per arm, 4 front diverter valves allow multitude of LC modes and/or chemistries, functionalities

4

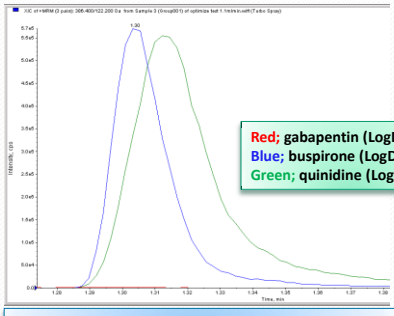
High-Throughput: "Trap-and-Elute"



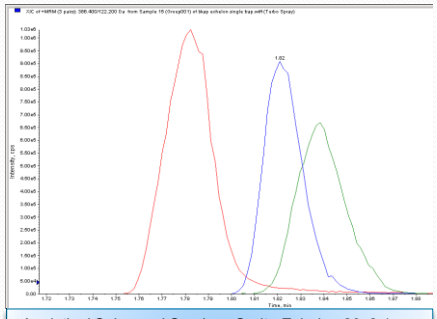


5

Continuous Improvement of High-Throughput Bioanalysis



-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
-Weaker eluent **("Hybrid" trap-and-elute)**

"Hybrid" trap-and-elute increases scope of retained compounds and provides separation without impacting throughput

6

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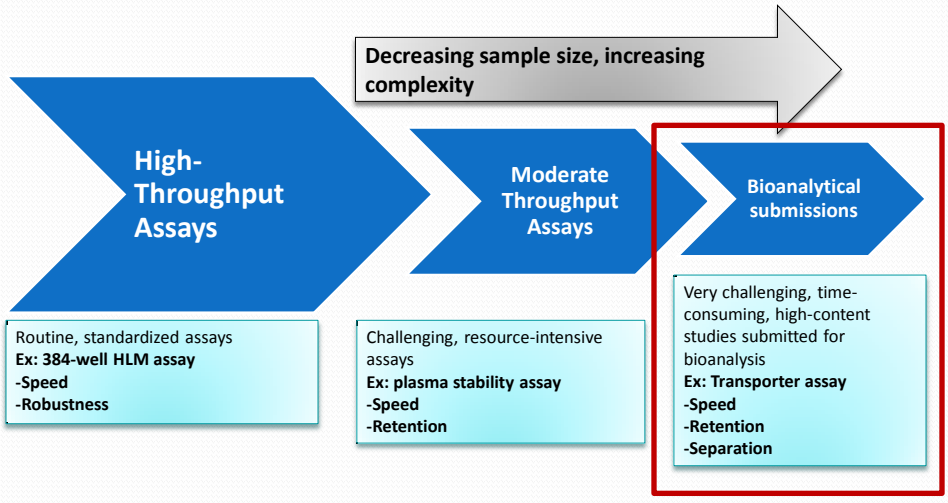


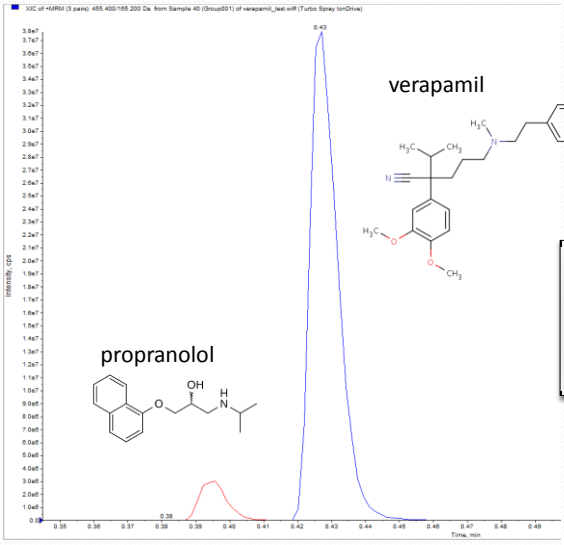
7

Case Study: Transporter Assay Bioanalysis

Challenge	Details
High sample burden	**~1200 samples/week** (32 compounds x 3 replicates x 6 plates x 2 studies)
Cassetted approach	**4 compounds/sample** (analyte, inhibitor, internal standard, assay control) different polarities, response, LC conditions, requires separation
Diversity of analytes	**Ionization efficiency varies**
Co-dosed inhibitors	**Extremely high concentrations** (10-100µM)
Membrane control	**Atenolol: small, polar, extreme difference in response across plates**
Rapid turnaround	**Traditional gradient HPLC: 2min/injection (previously 20 hour run-time)**

8

Compound-Dependent Ionization Efficiency

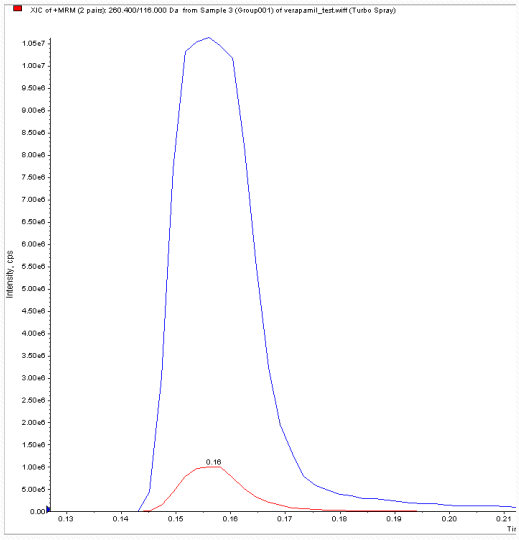


Gradient separation of propranolol (red) and verapamil (blue) **both at 0.1µM**

20-fold difference in response

9

Compound-Dependent Ionization Efficiency



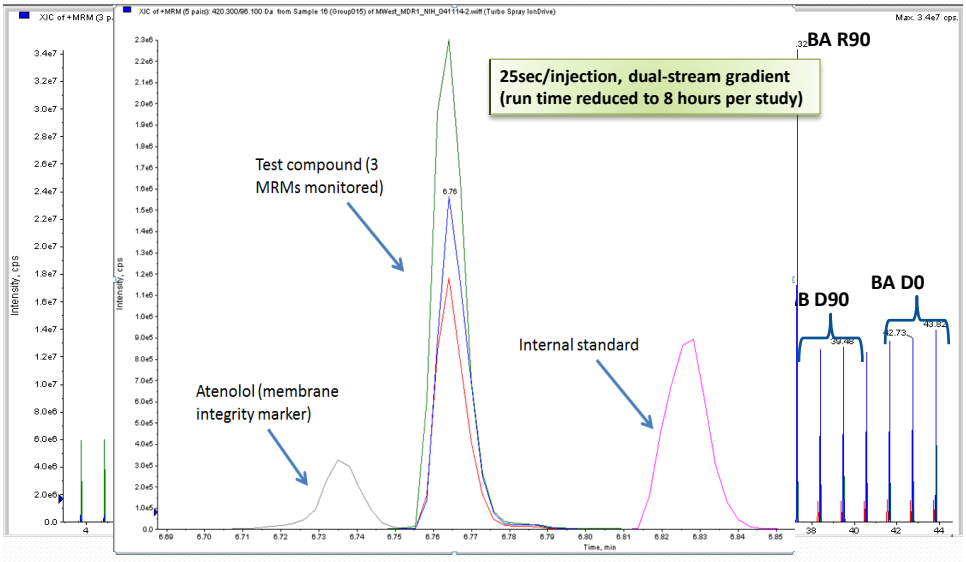
0.1µM verapamil (blue) and propranolol (red); co-elution in trap-and-elute mode

Co-elution may lead to suppression of poorly-ionizing compounds such as propranolol

Propranolol signal suppressed ~20% by good ionizers such as verapamil at 5µM

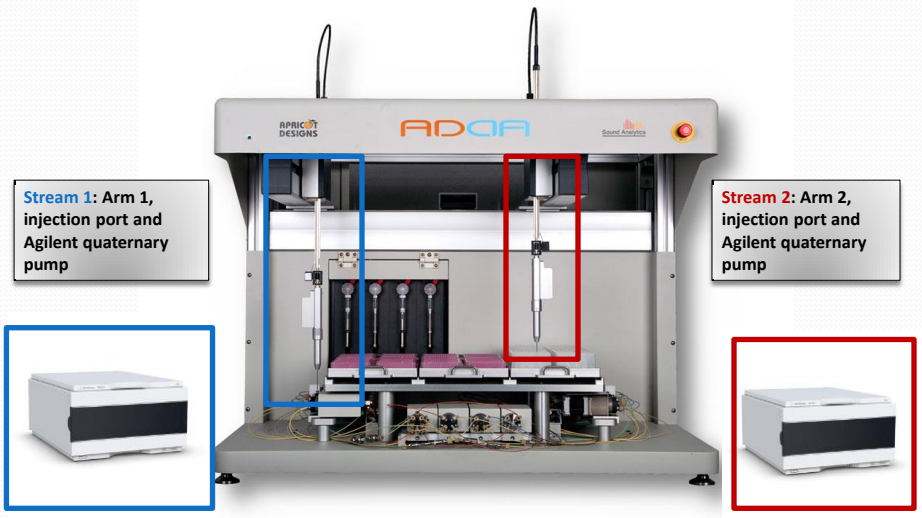
10

Case Study: Transporter Assay Bioanalysis



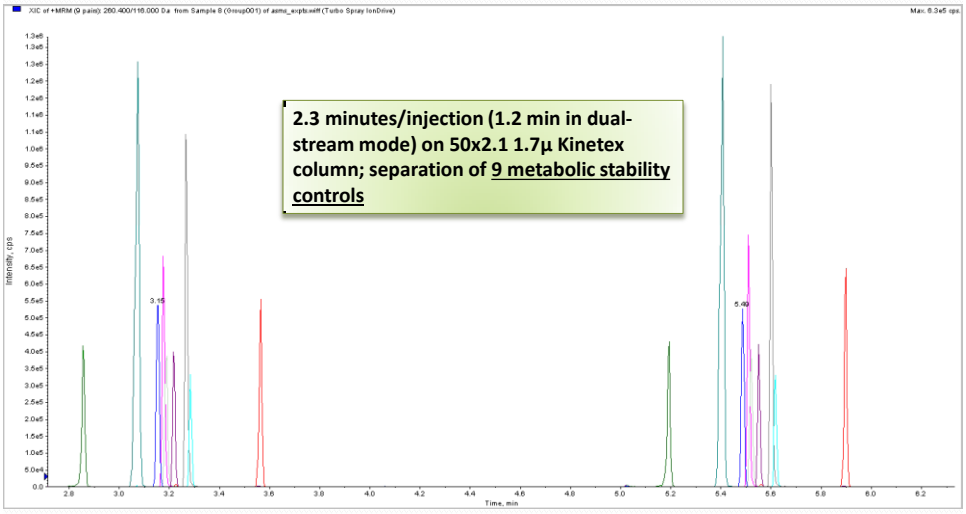
11

"Dual-stream" autosampling



12

Higher-Throughput Gradient Separation Via Dual-Stream UHPLC



13

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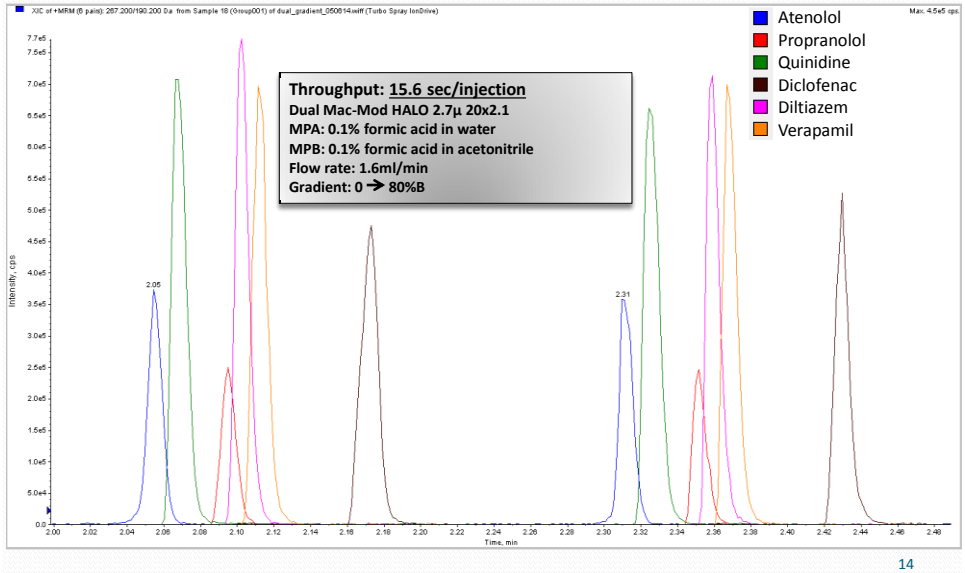
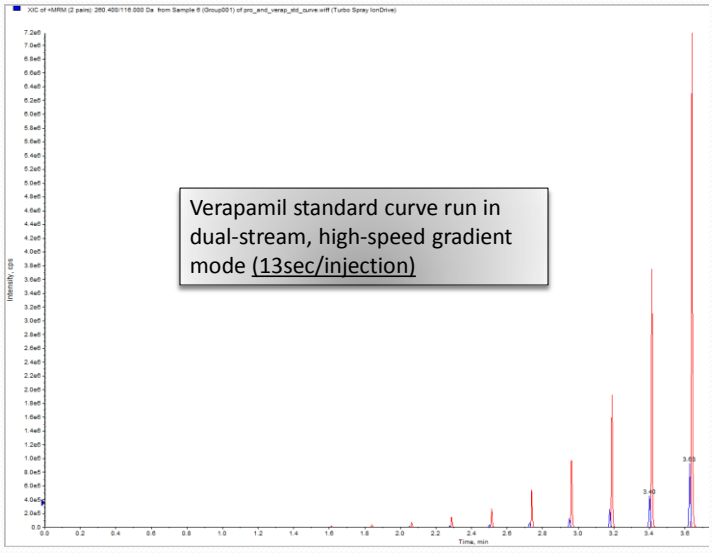


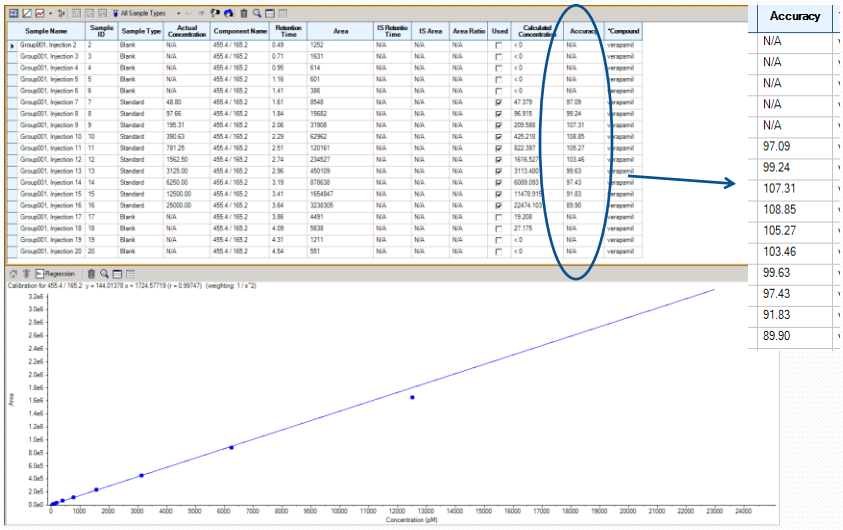
14

Higher-Throughput Gradient Separation Via Dual-Stream UHPLC



15

Higher-Throughput Gradient Separation Via Dual-Stream UHPLC



Sample Name	Sample ID	Sample Type	Actual Concentration	Component Name	Retention Time	Area	IS Retentio Time	IS Area	Area Ratio	Used	Calculated Concentration	Accuracy	*Compound
Group001, Injection 2	2	Blank	N/A	455.4 / 165.2	0.49	1252	N/A	N/A	N/A	☐	< 0	N/A	verapamil
Group001, Injection 3	3	Blank	N/A	455.4 / 165.2	0.71	1631	N/A	N/A	N/A	☐	< 0	N/A	verapamil
Group001, Injection 4	4	Blank	N/A	455.4 / 165.2	0.95	614	N/A	N/A	N/A	☐	< 0	N/A	verapamil
Group001, Injection 5	5	Blank	N/A	455.4 / 165.2	1.16	601	N/A	N/A	N/A	☐	< 0	N/A	verapamil
Group001, Injection 6	6	Blank	N/A	455.4 / 165.2	1.41	386	N/A	N/A	N/A	☐	< 0	N/A	verapamil
Group001, Injection 7	7	Standard	48.80	455.4 / 165.2	1.61	8548	N/A	N/A	N/A	☑	47.379	97.09	verapamil
Group001, Injection 8	8	Standard	97.66	455.4 / 165.2	1.84	15682	N/A	N/A	N/A	☑	96.915	99.24	verapamil
Group001, Injection 9	9	Standard	195.31	455.4 / 165.2	2.06	31908	N/A	N/A	N/A	☑	209.588	107.31	verapamil
Group001, Injection 10	10	Standard	390.63	455.4 / 165.2	2.29	62962	N/A	N/A	N/A	☑	425.218	108.85	verapamil
Group001, Injection 11	11	Standard	781.25	455.4 / 165.2	2.51	120161	N/A	N/A	N/A	☑	822.397	105.27	verapamil
Group001, Injection 12	12	Standard	1562.50	455.4 / 165.2	2.74	234527	N/A	N/A	N/A	☑	1616.521	103.46	verapamil
Group001, Injection 13	13	Standard	3125.00	455.4 / 165.2	2.96	450109	N/A	N/A	N/A	☑	3113.486	99.63	verapamil
Group001, Injection 14	14	Standard	6250.00	455.4 / 165.2	3.19	878638	N/A	N/A	N/A	☑	6089.093	97.43	verapamil
Group001, Injection 15	15	Standard	12500.00	455.4 / 165.2	3.41	1654847	N/A	N/A	N/A	☑	11478.910	91.83	verapamil
Group001, Injection 16	16	Standard	25000.00	455.4 / 165.2	3.64	3238305	N/A	N/A	N/A	☑	22474.160	89.90	verapamil
Group001, Injection 17	17	Blank	N/A	455.4 / 165.2	3.86	4491	N/A	N/A	N/A	☐	19.208	N/A	verapamil
Group001, Injection 18	18	Blank	N/A	455.4 / 165.2	4.09	5638	N/A	N/A	N/A	☐	27.175	N/A	verapamil
Group001, Injection 19	19	Blank	N/A	455.4 / 165.2	4.31	1211	N/A	N/A	N/A	☐	< 0	N/A	verapamil
Group001, Injection 20	20	Blank	N/A	455.4 / 165.2	4.54	581	N/A	N/A	N/A	☐	< 0	N/A	verapamil

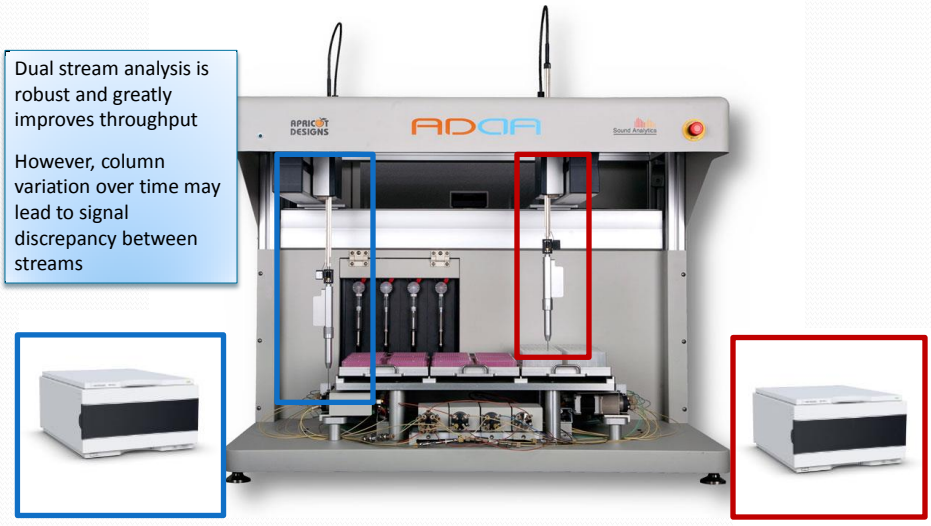
Accuracy
N/A
N/A
N/A
N/A
N/A
97.09
99.24
107.31
108.85
105.27
103.46
99.63
97.43
91.83
89.90

16

"Dual-Stream" Autosampling

Dual stream analysis is robust and greatly improves throughput

However, column variation over time may lead to signal discrepancy between streams

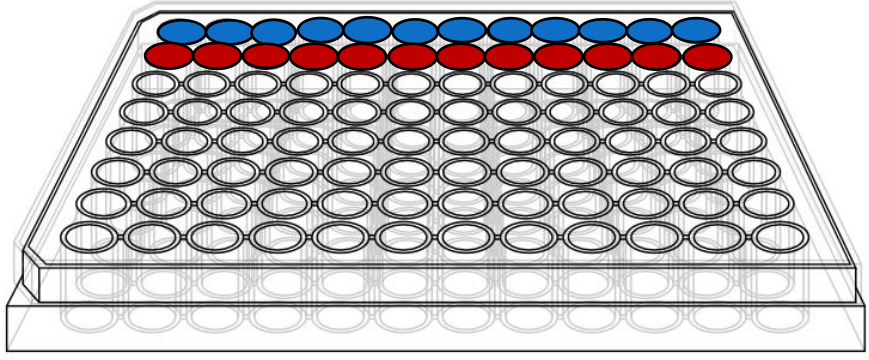


17

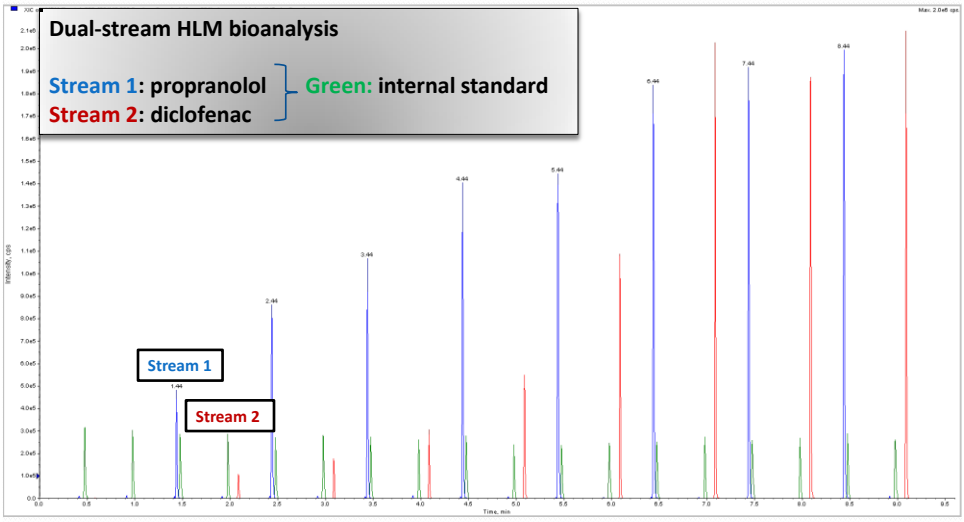
Leveraging Flexibility to Expedite Analysis

<u>Solution:</u> Maximize throughput and reproducibility via dual-stream analysis of 2 different analytes simultaneously

Stream 1 injects propranolol
Stream 2 injects diclofenac



Dual-Stream Metabolic Stability (HLM)



19

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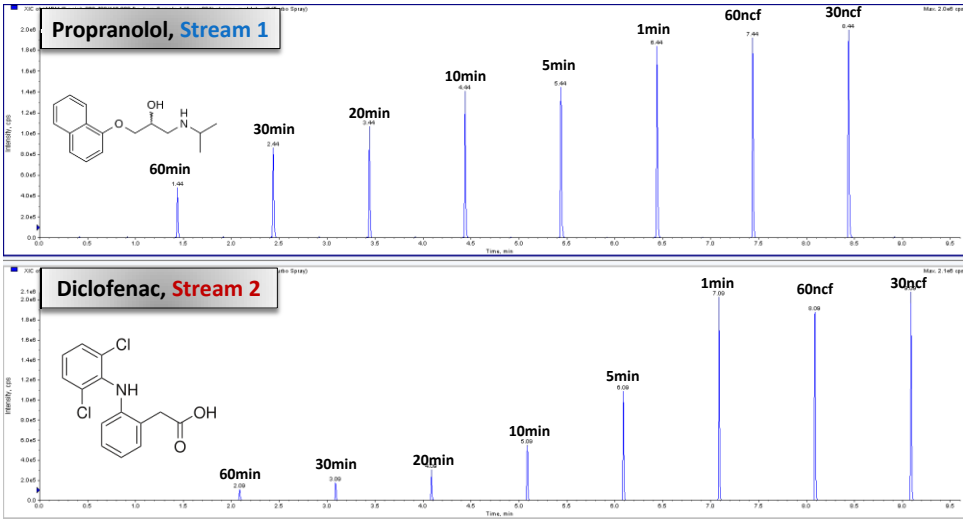
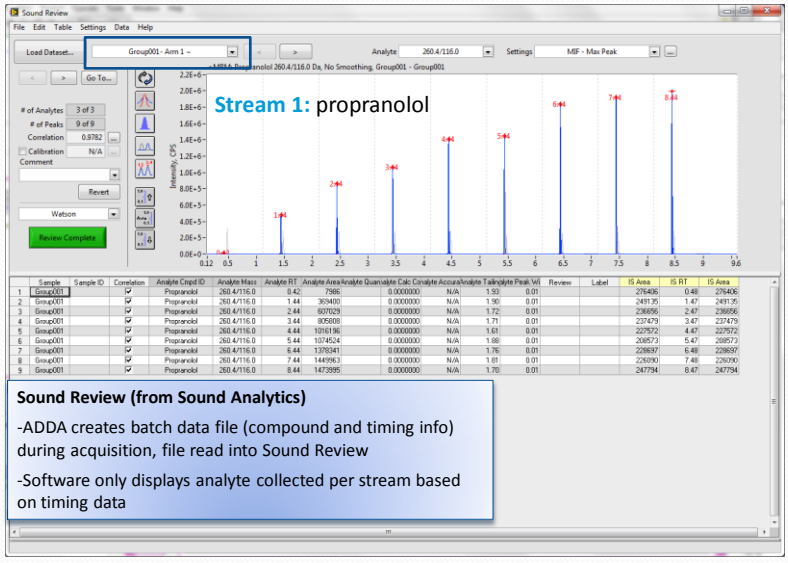


20

Dual Stream Analysis: Data Deconvolution



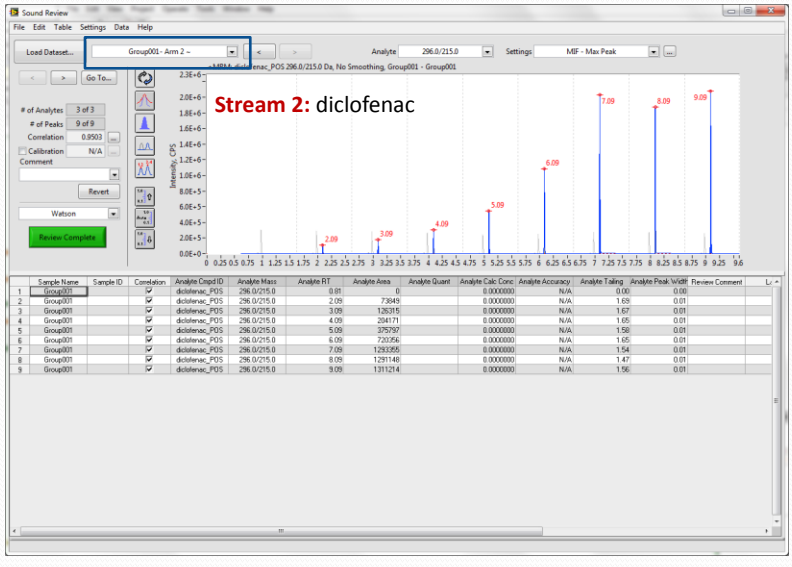
Sound Review (from Sound Analytics)

-ADDA creates batch data file (compound and timing info) during acquisition, file read into Sound Review

-Software only displays analyte collected per stream based on timing data

21

Dual Stream Analysis: Data Deconvolution



22

ADDA: Multiple Autosamplers in One


High-throughput


Screening binary gradient


Secondary binary gradient


Method development

4 injection ports/stream
4 front diverter valves

Flexibility
Multiple LC modes (isocratic, gradient)
Multiple chemistries per LC mode

Acknowledgements

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24