Application of High-Throughput Micro-Flow LC/MS/MS to a Metabolic Stability Screening Workflow

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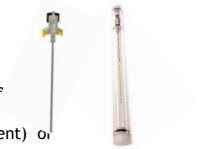
Introduction

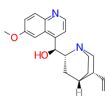
Research and development groups in the pharmaceutical industry can gain competitive advantage by accelerating time to market and reducing operating cost. Assay miniaturization and sample pooling approaches can effectively increase throughput, but add complexity and demand lowered limits of quantification. Micro-flow bioanalytical LC/MS/MS has proven to be successful in addressing these challenges, current applications use specialized systems that offer no throughput advantage, however, over standard UHPLC/HPLC methods (~3-5 minutes/ injection). Here we describe our recent development of an ultrafast microLC/MS/MS platform to support early ADME screening workflow. A high-performance, sample delivery system was integrated with a global bioanalytical "enterprise" database to enable high-throughput micro-flow bioanalysis. Ultra fast separation was achieved with superb separation power and peak capacity. When combined with post incubation sample pooling, analysis for regular in vitro HLM metabolic stability screen was achieved for <15sec/sample and 3 hrs for 384 test compounds.

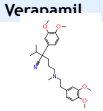
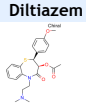
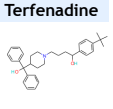
Instrumentation and Materials



- Mass spectrometer: SCIEX 5500 mass spectrometer with Analyst 1.6.3 software
- Autosampler: LeadSampler equipped with high-pressure Rheodyne injection ports
- LC: Agilent 1290 Infinity for high flow and Eksigent Expre micro-flow applications
- Electrospray probe: 65µ ESI electrode (from Sciex/Eksigent) o PicoFuze (from New Objective, Woburn MA) for direct spray
- 1/32"OD & 0.003" ID tubing from Analytical Sales and Services
- Human liver microsome (HLM) stability samples were generated by following protocol: Pooled human liver microsomes were incubated in the presence and absence of NADPH and 1uM control compound for one hour at 37°C. 20uL sample were taken at designated time and terminated with ACN. 10uL supernatant from each incubation per time point were pooled and dried under nitrogen. Samples were reconstituted in 5% methanol and vortexed prior to LC/MS/MS analysis.

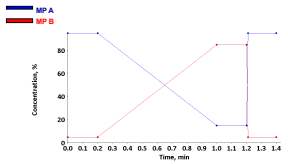
List of Standard Compounds Tested

	Propranolol	Quinidine	Diclofenac	Desipramine
				
MRM	260.1 / 116.1	325.1 / 184.1	296.0 / 215.0	267.3 → 208.2
LogD7.4	0.79	1.66	1.44	1.27
pKa (most acidic)	9.4	8.6	4.1	10.4

	Verapamil	Diltiazem	Terfenadine
			
MRM	455.4 / 165.1	415.2 / 178.2	472.4 / 436.3
LogD7.4	2.41	3.36	3.61
pKa (most acidic)	8.9	12.8	13.2

Bioanalytical Methods

Gradient Setting



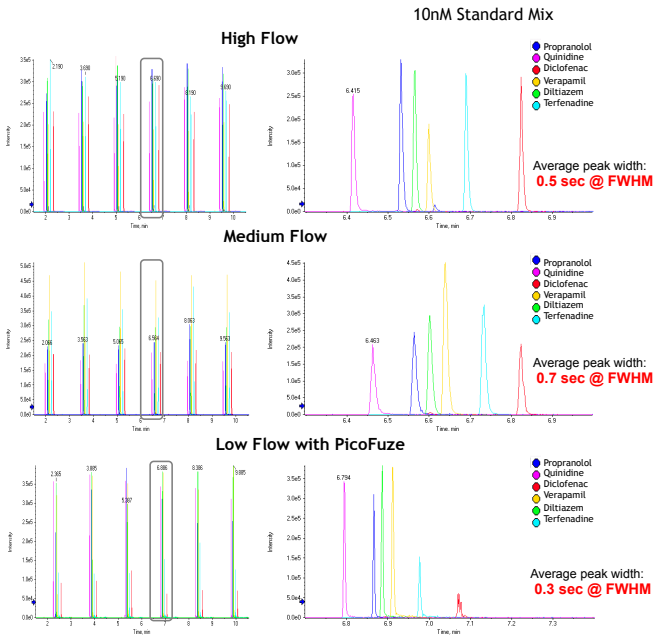
Mobile phase A: 2mM ammonium acetate with 0.1% formic acid in water

mobile phase B: 0.1% formic acid in acetonitrile

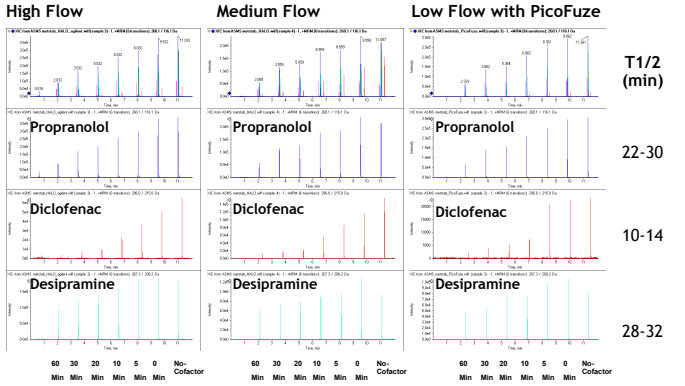
Column and other LC settings

Method	Column	Flow Rate	Cycle Time	Injection vol.	Column Temperature
High Flow	HALO C18 20x2.1 2.7µ	800 µL/min	90s	1µL	55°C
Medium Flow	HALO C18 30x0.5 2.7µ	75 µL/min	90s	1µL	55°C
Low Flow	Reprosil-PUR C18 50x0.2 3µ (PicoFuze)	18 µL/min	90s	1µL	N/A

LC/MS/MS performance



Micro-Flow Bioanalysis for HLM Metabolic Stability Samples



Conclusion

An LC/MS/MS system was designed for low delay and extra-column volume, and various micro-flow methods were tested alongside a standard, high-flow LC method using a cocktail of control compounds. The micro-flow methods delivered very good resolution, peak capacity, reproducibility and response at 90sec/injection.

While both micro-flow methods delivered a high degree of analyte separation at low flows, the PicoFuze probe assembly from New Objective generated very sharp peaks (**0.3sec @ FWHM**) at much lower flow rates (18 µl/min). Care has been taken to use small tubing and connectors to minimize the dead volume, while the main contributor of such superb separation came from the PicoFuze column design, by which post-column volume was minimized to nL range.

All methods delivered good separation despite dilution from pooling of samples. When normalized by injections per compound, both medium and low micro flow methods matched our routine high-throughput LC/MS/MS methods with on line SPE mode (without full separation). In addition, significant savings are realized in term of solvent consumption and analysis time.

Overall, micro-flow LC/MS/MS is a robust, resource-sparing bioanalytical technique that is capable of delivering good separation at low flows. Through post-assay sample pooling, analysis throughput were effectively enhanced to less than 15sec per sample or ~3 hrs for 384 test compounds with full time course HLM metabolic stability screen.

HLM Metabolic Stability Screen of 384 test compounds	Standard Gradient (800µL/min)	Microflow gradient (75 µL/ min)	Picofuze gradient (18µL/ min)	Picofuze gradient w/ Sample Pooling
Assay Throughput per cmpd	15 min	15 min	15 min	2.5 min
Total Runtime	96 hrs	96 hrs	96 hrs	16 hrs
Total Solvent usage	4.6 L	430 mL	104 mL	18 mL

Acknowledgements

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Reference

High-Throughput, Dual-Stream Micro-flow LC/MS/MS Bioanalysis, Kapinos B. et. al., 63th ASMS Conference, 2015, St. Louis, MO