Comparison of High-Throughput Separation Techniques Coupled to MS in a DMPK Screening Setting to Facilitate a Rapid Design-Make-Test-Learn Cycle

Santiago, Brandon G.; Liebhardt, Amanda M.; Eisennagel, Stephen H.; Peckham, Gregory, E.; Karlinsey, Molly Z.; Alburn, Chad A.; Berry, Shannon E.; Roethke, Theresa J.; Mahajan, Mukesh K., Sydor, Jens, Reilly, Michael A.; Reilly

Drug Metabolism and Pharmacokinetics, GlaxoSmithKline

Introduction

As the desire for a shortened design-and-test cycle increases in early drug discovery, the pressure to rapidly deliver DMPK data continues to rise. From a bioanalytical standpoint, *in vitro* assays are particularly challenging because they are amenable to automation and produce multiple samples/compound. To keep up with the ever-increasing analysis demand (without a substantial increase in resources) alternative approaches to traditional Ultra High Performance Liquid Chromatography (UPLC) separations need to be implemented. In this work, a series of commercially available compounds were tested in DMPK screening assays and samples were evaluated using both UPLC and a 20 second Trap-and-Elute (TnE) methodology. Furthermore, the current work looks to improve the TnE cycle time to 12 seconds/sample while maintaining equivalent data quality.

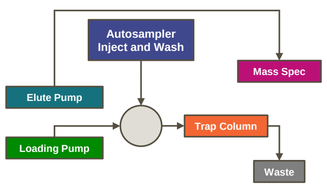
LS-1 Autosampler and LeadScape (Sound Analytics)

- Flexible system capable of both TnE and UPLC methodologies without re-plumbing
- 10-plate open architecture allows for rapid sampling across plates positions
- LeadScape™ software seamlessly accesses DiscoveryQuant SQL MS/MS MRM database
- User replaceable hardware minimizes system downtime despite high volume of use

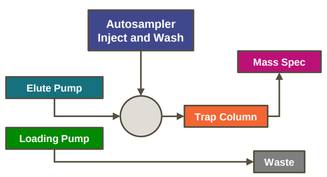
Analytical Methods

- MRM transitions for all analytes were developed automatically using DiscoveryQuant software
- Data was analysed with a custom MultiQuant query that allows a review-by-exception workflow
- UPLC analyses were performed using a generic 2-minute reverse phase gradient and a Waters BEH C18 column (2.1 x 50 mm) coupled to a Sciex 5000 triple quadrupole mass spectrometer
- TnE analysis were performed using the LS-1 autosampler coupled to a Sciex 5500 QTRAP mass spectrometer. A PS-DVB 1.5 X 5mm trap column was utilized for all studies
- The 20 s sample-to-sample (S2S) method utilized a flow rate of 0.8 mL/min whereas the 12 s method flow rate was 1.4 mL/min; both methods switch from 100% aqueous to 100% organic using the valves of the LS-1

Step 1: Load and Desalt



Step 2: Elute and Equilibrate



- The valve timing table below shows how the method was altered to minimize the arm idle time and maximize throughput
- Needle wash volumes were reduced to accommodate the higher sampling rate; however, sufficiently low carryover was still observed due to the LS-1 flow path
- The injection volume was determined by the 2 µL injection loop that was overfilled in both methods

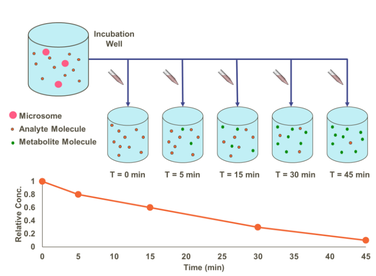
Valve Timing (s)	20 s S2S	12 s S2S
Desalt	5	3
Elute	10	6
Equilibrate	5	3
Total	20	12
Arm Idle Time	2.42	1.01

Comparison of UPLC and 20 s Trap-and-Elute

- *In vitro* assays were performed with a series of commercially available compounds using a Tecan Fluent® 1080 that runs custom scripts designed to handle the throughput necessary for GSK's screening DMPK assays (96 compounds x 2 species per run)
- Samples were analysed both by traditional UPLC-MS/MS and TnE-MS/MS, and the assay results were compared based on % LBF (clearance) or % Free (binding)

Microsomal Clearance Assay

- Microsomal clearance aliquots were taken at the appropriate timepoints and crashed 5:1 with acetonitrile containing a generic internal standard, vortexed, centrifuged, and injected
- Assay outcome for this comparison was the calculated % Liver Blood Flow (%LBF); values were capped at both the low and high ends (value based on species)



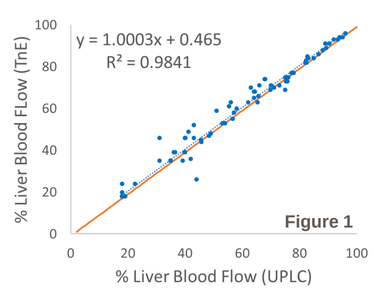
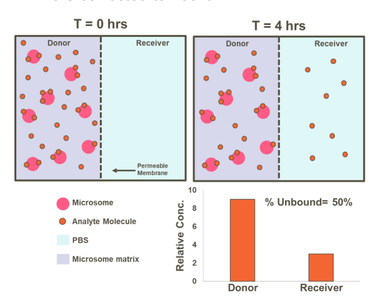


Figure 1

99% of TnE results within 25% of UPLC %LBF value

Microsomal Binding Assay

- The microsomal binding assay was run in triplicate during each Tecan run, and average fraction unbound (% free) are reported
- Samples from each well were matrix matched to the opposing well, and aliquots were crashed 4:1 with acetonitrile containing a generic internal standard, vortexed, centrifuged, and injected
- Assay outcome for this comparison was the fraction of unbound drug; % free values over 100% were corrected to 100%



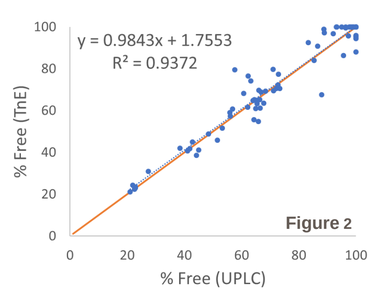


Figure 2

97% of TnE results within 25% of UPLC F_u value

Comparison of 20 s and 12 s Trap-and-Elute Results

- Samples from the weekly GSK screening assays were analysed on the LS-1 using both the 12 s and 20 s methods in rapid succession
- Clearance runs were performed with both rat and human microsomes, and % LBF values were capped as previously mentioned
- The binding assay was performed using only human microsomes, and % free values over 100% were corrected to 100%

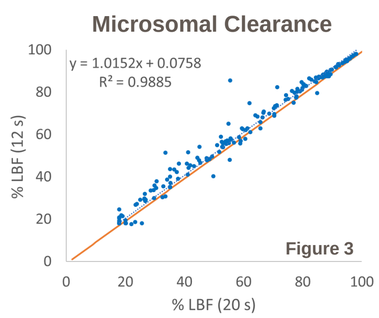


Figure 3

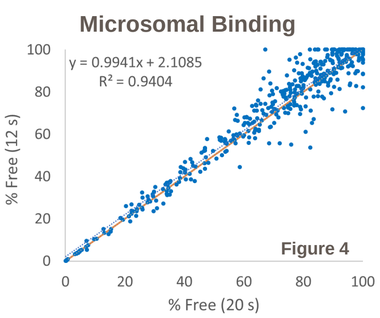


Figure 4

% of Results within 25%: 98% (Clearance) / 96% (Binding)

Robustness of the LS-1 for Screening Assays

- During the 13 months since the assays went 'live', over 400,000 samples from >13,000 compounds have been analysed on the LS-1 using the 20 s TnE method
- Open architecture leads to ease of user up-keep and maintenance, resulting in minimal instrument downtime despite the high usage rate
- Intra-sample set failure rate for internal standard (IS) signal (mean area ± 35%) is 0.16% for clearance assays and 0.86% for binding assays

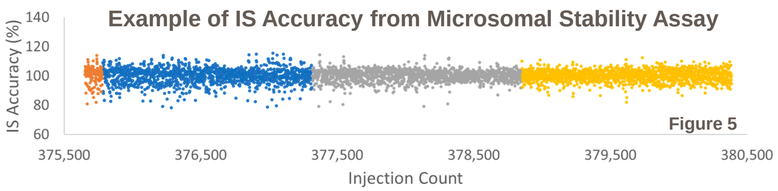


Figure 5

Conclusions

- A rapid 20 s trap-and-elute method was developed using a Sound Analytics LS-1 autosampler
- Results for two DMPK screening assays were in good agreement when analysed with both the TnE method and a traditional UPLC method (R^2 > 0.93)
- The TnE method was further refined to have a sample-to-sample time of 12 s, without a significant change to the outcome data
- The LS-1 in TnE mode has been fully implemented for 12+ months, successfully analysing over 350,000 samples, with a total sample IS failure rate of just 0.50%

1 Year IS Failure Rate of 0.5 %

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