Accelerated pharmacokinetic profiling and metabolite monitoring Using Echo® MS System

Acoustic Ejection Mass Spectrometry

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The generation of clearance profiles and calculation of intrinsic clearance (CL_{int}) values is an important stage in pre-clinical drug discovery.[1] The pharmacokinetic (PK) assessment of potential drug candidates is typically performed by a time-course incubation with human liver microsomes (HLM) before LC-MS analysis. The *in vitro* CL_{int} can be scaled to *in vivo* and used to predict human clearance which can be then used to inform dosing studies, as well as provide insight into enzymes responsible for metabolism of drug candidates.

As the process is performed during the early stage of drug discovery, the number of potential candidates can still be in the thousands, or even tens of thousands, and CL_{int} values will need to be calculated for each target. This will require multiple time-points for each target leading to a large analytical demand. This creates a significant bottleneck using current LC-MS solutions.

The Echo MS System using Acoustic Ejection Mass Spectrometry (AEMS) provides a sample introduction method capable of analyzing 1 sample per second and up to 3 samples per second when multiplexed.[2] With the Echo MS System, very small, reproducible droplets can be ejected from high-density well plates, and this ejection process is compatible with the wide range of solutions and complex matrices used in drug discovery. The ejected droplets are then captured by the Open Port Interface (OPI) device which uses a laminar flow of carrier solution that dilutes and transports the sample to the electrospray source, where it is ionized by conventional electrospray (Figure 2).[2] This process forms a contactless sample introduction method with minimal carry-over and minimal matrix effects.

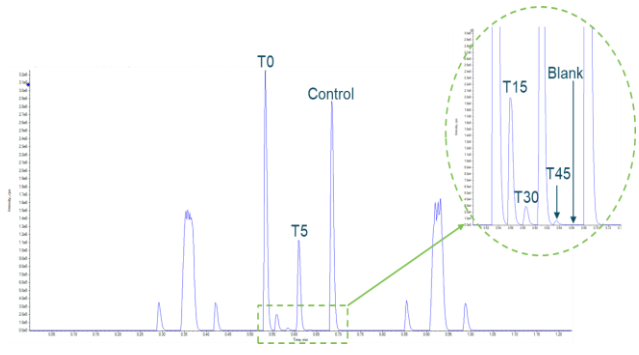

Figure 1. Microsomal incubation of buspirone. Example data using the Echo MS System were obtained from a microsomal incubation of buspirone with data acquired from 5 timepoints, a control and a blank reference, all completed in under 90 seconds. Sampling was done in a serpentine manner from the 384-well plate.

Here, the utility of this high-throughput solution was explored for use in candidate screening. The goal was to highlight the ability of the Echo MS System to alleviate the analytical bottleneck of traditional LC-MS solutions through speed and quantitative performance, and show how that correlates with current Cyprotex methodology.

Key features of the Echo® MS System

- Rapid sample analysis using Acoustic Droplet Ejection and Open Port Interface:
  - One sample per second for a single analyte, up to three samples per second in multiplex mode
  - Highly reproducible sample injection
  - Extremely low potential for carryover
- Broad compound coverage using electrospray ionization featuring the OptiFlow® Turbo V Ion Source for robust and efficient ionization
- Industry proven high sensitivity and quantitative robustness using the SCIEX Triple Quad™ 6500+ LC-MS/MS System
- Ease of operation using SCIEX OS Software
- Purpose built for incorporation into HTS workflows
  - Sample plate tray can be accessed by most robotic arms
- Open software API for incorporation of platform into existing automated HTS environments

Methods

Sample preparation: Microsomal incubations were performed in human liver microsomes (0.1M phosphate buffer, 0.5 mg/mL) initiated with NADPH (1mM) for 39 traditional small molecule APIs (1µM) and quenched in acetonitrile at 0, 5, 15, 30 and 45 time-points. These same samples were split for LC-MS analysis and Echo® MS System analysis.

Acoustic ejection: The Echo MS System was operated using 70% acetonitrile with 2mM ammonium formate and 0.1% formic acid at a flow rate of 450 µL/min. A total ejection volume of 25 nL was employed for each acoustic event at a frequency of 1.5 Hz.

Mass spectrometry: The SCIEX Triple Quad 6500+ System was operated in MRM mode monitoring a single transition with a total analytical cycle time of 100 msec. The ESI source was operated at 500 ºC with a capillary voltage of 5500 V and the GS1/GS2 gas flows at 90 and 50 psi, respectively.

Data processing: SCIEX OS Software 1.6 was employed for the optimization of the target MRM transitions, automatic integration of the MS peaks and the automatic creation of the results file and associated .txt file for LIMS integration.

Results

The Echo MS System is specifically calibrated to eject droplets of 2.5 nL per acoustic event. The effective ejection volume can be increased by firing multiple 2.5 nL droplets at a high frequency (20 Hz), which the MS system detects as a single peak. The process allows the user to optimize analytical sensitivity versus the matrix effects, because of the "larger"

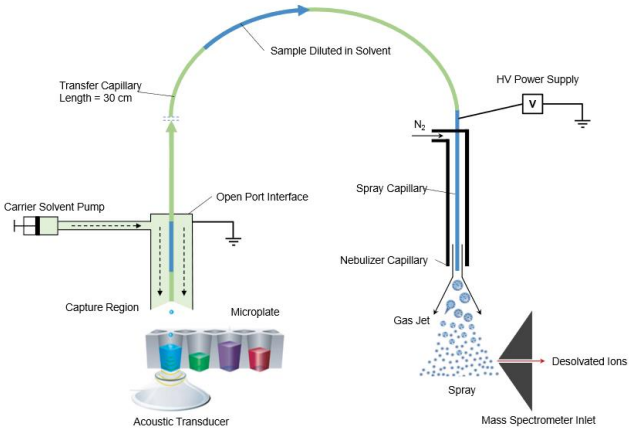

Figure 2. System schematic. Schematic of the Echo MS System showing the acoustic ejection of sample droplets and their capture and transfer to the OptiFlow Source by the OPI**.**

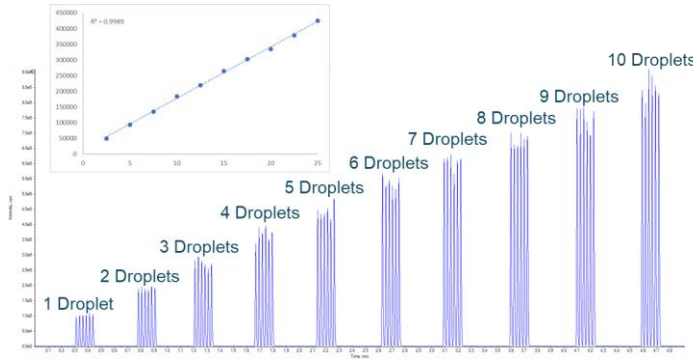

Figure 3. An example AEMS droplet ladder for lidocaine ranging from 1-10 droplets per MS measurement, equivalent to ejection volumes of 2.5-25 nL. The calibration line shown in insert with linear R^2 value >0.998.

ejection volumes. Figure 3 shows this process using lidocaine as the target analyte.

The "droplet ladder" shown in Figure 3 shows the typical response obtained from the Echo MS System producing peak widths of approximately 400 msec at 50% peak height. These sharp peaks enable the 1 sample per second acquisition rates.

To assess the quantitative performance of the Echo MS System, solvent standard calibration lines for each of the 39 analytes were created across a target concentration range of 0.01-100nM. The AEMS analysis of the solvent standard calibration line was performed using an ejection volume of 25 nL. This will allow maximum analyte sensitivity from the non-complex solvent

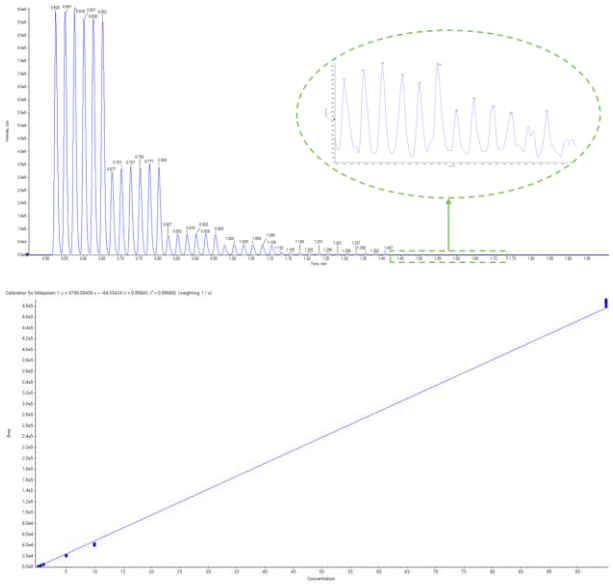

Figure 4. Concentration curve for Midazolam. (Top) Midazolam ejection peaks across the concentration range of 100-0.05nM ran from high to low with n=6 technical replicate ejections from each well position. (Bottom) Linear regression for midazolam ejections show good quantitative performance (R^2 >0.99).

Table 1. Quantitative performance of the Echo MS System for six representative marketed drugs (0.01-100nM).

Compound name	MRM transition (Q1-Q3)	Calibration range (nM)	Regression value (R2)	Accuracy >LLOQ/@LLOQ	%CV >LLOQ/@LLOQ
Acetaminophen	*152-110*	*1-100*	*0.994*	*108% / 97%*	*6% / 7%*
Codeine	*300-165*	*0.5-100*	*0.996*	*105% / 94%*	*3% / 18%*
Diphenhydramine	*256-167*	*0.1-100*	*0.991*	*95% / 98%*	*2% / 17%*
Midazolam	*326-291*	*0.05-100*	*0.996*	*93% / 100%*	*3% / 11%*
Zolpidem	*308-235*	*0.01-100*	*0.998*	*98% / 105%*	*2% / 20%*

standard solution. Figure 4 shows example data for midazolam across the calibration range.

Following the quantitative testing, the clearance profiles for each of the target analytes were determined. In all cases, a single MRM transition was monitored with no internal standard used. The timepoints provided were 0, 5, 15, 30, 45 min. The peak area values obtained from the analysis of T0 were set to 100% parent response, and the following time-points were provided as % parent remaining. Figure 5 provides three clearance regression profiles showing a range of clearance profiles.

Using the gradient of the clearance profiles, the elimination rate constant (k) and compound half-life ($t_{1/2}$) can be determined. The CL_{int} values for each of the target APIs can be calculated using the following equation

$$t_{1/2} = \frac{0.693}{k}$$

$$CL_{int}\ (\mu L\ /\mathrm{min}/mg) = \frac{0.693}{t_{1/2}} x \frac{\mu L\ of\ incubation}{mg\ microsomes}$$

Where; k = calculated elimination rate constant

A strong correlation (r^2= 0.985) of calculated CL_{int} is observed between the Echo MS System data and LC-MS data (Figure 6).

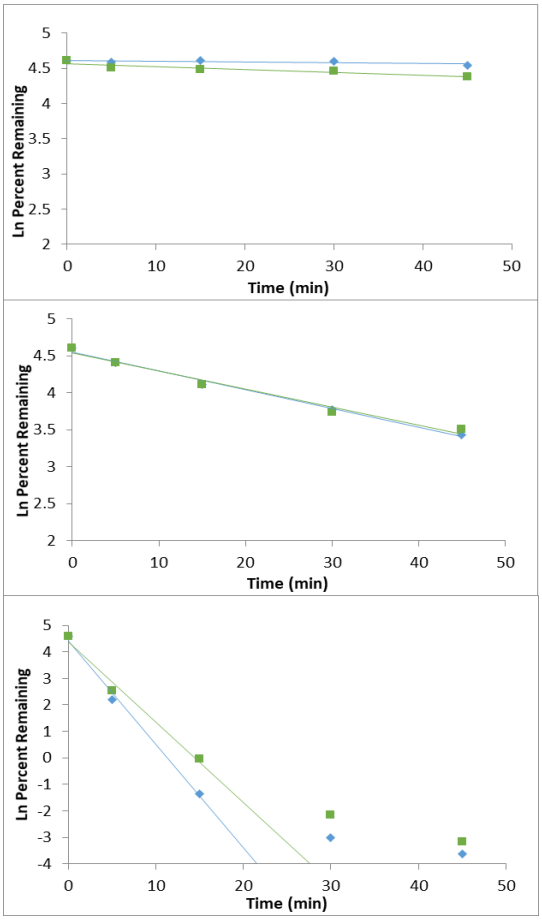

Figure 5. Clearance profiles. Clearance profiles for prazosin (top), erythromycin (center) and amodiaquine (bottom) are shown. The rapid acquisition approach using Echo MS System is shown as the blue trace vs. the same samples run with standard LC-MS analysis shown in the green trace.

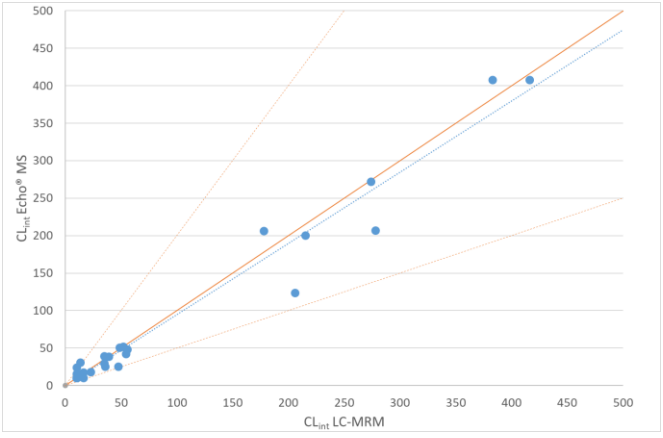

Figure 6: **Correlation analysis of Echo MS System data with LC-MRM data.** Blue dotted line represents correlation, solid orange is the line of unity, dashed orange line is +/- 2-fold of unity. CL_{int} values below the assay level of quantification for the assay (<10.3 µL/min/mg equivalent to $t_{1/2}$ of 3*incubation time) have been excluded.

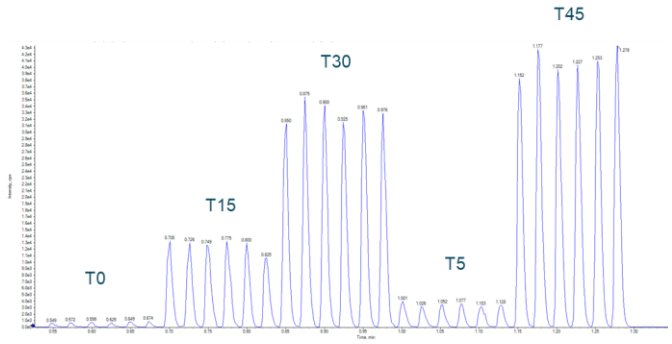

Figure 7. Incubation time course. Echo MS System data monitoring hydroxy-imipramine over the incubation time course with n=6 replicate measurements per time point.

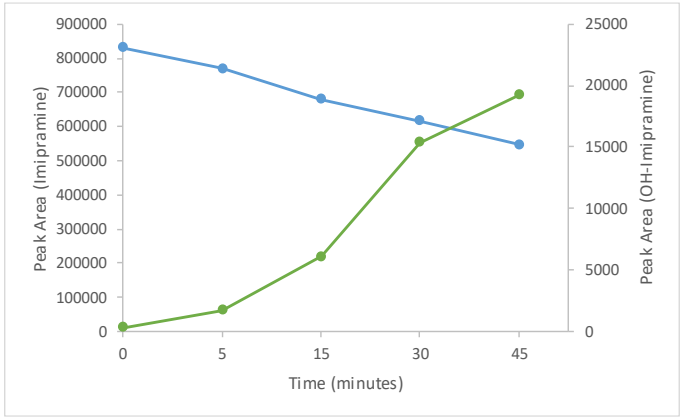

Figure 8. Clearance of imipramine. Clearance of imipramine parent (blue) overlaid with detection of hydroxy-imipramine metabolite (green) across the incubation time scale.

The data spans a range of clearance values and is scattered around the line of unity.

In addition to the clearance information, the ability to monitor suspected or known metabolites in a rapid manner might also be of benefit during late-discovery phase. The ability of the Echo MS System to provide this data was tested by repeating the measurement of the imipramine incubation with the additional MRM transition of the known hydroxy metabolite (297.1→86.1). The MS data produced from this scan is provided in Figure 7.

This metabolism data can then be related back to the clearance of the parent imipramine molecule (Figure 8) providing the analyst with DMPK information in rapid time frames suitable for the high through-put environment.

Conclusions

The data presented here confirms the viability of the use of the Echo MS System for early discovery phase studies providing DMPK data in seconds as opposed to the hours required when using traditional LC-MRM based scanning methodologies. The data generated from the Echo MS System was quantitative, with excellent %CV and accuracy values. The technology also provides clearance information with good agreement between AEMS and traditional LC-MS, as well as high-throughput metabolite monitoring, allowing decisions on candidate progression to be made quickly and accurately.

References

1. Chao P, Uss AS, Cheng KC. (2010) Use of Intrinsic Clearance for Prediction of Human Hepatic Clearance, *Expert Opinion on Drug Metabolism and Toxicology*, **6(2),** 189-98.
2. Rapid MS/MS analysis with Acoustic Ejection Mass Spectrometry (AEMS). SCIEX technical note, RUO-MKT-02-11385-A.

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