Is there a place for Echo-MS in high throughput ADME ?

Simon Wood, Mark Denn, Beth Raper

Cyprotex Discovery Ltd, Alderley Park, Macclesfield, SK10 4TG

Introduction

Echo MS (Acoustic Ejection Mass Spectrometry) is an emerging technology to increase MS throughput. With the potential to run 1 second cycle times this technology could have a significant impact on analytical throughput in the drug discovery environment, specifically screening of ADME (absorption, distribution, metabolism and excretion) properties. Improved throughput and removal of the LC-MS analysis as a bottleneck would allow faster generation of decision making ADME screening data and/or an increase in number of compounds that can be screened in a given time period. Accurate and robust methods are essential in this space to facilitate good decision making in compound selection. A couple of potential inadequacies of Echo-MS compared to LC-MS are lack of time separation of isobarics and potential for matrix effects given no time separation of ion suppressing/enhancing interferants. Here we describe the evaluation of Echo-MS for application of some common ADME assays for a set of benchmark compounds. Assay performance is compared to standard LC-MS analysis and the potential for Echo-MS to make an impact in this space is discussed.

Two common assays were evaluated to test the breadth of assay complexity required for ADME applications. Firstly metabolic stability where test compound depletion is followed in microsomes to generate intrinsic clearance (CL_{int}) values. Secondly CYP inhibition where the inhibition effect of test compound on the metabolism of a marker compound is measured by monitoring formation of a known metabolite. For both assays a panel of test compounds was used and the performance of Echo MS benchmarked against established LC-MS methods.

Echo MS Concept

Echo MS uses Acoustic Ejection Mass Spectrometry (AEMS) to introduce accurate small volumes (typically 2.5nL but scalable to 25 nL by using multiple ejections) of sample to the mass spectrometer. These are then sampled by the open port interface (OPI) and transferred to the ion source (Figure 1). This contactless technique is highly reproducible and gives zero carryover. Used in combination with a Sciex 6500+ triple quad instrument it provides sensitivity levels suitable for ADME applications.

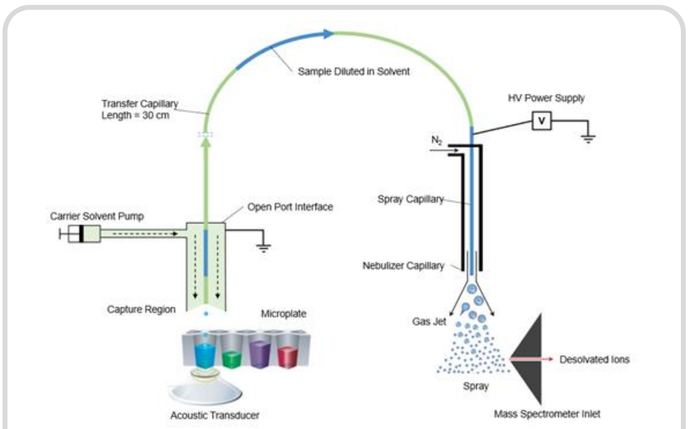


Figure 1: Schematic of the Echo MS System showing the acoustic ejection of sample droplets and their capture and transfer to the OptiFlow Source by the OPI.

The sample dilution effect in the OPI (typical dilution factor >1000) in theory leads to reduced matrix related ion suppression effects. This combined with the uniformity of control biological matrix used in in vitro ADME should make AEMS suitable for ADME applications. In addition where no isobaric separations are required (ie where MS/MS selectivity alone is sufficient) AEMS should provide a suitable analytical solution.

Prior to application to ADME samples the linearity and reproducibility of sample ejection volume in Echo MS was assessed. Results are presented in Figure 2.

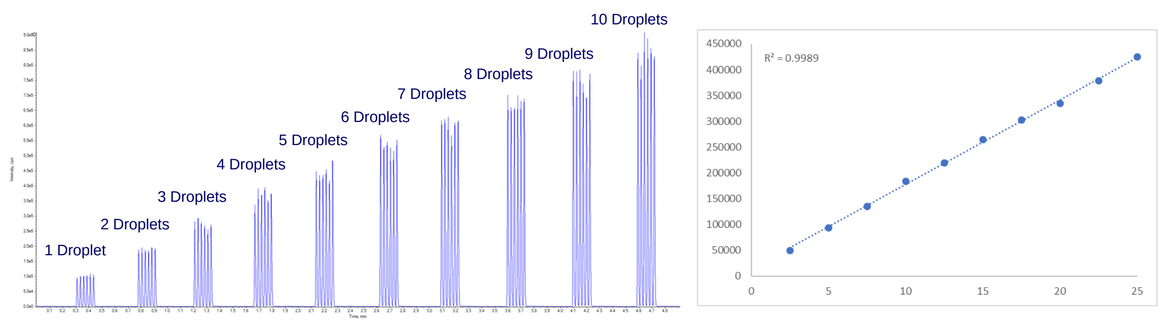


Figure 2: An example AEMS droplet ladder for lidocaine ranging from 1- 10 droplets per MS measurement, equivalent to ejection volumes of 2.5- 25 nL. The calibration line shown in insert with linear R^2 value >0.998

Droplet linearity was shown to be good (R^2 value >0.998). This allows increased sample ejection volumes to be used when sensitivity in biological matrix may be limited. The technique was also shown to be very reproducible with typical RSDs based on peak area response to be in the 5-10% range from samples from different wells,

Metabolic stability evaluation

A set of benchmark commercial compounds was used to assess this assay. The compound set included compounds with a range of CL_{int} values.
Human microsomes (final protein concentration 0.5 mg/mL), 0.1 M phosphate buffer pH 7.4 and test compound (final substrate concentration 1 µM; final DMSO concentration 0.25%) were preincubated at 37 °C prior to the addition of NADPH (final concentration 1 mM) to initiate the reaction. All incubations were performed singularly for each test compound. Each compound was incubated for 0, 5, 15, 30 and 45 min. The reactions were stopped by transferring incubate into acetonitrile at the appropriate time points, in a 1:3 ratio. The termination plates were centrifuged at 3,000 rpm for 20 min at 4 °C to precipitate the protein. Analysis was performed on the same sample set first using a generic LC-MS method then subsequently using Echo MS.
CL_{int} (Intrinsic clearance) values and compound responses vs time profiles were compared. Examples of profiles obtained for slow and rapidly clearing compounds are presented in Figures 3 and 4 respectively.

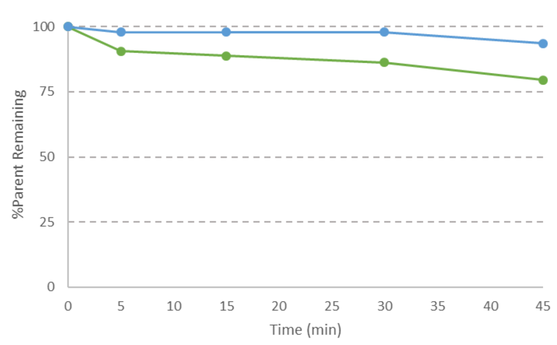


Figure 3: Response/time profiles for the clearance in human microsomes for Prazosin measured by LC-MS (green) and Echo MS (blue).

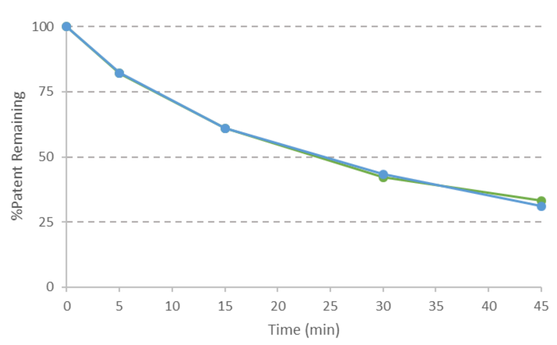
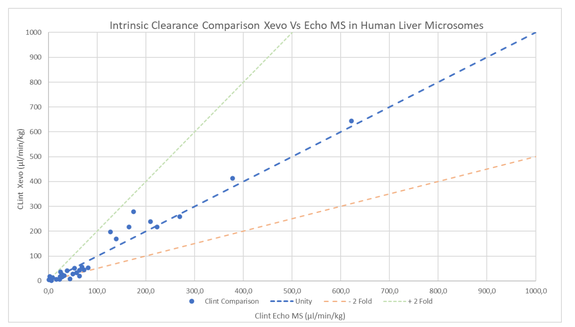


Figure 4: Response/time profiles for the clearance in human microsomes for Amodiaquine measured by LC-MS (green) and Echo MS (blue).



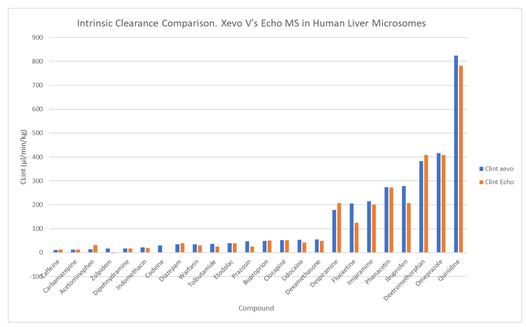


Figure 5: Comparison of intrinsic clearance values for the full compound test set derived from LC-MS and Echo MS

Clearance values were in excellent agreement across the range of values. Examination of LC-MS chromatograms indicated that no additional isobaric peaks were present. This removes the need for time separation and increases the applicability of Echo MS as a viable technique for this assay.

CYP inhibition evaluation

The following method was used to assess the inhibition potential of a set of validation compounds, with a range of IC_{50} values. This was performed for all 7 isoforms (8 substrates). See table 1.
Human liver microsomes were incubated with test compound at 6 concentrations, in the presence of NADPH (1mM) and a probe substrate, at K_m. Following incubation, the reaction was terminated by removing an aliquot of the incubate and quenching into methanol. Vehicle controls (0.25% DMSO) were included for all experimental conditions. The samples were centrifuged at 3000 rpm for 30 min at 4°C, and aliquots of the supernatant were diluted with formic acid in deionised water containing the internal standard (final formic acid concentration = 0.1%). Cyprotex generic LC-MS/MS conditions were used to monitor metabolite formation. Following this, the samples were analysed using Echo MS.

Isoform	Substrate	K_m (µM)	Protein concentration (mg/mL)	Incubation time (mins)	Metabolite
1A2	Phenacetin	18	0.1	5	Acetaminophen
2B6	Bupropion	150	0.1	5	Hydroxybupropion
2C8	Amodiaquine	2	0.05	5	N-desethylamodiaquine
2C9	Diclofenac	7	0.5	5	4-Hydroxydiclofenac
2C19	(S)-Mephenytoin	50	0.5	15	4-Hydroxymephenytoin
2D6	Dextromethorphan	2	0.1	5	Dextrorphan
3A4	Midazolam	2.5	0.1	5	1-Hydroxymidazolam
	Testosterone	50	0.1	5	6β-Hydroxytestosterone

Table 1:
Assay conditions for IC_{50} determination assay

CYP1A2 was found to be incompatible with Echo MS due to inability to separate isobaric peaks (insource conversion of Phenacetin to Acetaminophen). No inhibition was observed throughout the samples for this isoform.

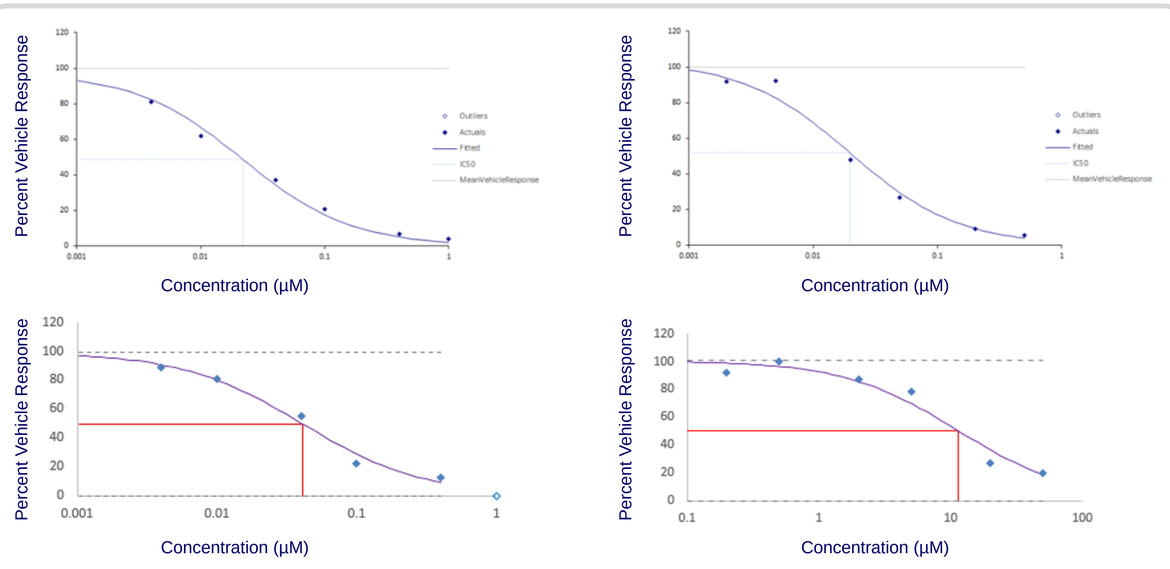


Figure 6: Inhibition profiles of quinidine in CYP2D6 on LC-MS (top) and Echo MS (bottom).

Figure 7: Inhibition profiles of disulfiram in CYP3A4 (testosterone) on LC-MS (top) and Echo MS (bottom).

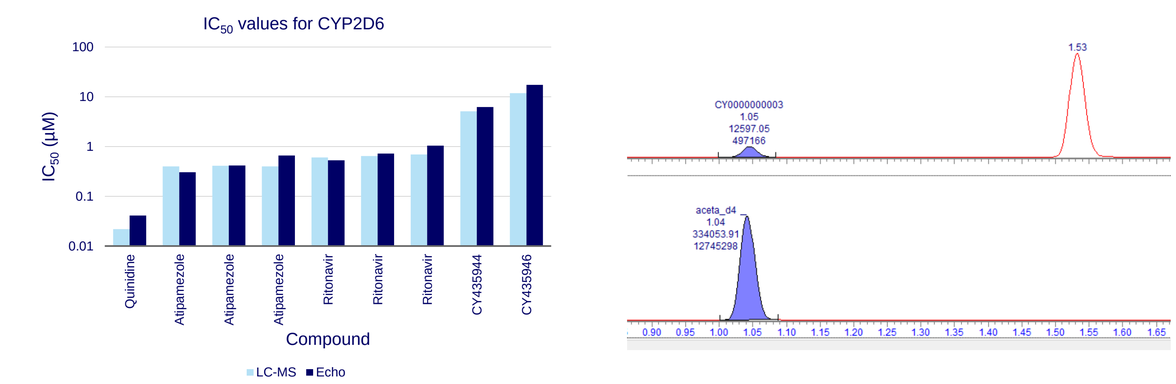


Figure 8: Comparison of all compounds where a predicted IC_{50} value was generated for CYP2D6

Figure 9: Chromatography for CYP1A2 metabolite acetaminophen and acetaminophen-d4 (internal standard).

The majority of isoforms showed IC_{50} values in good agreement between LC-MS and Echo MS generated data. CYP1A2 provides a good example where time separation of isobarics imply the non-applicability of Echo MS.

Summary/ conclusions

Overall we are very encouraged by the data generated in this evaluation. Clearly Echo MS is able to reduce cycle time for analytical determinations in HT ADME. The technique is probably very applicable to straightforward assays like metabolic stability but should be considered on a case by case basis for other ADME applications. Cyprotex is continuing to test its panel of ADME screening assays to assess the breadth of applicability.

Acknowledgements

The authors would like to acknowledge the kind collaboration of Sciex in running the Echo MS analyses and for providing graphics on Echo MS concept.