Ultilising Echo® MS as a mass spectrometry-based assay technique to screen for Acetylcholinesterase inhibition

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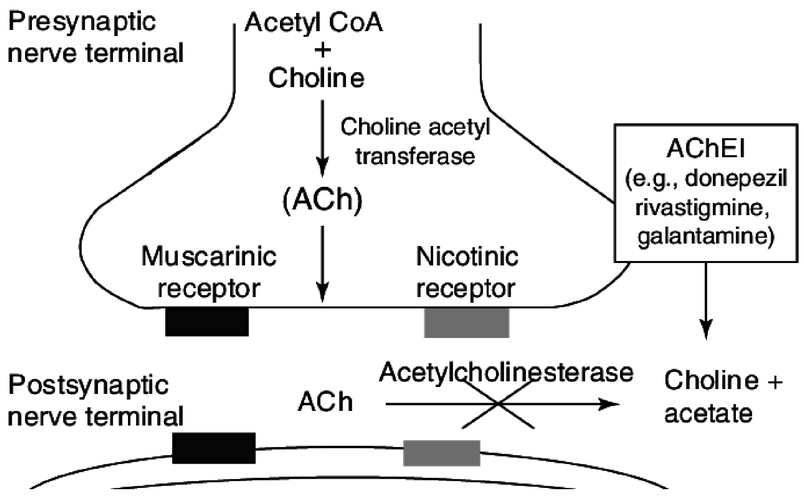
Background

In recent years, the use of mass spectrometry as a detection technique for biochemical enzymatic assays has significantly grown. Additional technological advances have also enabled the label free technique to improve throughput and therefore ability to be used as a screening tool for drug discovery. The acetylcholinesterase (AChE) inhibition assay is used as example to demonstrate the advantages of Echo® MS. The purpose of this screening assay is to identify candidate compounds with potential AChE liabilities.

Figure 1. Schematic representation of AChE



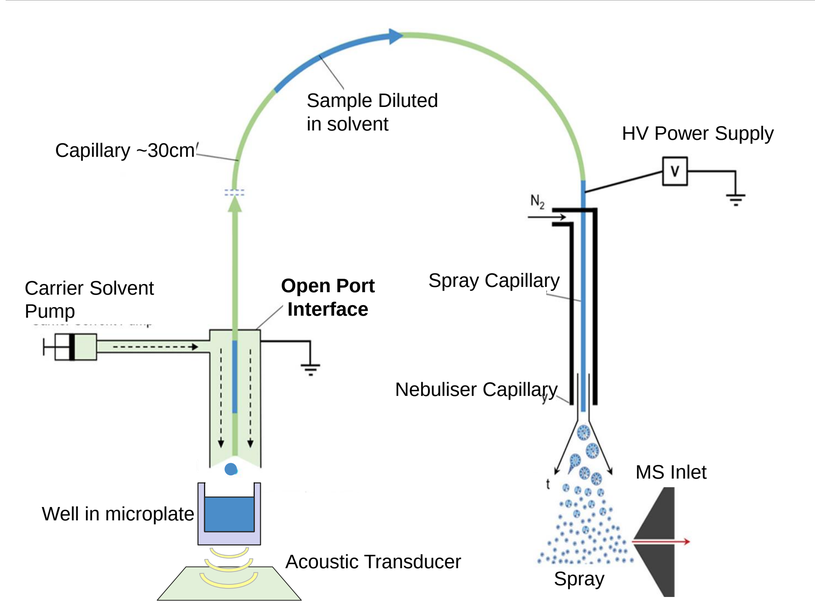
AChE is a serine protease that hydrolyses the neurotransmitter acetylcholine (ACh). AChE is found mainly at neuromuscular junctions and cholinergic brain synapses, where its activity serves to maintain a very low concentration of ACh in the synaptic cleft *(Figure 1).* Inhibition of AChE leads to accumulation of ACh in the synaptic cleft and consequently overstimulation of the receptors, impeding neurotransmission[1]. This may be therapeutically beneficial in some neurological diseases, or may be an adverse effect. Mode-of-action is important, because irreversible inhibitors cause significant adverse effects and/or toxicities, such as decreased blood pressure, decreased heart rate, bronchoconstriction and possibly death[2].

Figure 2. RapidFire MS (left) and Echo® MS (right)

Previous work for this assay was undertaken using another MS based screening platform from Agilent, RapidFire MS (RFMS) (*Figure 2, left*). This front end instrumentation uses online solid-phase extraction in place of standard chromatographic separation to allow for sample clean up and analysis within 10 seconds. However the RFMS had a high background signal for Choline, increasing the limit of detection to ~1uM and carryover was particularly high, requiring several additional wash steps, resulting in a 384 plate analysis time of ~90 minutes.

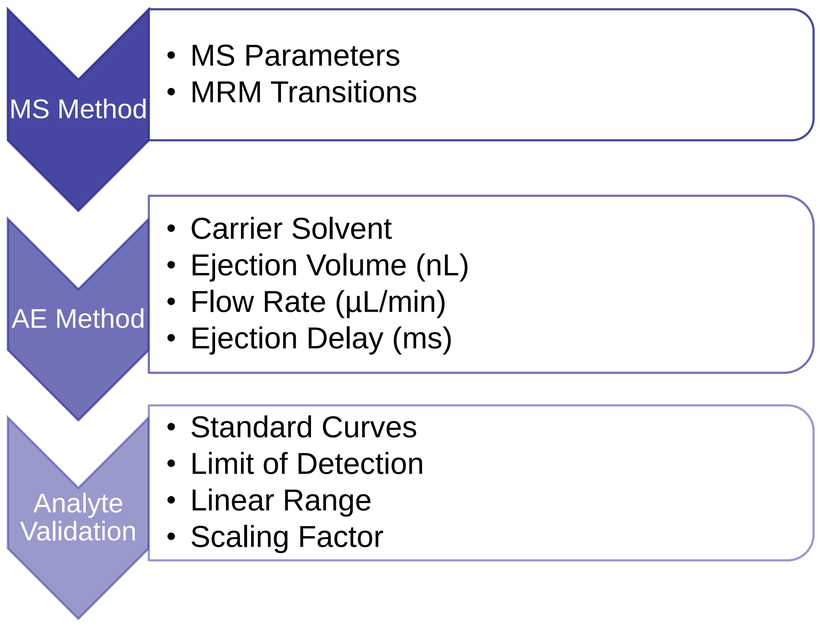
In 2020, SCIEX released the Echo® MS platform (*Figure 2, right*); an instrument that utilises acoustic ejection (AE) sampling coupled to a triple quadrupole mass spectrometer. These are coupled together with an open port interface (OPI) able to deliver nano-litre (nL) scale droplets from a well plate directly into the MS source as illustrated in *Figure 3*.

Figure 3. AE-OPI-MS Schematic [4]



Method Development

Figure 4. Method Development Workflow



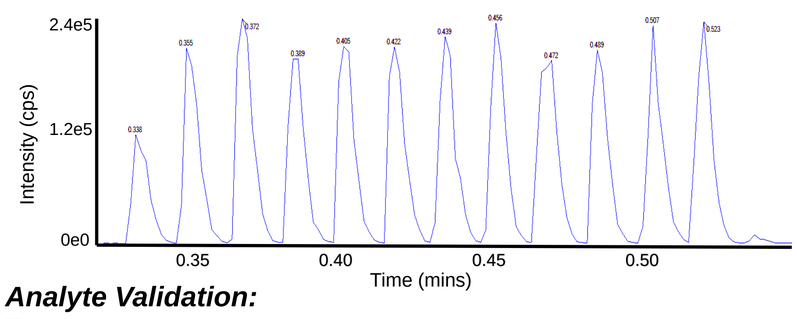
MS Method development:
Initial MS parameters were determined for the mass spectrometer (6500+, Sciex) to set up a multiple reaction monitoring (MRM) method with suitable transitions for both ACh and Choline (Ch). Both analytes contain a quaternary nitrogen so M+ ions were used. Multiple precursor to product ion transitions were monitored and one per analyte was chosen based on low background, high S/N and good intensity, these were *m/z* 146.2→87 for ACh and *m/z* 104.2→60 for Ch.

Acoustic Ejection (AE) Method development:

Carrier Solvent	70% acetonitrile/30% water – with 0.1% formic acid
Ejection Volume	2.5nL
Flow Rate	650µl/min
Ejection Delay	1000ms

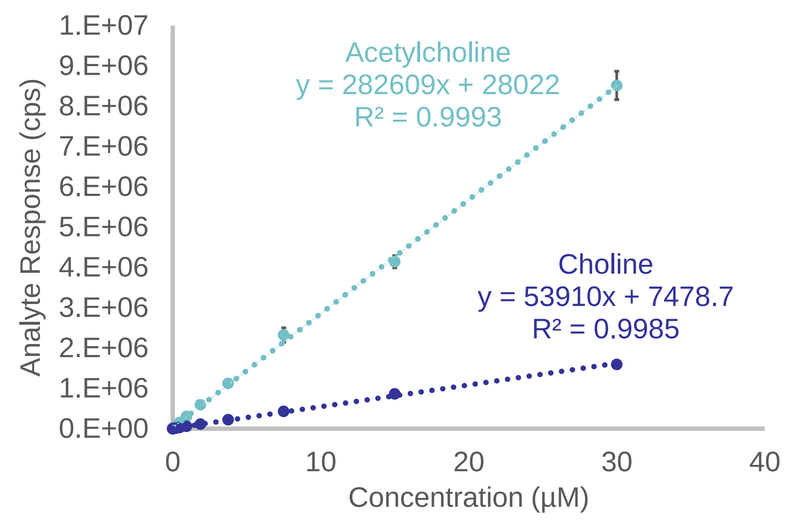
Sampling requirements for the Echo ® MS system also include an acoustic ejection method to determine suitable conditions to allow for ejection from well plates. Carrier solvent was chosen based on the initial RFMS solvent parameters. The high % of acetonitrile allowed for higher flow rates to be used, therefore peaks produced from ejections should be narrower and return to baseline sooner *(Figure 5),* reducing the delay time between ejections. The initial flow rate was set to ~650uL/min but this should be optimised before each run and will differ between electrodes. The smallest ejection volume available on the system was 2.5nL and this provided sufficient response intensity so was chosen for the AChE assay development.

Figure 5: XIC for Choline showing 12 individual ejections



Analyte Validation:
To determine linear range, comparative ionisation efficiency and limits of detection, standard curves for both analytes were performed in assay buffer conditions *(Figure 6)*. Due to the difference in ionisation of the analytes, a scaling factor of 0.19 was required to use percentage conversion as a normalisation for the assay results.

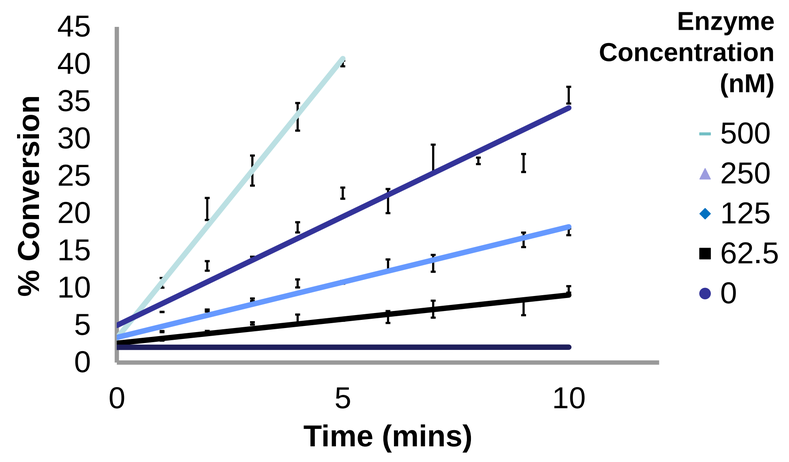
Figure 6 – Standard Curves for ACh and Ch



Assay Development and Validation

Enzyme titration *(Figure 7)* and kM experiments were undertaken to confirm final enzymatic conditions required for the biochemical assay. Final conditions to run a linear enzymatic reaction were 100pM AChE, 200µM ACh (substrate) and an 8 minute incubation time. Additional DMSO and validation plates were run before assay was deemed fit for routine screening.

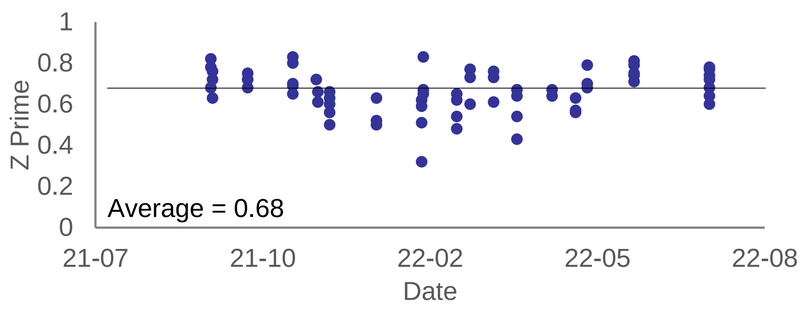
Figure 7. Enzyme titration in 0.1% DMSO for AChE assay



Assay Quality Metrics

Z Prime is a common assay quality metric used (in addition to standards on a dose response curve). Since moving to Echo® MS the Z prime metric has averaged 0.68*(Figure 8)*.

Figure 8. Z Prime over several months



Comparison and Conclusions

	RapidFire MS	Echo® MS
Sample analysis time	14 sec (90 mins per plate)	~1 sec (8 mins per plate)
Inject volume	30-40 **µL**	2.5 **nL**
Carrier Solvent	0.1% Ammonium Acetate (aq) and 0.1% TFA in MeCN	70% MeCN (aq) + 0.1% HCOOH
Flow rates	1 – 1.5 mL/min	0.65 mL/min
ESI source temperature	650°C	300°C

Overall, the transition from RFMS to Echo® MS for the AChE biochemical assay had several improvements, especially in throughput. There were also significant energy (ESI source temperature) and solvent (flow rate) savings. The low sample volumes required also allowed for repeat analysis if required. Echo® MS provided a useful platform for biochemical assay screening, especially in development as only a simple method development workflow was required (no chromatography or other online sample clean up optimisation). However use of the instrument should be considered on a analyte specific basis, especially when higher requirements of sensitivity are required at a low concentration. Future work will include additional biochemical assay development work for other targets using the Echo® MS.

References

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Acknowledgments

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