Enhancing Throughput of In Vitro DDI Assays with Echo®MS+ Platform: Efflux Transporter Case Study

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INTRODUCTION

High throughput screening (HTS) of typical in-vitro drug-drug interaction (DDI) can greatly reduce assay turn-around time, thereby affording faster decision making. A typical pain point is the liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) detection of the substrates and/or metabolites used in these assays. With run times in minutes coupled with issues of matrix effects, carryover and the difficulty in analyzing small polar analytes, development time is also a factor. Use of acoustic ejection mass spectrometry alleviates many of these issues (1). The rapid sample analysis (seconds per injection) and robust sensitivity make this platform ideal for DDI studies (2). MDR1/P-gp and BCRP are members of the ATP-binding cassette (ABC) family of transporters that use ATP to actively transport xenobiotics across cell membranes. The FDA and ICH M12 guidelines (3,4) outline the recommended substrates and inhibitors for use in in-vitro transporter studies. Here we present data from our Echo®MS+ system coupled to a ZenoTOF 7600 demonstrating a robust linear range for a variety of ABC transporter substrates and IC_{50} data for BCRP and MDR1/P-gp.

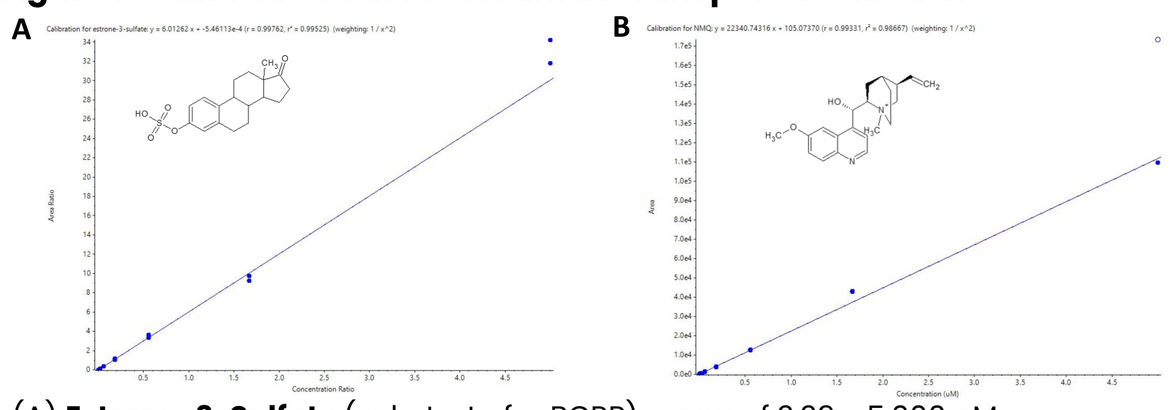
METHODS

Materials: HEK293-derived human MDR1/P-gp and BCRP vesicles (Corning® TransportoCells™) and MRP/BCRP vesicle assay kit were purchased from Discovery Life Sciences. Substrate and inhibitor compounds, and internal standards were purchased from Toronto Research Chemicals, Millipore Sigma, or Santa Cruz Biotechnology.
Uptake Assay: Utilizing a 96-well format, the prepared Corning® TransportoCells™ MDR1/P-gp or BCRP vesicles (containing substrate with and without inhibitor) were preincubated in assay buffer at 37°C for 10 minutes prior to addition of ATP or AMP(5). After addition of ATP or AMP the reactions were incubated for 2 minutes at 37°C and then terminated by addition of ice-cold washing buffer followed by filtering through a glass fiber filter plate (MilliporeSigma MSFBN6B10). The filter plate was washed 5 times with ice cold buffer and then dried. The samples were eluted from the glass fiber filter plate by 2 x 50 µL volumes of acetonitrile:water (80:20, v/v) and then processed by solid phase extraction (Wates Oasis HLB Prime µElution or WCX µElution). After extraction the samples were transferred to a 384-well plate using an Integra Assist Plus and then analyzed using a Sciex Echo®MS+ system coupled to a Sciex ZenoTOF 7600. Methanol with 0.1% formic acid (450 µL/min) was used as the carrier solvent, SP fluid class with 100 nL injection volume at 50 Hz in wide acquisition mode. The compounds were detected in ZenoTOF MRM^{HR} mode.

Parameter	Value
Ion source gas 1/gas 2/curtain gas	90/40/35 psi
Source Temperature	300°C
CAD gas	7
Ion Spray Voltage	4500

RESULTS

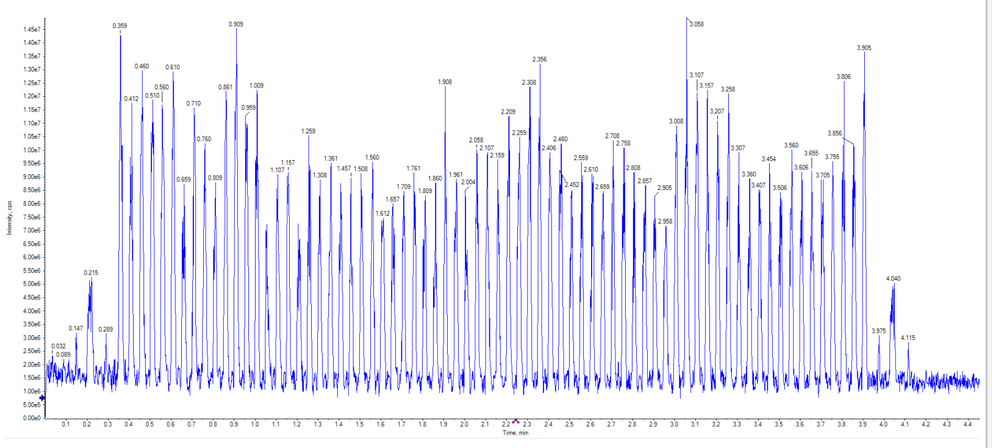
Figure 1: Calibration Curves for Efflux Transporter Substrates



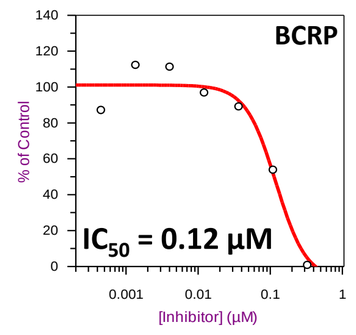
(A) **Estrone-3-Sulfate** (substrate for BCRP) range of 2.29 - 5,000 nM
(B) **N-Methyl Quinidine** (substrate for P-gp) range of 2.29 - 5,000 nM

Analytical performance was similar to that of liquid chromatography coupled to a tandem mass spectrometer. Lower limit of quantitation was suitable for the assay requirements.

Figure 2: EchoMS+ ejections An example series of ejections marked at beginning and end with marker ejections. 384 ejections takes about 10 minutes.



BCRP		P-gp	
Estrone-3-Sulfate Uptake		N-Methyl Quinidine Uptake	
Ko143 (µM)	Average % Control	Elacridar (µM)	Average % Control
1.00	-3.70	3.00	0.561
0.333	0.482	1.00	6.73
0.111	53.6	0.333	94.8
0.0370	88.8	0.111	109
0.0123	96.6	0.0370	106
0.00412	111	0.0123	92.7
0.00137	112	0.00412	95.5
0.000457	86.9	0.00137	103
0	100		100



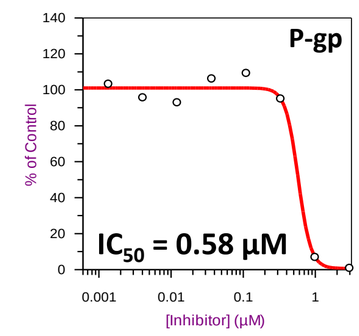


Figure 3: Uptake Inhibition Profiles
Prototypical inhibitors were used to demonstrate concentration-dependent inhibition of substrate transport. Ko143 stopped transport of E3S by BCRP with an IC_{50} of 0.12 µM. Elacridar stopped transport of NMQ with an IC_{50} of 0.58 µM. These values compared with values in literature.

CONCLUSION

Using the Echo®MS+ system coupled to a ZenoTOF 7600, we demonstrated a robust linear range for various transporter substrates for BCRP and MDR1/P-gp. This system reproduced IC_{50} data that agrees with reported values and confirms the validity of this approach. With suitable performance and speed, the Echo®MS+ platform is a powerful tool for transporter and other DDI assays.

REFERENCES

1. Wagner A. et al. Anal Chem. 2020; 92:13525-13531.
2. McCabe J et al. Rapid and accurate bioanalysis for drug screening. Sciex technical note.
3. FDA Guidance: In Vitro Drug Interaction Studies — P450 Enzyme- and Transporter-Mediated Drug Interactions. 2020.
4. ICH M12: Drug Interaction Studies. 2024.
5. Corning TransportoCells HEK-293 Derived ABC Transporter Membrane Vesicles. Instructions for Use.
6. Shen et al. DMD. 2022; 50:299-319.
7. Wolff et al. XenoTech Poster. ISSX. 2013.