A novel high duty cycle data independent technique for metabolite identification in drug metabolism

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ABSTRACT

Drug metabolism is an integral part of the drug discovery process. Many advancements have taken place with respect to faster and more selective mass spectrometers but sensitivity in MS/MS mode has not been fully addressed specially when using a data independent acquisition strategy. Detecting and identifying low level metabolites is important as not all metabolites will ionize in a similar manner and some of these metabolites can be very relevant for toxicity and pharmacokinetic purposes.

A unique workflow that fully leverages a Zeno trap (figure1) coupled to a novel QTOF in which >90% duty cycle is achieved in MS/MS mode is demonstrated [1] and when coupled to a data independent acquisition using variable windows, we observed a higher percentage of metabolites detected.

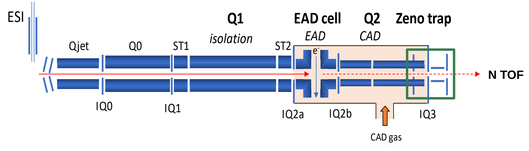

Figure 1. ZenoTOF 7600 system ion path with highlighted collision cell region with the Zeno trap.

MATERIALS AND METHODS

Sample preparation: Buspirone was incubated in rat hepatocytes at 1 µM starting concentration. Time points of 0, 30 min and 120 min were pulled from the incubation and quenched with 1 volume of CH_3CN. Samples were vortexed, centrifuged, and the supernatant transferred to an HPLC vial for analysis.

HPLC conditions: An ExionLC AD system with a Phenomenex Luna Omega Polar C18, 150x2.1mm, 1.8 µm column at 40ºC. Gradient separation using 0.1% formic acid in water and 0.1% formic acid in acetonitrile was used at a flow rate of 400 µL/min. Separation was performed over 4.75 minutes from 5% B to 95% B. The injection volume was set to 0.5 µL.

MS/MS Conditions: A SCIEX ZenoTOF 7600 system with Turbo V ion source and electrospray ionization (ESI) was used.

The samples were analyzed in data independent mode, using SWATH acquisition with CID and with and without Zeno trap activation. The TOF MS scan was scanned between *m/z* 100-1000, the SWATH acquisition MS/MS scans were 22 variable windows covering *m/z* 100 to 980 and scanned between *m/z* 60 – 1000.

CID conditions were set to generic collision energy using a value of 45 V and a collision energy spread of 15V.

RESULTS

From the CID SWATH acquisition for the 30-minute timepoint unmetabolized Buspirone was not detected. The detected metabolites represented 2 phase 2 conjugations with glucuronic acid and the remainder phase I metabolites. Shown in Table 1 are all metabolites. For the metabolites to be identified, both MS and MS/MS are needed from the SWATH acquisition data to meet the minimum criteria as set in the defined processing method.

Table 1. Parent and metabolites detected from t-30-minute timepoint at greater than 0.1% peak area from SWATH acquisition.

Metabolite name	R.T	% Area	Mass Accuracy
Oxidation 1	3.43	36.13	0.4
Di-oxidation 1	3.27	22.43	-0.4
Oxidation 2	3.03	10.51	-0.6
Oxidation 3	3.13	8.08	0.7
Di-oxidation 2	2.64	4.99	1.0
Di-Ox + glucuronidation	2.95	4.18	2.8

Metabolite name	R.T	% Area	Mass Accuracy
Ketone formation	3.01	4.09	0.4
Di-oxidation 3	2.79	4.06	-0.9
Di-oxidation 4	3.53	1.94	-0.9
Ox + glucuronidation 1	3.32	1.60	-0.6
Oxidation 4	3.58	1.38	-4.7

From the Zeno SWATH acquisition using CID fragmentation for the 30-minute timepoint unmetabolized Buspirone was detected in both MS and MS/MS representing 0.7% of total MS area. The detected metabolites, 18 in total represented 2 phase 2 conjugations with glucuronic acid and the remainder phase I metabolites. Shown in table 2 are all metabolites. The same processing method and therefore scoring parameters were utilized as with the standard SWATH acquisition.

Table 2. Parent and metabolites detected from t-30-minute timepoint at greater than 0.1% peak area from Zeno SWATH acquisition.

Metabolite name	R.T	% Area	Mass Accuracy
Oxidation 1	3.44	30.20	2.0 ppm
Di-oxidation 1	3.28	20.06	2.1 ppm
Oxidation 2	3.04	10.49	1.6 ppm
Oxidation 3	3.15	7.64	1.3 ppm
Di-oxidation 2	2.65	4.48	1.8 ppm
Oxidation 4	3.34	4.26	1.0 ppm
Ketone formation	3.02	4.14	1.5 ppm
Di-Ox + glucuronidation	2.96	2.96	0.9 ppm
Di-oxidation 3	2.81	2.82	0.0 ppm

Metabolite name	R.T	% Area	Mass Accuracy
Di-oxidation 4	3.15	2.54	2.3 ppm
Di-oxidation 5	3.54	1.96	-1.5 ppm
Ox + glucuronidation 1	3.32	1.93	1.0 ppm
Oxidation 5	3.58	1.93	-2.0 ppm
Ketone 2	3.31	1.77	1.2 ppm
Di-oxidation 6	2.97	0.81	-0.5 ppm
Tri-Ox 1	2.86	0.75	-4.3 ppm
Parent	3.76	0.72	0.3 ppm
Tri-ox 2	3.02	0.51	0.8 ppm

Table 1 and 2 show the increase in putative metabolites identified with Zeno SWATH. This shows a greater than 50% increase in the number identified. This comes from the MS/MS driven processing whereby each metabolite is scored at the MS and MS/MS level. Where fragment ions that would be expected for that modification are not observed in the experimental data these metabolites are therefore not identified.

The Zeno trap is designed to drive MS/MS duty cycle above 90%. Figure 2 shows the impact of the Zeno trap when activated or deactivated.

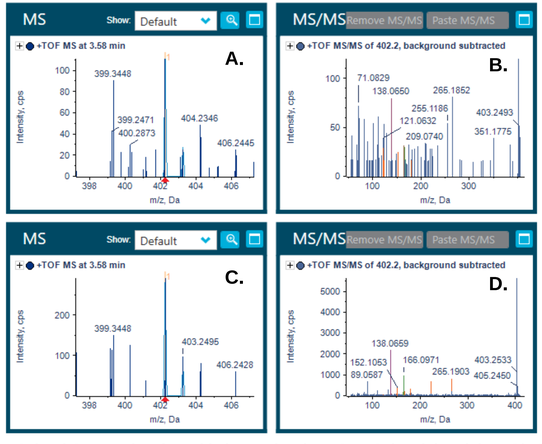

Figure 2. (Top) SWATH data for oxidation 5, showing TOF MS data (A) and the MS/MS data (B). (Bottom) SWATH acquisition data for oxidation 5, showing TOF MS data (C) and the MS/MS data (D).

The key driver for the improvement of metabolite coverage is the increased sensitivity at the MS/MS level from the SWATH acquisition data. The Zeno trap when activated delivers an average of 7-15-fold increase in signal across the fragment mass range. This is shown in Figure 2 b and d where each of the MS/MS spectra have been extracted. On average the low mass fragments are enhanced by a factor of 10. This leads to unambiguous spectra which can be assigned based on similarity to parent including common fragments and neutral losses. This impact to the MS/MS spectra is what in turn leads to the deeper coverage of metabolites.

A key advantage of SWATH acquisition is the ability to extract and quantify at both the MS and MS/MS level. Here the impact is further enhanced with an increase in signal to noise of 10x when the Zeno trap is enabled over MS.

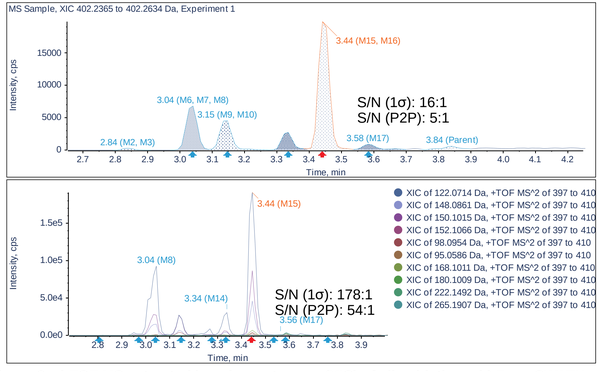

Figure 3. a) TOF MS extracted ion chromatogram (XIC) of all oxidations, b) Zeno SWATH MS/MS XICs for all oxidations

CONCLUSIONS

The enablement of the Zeno trap in operation with SWATH acquisition leads to a significant increase in sensitivity. The impact to the detection of metabolites is under the conditions tested is a greater than 50% increase in coverage.

Zeno SWATH shows the potential to dig deeper where MS sensitivity and/or selectivity limit the detection of metabolites in complex matrices.

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