High-Throughput Soft Spot Identification with SWATH–MS and MassMetasite: untargeted data acquisition and automatic data processing

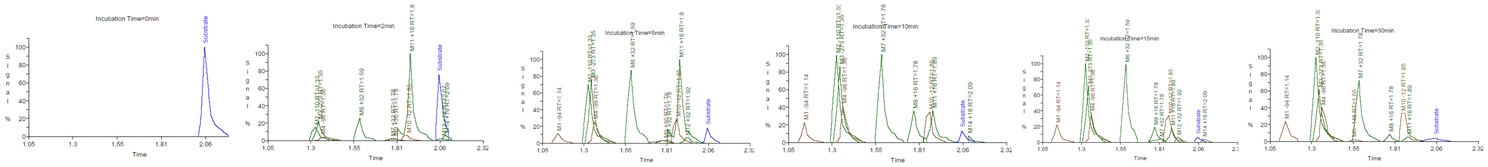
Ismael Zamora[1, 3]; Fabien Fontaine[1, 2]; Natasha Penner[4]
[1]Lead Molecular Design, S.L., Sant Cugat Del Valles, SPAIN; [2]Molecular Discovery, London, UK; [3]Pompeu Fabra University, Barcelona, Spain; [4]Biogen-Idec, Cambridge, MA

Introduction

Last year a study was presented at the ASMS comparing the Soft Spot Identification for a set of 9 compounds using different acquisition modes (targeted and untargeted) showing similar results in all different cases. Also, new acquisition modes have been developed recently like the SWATH untargeted mode where the MSMS is triggered using different mass window. The aim of this presentation is to show how the same incubation samples that were presented before perform under this new acquisition mode.

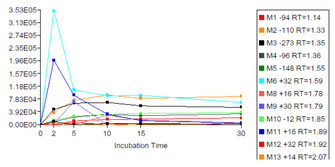
Results

The preliminary results show that the untargeted SWATH data yield the similar soft spot than the previously reported studies. Which means that similar atoms in the parent structures of verapamil, Saquinavir, nefazodone, dextromethorphan, 2N-569S, 7Z-0822,10P-909 and 10R-0650 were pointed as the labile place for oxidative metabolism. The interpretation of the SWATH spectra in terms of fragmentation analysis gave similar quality in the scoring analysis than the Data Dependent scan methods using the same or different equipment with different acquisition modes. In addition it reduced the set of potential metabolites peaks when compared to the more traditional non targeted methods. Results show for Nefazodone.

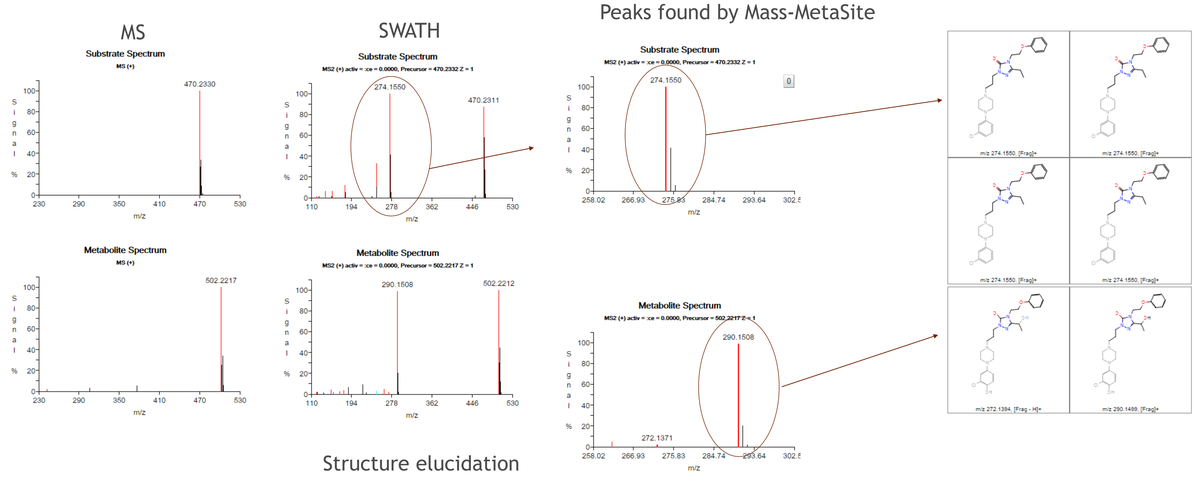


HPLC analysis: Chromatogram for the different time points

Name	RT	m/z	m/z diff (ppm)	Area ABS	Area %	ion formula	Z	Groups	Max score
M1 -94 RT=1.14	1.14	376.2343–376.2356	0.05–-3.29	1.09E04–2.11E04	2.27–6.67	$[C_{19}H_{28}N_5O_3 + H]^+$	1		558.9
M2 -110 RT=1.33	1.33	360.2396–360.2403	-0.55–-2.60	3.72E04–9.11E04	5.65–27.42	$[C_{19}H_{28}N_5O_2 + H]^+$	1		740.5
M3 -273 RT=1.35	1.35	197.0835–197.0839	0.49–2.46	4.65E04–6.84E04	7.07–17.02	$[C_{10}H_{13}ClN_2 + H]^+$	1		386.8
M4 -96 RT=1.36	1.36	374.2187–374.2195	-0.02–-2.34	9.11E03–3.34E04	1.38–10.54	$[C_{19}H_{27}N_5O_3 + H]^+$	1		464.1
M5 -148 RT=1.55	1.55	322.1386	3.46	3.76E03	1.19	$[C_{15}H_{19}N_3O_5 + H]^+$	1		270.0
M6 +32 RT=1.59	1.59	502.2208–502.2235	-0.24–-3.77	6.82E04–3.53E05	21.53–53.58	$[C_{26}H_{32}ClN_5O_4 + H]^+$	1		837.4
M8 +16 RT=1.78	1.78	486.2262–486.2269	-0.14–1.00	5.56E03–2.93E04	1.75–7.29	$[C_{26}H_{32}ClN_5O_3 + H]^+$	1		672.5
M9 +30 RT=1.79	1.79	500.2058–500.2064	0.32–-0.90	4.37E03–7.01E04	0.66–14.54	$[C_{26}H_{30}ClN_5O_4 + H]^+$	1		87.5
M10 -12 RT=1.85	1.85–1.87	458.1943–458.1977	-0.98–-5.05	1.37E04–3.77E04	2.09–11.90	$[C_{23}H_{28}ClN_5O_3 + H]^+$	1		203.9
M11 +16 RT=1.89	1.89	486.2260–486.2273	0.32–-1.45	4.87E03–1.98E05	1.54–30.12	$[C_{26}H_{32}ClN_5O_3 + H]^+$	1		633.8
M12 +32 RT=1.92	1.92	502.2209–502.2222	1.25–-1.28	4.41E03–6.52E03	1.29–1.35	$[C_{26}H_{32}ClN_5O_4 + H]^+$	1		537.9
Substrate	2.06	470.2316–470.2342	-0.15–-5.19	2.13E03–1.20E06	0.67–100.00	$[C_{26}H_{32}ClN_5O_2 + H]^+$	1		
M13 +14 RT=2.07	2.07	484.2118	-1.61	5.43E03	0.82	$[C_{26}H_{30}ClN_5O_3 + H]^+$	1		306.7
M14 +16 RT=2.09	2.09	486.2247–486.2279	-0.21–-4.09	2.54E03–9.81E03	0.75–1.49	$[C_{26}H_{32}ClN_5O_3 + H]^+$	1		594.2

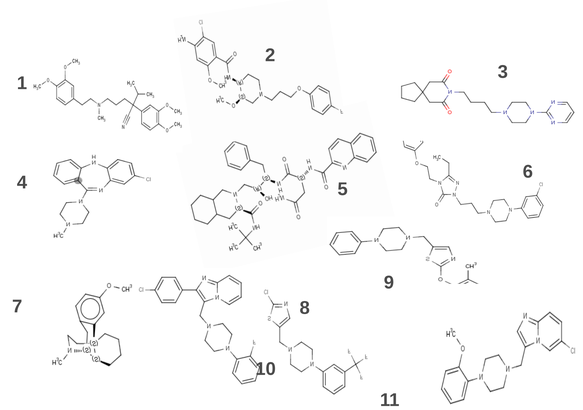


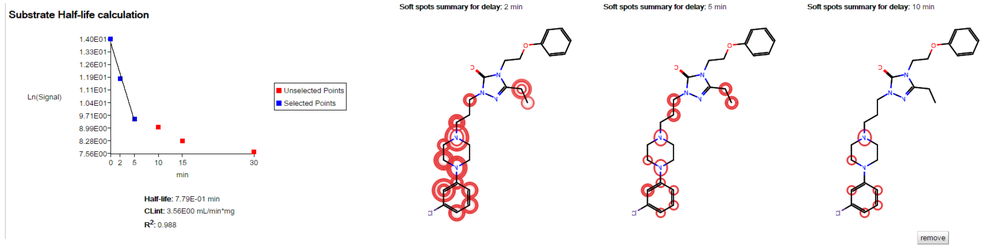
Peaks found by Mass-MetaSite



Methods

Human Liver Microsomes (HLM) at concentration of 0.5 mg/ml were incubated with 1 µM drug substrates in 0.1M sodium-phosphate buffer substituted with 1mM NADPH. Samples were taken after 2, 5, 10, 15 and 30 minutes and reaction was stopped by adding one volume acetonitrile. Samples analyzed by Waters-UPLC-system coupled ABSciexTripleTOF™5600 mass-spectrometer. Generic, targeted LC-HRMS method was applied to 9 compounds. Data analysis was performed with MassMetaSite and the pre-process data was uploaded automatically into WebMetabase for expert approval a visualization.





Soft Spot Analysis

Conclusions

- Mass-MetaSite can effectively process SWATH data for:
  - Metabolite Identification
    - Peak finding
    - Structure Elucidation
  - Soft Spot Analysis