PosterREPRINT

Waters

HIGH MASS ACCURACY MEASUREMENTS IN W-OPTICS™ USING AN ORTHOGONAL HYBRID QUADRUPOLE TIME OF FLIGHT MASS SPECTROMETER FOR *IN VITRO* METABOLISM STUDIES

*Jose Castro-Perez[1] *, Gary Harland[1], Jonathan Pugh[1], Carina Leandersson[2] , Richard Thompson[2]*
[1]Waters Corporation, UK; [2]AstraZeneca R&D, DMPK & Bioanalytical Chemistry, S-431 83 Mölndal, Sweden

Presented at ASMS, Montréal, Canada, June 8-12, 2003

OVERVIEW

Waters® Micromass® Q-Tof Ultima™ Mass Spectrometer in W-Optics (Figure 1) at a resolution of FWHM 17,000 with a new modification on the TOF to improve exact mass was used. The main aim of this experiment was to run microsomal incubations at a very low level ranging 0.5-1µM in both modes full scan MS and MS/MS with exact mass and show the improvement in exact mass and quality of the data. LockSpray™ was used for exact mass measurements. The mass measurments errors were better than 0.8 ppm RMS in full scan MS mode and 1 ppm RMS in full scan MS/MS mode.

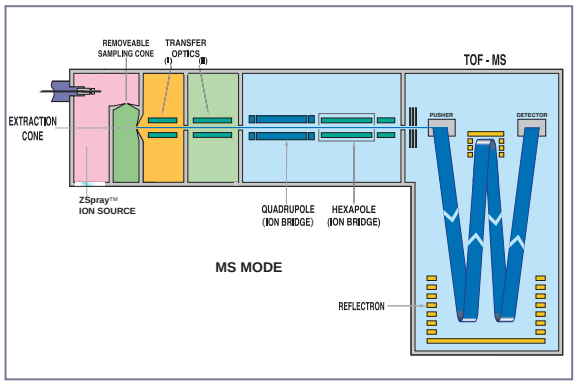

Figure 1. Q-Tof Ultima in W-Optics mode.

INTRODUCTION

An important area in the drug discovery and development process is the identification of drug metabolites in both *in vitro* and *in vitro* samples. Plasma metabolites are often difficult to detect by typical screening methods due to their low levels in circulation. The use of TOF technology has made this an easier task due to the high sensitivity in full scan mode in both MS and MS/MS. Also, good exact mass measurements in MS and MS/MS are important to detect and highlight biological changes which may occur as a result of metabolism. Moreover, during drug discovery accuracy of results will mean faster decision making for changes in the structure of the candidate compound so that it passes the screening criteria and can move onto the next stage. In this poster, we will show data obtained from different compounds following a microsomal incubation at 0.5 µM and 1 µM levels.

METHODS

Mass Spectrometer:	Q-Tof Ultima in W-mode
Ionization Mode:	ESI +ve ion
Capillary Voltage:	3.2 kV
Cone Voltage:	35 V
Source Temperature:	120 °C
Desolvation Temperature:	250 °C
Acquisition Mass Range:	100-850 amu
Lock Mass:	Leucine Enkephalin m/z 556.2771
Solvent Delivery System:	Waters 2795 Separations Module
Column:	Waters Atlantis™ dC_{18} 150 x 2.1 mm id 3.5 µm
Flow Rate:	200 µL/min
Mobile Phase A:	Water + 0.1 % Formic Acid
Mobile Phase B:	Acetonitrile + 0.1 % Formic Acid

Gradient

Time (min)	A%	B%	Flow (µL/min)
0.00	95.0	5.0	0.200
2.00	95.0	5.0	0.200
10.00	20.0	80.0	0.200
12.00	20.0	80.0	0.200
12.10	95.0	5.0	0.200
15.00	95.0	5.0	0.200

Injection Volume: 10 µL

Samples

Rat liver microsomes with a protein content of 0.5mg/mL was used to incubate Verapamil, Midazolam, Dextromethorphan, Metoprolol, Praziquantel, and Diazepam (Figure 2) at 0.5 and 1µM level. The reaction was stopped by adding 1 part of ice cold Acetonitrile with 2 parts of sample after a 60 min incubation. Then, the sample was centrifuged at 15,000 rpm and the supernatant was collected for subsequent LC/MS/MS analysis.

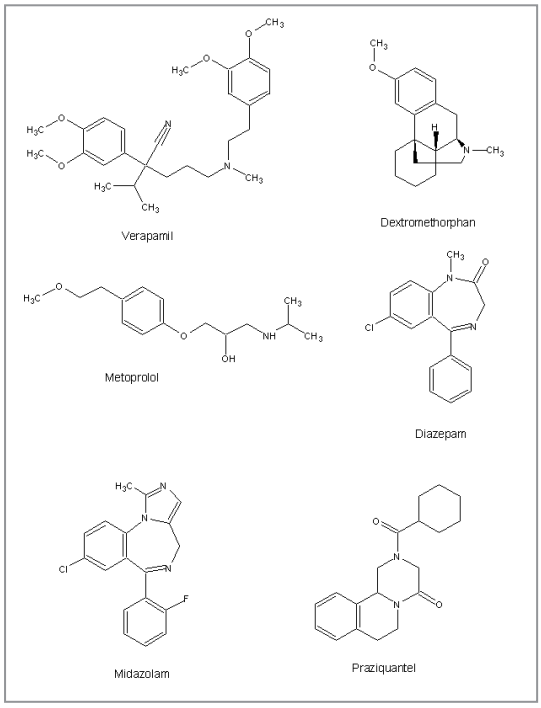

Figure 2. Compounds used for the Microsomal incubations.

RESULTS

- Good sensitivity was achieved for all the compounds incubated at 0.5 and 1µM in MS mode (Figure 3)
- The use of W-Optics provided greater specificity and selectivity for co-eluting interferences and exact mass measurements (Figure 4)
- Exact mass better than 0.8 ppm RMS was obtained in MS mode (Table 1) for all the parent compounds incubated and corresponding xenobiotics
- Subsequent MS/MS acquisitions were performed after detection of putative metabolites for structural ID (Figure 5 and 6)
- Excellent MS/MS data with exact mass was obtained which helped to elucidate the structure of several metabolites of interest. The mass measurements for the fragments of Dextromethorphan and the N-dealkylated metabolite were better than 1 ppm RMS (Table 2).

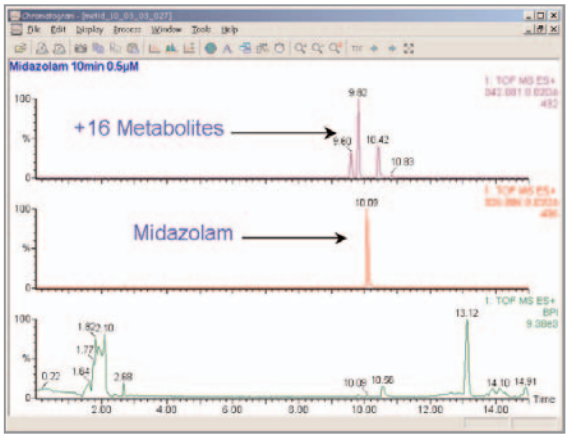

Figure 3. Midazolam and +16 Metabolites at 0.5 µM incubations.

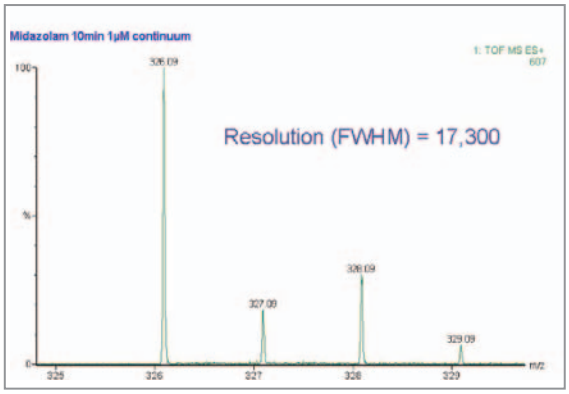

Figure 4. W-Optics resolution for Midazolam.

Compound name	m/z observed	m/z theoretical	mDa +/-	ppm +/-
Verapamil	455.2914	455.2910	0.4	1.0
M-14	441.2750	441.2753	0.3	0.7
M-14	441.2755	441.2753	0.2	0.5
M-28	427.2597	427.2597	0.0	0.0
M-164	291.2063	291.2073	1.0	3.4
Diazepam	285.0791	285.0795	0.3	1.2
M+16	301.0742	301.0744	0.2	0.7
M-14	271.0637	271.0638	0.1	0.5
Praziquantel	313.1918	313.1916	0.2	0.6
M+16	329.1874	329.1865	0.9	2.8
M-2	311.1763	311.1760	0.3	1.2
M-2	311.1759	311.1760	0.1	0.2
Metoprolol	268.1915	268.1913	0.2	0.7
M+16	284.1863	284.1862	0.1	0.4
M-14	254.1757	254.1756	0.1	0.4
Midazolam	326.0858	326.0860	0.2	0.8
M+16	342.0810	342.0809	0.1	0.1
M+16	342.0812	342.0809	0.3	0.7
M+16	342.0810	342.0809	0.1	0.1
Dextromethorphan	272.2017	272.2014	0.3	1.0
M-14	258.1855	258.1858	0.3	1.0
M-28	244.1704	244.1701	0.3	1.1
			Average mDa	RMS ppm
			0.3	0.8

Table 1. MS results.

PosterREPRINT

Waters

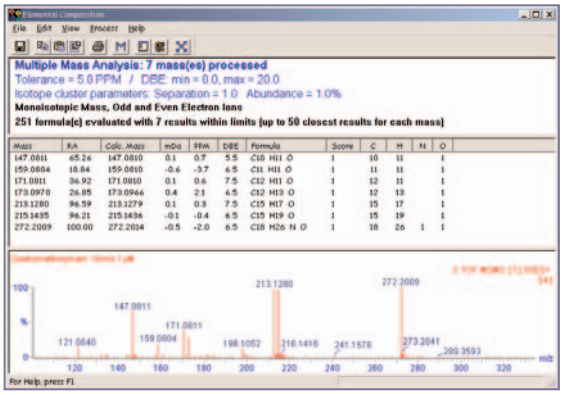

Figure 5. LC/MS/MS exact mass for Dextromethorphan.

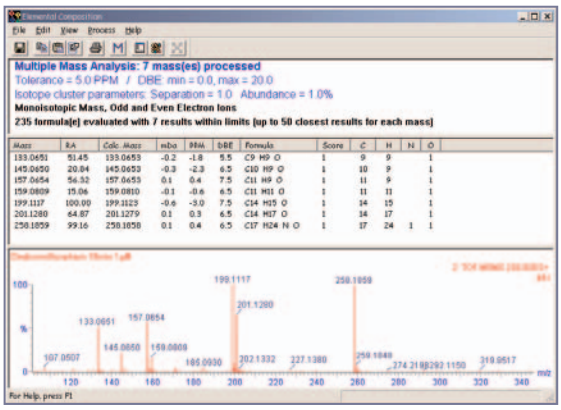

Figure 6. LC/MS/MS exact mass for N-Dealkylated metabolite of Dextromethorphan.

To whom all correspondence should be addressed:
e-mail: Jose_Castro_Perez@waters.com

Fragments for N-Dealkylation metabolite for Dextromethorphan

m/z observed	m/z theoretical	mDa +/-	ppm +/-	Formula
133.0651	133.0653	-0.2	-1.8	C9 H9 O
145.0650	145.0653	-0.3	-2.3	C10 H9 O
157.0656	157.0653	0.3	1.7	C11 H9 O
159.0810	159.0810	0.0	0.1	C11 H11 O
199.1117	199.1123	-0.6	-3.0	C14 H15 O
201.1279	201.1279	0.0	-0.2	C14 H17 O
258.1859	258.1858	0.1	0.4	C17 H24 N O

Fragments for Dextromethorphan

m/z observed	m/z theoretical	mDa +/-	ppm +/-	Formula
147.0811	147.0810	0.1	0.7	C10 H11 O
159.0804	159.0810	-0.6	-3.7	C11 H11 O
171.0811	171.0810	0.1	0.6	C12 H11 O
173.0970	173.0966	0.4	2.1	C12 H13 O
213.1280	213.1279	0.1	0.3	C15 H17 O
215.1435	215.1436	-0.1	-0.4	C15 H19 O
272.2009	272.2014	-0.5	-2.0	C18 H26 N O

Average mDa	RMS ppm
0.10	1.8

Table 2. MS/MS results.

CONCLUSIONS

- This new modification on the Q-Tof Ultima allowed much better exact mass to be obtained for metabolism studies
- Greater confidence in assigning elemental compositions-structures and especially for 'unknowns' and elimination of false positives. Moreover, the added advantage of obtaining an elemental composition is that it also provides Double Bond Equivalances, which are very important when trying to decipher a structure.
- Good resolution and exact mass makes it easier to distinguish between drug-related and isobaric compounds
- When trying to confirm a particular structure or identifying an 'unknown' exact mass MS/MS also plays a vital role in the identification process

WATERS CORPORATION
34 Maple St.
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com

Waters

MICROMASS MS TECHNOLOGIES

Opt-In! My Profile www.waters.com

LLOYD'S REGISTER QUALITY ASSURANCE ISO9001

UKAS QUALITY MANAGEMENT 001

VIT