アプリケーションノート

Evaluation of Improvements in Mass Accuracy of Q-Tof Ultima API for the Rapid and Automated Screening of Compound Libraries

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Abstract

The overall exact mass measurement accuracy and precision were investigated for three Waters Micromass Q-Tof Ultima API instruments on three separate sites, each equipped with the LockSpray interface, modified electronics, and operated in W-Optics mode.

Benefits

The overall aim of reliably producing exact mass confirmation for, on average, greater than 90% of the 'found' target components to better than 2 ppm was achieved

Introduction

The overall exact mass measurement accuracy and precision were investigated for three Waters Micromass Q-Tof Ultima API instruments on three separate sites, each equipped with the LockSpray interface, modified electronics and operated in W-Optics mode.

Data were acquired in real-time exact-mass centroid mode and processed automatically using the OpenLynx Application Manager post-acquisition processing software.

The sample set consisted of a GSK test plate containing 96 samples chosen to be representative of a larger pharmaceutical compound library. The test plate was analyzed a total of eleven times using the same generic high throughput LC-MS method.

The results obtained show that overall, in excess of 90% of detected target masses were exact mass measured to within 2 ppm of the theoretical value and 100% within 5 ppm.

The overall RMS mass measurement error was found to be better than 1 ppm for the entire sample set.

Experimental

LC Conditions

Liquid chromatograph:	Waters Alliance HT 2795 Separations Module
Column:	Waters Symmetry C_{18} 30 × 2.1 mm, 3.5 µm
Flow rate:	0.6 mL/min
Mobile phase A:	Water 0.1% formic acid
Mobile phase B:	Acetonitrile 0.1% formic acid
Injection volume:	1 µL

Gradient

Time (min)	%B
0	0
0.1	0
3.1	100
3.8	100
3.9	0
5.5	0

MS Conditions

Mass spectrometer:	Waters Q-Tof Ultima API
Tof resolution:	17000 FWHM
Ion mode:	ES+ (LockSpray)
Scan time:	1.0 s
Inter scan time:	0.1 s
Reference (lockmass):	Reserpine 609.2812 *m/z*, sampling rate 1:3 sec
Acquisition range:	*m/z* 100 to 800 *m/z*
Acquisition mode:	Realtime Centroid Exact Mass

Data Processing

Each 96-well plate analysis was processed using OpenLynx set to automatically select and average target ion spectra with the optimum signal intensity from the real-time mass measured spectra from the total ion chromatograms.

Results and Discussion

The results were compiled in the form of electronic browser reports, as in the example below, to generate an integrated chromatogram, exact mass measured spectrum with the mass measurement error annotated and the associated elemental composition search report for each well in the plate.

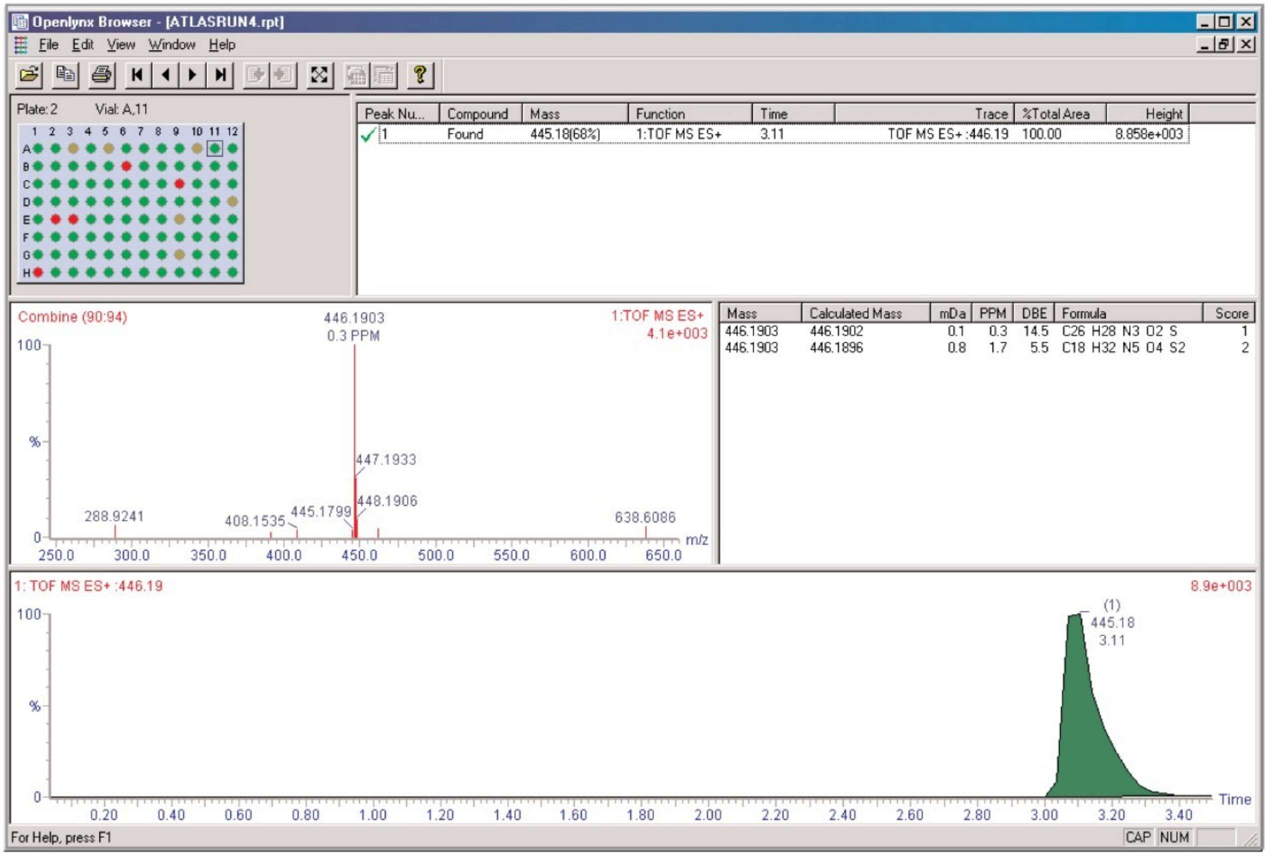


Plate: 2 Vial: A,11

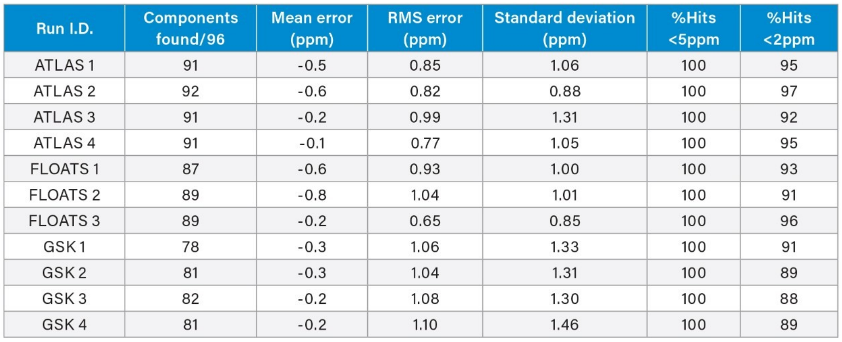
Peak Nu...	Compound	Mass	Function	Time	Trace	%Total Area	Height
✓ 1	Found	445.18(68%)	1:TOF MS ES+	3.11	TOF MS ES+ :446.19	100.00	8.858e+003

Mass	Calculated Mass	mDa	PPM	DBE	Formula	Score
446.1903	446.1902	0.1	0.3	14.5	C26 H28 N3 O2 S	1
446.1903	446.1896	0.8	1.7	5.5	C18 H32 N5 O4 S2	2

Browser Report.

The browser report for each plate was exported to produce a listing of individual mass measurement errors for each target component.

The table below is a summary of the average mass measurement accuracy and reproducibility for the overall sample set.



Run I.D.	Components found/96	Mean error (ppm)	RMS error (ppm)	Standard deviation (ppm)	%Hits <5ppm	%Hits <2ppm
ATLAS 1	91	-0.5	0.85	1.06	100	95
ATLAS 2	92	-0.6	0.82	0.88	100	97
ATLAS 3	91	-0.2	0.99	1.31	100	92
ATLAS 4	91	-0.1	0.77	1.05	100	95
FLOATS 1	87	-0.6	0.93	1.00	100	93
FLOATS 2	89	-0.8	1.04	1.01	100	91
FLOATS 3	89	-0.2	0.65	0.85	100	96
GSK 1	78	-0.3	1.06	1.33	100	91
GSK 2	81	-0.3	1.04	1.31	100	89
GSK 3	82	-0.2	1.08	1.30	100	88
GSK 4	81	-0.2	1.10	1.46	100	89

Conclusion

W-Optics mode operating at an elevated resolution (>17,000 FWHM) in combination with electronic modifications to stabilize the Tof pusher characteristics confer significant improvements in exact mass measurement performance. These modifications are now a standard feature.

The repeat analysis of the 96-well plate over 48 hours on each of three different instruments produced very consistent results across the study set. The overall aim of reliably producing exact mass confirmation for, on average, greater than 90% of the 'found' target components to better than 2 ppm was achieved.

The overall RMS error of less than 1 ppm and a standard deviation for any individual plate not greater than 1.5 ppm represents a considerable improvement over the currently stated instrument specification of less than 5 ppm RMS for Q-Tofs operated in V mode.

None of the 'found' target masses were measured with an error greater than 5 ppm compared to the expected mass. This allows a greater constraint to be put on elemental composition searches therefore producing considerably reduced elemental composition listings and greater confidence in the validity of the screening of compound libraries.

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