Waters™

Applikationsbericht

SYNAPT G2: Breakthrough Quantitative and Qualitative Performance for UPLC/MS and MS/MS (MS^E) Applications

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Introduction

We demonstrate the ability of SYNAPT™ G2, with its innovative QuanTof™ Technology, to provide high-resolution, exact mass measurement, accurate isotope ratios, enhanced dynamic range, and comprehensive MS and MS/MS information, all at acquisition rates compatible with ACQUITY UPLC® separations.

Instrumentation

The SYNAPT G2 System is an innovative hybrid quadrupole IMS orthogonal acceleration time-of-flight (oa-Tof) mass spectrometer providing a new level of high-resolution, exact mass, tandem MS performance, and the option to combine this with high-efficiency ion mobility separations, as shown in Figure 1A.

SYNAPT G2 employs QuanTof Technology – a nextgeneration oa-Tof architecture that integrates a series of technological advancements, as shown in Figure 1B. QuanTof combines innovative high field pusher and dual-stage reflectron designs with a novel ion detection system in an optimized, folded, Tof geometry. This provides a new dimension of high-resolution, exact mass, quantitative performance, which, crucially, is available at acquisition rates compatible with UPLC® separations.

This new level of Tof performance means SYNAPT G2 is the ideal platform for the most analytically-challenging samples, for example in the analysis of complex mixtures for proteomics and biomarker discovery; or metabolite, impurity, and lipid profiling studies.

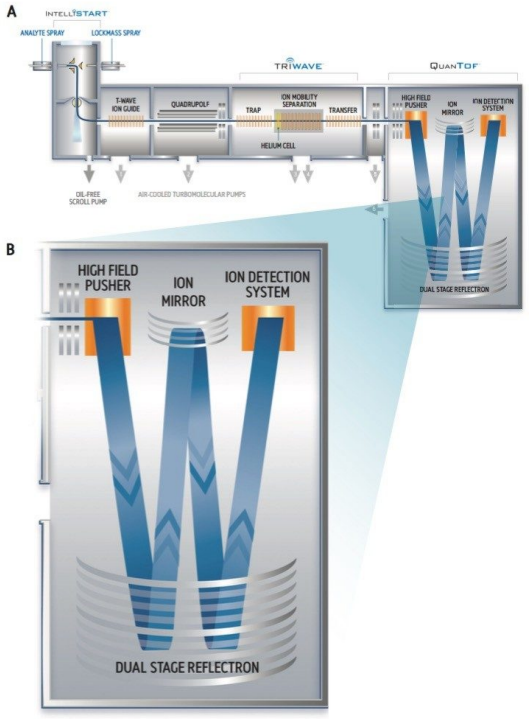
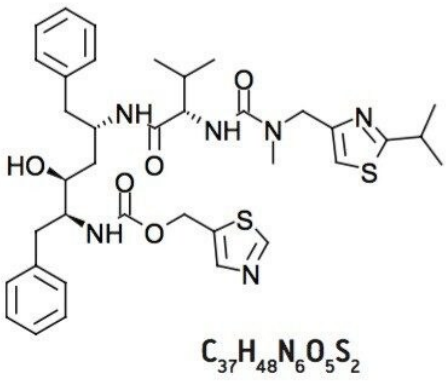


Figure 1. (A) Schematic of the SYNAPT G2 System. (B) QuanTof Technology, the enabling next generation Tof technology of SYNAPT G2. QuanTof's high field pusher and dual-stage reflectron, which incorporates high-transmission parallel wire grids, reduces ion turnaround times due to pre-push kinetic energy spread, and improves focusing of high energy ions, respectively. These innovative technologies combine to provide the highest levels of Tof performance. The novel ion detection system combines an ultra-fast electron multiplier and hybrid ADC detector electronics to provide outstanding sensitivity and quantitative performance for both MS and the elevated data acquisition rates of HDMS™ analysis.

Experimental

Samples analyzed:	Bile samples from rat dosed with Ritonavir at 10 mg/kg



$C_{37}H_{48}N_6O_5S_2$

UPLC Conditions

LC system:	ACQUITY UPLC System
Column:	ACQUITY UPLC HSS T3, 2.1 x 100 mm, I.D. 1.7 µm
Mobile phase A:	5 mM Ammonium Acetate, pH 5
Mobile phase B:	MeCN

Gradient Table

Time (min)	Flow rate (mL/min)	%A	%B	Curve
1. Initial	0.5	98.0	2.0	n/a
2. 5.0	0.5	50.0	50.0	6
3. 9.0	0.5	40.0	60.0	6
4. 9.1	0.5	1.0	99.0	1
5. 12.9	0.5	1.0	99.0	1
6. 13.0	0.5	98.0	2.0	1

MS Conditions

MS system:	SYNAPT G2
Ionization mode:	ESI positive
Acquisition mode:	MS^E
Capillary voltage:	1.5 kV
Cone voltage:	40.0 V
Trap collision energy:	6.0 V
Transfer collision energy:	4.0 V
Collision energy ramp:	15.0 to 25.0 eV
Trap/Transfer gas:	Argon
Acquisition range:	100 to 1200 *m/z*

Results and Discussion

The combination of high chromatographic and mass resolution is essential for the comprehensive, confident analysis of very complex matrices, for example in profiling of metabolites in biological (*in vivo*) samples.

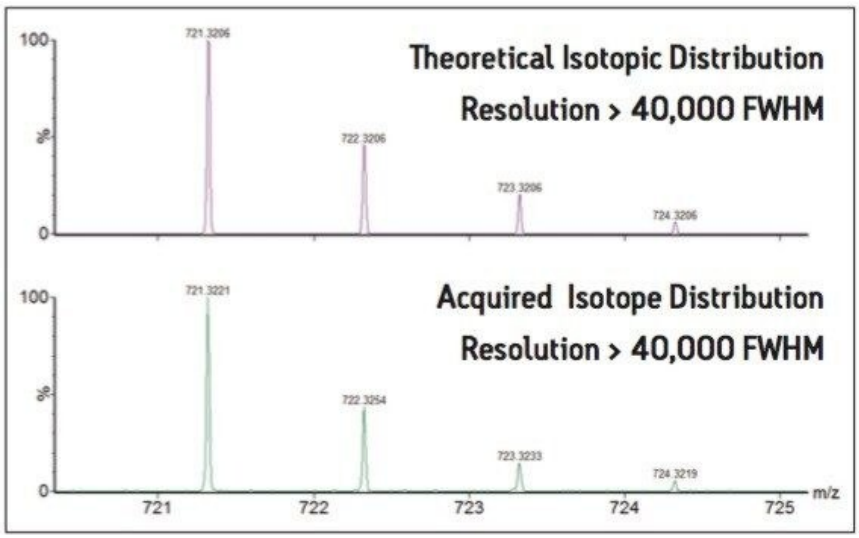


Figure 2. High resolution at 20 spectra/sec.

High Resolution and Mass Accuracy at the Highest Acquisition Rates

Figure 2 demonstrates the ability of SYNAPT G2 to provide high resolution (>40,000 FWHM) at the fast spectral acquisition rates required to keep pace with ACQUITY UPLC separations, which typically deliver peak widths of less than 2 sec at half height. By delivering up to 20 spectra/second, SYNAPT G2 ensures sufficient points can be obtained to generate accurate LC peak profiles with mass resolutions over 40,000 FWHM to maximize the ability to better resolve compounds and provide exact mass measurement. The ability of SYNAPT G2 to deliver high mass accuracy and accurate isotope ratios significantly aids the confident identification of small molecules through elimination of false positives, as shown in Figure 3.

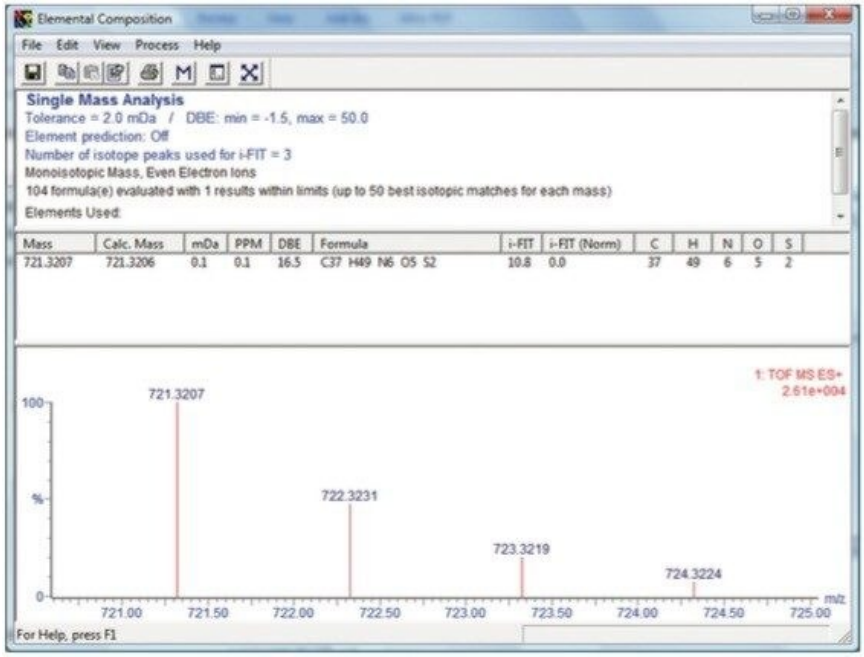


Figure 3. Elemental composition calculation for the parent drug Ritonavir showing accurate isotopic ratios reflected by the i-FIT™ (norm) value of 0, and high mass accuracy.

Precision

QuanTof delivers exact mass accuracy with high precision across LC peaks, which in turn provides high selectivity and confidence for the detection and identification of components in complex mixtures. In the case of complex (*in vivo*) matrices such as bile, urine, and plasma, QuanTof's selectivity provides more confident detection of components in the presence of endogenous metabolites and the dosing vehicle. This is demonstrated in Figure 4 where a window of <5 mDa is typically used to generate an extracted ion chromatogram for a drug and its metabolites.

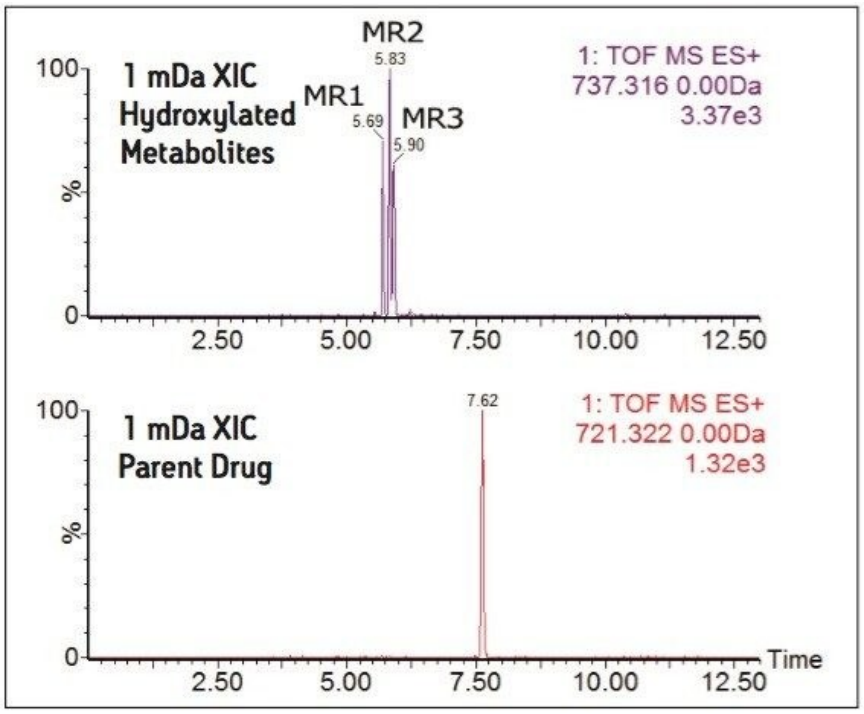


Figure 4. Extracted ion chromatogram windows at 1 mDa of parent drug and hydroxylated metabolites using profile data.

Dynamic Range

Since complex (*in vivo*) samples can contain thousands of components (drug-related metabolites and endogenous peaks) over a wide dynamic range, it is important that high mass accuracy is maintained across the concentration range. Figure 5 demonstrates that SYNAPT G2 provides an in-spectrum dynamic range of more than 4 orders of magnitude where mass accuracies for caffeine (low concentration) and verapamil (high concentration) are <0.1 ppm and 1.5 ppm, respectively.

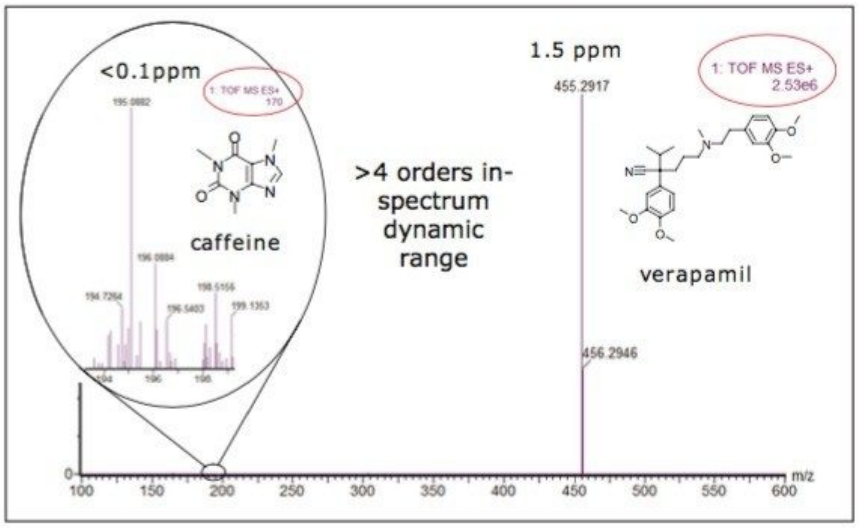


Figure 5. In-spectral dynamic range. Exact mass measurement of verapamil and caffeine at concentrations that differ by more than 4 orders of magnitude.

Comprehensive Fragment Ion Analysis

MS^E is a patented data independent acquisition method, which provides a simple route to delivering comprehensive molecular (MS) and fragment ion (MS^E) information from every detectable component in a complex mixture. The use of this rapid, information-rich approach on SYNAPT G2 ensures high selectivity and accuracy (in MS mode) for quantitative profiling and high mass resolution and accuracy (in MS^E mode) for identification and characterization, all at acquisition rates of up to 20 spectra/sec. This is demonstrated in Figure 6 for the analysis of Ritonavir in bile (from an *in vivo* metabolite profiling study). All mass accuracies are <0.5 mDa (1.3 ppm RMS). A 1.0 mDa extracted ion chromatogram window demonstrates the high precision across the entire chromatographic peak. The accuracy of fragment ion data enabled unambiguous structure assignment with MassFragment™ Software.

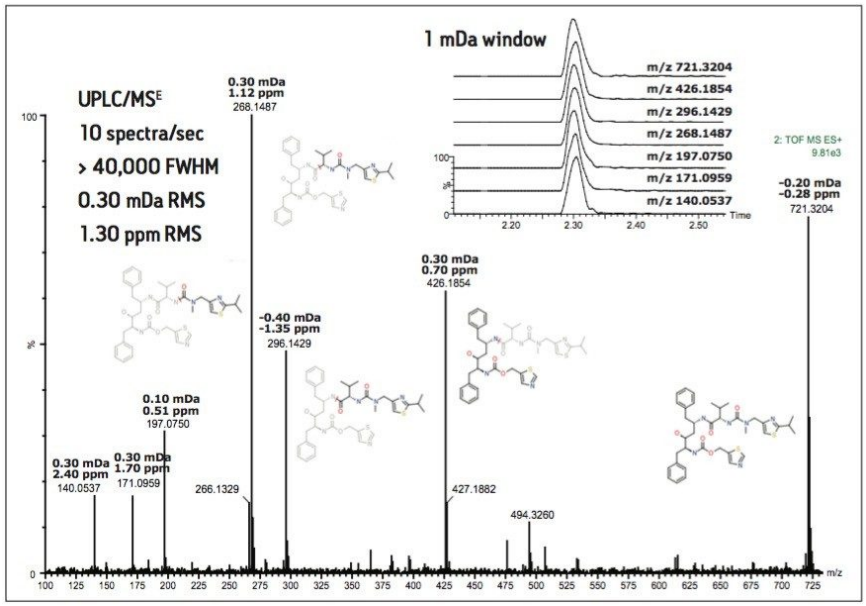


Figure 6. UPLC/MS^E fragment ion spectrum of Ritonavir (C37H49N6O5S2) from a complex rat bile sample. Data was acquired from a UPLC peak width of 1.5 sec at half height. A 1 mDa window was used to generate extracted ion chromatograms (inset) and structures were automatically determined (MassLynx™ Application Manager, MassFragment Software) for each individual fragment ion.

Conclusion

- The SYNAPT G2 System, with QuanTof Technology provides high-resolution (above 40,000 FWHM) at the high spectral acquisition rates required for UPLC/MS analysis, unlike electrostatic ion trap or FT-MS-based mass analyzers
- The exact mass, accurate isotope ratios, and wide dynamic range significantly aid the detection, quantitation, confirmation, and identification of compounds from complex biological samples using UPLC/MS
- SYNAPT G2, with its patented MS^E data-independent acquisition strategy, provides a simple route to comprehensive exact mass fragment ion information for every detectable precursor in UPLC separations.

- Unlike other quadrupole time-of-flight or ion trap-based instruments, the application of MS^E and MassLynx Informatics (MassFragment Software and MS^E-dedicated application managers) with SYNAPT G2 provides a unique route to simple, rapid, and comprehensive exact mass MS/MS analysis for the quantitation, identification, and characterization of peptides, lipids, and small molecule samples

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ACQUITY UPLC Columns <https://www.waters.com/nextgen/global/products/columns/acquity-uplc-columns.html>

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