Applikationsbericht

Metabolic Profiling of Urine Samples with the SYNAPT XS High Definition (HDMS)

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This Application brief demonstrates the increased performance of the SYNAPT XS HDMS compared to the SYNAPT G2-*Si* in terms of sensitivity, resolution, and labile ion transmission for metabolic profiling.

Benefits

The SYNAPT XS HDMS is the newest addition to Waters high resolution portfolio. The instrument benefits from improved sensitivity and resolution providing high quality quantitative data for metabolic profiling.

Introduction

Metabolic profiling is a fast-emerging science which provides insight into changes in biochemical pathways as a response to disease state, toxicological insult or other environmental factor. Successful metabolomic methodologies involve the detection and quantification of hundreds or thousands of metabolites in biochemical fluids such as plasma and urine.

Accurate mass LC-MS has become a mainstay of metabolomic research due to its analytical sensitivity, specificity, speed of analysis, and structural identification abilities. To provide reliable quantitative data, the mass

spectrometry system must be analytically sensitive, accurate, able to resolve compounds, and have a linear response over the systemic concentration range of the metabolites.

The SYNAPT XS HDMS System is equipped with a StepWave XS for improved analytical sensitivity and enhanced ion transmission as well as an extended flight tube for improved resolution. Here we demonstrate the resolution, analytical sensitivity, labile transmission, and dynamic range of the SYNAPT XS HDMS on a series of standard compounds spiked into human urine.

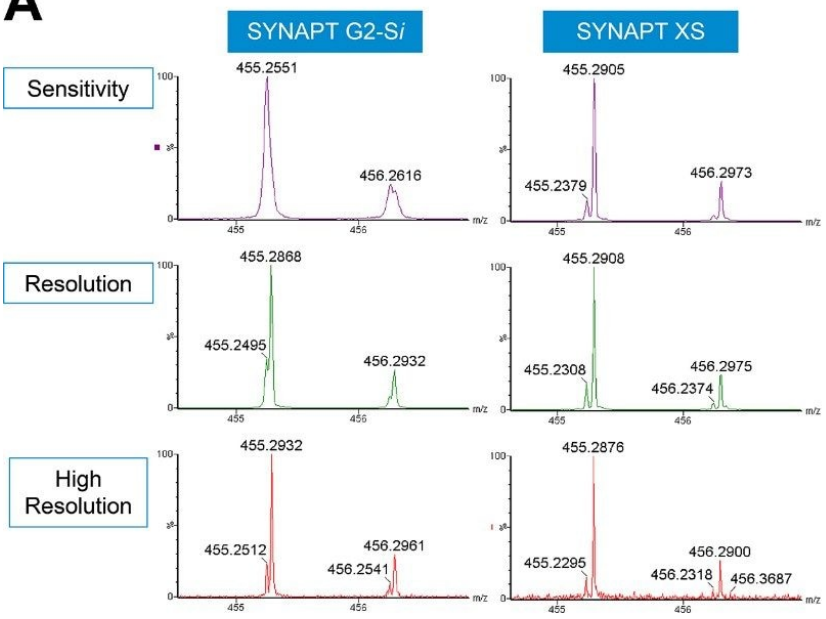
Results and Discussion

The performance of the SYNAPT XS HDMS was evaluated using urine spiked with the Waters LC-MS test mix, containing 9 non-endogenous compounds to achieve a range over seven orders of magnitude. The urine samples were diluted 1:10 with a mixture of 25:75 water:acetonitrile and centrifuged. Additionally, 11 standard labile compounds (metamphetamine, amphetamine, methylenedioxyamphetamine (MDA), hexazinone, metolachlor, tryptophan, phenylalanine, MCPA, MCPB, mecoprop, and kynurenic acid) were prepared at a concentration of 1 µg/mL in 5:95 water:acetonitrile.

The samples were chromatographically separated using a 2.1 x 100 mm ACQUITY BEH Amide Column operated under HILIC gradient conditions over 10 min. The mobile phases consisted of 10 mM ammonium formate and formic acid in 95:5 water:acetonitrile (mobile phase A) and 5:95 water:acetonitrile (mobile phase B). The column effluent was monitored using a SYNAPT G2-*Si* or SYNAPT XS HDMS operating in MS^E positive/negative ion mode. Data were collected using a collision energy ramp from 20–35 eV and a mass range of *m/z* 50–1200.

The mass resolution demonstrated by the SYNAPT XS was in the region of 20,000, 35,000, and 40,000 (FWHM) for sensitivity/resolution, high resolution and enhanced resolution modes respectively. The additional resolution provided by the extended flight tube, increases the specificity, helping to separate the compounds of interest from interfering matrix components (Figure 1).

A



B

	Mass error (ppm) SYNAPT G2-S*i*	Mass error (ppm) SYNAPT XS
Sensitivity[1]	-79	-1.1
Resolution	-4.8	0.2
High Resolution	-1.8	-2.0

Figure 1. Benefit of increased mass resolution with SYNAPT XS when compared against SYNAPT G2-Si. Verapamil is shown to be separated from the additional interfering components in all acquisition modes for SYNAPT XS, along with mass accuracies <2 ppm. A) Mass spectra of Verapamil (455.2910 m/z) from the various acquisition modes on both SYNAPT XS and SYNAPT G2-Si; B) Mass accuracy of Verapamil for all acquisition modes on both instruments.

The StepWave XS implemented within the SYNAPT XS HDMS provides improved transmission of ions compared to the previous generation of Stepwave. Thus, depending on the compound of interest, the analytical sensitivity of the SYNAPT XS was observed to be increased between a factor of 2–18, when compared with the SYNAPT G2-*Si* for sensitivity and resolution mode (Figure 2). Transmission of the labile compounds was also observed to be improved for SYNAPT XS HDMS by a factor of 1.2 to 13 (Figure 3), while precursor to fragment ion area ratios also increased (Figure 4).

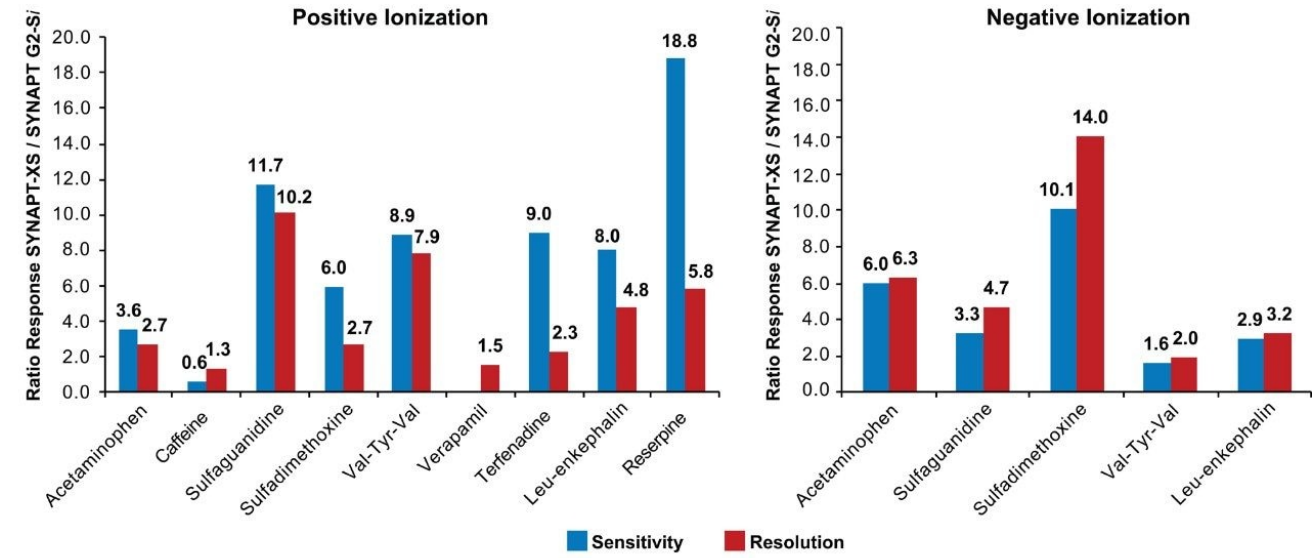


Figure 2. Response ratio between SYNAPT XS and SYNAPT G2-Si for the 9 compounds spiked into urine for positive and negative ionization. Sensitivity and resolution modes were utilized for both polarities.

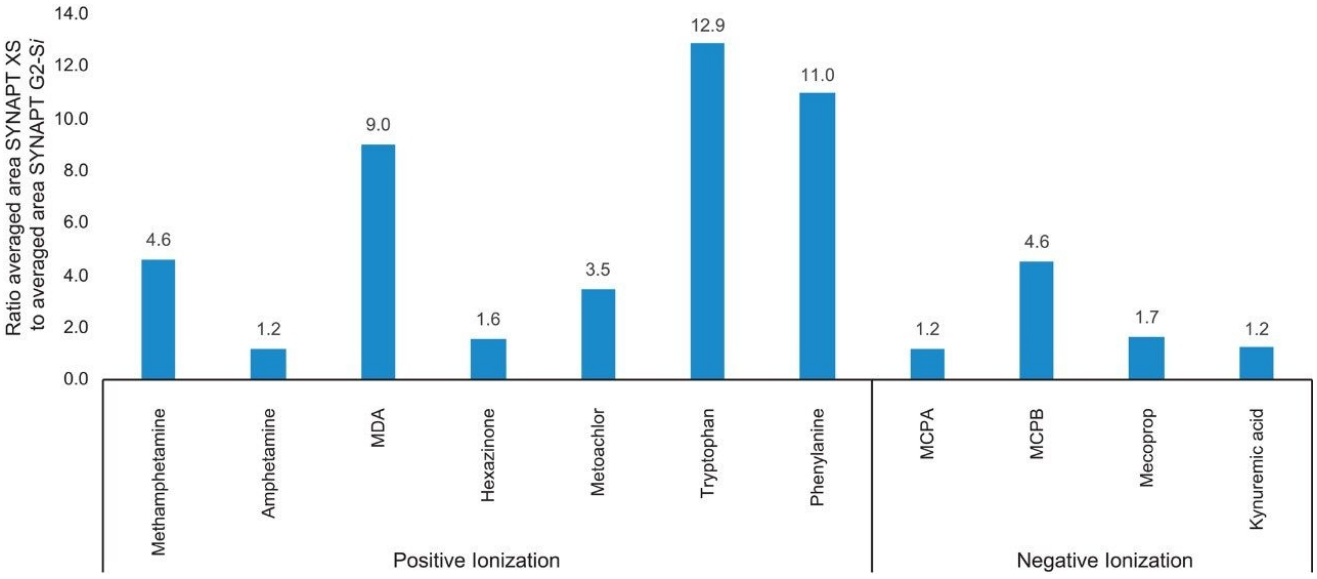


Figure 3. Response ratio between SYNAPT XS and SYNAPT G2-Si for 11 labile compounds (negative and positive ionization modes).

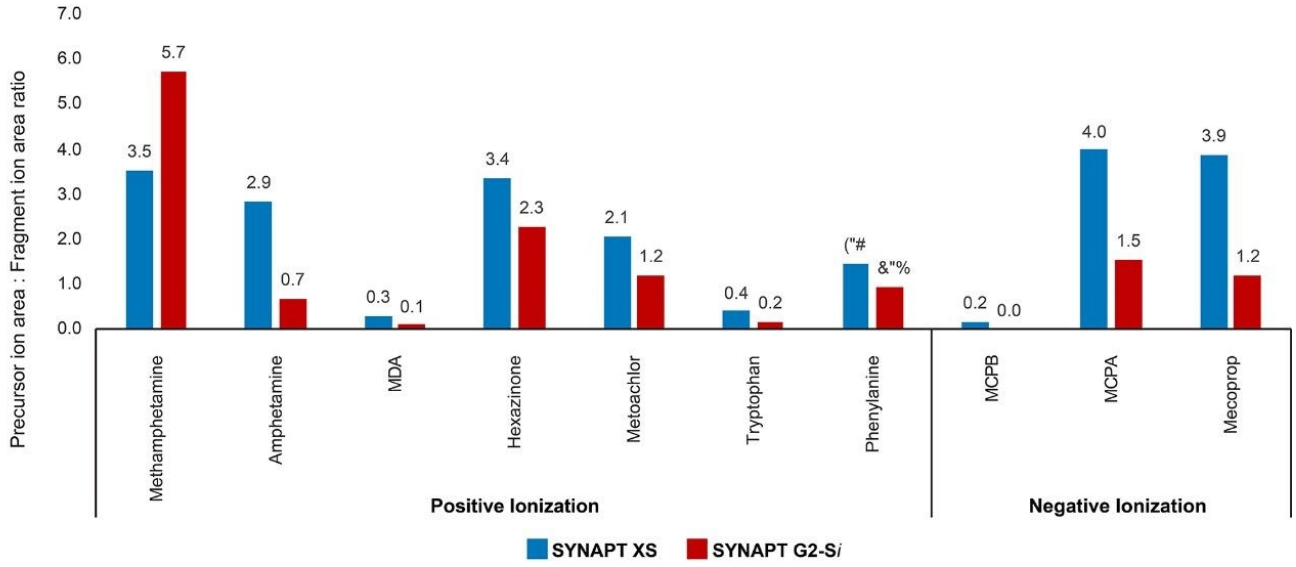


Figure 4. Precursor ion to fragment ion area ratio for 11 labile compounds (negative and positive ionization modes).

The data acquired provided a linear response over 3 orders of magnitude. A typical calibration line obtained for sulfaguanidine is shown in Figure 5. It should be noted that the upper limit was not reached.

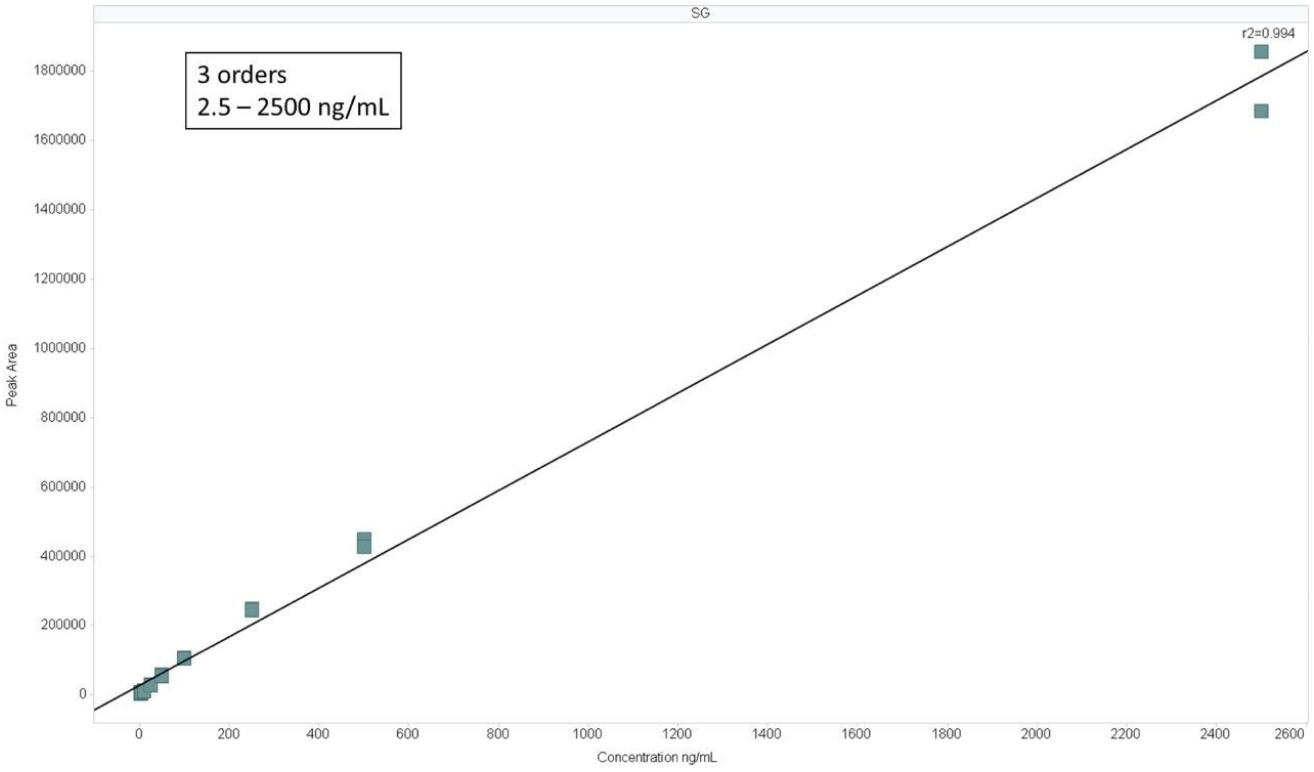


Figure 5. Example linear dynamic range for the compound sulfaguanidine, which shows 3 orders of magnitude from 2.5 to 2500 ng/mL (r^2 = 0.994).

Conclusion

The SYNAPT XS has been shown to provide high quality data for metabolic profiling of urine samples in biomedical research. Thus, depending on the acquisition mode, the mass resolution of representative singly charged compounds is demonstrated to be between 20,000 and 40,000 at FWHM. In addition to the increased resolution, an increase in analytical sensitivity between a factor of two and 18 was also observed, when comparing SYNAPT G2-*Si* with SYNAPT XS for sensitivity and resolution modes. Implementation of the StepWave XS has shown that the transmission of labile ions is also improved by a factor of 1.2 to 13, with improved precursor to fragment ion ratio. For the spiked components in urine, a typical dynamic range of 3 was also observed.

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SYNAPT G2-Si High Definition Mass Spectrometry <https://www.waters.com/134740622>

720006723, November 2019