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Nota applicativa

The Ultimate Forensic Toxicology Screening Companion – Xevo G3 QTof

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

For forensic toxicology use only.

Abstract

This application brief describes the evaluation of the existing Forensic Toxicology high-resolution mass spectrometry (HRMS) Screening Solution for use with the Xevo G3 QTof (Figure 1).

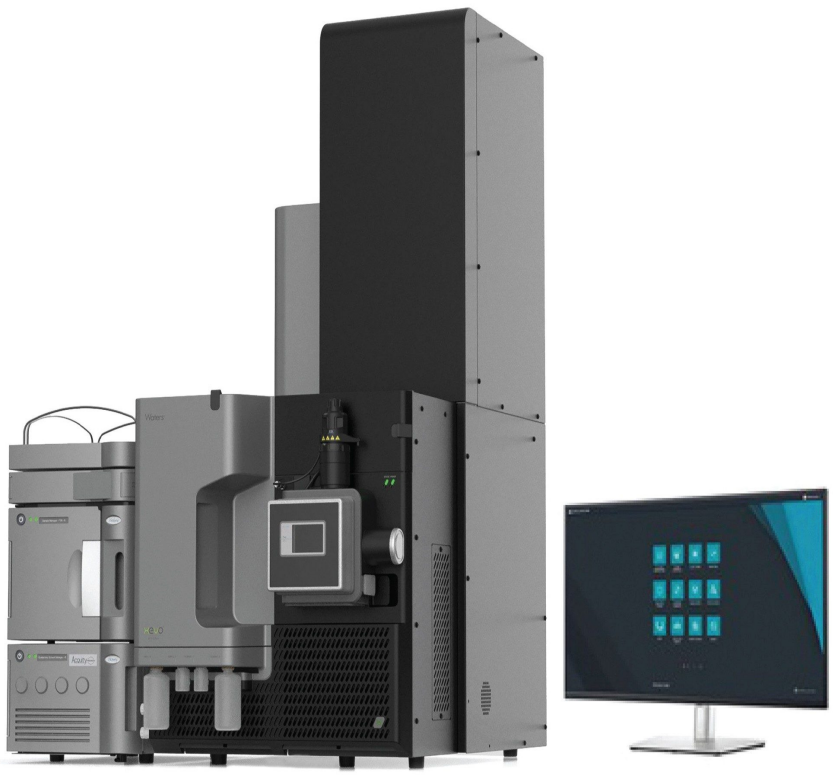

Figure 1. ACQUITY UPLC I-Class with the Xevo G3 QTof.

Benefits

Utilizing the technology enhancements of the Xevo G3 QTof with the ready to implement forensic toxicology screening solution that includes a library, containing over 2000 parent drugs and metabolites, was evaluated using authentic urine samples.

Introduction

High-resolution mass spectrometry (HRMS) has gained popularity for broad toxicological screening. Techniques that can detect a wide variety of toxicants in highly complex biological matrices are very much in demand in forensic toxicology laboratories. At Waters™, we have provided and supported, ready-to-implement toxicology screening solutions for over 15 years, which have been applied on a number of HRMS systems, including the

Xevo G2-XS QTof and now the Xevo G3 QTof. The HRMS Screening Solution comprises dedicated chromatographic methods that maximize sensitivity for positive and negative ionizing analytes and processed against the comprehensive data rich libraries that consists of reference retention time (RT), accurate mass for the precursor, and fragment ion information, generated through data-independent analysis (DIA) using MS^E.

For this study, system test solutions and a selection of authentic urine samples from a drugs cohort were analyzed in order to evaluate the Forensic Toxicology HRMS Screening Solution for use with the Xevo G3 QTof mass spectrometer.

Experimental

Materials

Testing was performed using two Test Solutions. Test Solution 1 is a commercially available standard, containing 10 compounds, which was obtained from Waters, UK (Forensic Tox Installation Standards Kit p/n: 186007361 < https://www.waters.com/nextgen/global/shop/standards--reagents/186007361-forensic-tox-installation-standards-kit.html>). This Test Solution 1 is regularly analyzed as a daily system suitability check.[1] Test Solution 2 is an in-house sample, containing 6 compounds prepared from Certified Reference Material obtained from Merck (Dorset, UK). The list of compounds contained in the Test Solutions 1 and 2 are shown in Table 1.

In addition, 20 authentic urine samples, were selected at random from a drugs cohort. These urine samples had been previously characterized on the ACQUITY™ UPLC™ I-Class (FTN) with the Xevo G2-XS QTof using the Forensic Toxicology Screening Solutions (ESI positive and ESI negative modes).[2]

System test solution 1	System test solution 2
Buflomedil	Amiloride
Clozapine	Chlorthalidone
Milnacipran	Hydrochlorothiazide
Nicotine	Metolazone
Perphenazine	Tolvaptan
Scopolamine	Xipamide
Tianeptine	
Tiapride	
Trazodone	
Triprolidine	

Table 1. List of compounds in Test Solution 1 and 2, used for the evaluation of the Forensic Toxicology HRMS Screening Solution.

Sample Preparation

Test Solution 1 (ESI positive): The 500 ng/mL ten compound mixture was diluted 20-fold using 5 mM ammonium formate pH3.0 (mobile phase A1) to achieve a concentration of 25 ng/mL.

Test Solution 2 (ESI negative): A 2500 ng/mL stock solution, consisting of six compounds, was prepared in methanol, and subsequently diluted 100-fold using 0.001% formic acid in water (mobile phase A2) to achieve a concentration of 25 ng/mL.

Authentic urine samples were prepared following a 5-fold dilution using mobile phase A1, for positive ionization analysis, and a 5-fold dilution using mobile phase A2, for negative ionization analysis. All samples were vortex mixed prior to analysis.

Data Acquisition

Data were acquired using the ACQUITY UPLC I-Class (FTN) system in combination with the Xevo G3 QTof based on established techniques. Sample information were subsequently processed using the waters_connect™ informatics package and compared with the Waters forensic toxicology database, which was previously prepared under the same conditions, containing in excess of 2000 drugs and metabolites. Samples are screened using

dedicated positive (ESI positive) and negative (ESI negative) ionization methods.[3,4]

Analysis was achieved in 15 minutes (ESI positive) and 7.5 minutes (ESI negative) using their respective established toxicology screening gradients and mobile phases.[2] The Xevo G3 QTof mass spectrometer was operated in MS^E acquisition mode. This mode of acquisition facilitates collection of full MS spectra and involves the rapid alternations between two collision-cell voltages: the first, acquired at a low voltage, provides accurate mass of the precursor ion; the second, as a ramped voltage (10-40 eV), provides accurate mass of the fragment ions for additional confirmatory purposes.

Results and Discussion

The acceptance criteria for a positive identification, in both ESI positive and ESI negative mode, of each analyte was as follows: three dimensional (3D) low energy ion count intensity greater than 250, retention time to be within 0.35 minutes of the reference retention time, and the observed precursor mass to be within 5 ppm of expected exact mass. For additional confirmation, a minimum of one diagnostic fragment ion in the high energy function must be detected. All 10 compounds (25 ng/mL) were identified, for Test Solution 1 (ESI positive), the results for clozapine are shown in the waters_connect™ Review Tab (Figure 2).

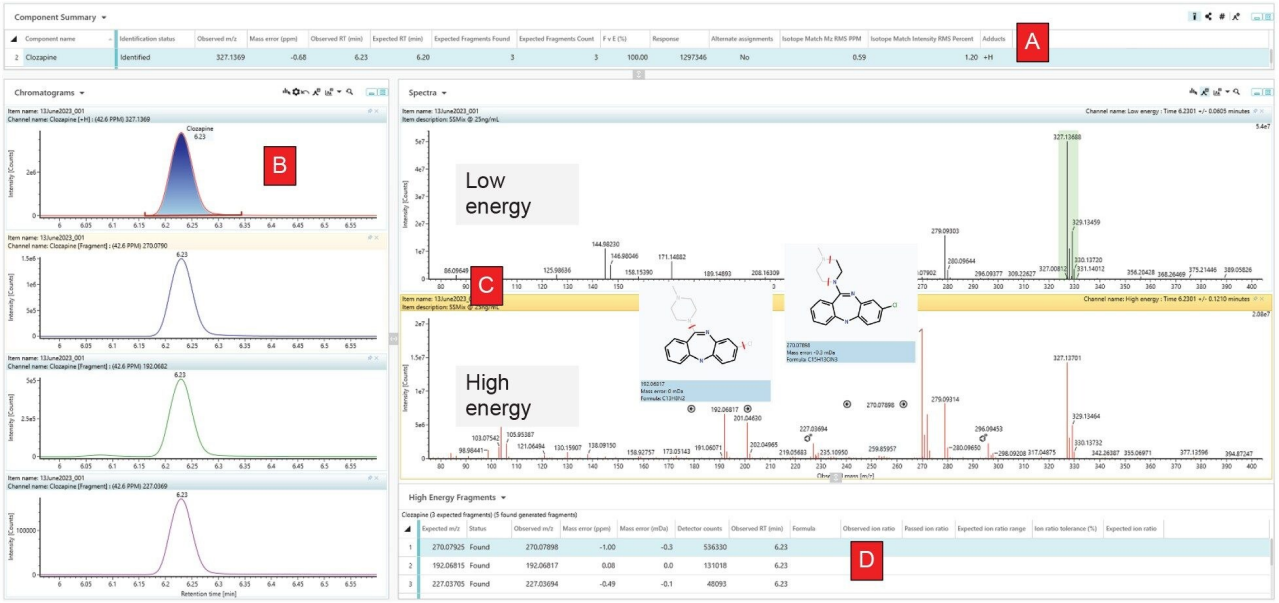

Figure 2. Detection of Clozapine identified in Test Solution 1 (System Suitability Mix), acquired in ESI positive. In this figure the upper table (A) details the results of the comparison of acquired data against the reference information contained in the Toxicology Library. Panel B displays the extracted ion chromatograms for the targeted precursor and the 3 diagnostic fragment ions and demonstrates that all ions are time-aligned at 6.23 minutes. The low and high energy spectra are shown in Panel C, and details of the fragment ions are listed in the lower table, Panel D.

A total of 27 parent drugs and/or their metabolites were detected, in the 20 urine samples for the ESI positive analysis, using the acceptance criteria. The detected drugs could be broadly divided into the following drug classes: amphetamines, antidepressant, antihypertensive, antimalarial, opiate/opioid, prescription/over-the-counter (OTC) medication, and sedatives. Their distributions are shown in figure 3. Overall, the data demonstrated excellent concordance with the previously characterized data on the Xevo G2-XS QTof.

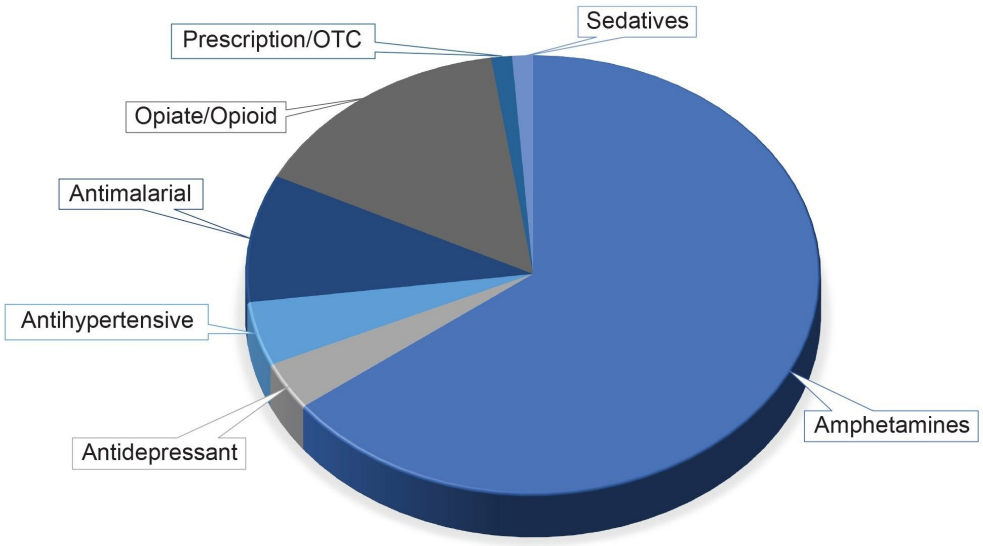

Figure 3. Results summary following the analysis of 20 authentic urine samples.

Furthermore, the ESI negative method correctly detected and assigned the 6 target compounds contained within Test Solution 2 (25 ng/mL). Results obtained for the urine samples using this method were comparable with the results obtained previously on the Xevo G2-XS QTof and included the presence of ethyl-glucuronide, ethyl-sulphate, furosemide, naproxen, salicylic acid, and carboxy-THC. The results for carboxy-THC are shown in the Review Tab (Figure 4).

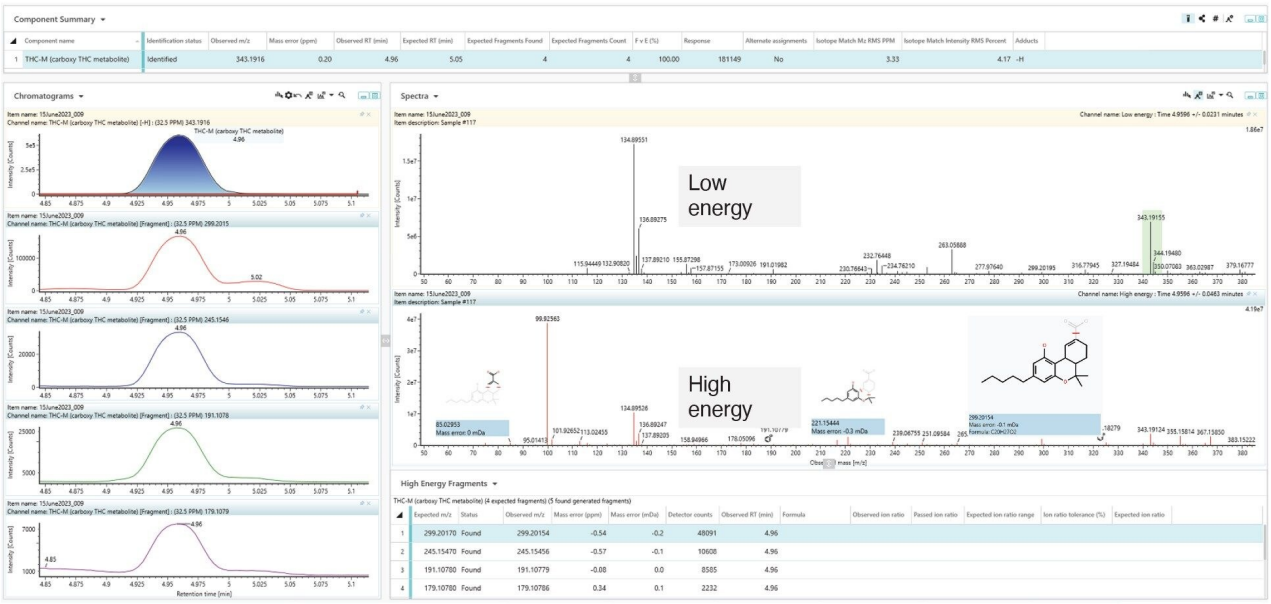

Figure 4. Detection of carboxy-THC identified in an authentic urine sample (ESI negative). Identified with a mass error of 0.20 ppm, within 0.09 min of the reference retention time, and with all expected fragment ions detected.

Conclusion

In toxicology laboratories, it remains a key analytical challenge to be able to confidently identify analytes in complex biological matrix samples. Here we have demonstrated the successful implementation of the Forensic Toxicology HRMS Screening Solution for use with the Xevo G3 QTof using the waters_connect informatics platform.

The ready to use HRMS Screening Solution, with over 2000 library entries, was proven to be compatible through the analysis of a system suitability mix containing known compounds and by the consistent detection of compounds present in authentic urine samples, which were previously characterized using the Xevo G2-XS QTof.

References

1. Care and Use Manual; Forensic Toxicology Installation Standards Kit, Waters User Manual 715004381 < https://www.waters.com/webassets/cms/support/docs/715004381.pdf> , Nov 2013.
2. HRMS Forensic Toxicology Screening solution media available at Forensic Toxicology Application Solution Media by Waters | Marketplace <https://marketplace.waters.com/apps/159226/forensic-toxicology-application-solution-media#!overview> .
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4. R. Lee, S. Freeto, M. Wakefield and M. Wood. No Compromise! Improved Sensitivity for Negatively-Ionizing Substances. Waters Application, 720005479, Aug 2015.

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ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Xevo G3 QTof </nextgen/it/en/products/mass-spectrometry-systems/xevo-g3-qtof.html>

Xevo G2-XS QTof <https://www.waters.com/waters/nav.htm?cid=134798222>

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