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Application Note

Targeted MRM Screening for Forensic Toxicology in Negative Electrospray Ionization Mode Using the Xevo TQD or Xevo TQ-S micro

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For forensic toxicology use only.

Abstract

This application brief evaluates the performance of previously published methodology by using either the Xevo TQD or Xevo TQ-S micro in MRM mode to detect negative ionizing compounds.

The Xevo TQD and Xevo TQ-S micro Mass Spectrometers are designed to provide rapid, reliable, and reproducible data to deliver consistent low levels of quantitation over a wide dynamic range. When coupled with the ACQUITY UPLC I-Class System, the Xevo TQD and the Xevo TQ-S micro show excellent sensitivity when using a preconfigured targeted MRM qualitative screening method for the analysis of negatively ionizing compounds, such as barbiturates, diuretics, and NSAIDs in urine. The use of dedicated chromatography conditions for negative ionizing compounds allowed for the detection of some compounds, at toxicologically relevant concentrations, which would not have been possible using standard screening conditions. The described sample preparation method can also be applied to other biological matrices such as serum or plasma.

Benefits

A simple and sensitive UPLC-MS/MS method for the targeted MRM screening of negatively ionizing compounds in biological matrices for use in forensic toxicology laboratories.

Introduction

Forensic toxicology laboratories require reliable screening methods that can detect a wide variety of toxicants in highly complex biological matrices, such as ante and postmortem specimens. In 2009, Waters released a targeted toxicology screening application using the ACQUITY TQD System.[1] This approach has been used routinely by many groups including Rosano et al. for the analysis of postmortem blood samples[2] and Lee for the analysis of urine in drug intoxicated patients.[3] This method was configured to detect compounds in positive electrospray ionization (ESI) mode; however there are classes of compounds, relevant to forensic toxicologists, such as barbiturates, diuretics, and non-steroidal anti-inflammatory drugs (NSAIDs) that can only be detected, or have improved sensitivity, in negative ESI mode.

In 2015, a negatively ionizing method and associated chromatographic procedure, was reported for toxicology screening using a time-of-flight mass spectrometer, which showed that the chromatographic

conditions can have a significant effect on analytical performance and sensitivity; 75% of tested analytes demonstrated increased peak responses, ranging from 2 to 50-fold.[4] In this current study, the same method has been modified for use on two of the tandem mass spectrometers available from Waters (Xevo TQD and Xevo TQ-S micro), and has been evaluated for targeted MRM screening in negative ESI.

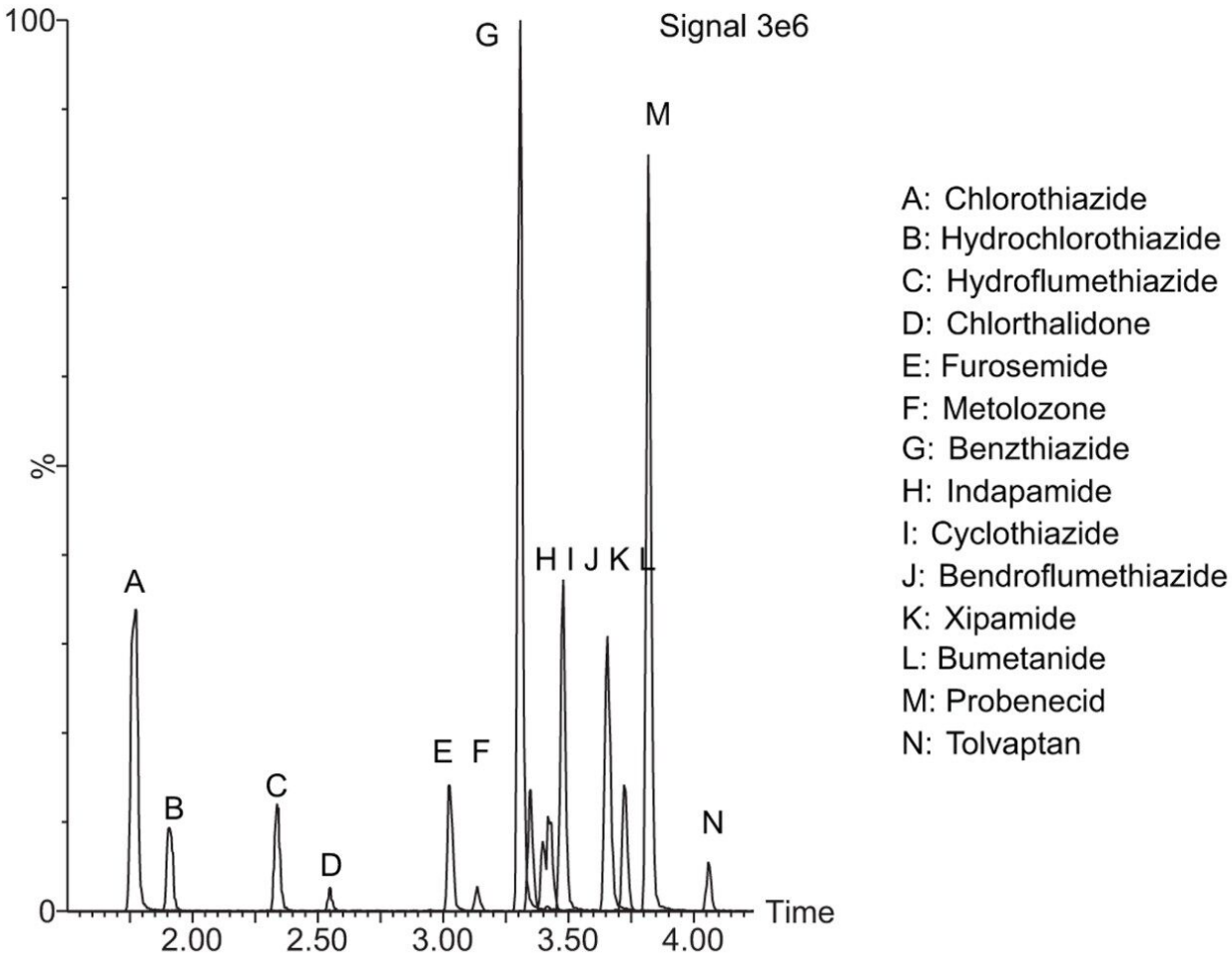

Figure 1. Chromatogram showing a selection of diuretics spiked into control urine at the WADA MRPL (200 ng/mL) detected by the Xevo TQD using the supplied sample preparation and the developed targeted MRM method. The quantifier ion transition only is displayed.

Experimental

Test substances

The following commercial human urine reference controls were obtained: DCT-25% (UR22020A) from ACQ Science; Urine Toxicology Control DAU HC2 (50701) from UTAK; and the following Liquichek Urine Toxicology Quality Controls from Bio-Rad – Negative Control (460), C2 (442), and S10 (673).

Spiked urine preparation

Fifty-three toxicologically relevant analytes, including NSAIDS (17), diuretics (16), barbiturates (8), cannabinoids (3) etc, were split into eight mixtures, containing a maximum of eight analytes, and prepared at 10 µg/mL in methanol. Each mixture was spiked into Bio-Rad Negative Control urine at 200 ng/mL which is the minimum required performance levels (MRPL) recommended by World Anti-Doping Agency (WADA) for urine analysis.[5]

Sample preparation

Two hundred microliters of each spiked or commercial reference urine was diluted with 200 µL 4% phosphoric acid and vortex-mixed. Following centrifugation 200 µL of diluted sample was loaded onto an Oasis PRiME HLB µElution plate (p/n 186008052). After loading, the wells were washed with 200 µL 5% methanol and the analytes were eluted with 2 x 25 µL acetonitrile/methanol (90/10 v/v). The samples were evaporated to dryness under a stream of nitrogen at 50 °C using a Techne sample concentrator and reconstituted in 100 µL 0.001% formic acid containing 10 % acetonitrile. The collection plate was covered with a Waters silicone/PTFE treated cap mat and vortex-mixed for two minutes.

LC conditions

System:	ACQUITY UPLC I-Class with flow-through-needle (FTN)
Column:	ACQUITY UPLC HSS C_{18}, 100 Å, 1.8 µm, 2.1 mm x 150 mm (p/n 186003534)
Column temp.:	50 °C
Sample temp.:	10 °C
Injection volume:	10 µL
Wash solvent:	Acetonitrile/water (95:5 v/v)

Purge solvent:	0.001% formic acid in water
Flow rate:	0.4 mL/min
Mobile phase A:	0.001% formic acid in water
Mobile phase B:	0.001% formic acid in acetonitrile

MS conditions

System:	Xevo TQD or Xevo TQ-S micro
Ionization mode:	ESI
Capillary voltage:	2.5 KV
Source temp.:	150 °C
Desolvation temp.:	400 °C
Desolvation gas:	800 L/Hr
Cone gas:	20 L/Hr
Cone voltages:	Preconfigured in provided MRM method
Collision energies:	Preconfigured in provided MRM method

Results and Discussion

The data was collected using the MRM method supplied, which contains two transitions (quantifier and where possible, a qualifier) per compound, with associated preconfigured parameters for cone voltage and

collision energies for the 53 compounds. The data was automatically processed using the TargetLynx Application Manager and the screening results were compared for equivalence on the two UPLC-MS/MS platforms.

Of the 53 compounds spiked into urine at 200 ng/mL, 51 were detected using the methodology described above, using both LC-MS/MS platforms. Amiloride and cannrenoic acid were not detected using the reported sample preparation method, but could be detected at this concentration if the urine was diluted 5-fold in 0.001% formic acid containing 10% acetonitrile.

The Negative Control reference urine and four positive control reference urines (C2, S10, DAU HC2, and DCT -25%) containing certified levels of analytes, were assayed using the methods described above on the two UPLC-MS/MS systems. A number of compounds, which are routinely observed in urine screens, were detected in the negative control reference urine on both platforms, i.e. the metabolites of aspirin (salicylic acid and gentisic acid) as well as naproxen (a commonly prescribed NSAID). For the certified positive control reference urines, both platforms detected all the expected analytes, including several barbiturates and carboxy-THC (the major metabolite of tetrahydrocannabinol (THC) which is found in urine (Figure 2). These analytes were detected at concentrations equivalent to the maximum cut-off concentration currently recommended by the European Workplace Drug Testing Society (EWDTS) for both screening and confirmation tests in urine.[6]

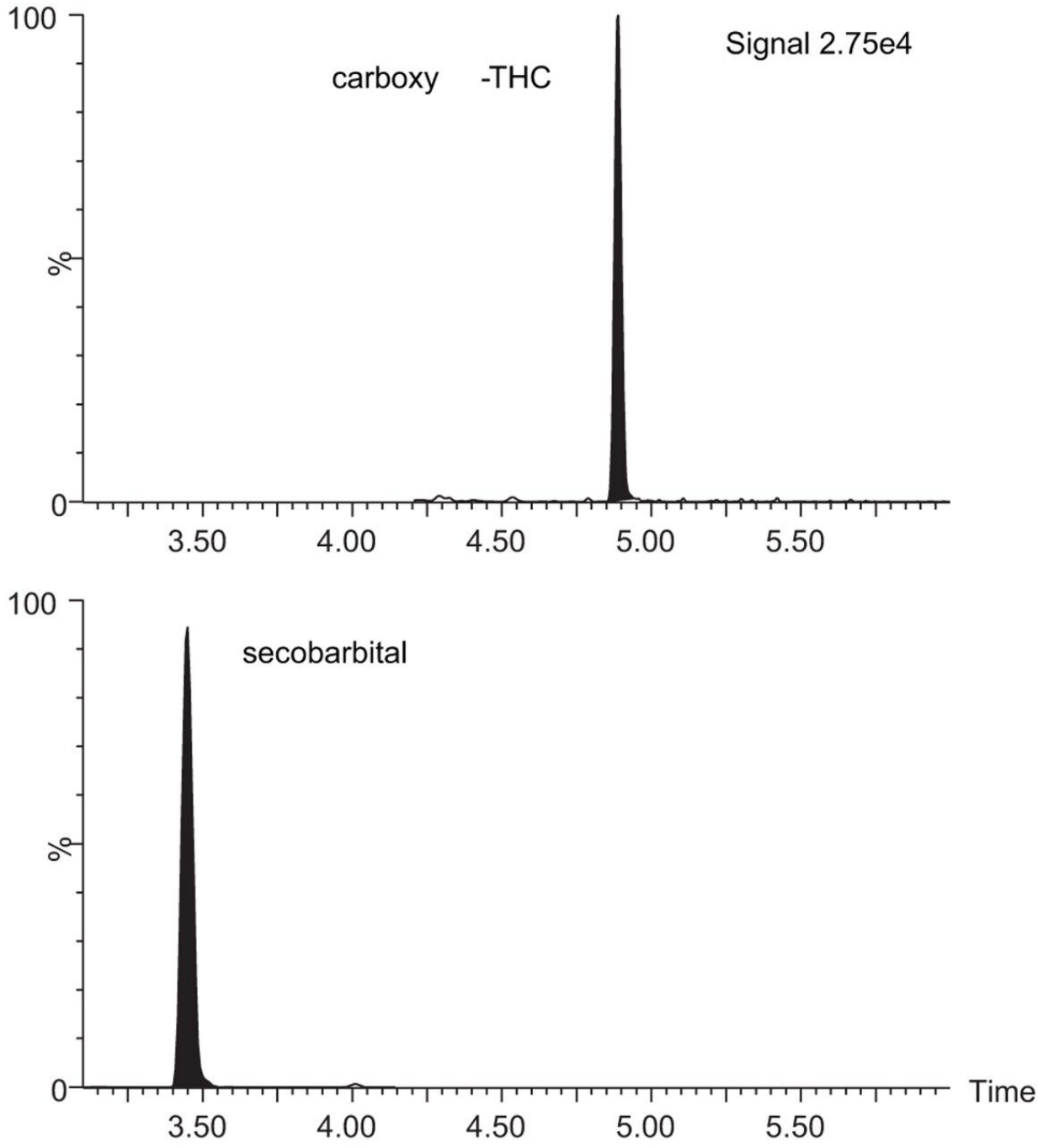

Figure 2. Chromatogram showing secobarbital (150 ng/mL) and carboxy-THC (37.5 ng/mL) in the Bio-Rad S10 commercial reference urine detected by the Xevo TQD using the supplied sample preparation and the developed targeted MRM method. The quantifier ion transition only is displayed.

Conclusion

The Xevo TQD and Xevo TQ-S micro Mass Spectrometers are designed to provide rapid, reliable, and reproducible data to deliver consistent low levels of quantitation over a wide dynamic range. When coupled with the ACQUITY UPLC I-Class System, the Xevo TQD and the Xevo TQ-S micro show excellent sensitivity when using a preconfigured targeted MRM qualitative screening method for the analysis of negatively ionizing compounds, such as barbiturates, diuretics, and NSAIDs in urine. The use of dedicated chromatography conditions for negative ionizing compounds allowed for the detection of some compounds, at toxicologically relevant concentrations, which would not have been possible using standard screening conditions. The described sample preparation method can also be applied to other biological matrices such as serum or plasma.

References

1. Roberts M, Lee R, and Wood M. Targeted MRM Screening for Toxicants in Biological Samples by UPLC/MS/MS. Waters Application Note. 720002749EN. 2009
2. Rosano T, Wood M, and Swift T. Postmortem Drug Screening by Non-Targeted and Targeted Ultra-Performance Liquid Chromatography-Mass Spectrometry Technology. Journal of Analytical Toxicology. 35: 411–423. 2011.
3. Lee YW. Simultaneous Screening of 177 Drugs of Abuse Using Ultra-Performance Liquid Chromatography with Tandem Mass Spectrometry in Drug-Intoxicated Patients. Clinical Psychopharmacology and Neuroscience. 11(3): 158–164. 2013.
4. Lee R, Freeto S, Wakefield M, and Wood M. No Compromise! Improved Sensitivity for Negatively Ionising Substances. Waters Application Note. 720005479EN. 2015.
5. The World Anti-Doping Code. WADA Technical Document – TD2017MRPL. https://www.wada-ama.org (accessed 11 Dec 2018).
6. European Workplace Drug Testing Society Guidelines. http://www.ewdts.org (accessed 11 Dec 2018).

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