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Screening Environmental Samples for a Diverse Range of Compounds With Accurate Mass LC-MS and an Integrated Scientific Information System

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Abstract

The presence of an increasingly complex array of pharmaceuticals and personal care products (PPCPs) in water bodies throughout the world is placing a greater demand on techniques used to screen for these compounds. In combination with UNIFI, an integrated scientific information system, it is now possible to screen for the presence of PPCPs, their fragments, adducts, and potential metabolites in a routine laboratory environment. This application note describes the use of Waters Screening Platform Solution in combination with the UNIFI Toxicology library to screen a local well water sample for the presence of a large number (>1000) of PPCPs, pesticides, and drugs of abuse.

Benefits

- High resolution mass spectrometry (HRMS) screening of a large target list of diverse compound classes and structures.

- Faster UPLC analysis with the ACQUITY UPLC HSS C_{18} Column.

- Accurate mass precursor and fragment ion information integrated into the identification and review process

Introduction

The presence of an increasingly complex array of pharmaceuticals and personal care products (PPCPs)[1] in water bodies throughout the world is placing a greater demand on techniques used to screen for these compounds. Figure 1 illustrates an example of the range of PPCP compounds and classes.
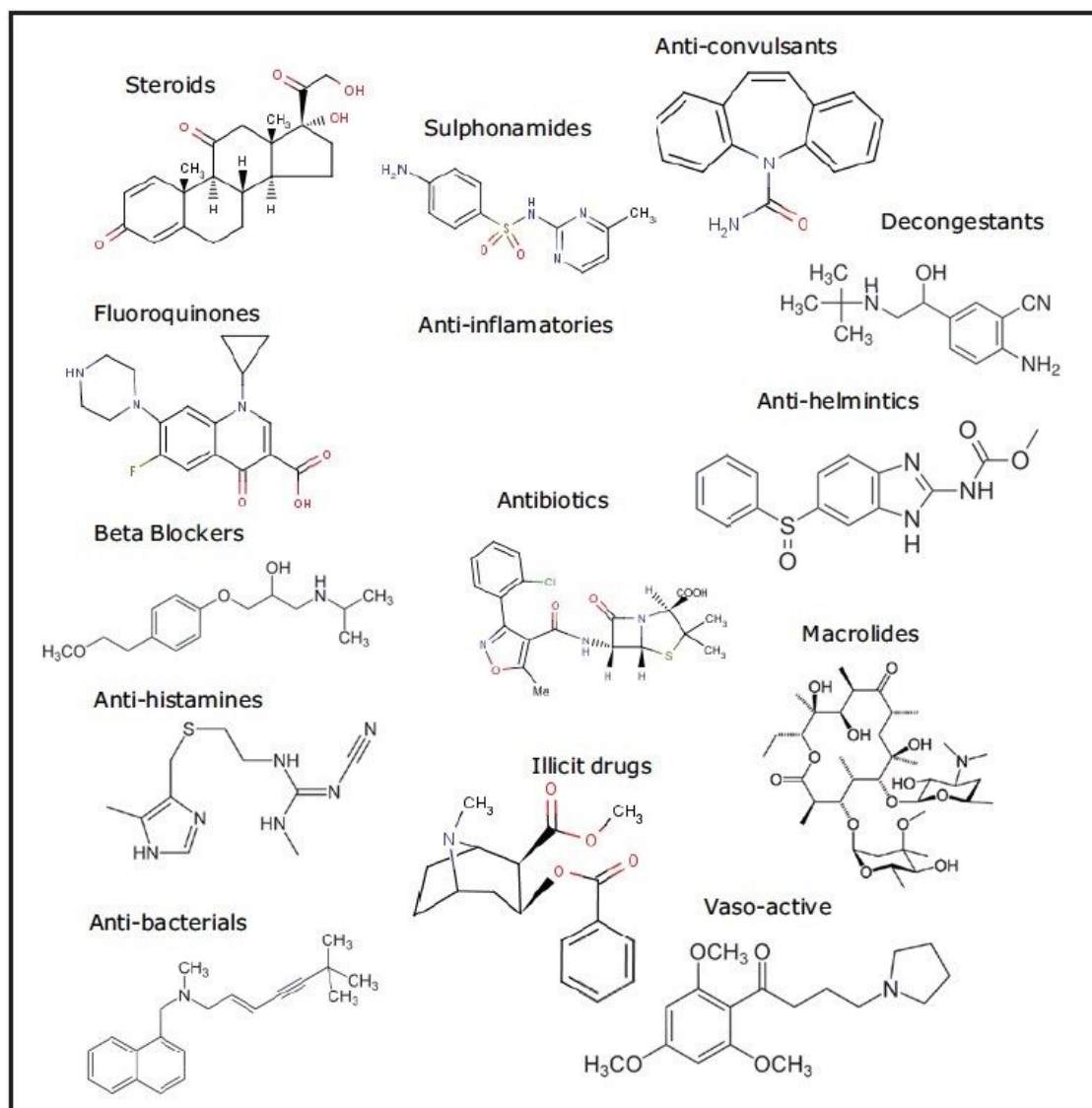
Figure 1. Examples of the range of compounds and classes describing PPCPs.

Conventional MS/MS screening methods, utilizing a tandem mass spectrometer, have target list limitations as the maximum duty cycle of the instrument is achieved. Data obtained from a non-targeted acquisition on a high resolution mass spectrometer can be used to target a theoretical unlimited number of compounds. Moreover, information rich datasets collected using UPLC-MS^E can be used to reduce the large number of false detects that arise when targeting a large number of compounds with accurate mass as the sole point of contaminant identification. MS^E provides accurate mass measurements for both precursor and fragment ion information in a single experiment.

In combination with UNIFI, an integrated scientific information system, it is now possible to screen for the

presence of PPCPs, their fragments, adducts, and potential metabolites in a routine laboratory environment. This application note describes the use of Waters Screening Platform Solution in combination with the UNIFI Toxicology library to screen a local well water sample for the presence of a large number (>1000) of PPCPs, pesticides, and drugs of abuse.

Experimental

Sample preparation

The following samples were prepared.

PPCP standards: High level standards (10 μg/L) in UHPLC-grade water

Extracted blank: Treated as a reference. A UHPLC-grade water sample (Fisher Optima), enriched using the protocol in Figure 2.

Extracted well water sample: Treated as the unknown. A well water sample enriched as above.

Extracted well water sample, post spike: Enriched as above and post spiked with the 35 PPCPs in Table 1 at a level of 1 μg/L.

Extracted well water sample, pre spike: Well water sample pre-spiked with the 35 PPCPs described in Table 1 (at 1 ng/L) then enriched as above. Pre-spike samples were prepared in duplicate.

Non-extracted well water: Well water sample neither enriched nor spiked.

Extracted calibration standards: Eight levels (1.0, 2.0, 2.5, 5.0, 10.0, 25.0, and 50.0 ng/L) of the 35 PPCPs in Table 1 spiked into the UHPLC-grade water and enriched as above.
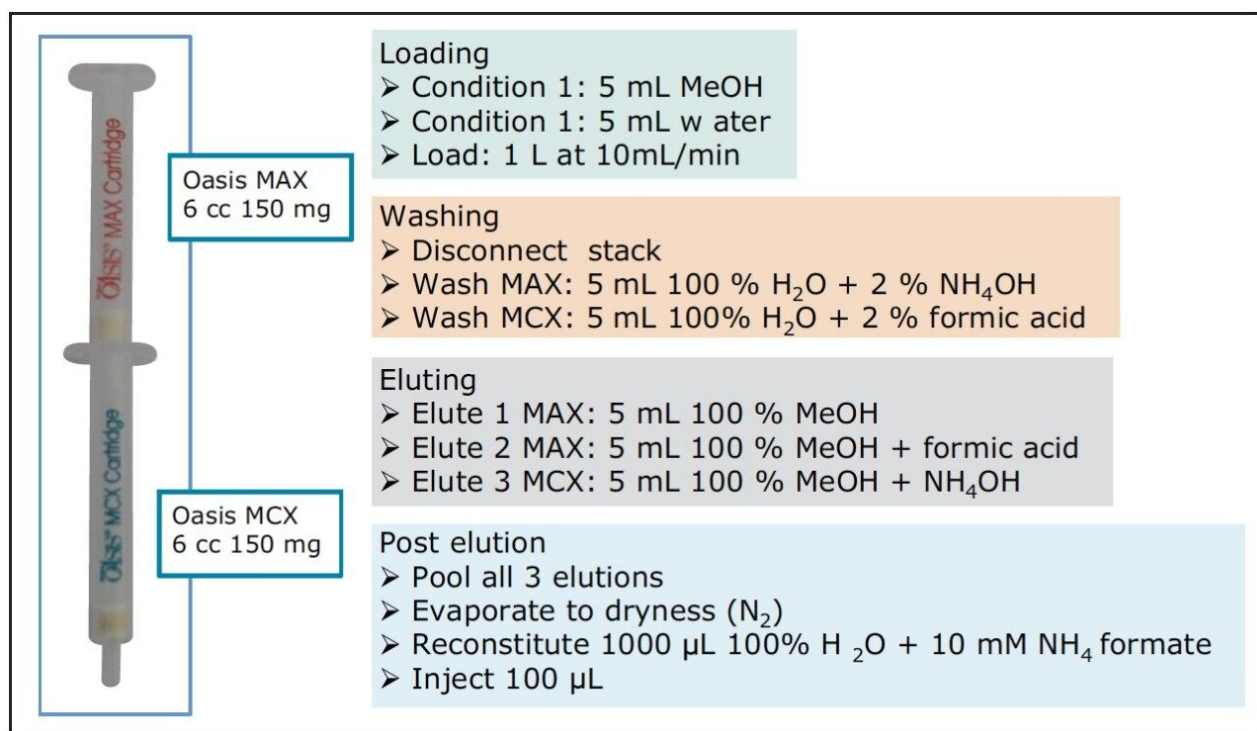
Figure 2. Sample preparation protocol used to extract water samples. Sample preparation resulted in a 1000:1 enrichment.

Acetaminophen	Codine	Naproxen	Sulfamethoxazole
Atenolol	Corticosterone	Ofloxacin	Ticlopidine
Azithromycin	Cortisone	Oxprenolol	Tolbutamide
Benzocaine	Cotinine	Procaine	Triamcinolone acetonide
Bromhexine	Digoxigenin	Promethazine	Trimethoprim
Buflomedil HCl	Ketoprofen	Pyrimethamine	Tripolidine
Chlorpheniramine	Levamisole	Ranitidine	Warfarin
Cimetidine	Metoprolol	Roxithromycin	Xylazine
Cocaine	Miconazole	Salbutamol (albuterol)	

Table 1. 35 PPCPs for the analyses performed.

LC-MS conditions

Comprehensive datasets were collected using UPLC-MS^E data acquisition. MS^E uses parallel low and elevated collision energy MS acquisition to provide accurate mass precursor and product ion information in a

single injection.

UPLC conditions

LC system:	ACQUITY UPLC I-Class
Runtime:	15.00 min
Flow rate:	0.40 mL/min
Injection:	100.0 μL
Column:	ACQUITY UPLC HSS C$_{18}$ 1.8 μm, 2.1 x 150 mm
Column temp.:	50 °C
Mobile phase A:	Water with 5 mM NH4HCO2 adjusted to pH 3.0 with formic acid
Mobile phase B:	Acetonitrile with 0.1% (v/v) formic acid

Gradient table:

Time	Flow rate mL/min	Composition A	Composition B	Curve
0.00	0.400	87.0	13.0	Initial
0.50	0.400	87.0	13.0	6
10.00	0.400	50.0	50.0	6
10.75	0.400	5.0	95.0	6

Time	Flow rate mL/min	Composition A	Composition B	Curve
12.25	0.400	5.0	95.0	6
12.50	0.400	87.0	13.0	6
15.00	0.400	87.0	13.0	6

MS conditions

MS system:	Xevo G2-S QTof
Ionization mode:	ESI+
Scan time:	0.2 seconds
Capillary voltage:	1.0 kV
Sampling cone:	20.0 V
Source temp.:	120 °C
Desolvation temp./gas:	550 °C/1000 L/H
Mass range:	m/z 50 to 1200
MS^E low energy:	4.0 V
MS^E high energy:	10.0 to 45.0 V
LockSpray solution:	Leucine enkephalin
LockSpray mass:	m/z 556.2766

Due to the wide chemical diversity of compounds described as PPCPs, the extraction and separation of the many classes and structures poses a major analytical challenge. A method employing mixed mode solid phase extraction was used to prepare samples, as shown in Figure 2 and described in a previous work.[2]

The concentration level of the post spiked compounds was chosen to account for the 1000-fold enrichment achieved in the sample preparation. A concentration of 1 ppt (1 ng/L) in the sample equated to an in-vial concentration at the same level as the post spike sample, 1 ppb (1 µg/L).

Data processing

All MS[E] data were collected and processed within the UNIFI, Scientific Information System. Data within UNIFI is passed through the apex peak detection and alignment processing algorithms.[3] This enables related ion components to be grouped together and analyzed as a single entity. Charged species, salt adducts, and fragments are all automatically aligned and grouped, so that all this information can be used to automatically interpret the data.

The Forensic Toxicology Screening Application Solution with UNIFI comes with pre-defined LC-MS conditions and processing parameters encompassed within an analysis method.[4] The Toxicology Library in UNIFI is comprised of over 1000 compounds including many PPCPs, such as drugs of abuse, veterinary medicines, and pharmaceuticals. Library entries contain names, formula, mol files, retention times, and theoretical accurate mass fragment ions.

Results and Discussion

The analysis of compounds spiked into the well water sample demonstrates the applicability of the method for targeted analysis. An example of a calibration curve for one of the standards is shown in Figure 3. Of the 35 standards included, none were detected as incurred residues in the well water sample.
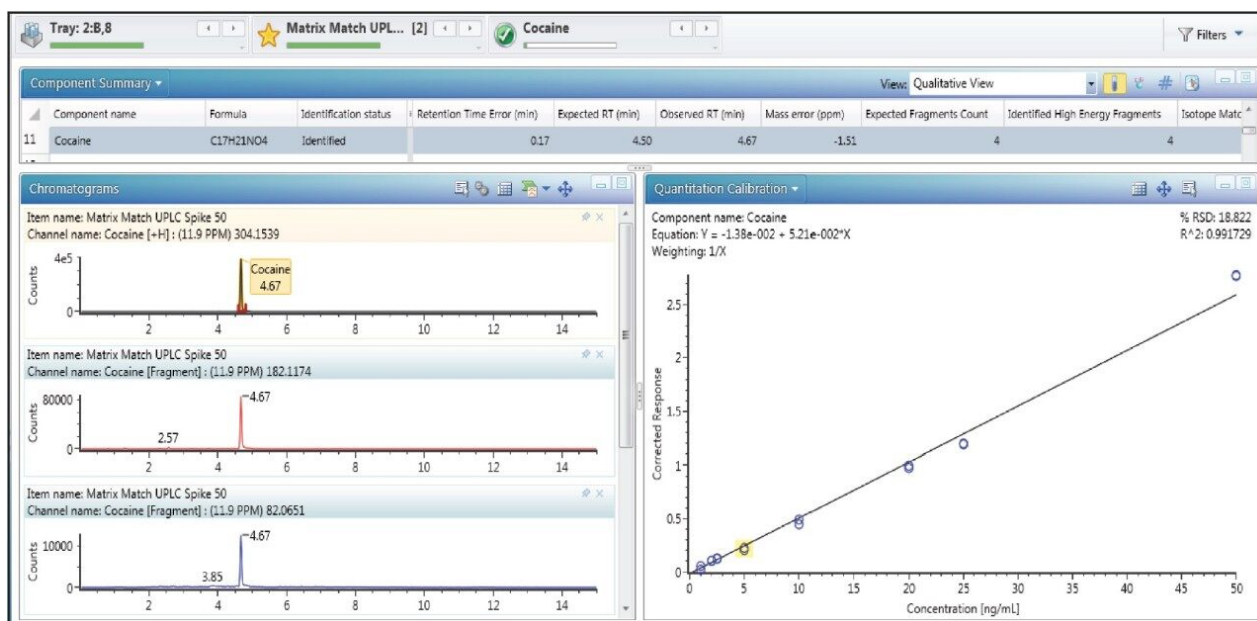
Figure 3. An example of the quantification aspects of UNIFI for a matrix matched cocaine standard. Identification details are provided in the component summary section. Extracted ion chromatograms for precursor and fragments are shown in the chromatograms section, and the calibration curve with replicate injections is shown in the quantitation calibration section.

In order to determine whether other compounds were present, the Toxicology Library with up to three adducts (H^+, Na^+, K^+) for each entry was employed. It has previously been demonstrated that screening against a large library using the accurate mass of only one diagnostic ion can result in an unreasonable number of false detects.[5] The importance of well resolved, reproducible chromatography in order to reduce the false detect rate has also been highlighted in previous work.[6] In the approach used here, full advantage was taken of both retention times and accurate mass fragment ions that are stored in UNIFI's scientific library. With a mass accuracy tolerance of 5 ppm, a retention time tolerance of 0.5 minutes, and the requirement of at least one fragment ion detection, four incurred residues were found to be present in the well water sample. These are shown in Table 2 and Figure 4.

Table 2. Component summary table in UNIFI showing details of each compound identified by screening the extracted well water sample against a library of over 1000 compounds.

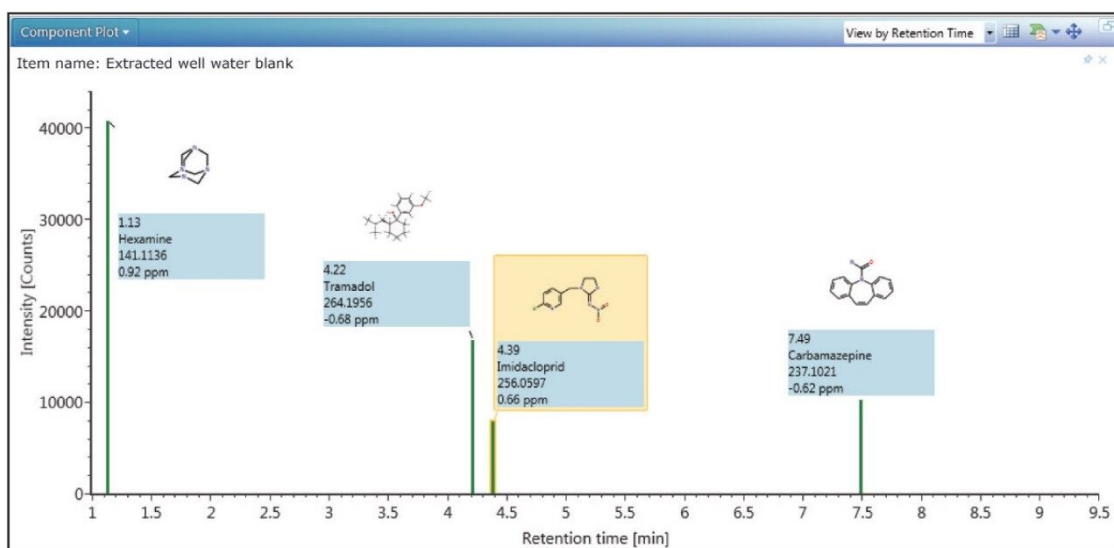|	Component na... ↓▲	Formula	m/z	Retention Time Error (min)	Mass error (ppm)	Identified High Energy Fragments	Response	Adducts
1	Carbamazepine	C15H12N2O	237.1021	0.21	-0.62	3	10282	+H
2	Hexamine	C6H12N4	141.1136	0.38	0.92	3	40806	+H
3	Imidacloprid	C9H10ClN5O2	256.0597	0.18	0.66	1	7907	+H
4	Tramadol	C16H25NO2	264.1956	0.42	-0.68	1	16859	+H

Figure 4. Component plot in UNIFI showing all identifications with respect to retention time. Each identification can be annotated as defined by the user. In this example, structure, retention time, compound name, expected m/z, and mass error (ppm) were labeled.

Readily available standards and an existing MS/MS tandem quadrupole method enabled the confirmation and quantification of carbamazepine and imidacloprid. This was achieved using standard addition in a non-extracted well water sample, which was then injected directly for analysis using the ACQUITY UPLC I-Class System with the Xevo TQ-S tandem mass spectrometer (data not shown). Concentrations were found to be 0.31 ng/L and 0.58 ng/L respectively. Tramadol and hexamine were neither confirmed nor quantified.

In UNIFI, the criteria set for identification is completely customizable, giving maximum flexibility to users. The bar chart in Figure 5 shows the effect of changing the retention time tolerances for identification with the sample containing the 35 spiked compounds shown in Table 1. With a mass accuracy tolerances of 5 ppm (SANCO 12495 guidelines) and no retention time or fragment ion criteria, 735 detections were returned (red

bars at the far right ofFigure 5). A reduction in false detects was observed when incorporating and decreasing a retention time tolerance, (red bars in Figure 5). Even with a retention time tolerance of 1 minute, the number of detects are reduced by over 65%. However, if this tolerance is set too low, false negatives may arise, as shown for the 35 spiked compounds as shown for the 35 compounds at a tolerance of 0.25 minutes or below (purple bars in Figure 5). A considerable decrease in false detects is observed when fragment ion presence is used to enhance the identification (green bars in Figure 5).
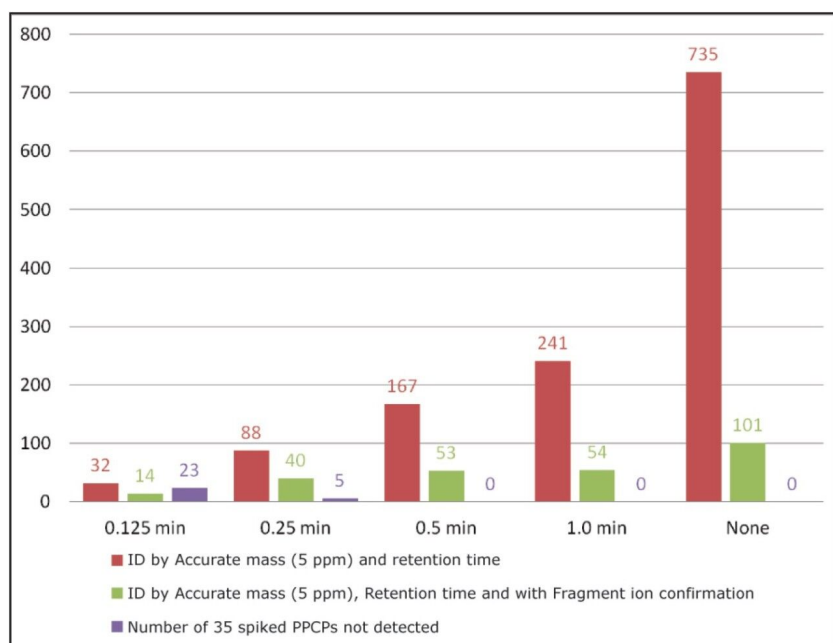

Figure 5. Chart showing the reduced number of false detects observed when using retention time and fragment ions in the identification criteria.

The key to success with HRMS screening is to use wider tolerances for multiple identification criteria. This results in a considerable reduction of false detects while minimizing false negatives. UNIFI provides the flexibility for users to combine criteria in a simplified workflow, enabling rapid data review and improved analyst efficiency.

Conclusion

Four compounds of interest were detected during an HRMS screening of a locally sourced well water sample enriched using an SPE protocol.

· Mixed-mode SPE allowed for the analysis of acidic, basic, and neutral compounds of interest in a single sample.

· The inclusion of both the retention times and accurate mass fragment ions in the Toxicology Library reduced the false detection rates, which is critical for rapid data review in screening experiments.

· Using multiple criteria with wider tolerances controls the number of false detects and minimizes the number of false negatives in HRMS screening.

References

1. S Richardson. Environmental Mass Spectrometry: Emerging Contaminants and Current Issues. *Anal Chem*. (2012), 84: 747-778.

2. C Mallet, G Cleland, J A Burgess. Multi-Residue Analysis of Pharmaceuticals and Personal Care Products (PPCPs) in Water Using the ACQUITY UPLC H-Class System and the Xevo TQD Tandem Mass Spectrometer. Waters Application Note No. 720004813en, October 2013.

3. Geromanos et al., The detection, correlation, and comparison of peptide precursor and product ions from data independent LC-MS with data dependant LC-MS/MS. *Proteomics* (2009) 9: 1683-1695.

4. J Archer, P Dargan, D Wood, N Mistry, M Wood. Screening for Classical and Novel Drugs in pooled urine using UPLC-TOF-MSE). Waters Application Note No. 720004583en, June 2013.

5. Mol *et al. Anal Bioanal Chem*. (2012) July; 403(10): 2891-2908.

6. Croley *et al. J Am Soc Mass Spectrum*. (2012), 23: 1569-1578.

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