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Forensic Toxicology Screening Using the ACQUITY UPLC I-Class System with the Xevo TQD

Mark Roberts, Michelle Wood

Waters Corporation

For forensic toxicology use only.

Abstract

The aim of this study was to evaluate the utility of the ACQUITY UPLC I-Class System and Xevo TQD for forensic toxicology screening and to assess the applicability of the existing chromatographic method combined with the existing full scan MS and MRM methodologies.

Benefits

Two complementary methodologies for comprehensive toxicological screening using the latest generation of instrumentation. The ACQUITY UPLC I-Class System and Xevo TQD MS:

- A targeted MRM method utilizing two transitions per compound that can screen for a panel of 178 key analytes with excellent sensitivity and selectivity
- A full scan MS method incorporating a spectral library for over 950 toxicologically relevant substances that can be easily appended by the user

Introduction

Forensic toxicology laboratories require broad screening techniques that can detect toxicants in highly complex biological matrices, such as ante- and post-mortem specimens. Two alternative toxicological screening methods, each utilizing the ACQUITY UPLC System with ACQUITY TQD, have been previously described.[1-3] These two complementary approaches allow the user to take full advantage of the benefits associated with full scan data acquisition and the improved sensitivity associated with targeted MRM screening.[4]

These methods have been successfully implemented in over one hundred laboratories worldwide, including those with little or no previous LC-MS experience. With the availability of a new generation of instruments offering improved functionality and performance, there is an interest in applying these powerful and proven screening methods to the new systems. This application note presents a preliminary evaluation of the applicability of the existing two screening methods to the ACQUITY UPLC I-Class System and Xevo TQD Mass

Spectrometer.

The goals of this study were to evaluate the utility of the ACQUITY UPLC I-Class System and Xevo TQD for forensic toxicology screening and to assess the applicability of the existing chromatographic method combined with the existing full scan MS and MRM methodologies[1-3] on this new platform.

Experimental

Sample Description

Drug standards were obtained from Sigma-Aldrich (Poole, Dorset, UK) and LGC Standards (Teddington, Surrey, UK) and were either solid chemicals or certified solutions at concentrations of 1 mg/mL. Ammonium formate and formic acid were from Sigma-Aldrich. Acetonitrile was obtained from Greyhound Chromatography (Birkenhead, UK). Authentic urine samples were obtained from collaborators for routine screening.

Authentic urine samples were extracted by liquid/liquid extraction and transferred to Waters Total Recovery Vials. Extracts were injected onto both the ACQUITY UPLC I-Class/Xevo TQD and the original configuration of the classic ACQUITY UPLC/TQD.

UPLC conditions

System:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC HSS C_{18}, 2.1 x 150 mm, 1.8 µm, part number 186003534
Column temp.:	50 °C
Sample temp.:	10 °C
Injection volume:	5 µL (MRM) or 10 µL (full scan)

Needle wash solvent:	5 mM ammonium formate, pH 3.0
Purge solvent:	5 mM ammonium formate, pH 3.0
Flow rate:	400 µL/min
Mobile phase A:	5 mM ammonium formate, pH 3.0
Mobile phase B:	0.1% formic acid in acetonitrile
Gradient:	13% mobile phase B increasing to 95%, with 15-minute analysis time

These are the same conditions that were previously used with the ACQUITY UPLC System.[1,2]

MS conditions

Mass spectrometer:	Xevo TQD
Ionization mode:	ESI positive (and ESI negative in full scan)
Capillary voltage:	3.0 kV
Cone voltage:	Varies according to method
Collision energy:	Varies according to method
Desolvation temp.:	400 °C
Desolvation gas:	800 L/h

Cone gas:	20 L/h
Acquisition mode:	Multiple Reaction Monitoring (MRM) or full scan MS
Data management:	MassLynx incorporating TargetLynx and ChromaLynx application managers

These are the same conditions that were previously used on the ACQUITY TQD.[1,2]

Innovative Technologies

The ACQUITY UPLC I-Class System with Flow-Through Needle (FTN)[5] design, shown in Figure 1, ensures that the sample comes into contact with only the needle and the direct flow path to the UPLC column. The sample is not transferred to a loop, which minimizes sample carryover; therefore, confidence in results is improved. In addition, the system volume has been reduced to 100 µL, which produces less analyte dispersion and improves peak shape. The column heater has also been improved with the inclusion of the active pre-heater (APH) reducing gradient delay and extra-column band-spreading.

The latest Xevo TQD,[6] shown in Figure 1, incorporates additional instrument features which could be highly advantageous to forensic analysis, such as RADAR and PICs. RADAR offers the capability to acquire full scan information while performing MRM analysis, which is a very useful tool for method development and troubleshooting. Product Ion Confirmation scanning (PICs) provides the option to automatically trigger a product ion scan when a particular MRM peak is detected. This allows the analyst to view additional confirmatory data and improve analyte identification.

Figure 1. The ACQUITY UPLC I-Class with Xevo TQD Mass Spectrometer.

Results and Discussion

Overview of the MRM and full scan MS techniques

The original MRM method screened for 178 commonly encountered substances. The acquisition method was arranged into 30 time windows over the chromatographic elution range in order to improve the efficiency of data collection and to ensure sufficient data points for peak characterization. Each MRM time window was configured so that the start of the window was 0.5 minutes before the first eluting compound, and the end of the window was 0.5 minutes after the last eluting compound. Therefore, a key element of this study was an evaluation of the transferability of retention time (RT) and to assess whether the original MRM time windows were still applicable on the ACQUITY UPLC I-Class System.

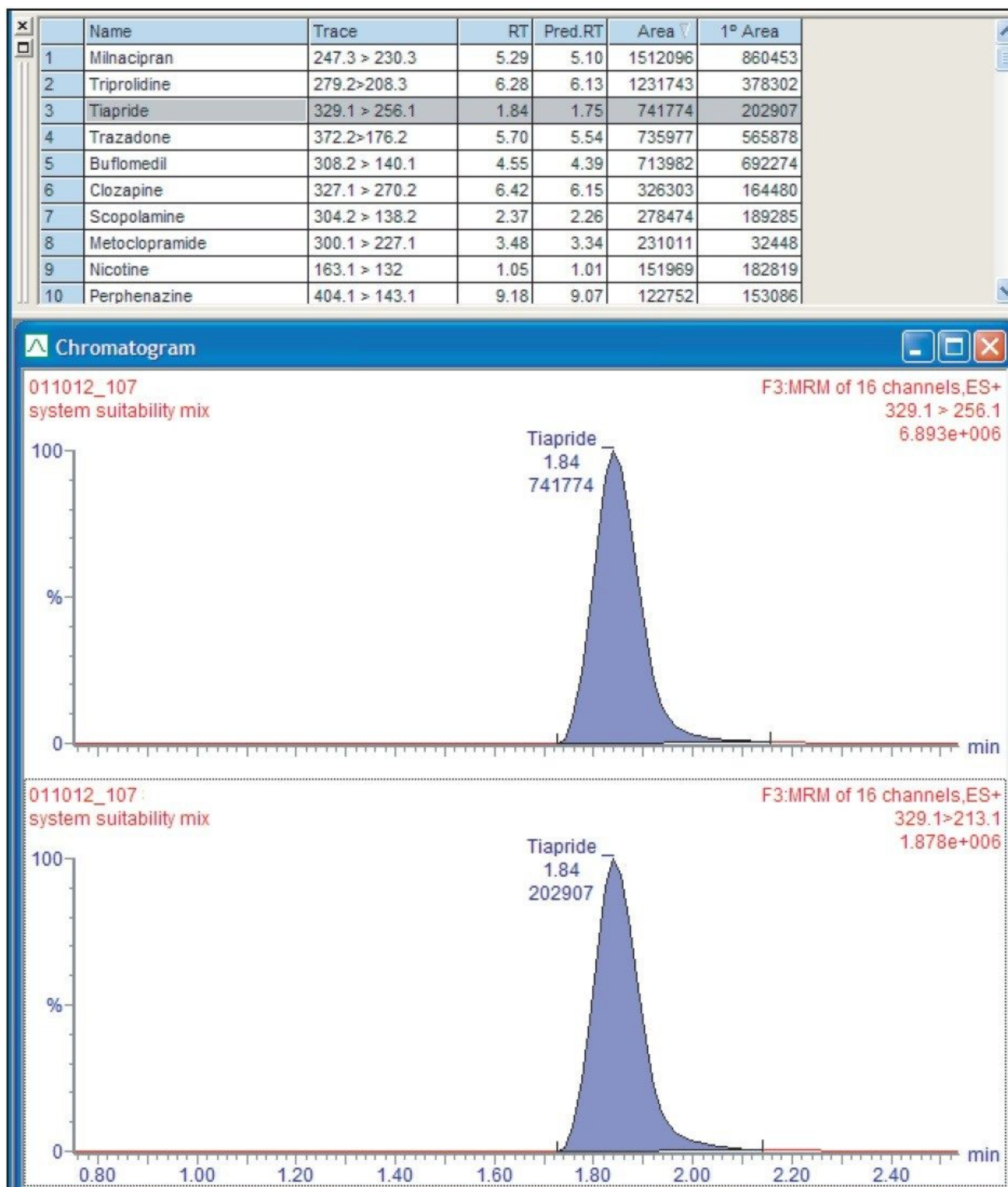
The original full scan MS method generated a comprehensive catalogue of mass spectral data by acquiring data at seven different cone voltages (+20 V, +35 V, +50 V, +65 V, +80 V, +95 V, and -20 V). This data was

automatically compared to a library comprised of spectral data for more than 950 substances using the ChromaLynx Application Manager.

In this study, ChromaLynx was used to compare spectral data acquired on the new MS system with the spectra contained in the original ACQUITY TQD library.

System suitability mixture injections using MRM and full scan acquisition modes

It is good laboratory practice to verify the performance of any analytical system prior to acquiring authentic sample data. This is commonly achieved by injecting a system suitability mixture (SSM) that contains a combination of substances that elute over the entire chromatographic range.



	Name	Trace	RT	Pred.RT	Area	1° Area
1	Milnacipran	247.3 > 230.3	5.29	5.10	1512096	860453
2	Triprolidine	279.2>208.3	6.28	6.13	1231743	378302
3	Tiapride	329.1 > 256.1	1.84	1.75	741774	202907
4	Trazadone	372.2>176.2	5.70	5.54	735977	565878
5	Buflomedil	308.2 > 140.1	4.55	4.39	713982	692274
6	Clozapine	327.1 > 270.2	6.42	6.15	326303	164480
7	Scopolamine	304.2 > 138.2	2.37	2.26	278474	189285
8	Metoclopramide	300.1 > 227.1	3.48	3.34	231011	32448
9	Nicotine	163.1 > 132	1.05	1.01	151969	182819
10	Perphenazine	404.1 > 143.1	9.18	9.07	122752	153086

Figure 2. TargetLynx browser displaying the results obtained with a typical SSM following targeted MRM

screening using an ACQUITY UPLC I-Class/Xevo TQD. The RTs for all ten components detected were within 0.3 minutes of the expected RT and well within the original MRM time windows. This indicates successful transfer of the chromatography method to the ACQUITY UPLC I Class System.

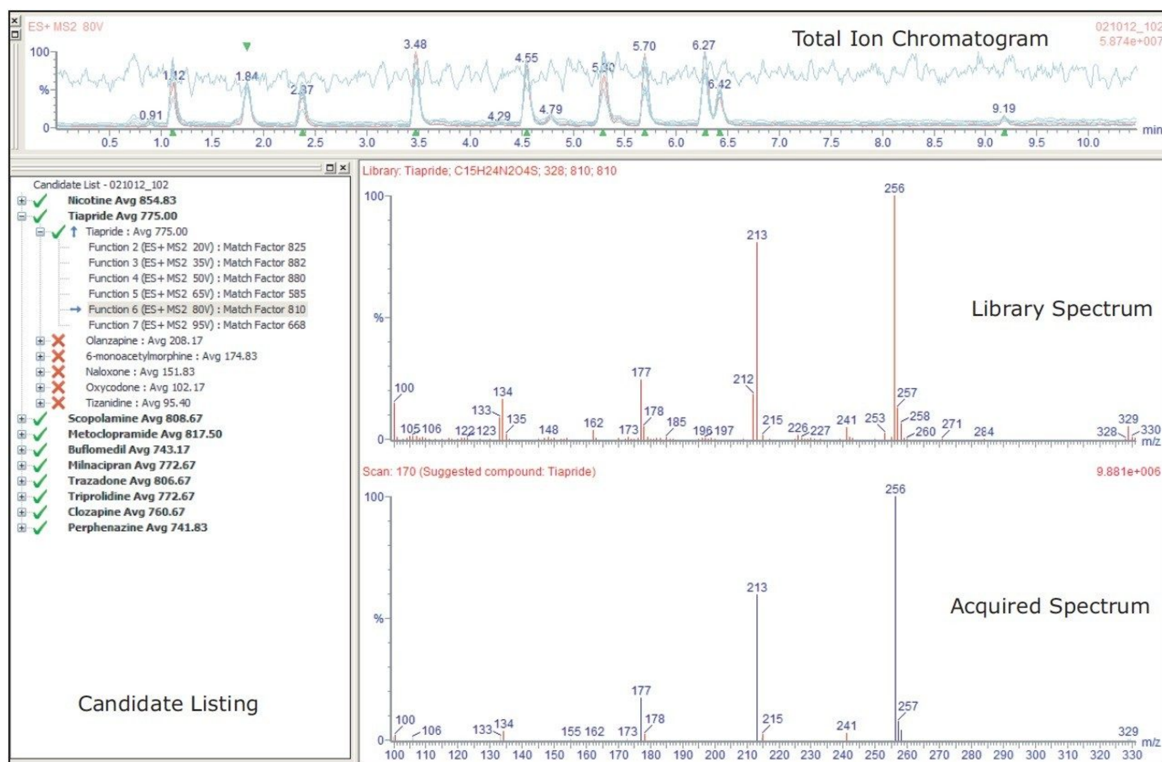


Figure 3. ChromaLynx browser displaying results of the full scan screening analysis of the SSM. Again, all ten components were detected, each one with a high average (Avg) match factor, indicating excellent agreement with the existing spectral library.

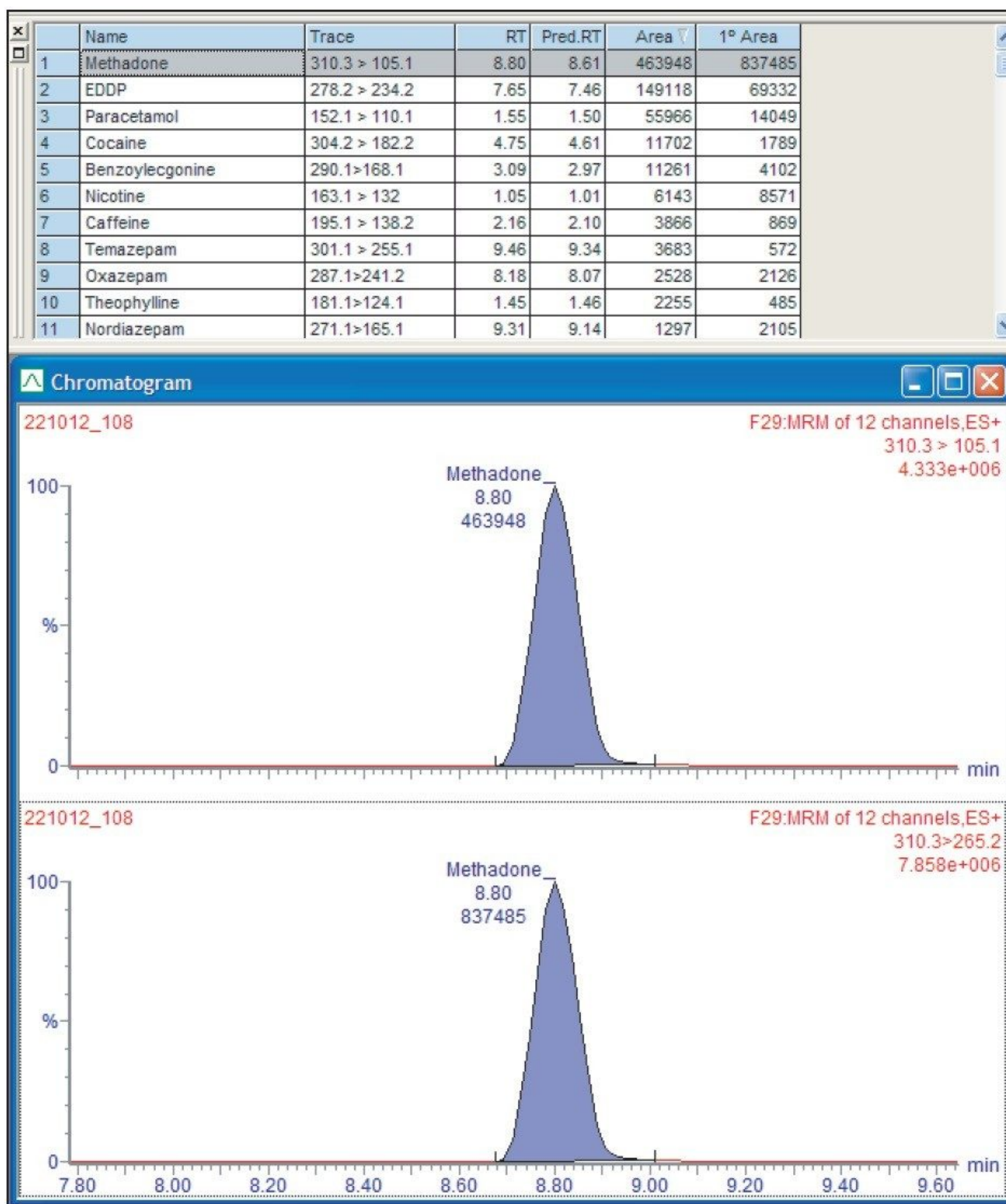
Analysis of an authentic urine sample by targeted MRM screening

The results for a urine sample submitted for routine forensic toxicological screening are shown in Table 1. The RTs of all identified substances were within 0.19 minutes of the expected RT, again supporting excellent transferability of the existing chromatographic method to the ACQUITY UPLC I-Class System.

		ACQUITY UPLC/TQD (original configuration)		ACQUITY UPLC I-Class/Xevo TQD (new configuration)	
Compound Name	**Predicted RT**	**Found RT**	**Peak Area**	**Found RT**	**Peak Area**
Methadone	8.61	8.77	434734	8.80	463948
EDDP	7.46	7.67	145310	7.65	149118
Paracetamol	1.50	1.58	5703	1.55	55966
Cocaine	4.61	4.80	11644	4.75	11702
Benzoylecgonine	2.97	3.13	10500	3.09	11261
Nicotine	1.01	1.02	3284	1.05	6143
Caffeine	2.10	2.19	1499	2.16	3866
Temazepam	9.34	–	–	9.46	3683
Oxazepam	8.07	8.17	1853	8.18	2528
Theophylline	1.46	–	–	1.45	2255
Nordiazepam	9.14	–	–	9.31	1297

Table 1. Results for an authentic urine sample analyzed by MRM targeted screening using both original and newer instrument configurations.

The identification of drug metabolites within a biological specimen can be highly beneficial for the following reasons: they can be used to extend the window of drug detection; provide additional confirmation of drug intake; and generally assist in data interpretation. In this sample, methadone and its metabolite EDDP, as well as cocaine and its metabolite, benzoylecgonine, were identified by both systems. Figure 4 shows the TargetLynx browser detailing the results from the ACQUITY UPLC I-Class/Xevo TQD MRM analysis. Some additional substances were detected with the new system configuration suggesting enhanced sensitivity; this was also supported by the observation that the peak areas obtained with the new configuration were generally larger than those seen with the ACQUITY UPLC/TQD. This may be as a result of the new ACQUITY UPLC I Class FTN, designed to minimize peak dispersion and maximize peak response.



	Name	Trace	RT	Pred.RT	Area	1° Area
1	Methadone	310.3 > 105.1	8.80	8.61	463948	837485
2	EDDP	278.2 > 234.2	7.65	7.46	149118	69332
3	Paracetamol	152.1 > 110.1	1.55	1.50	55966	14049
4	Cocaine	304.2 > 182.2	4.75	4.61	11702	1789
5	Benzoylecgonine	290.1>168.1	3.09	2.97	11261	4102
6	Nicotine	163.1 > 132	1.05	1.01	6143	8571
7	Caffeine	195.1 > 138.2	2.16	2.10	3866	869
8	Temazepam	301.1 > 255.1	9.46	9.34	3683	572
9	Oxazepam	287.1>241.2	8.18	8.07	2528	2126
10	Theophylline	181.1>124.1	1.45	1.46	2255	485
11	Nordiazepam	271.1>165.1	9.31	9.14	1297	2105

Figure 4. TargetLynx browser displaying processed results from a urine sample extract showing result for

methadone.

Analysis of an authentic toxicology urine sample by full scan MS

A major advantage of the full scan MS approach is the ability to simultaneously screen for extremely large numbers of substances (limited only by the size of the spectral library). This is in contrast to the targeted MRM approach which is restricted to a panel of key analytes. At this time, a library comprising spectral data formore than 950 substances is available from Waters. Additionally, this library can be very easily expanded by the user. Alternatively, new libraries may be created and multiple libraries searched using the ChromaLynx Application Manager. Another benefit of this approach is that the data is not restricted to specific channels; thus the complete data set is available for retrospective analysis.

Figure 5 shows the results for another urine sample screened using the full scan MS method on the new system. In this sample the metabolite mirtazapine N-desmethyl was found, as well as the parent compound, mirtazapine. The spectrum window within the ChromaLynx browser clearly shows a good match for the fragments of mirtazapine N-desmethyl compared with the library spectrum that was originally acquired using the ACQUITY TQD. This indicates that the existing library acquired on ACQUITY TQD can be used with the newer Xevo TQD.

The full scan MS method also detects another compound, xylometazoline. This nasal decongestant would not be identified by the targeted MRM method because the substance is not currently included in the targeted panel.

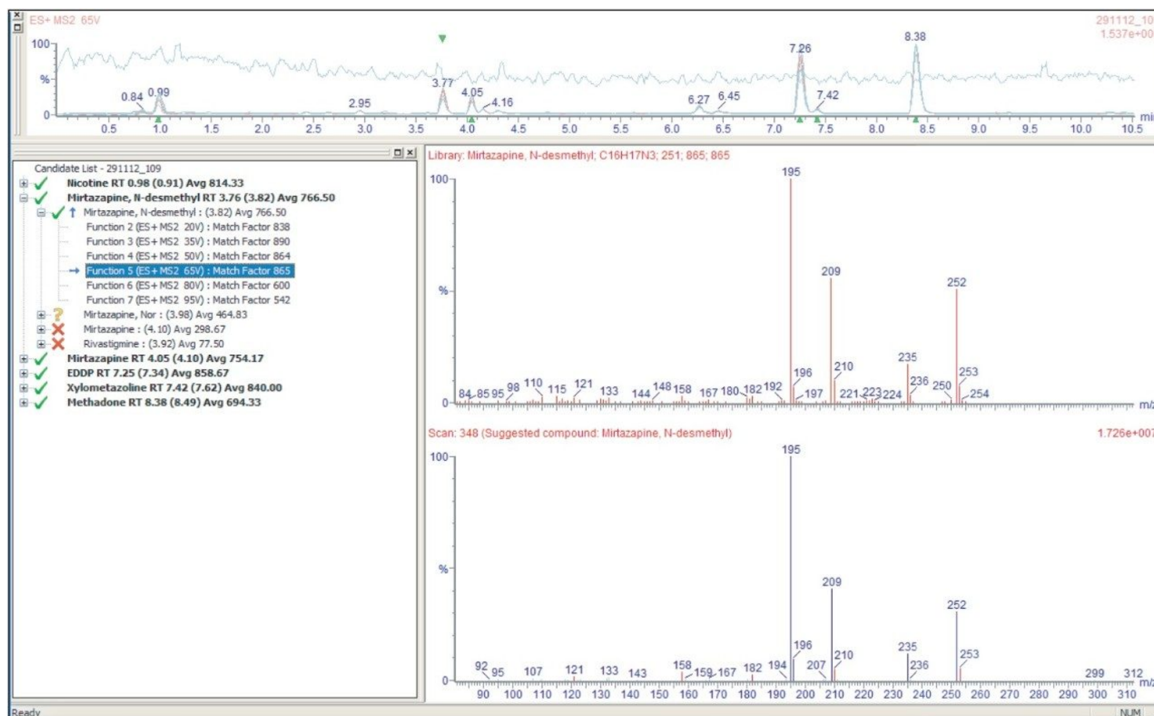


Figure 5. ChromaLynx browser displaying results from an analysis of urine sample extract showing results for the N-desmethyl metabolite of mirtazapine.

Using PICs to distinguish between hydromorphone and morphine

A useful feature of the new Xevo TQD is the ability to trigger data-directed Product Ion Confirmation scanning (PICs) during MRM analysis. This enables very similar compounds to be distinguished from one another by the pattern of their fragmentation from precursor into product ions.

Figure 6 shows the data obtained following analysis of hydromorphone using the MRM screening method. Responses were obtained in the two MRM channels for hydromorphone but also for morphine. This is not surprising, as these two substances are isomers sharing several MRM transitions. Furthermore, they elute within 0.2 minutes of one another using the chromatography described in this application note. Data generated from PIC scanning, however, may be used to provide additional information, which may assist in the differentiation of similar substances.

In this example, the PICs data produced a better match with a previously saved reference spectrum for hydromorphone than the one for morphine. The analyst is able to select both reference spectra from within the

TargetLynx method to allow visual comparison of the spectra.

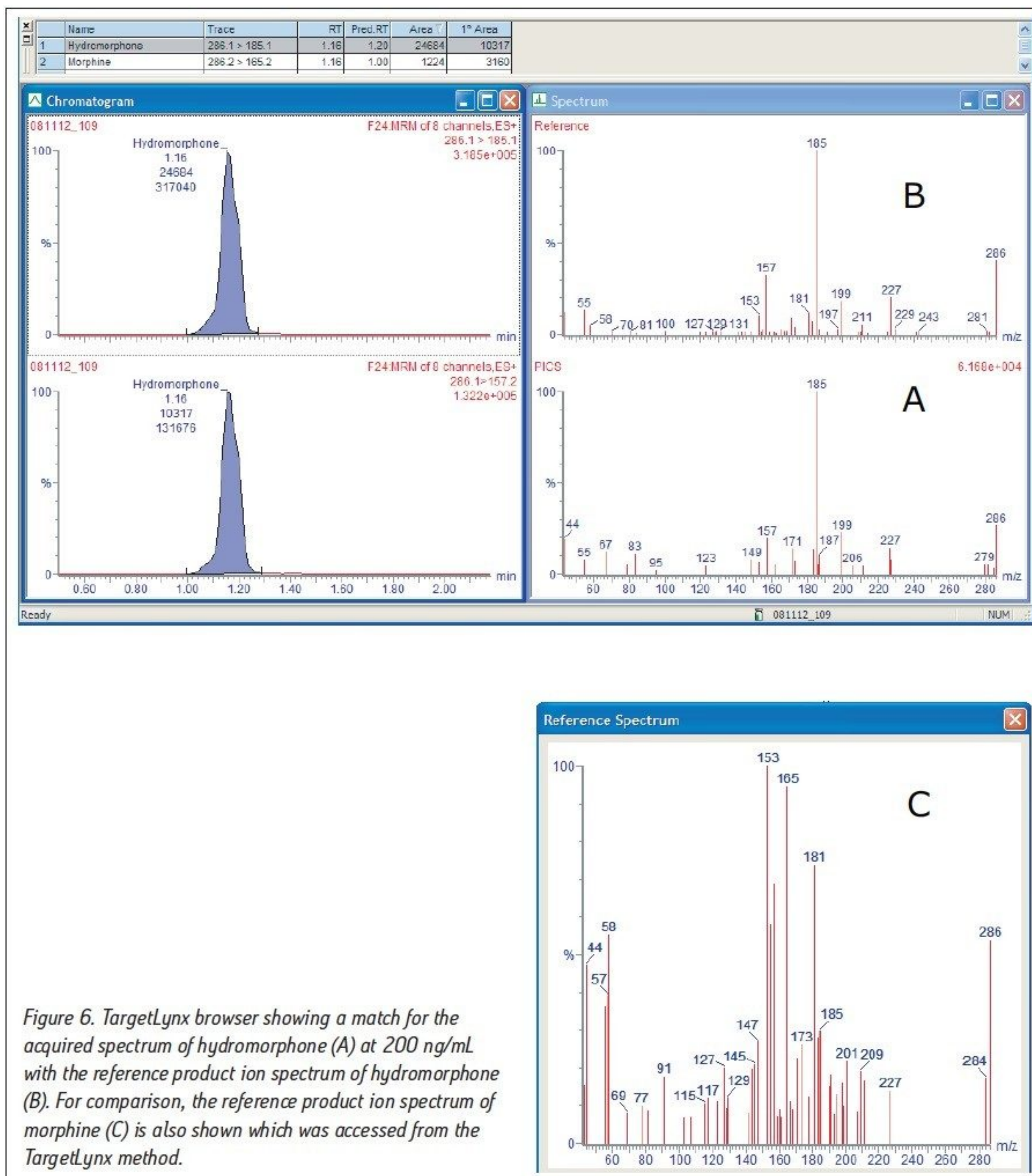


Figure 6. TargetLynx browser showing a match for the acquired spectrum of hydromorphone (A) at 200 ng/mL with the reference product ion spectrum of hydromorphone (B). For comparison, the reference product ion spectrum of morphine (C) is also shown which was accessed from the TargetLynx method.

Using RADAR to indicate the presence of extra analytes

The urine sample analyzed by full scan MS was also analyzed using the MRM targeted screening method with

RADAR enabled. This allows full scan data to be collected while performing conventional MRM analysis and will show peaks that would potentially be missed if the analytes were not in the MRM method. Figure 7 shows the full scan data from this analysis with a selection of the MRM data that was simultaneously acquired. The full scan peaks for the metabolites N-desmethyl mirtazapine, desmethyl citalopram, and the drug, xylometazoline, are clearly visible at 3.79, 6.47, and 7.42 minutes, respectively; however, they were not detected by the MRM method as these substances were not included in this targeted assay. Figure 8 shows the mass spectra of the two metabolite peaks acquired at a cone voltage of 30 V. This extra information can be very useful, particularly in complex biological specimens, as it can indicate the presence of unknown components that would typically remain undetectable in a targeted screening scenario. Moreover, it can be an invaluable tool for troubleshooting during method development and validation as it may be used to identify and resolve issues with co-eluting compounds.

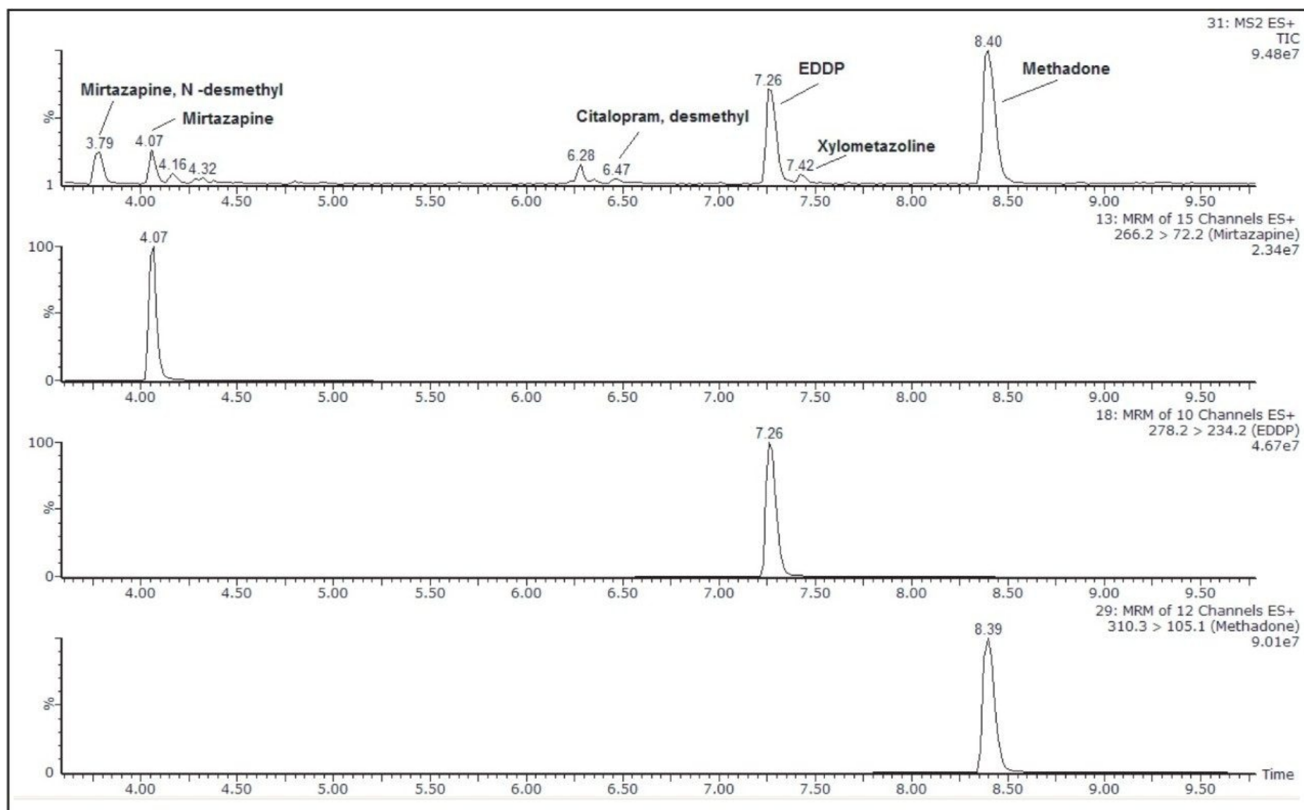


Figure 7. Full scan data (A) that was acquired simultaneously with MRM data for the hydrolyzed urine sample using RADAR. The full scan peak at 3.79 minutes was N-desmethyl mirtazapine, at 6.47 minutes desmethyl citalopram, and at 7.42 minutes was xylometazoline which are not currently included in this targeted MRM method.

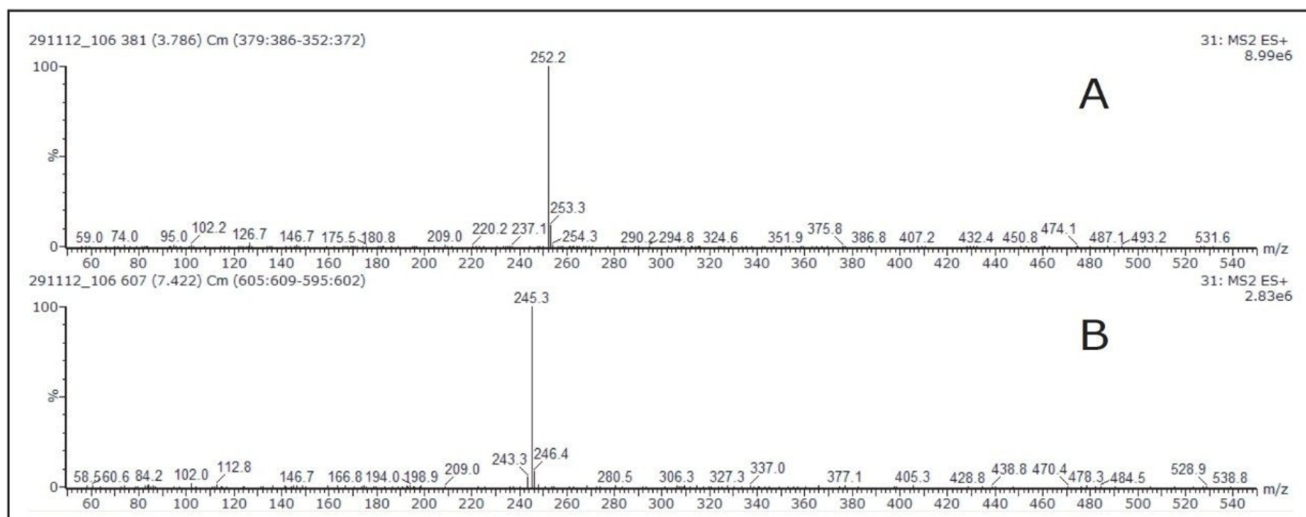


Figure 8. Full scan mass spectra (cone voltage 30 V) collected in RADAR mode for N-desmethyl mirtazapine at 3.79 minutes (A) and xylometazoline at 7.42 minutes (B).

Conclusion

Successful transfer of the toxicology screening methods to the next generation of the UPLC/MS system enables forensic analysts to access the latest analytical tools for their laboratories. The ACQUITY UPLC I Class System has reduced peak dispersion and small system volume, thereby improving the sensitivity of the MS used in the assay. The Xevo TQD has the new features of RADAR and PICs, which can be used to enhance the information that is available to the analyst.

Starter projects are available that contain all the necessary methods to both acquire and process the data. The preconfigured methods contain a large number of the most commonly encountered toxicants and are ready for laboratory implementation with minimal user intervention.

The methods are fully customizable and can be easily appended to meet the scientists' needs. For example, additional compounds can be added to the databases. This ensures that the methods are versatile and will remain relevant for the future.

A full validation by the user would be necessary prior to adoption in a laboratory.

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