

Targeted MRM Screening for Toxicants in Biological Samples By UPLC-MS/MS

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Abstract

We present a preconfigured broad screening method using the ACQUITY TQD System, which combines rapid

analysis time with the high sensitivity and selectivity of MRM acquisition.

Introduction

Toxicology laboratories are frequently required to perform broad screening techniques on complex biological samples in order to identify drugs of abuse and other toxicants. Traditional screening methods include immunoassay and chromatographic techniques such as LC/UV and GC-MS. Although these techniques are well-established, they are not without their disadvantages, such as the cost and cross-reactivity often associated with immunoassay, the sometimes limited selectivity and sensitivity of LC/UV and the poor performance of GC-MS with involatile or thermally labile compounds. LC-MS can overcome some of these potential limitations.

Previously we have described a LC-MS broad screen based on full scan acquisition¹ and suitable for use in emergency toxicology. The use of LC-MS/MS, in particular multiple reaction monitoring (MRM) analysis, can offer further increases in specificity and sensitivity. However, methods based on MRM can sometimes be compromised, either by the number of analytes that can be simultaneously screened for, or by the total analysis time.

Recent advances in both UPLC and MS technologies^{2,3} have culminated in increased analytical capabilities, now making it possible to monitor extremely large panels of analytes within a very short time.

Innovative Technologies

ACQUITY UPLC and the Waters TQ Detector

The introduction of UltraPerformance LC (UPLC) has brought many improvements to the analyst; the smaller particle sizes of ACQUITY UPLC Columns enhance chromatographic peak resolution, resulting in sharper, narrower peaks which provide better signal to noise and improved integration which can help to resolve analytes from background matrix. Low system volumes allow a precise and rapid delivery of mobile phase gradients and column equilibration. Consequently, the overall cycle time for a comprehensive screening can be reduced significantly, thereby greatly increasing sample throughput.

The Waters TQ Detector (Figure 1) can operate in MRM mode at very low dwell times without compromising sensitivity. Patented T-Wave technology⁴ reduces ion transit time through the collision cell (Figure 2) and minimises cross-talk between MRM channels. Thus, short dwell times combined with brief inter-channel delays of 5 ms result in the ability to monitor large panels of MRMs whilst still generating sufficient data points across the narrow UPLC peaks.



Figure 1. Waters ACQUITY TQD System.

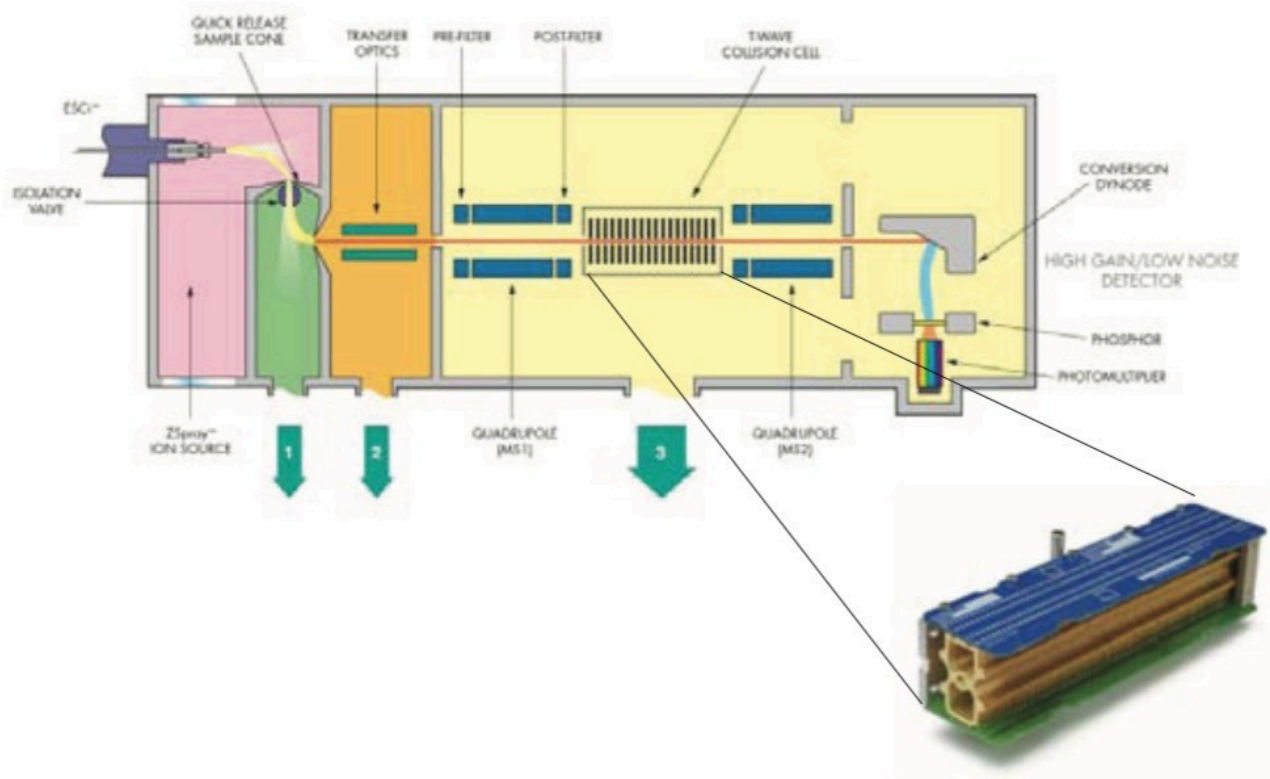


Figure 2. Schematic of TQ Detector with T-Wave Collision Cell.

Targeted Analysis by UPLC-MS/MS

By targeting those drugs most often identified in numerous settings e.g., emergency room admissions, this screening method offers excellent sensitivity and selectivity. The UPLC-MS/MS method utilises a single MRM for each compound. Preconfigured MRM conditions (Figure 3) are provided for each analyte in the compound database to ensure excellent sensitivity.

Channels						
	Compound Name	Parent (m/z)	Daughter (m/z)	Dwell (s)	Cone (V)	Collision (V)
1	Nicotine	163.1	130	0.02	35	25
2	Salbutamol	240.1	148.1	0.02	35	25
3	Atenolol	267.2	145.1	0.02	40	25
4	Morphine	286.2	165.2	0.02	55	40

Figure 3. First function showing MRM transitions and collision energies for four of the toxicants being screened.

MRM time-windows can be set which specify the time that a particular MRM is acquired (Figure 4). The ability to overlap these windows allows for a finer control over acquisition times, thereby increasing specificity and maintaining maximum sensitivity.

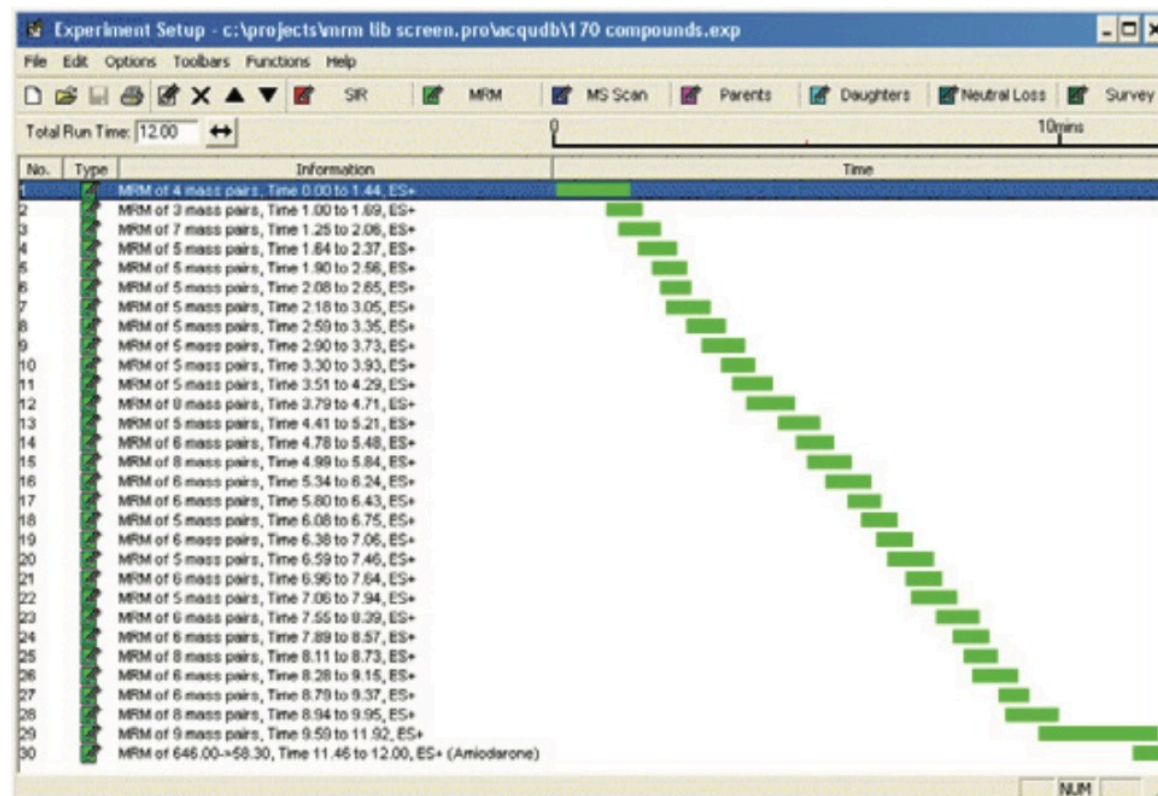


Figure 4. 170 Transitions divided into 30 separate MRM windows.

A starter project is provided which includes all of the necessary files for the comprehensive screen *i.e.*, 15 min UPLC method, MRM parameters and a data processing method. Supporting documentation is also provided enabling a laboratory to load and rapidly implement this method.

Experimental

LC Conditions

LC system:	Waters ACQUITY UPLC
Column:	ACQUITY UPLC HSS C ₁₈ Column, 2.1 x 150 mm, 1.8 µm
Column temp:	50 °C
Flow rate:	400 µL/min.
Mobile phase A:	5 mM Ammonium Formate, pH 3.0
Mobile phase B:	Acetonitrile with 0.1% formic acid
Gradient:	<i>Defined UPLC method provided</i>
Injection volume:	5 µL

MS/MS Conditions

MS system:	Waters TQ Detector
Ionization mode:	ESI Positive
Capillary voltage:	3000 V

Cone voltage:	<i>Preconfigured in MS method provided</i>
Desolvation temp:	400 °C
Desolvation gas:	800 L/Hr
Source temp:	150 °C
Collision energies:	<i>Preconfigured in MS method provided</i>

Software

Waters MassLynx Software v4.1 was used for data acquisition and the TargetLynx Application Manager⁵ was used for data processing.

Results and Discussion

Good laboratory practice often includes the use of a 'system suitability' (or check-mix) test to confirm and verify that the analytical equipment is performing as expected. By routinely injecting this mixture, it is possible to check that retention times are consistent and signal to noise ratios for peaks are sufficient. In this example, a mixture of 8 compounds that elute across the full chromatographic run was prepared in mobile phase A and analysed by the preconfigured method.

The results for 10 replicate injections of the system suitability mix are shown in Figures 5a and 5b. They illustrate the excellent chromatographic resolution and reproducibility (retention times and peak intensity) associated with UPLC-MS/MS.

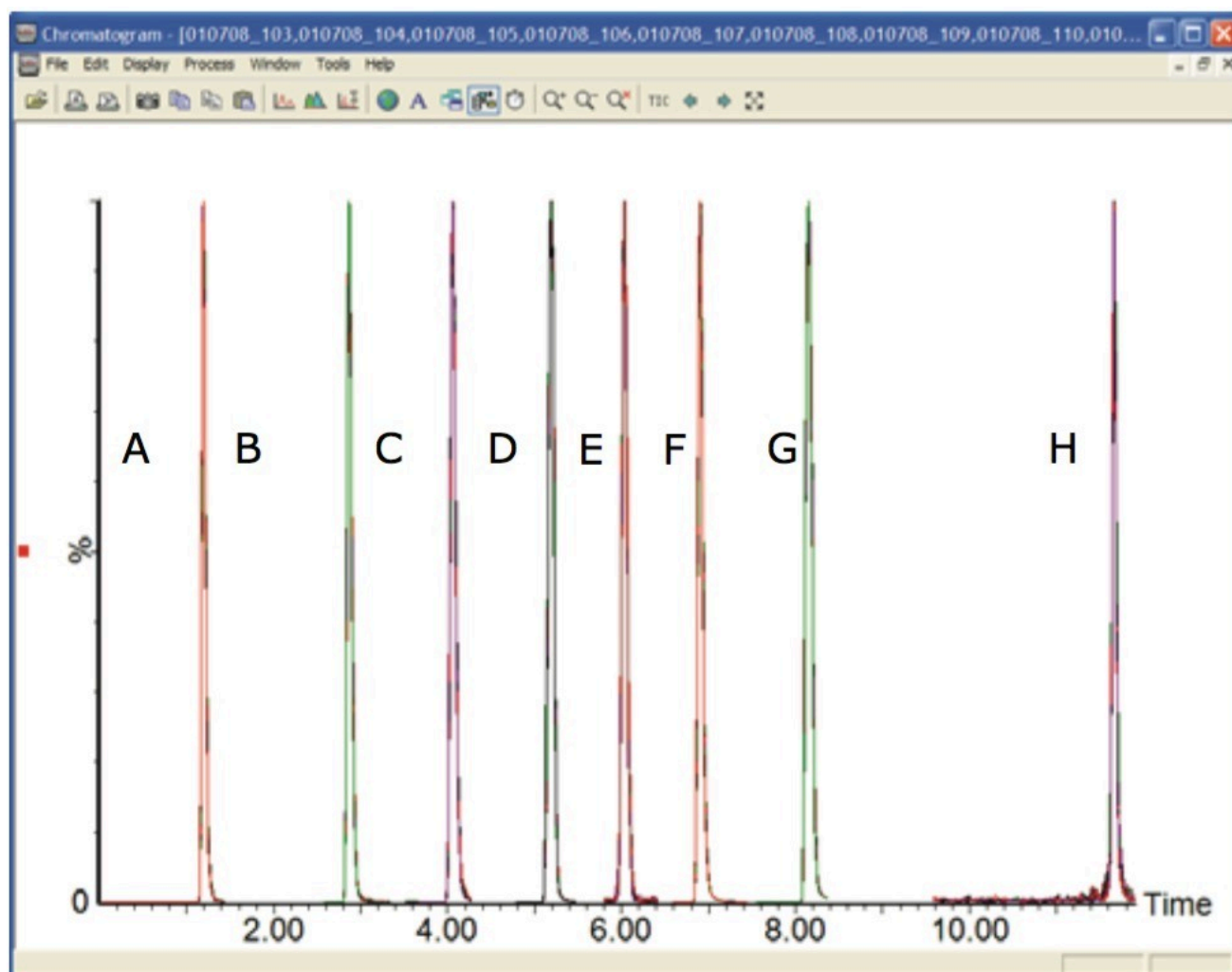


Figure 5a. Overlaid traces from 10 injections of the 'system suitability' mix (1 µg/mL) showing peaks for (A) Atenolol, (B) Amisulpride, (C) Milnacipran, (D) Citalopram, (E) Colchicine, (F) Imipramine, (G) Metoprolol and (H) Prazepam.

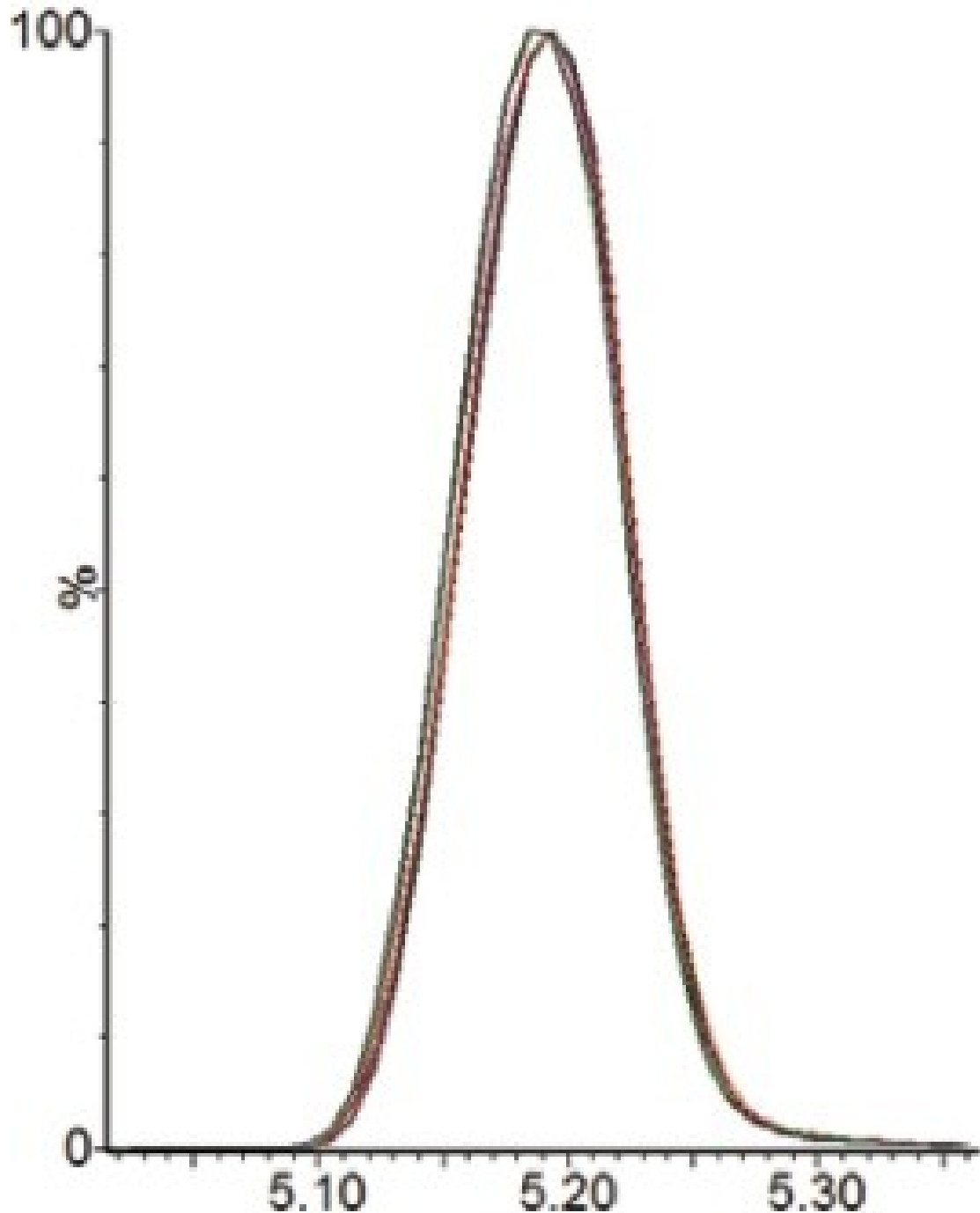


Figure 5b. Expansion of the data presented in Fig. 5a. Results shows reproducibility for 10 replicate injections of Citalopram. The data obtained following the analysis of an authentic sample is given in Figure 6. The sample was prepared using a simple protein precipitation procedure; briefly, 100 μ L serum was precipitated by the addition of 100 μ L acetonitrile. Following centrifugation, the supernatant was diluted 5-fold using water prior to analysis by the

UPLC-MS/MS targeted screen. The method has also been successfully applied to serum samples prepared using a liquid:liquid extraction (LLE) protocol performed at basic pH. Following analysis, the data was processed using the TargetLynx Application Manager.

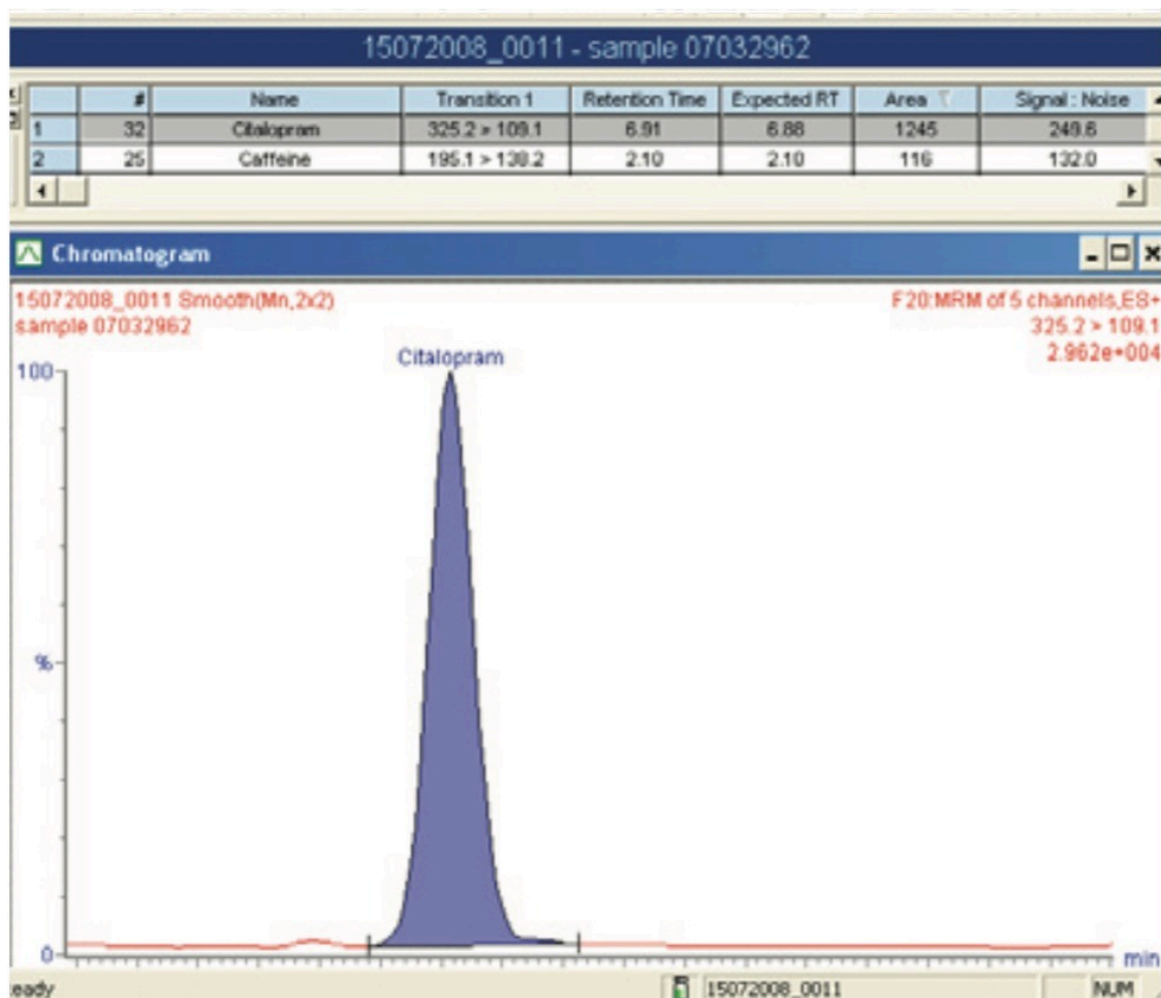


Figure 6. TargetLynx browser showing the results following the analysis of an authentic serum sample. The screening method identified 2 components i.e., citalopram and caffeine. The citalopram result was subsequently confirmed by a separate rapid, quantitative method for multiple psychotherapeutic drugs⁶; citalopram concentration was 166 µg/L.

Data Processing: TargetLynx Application Manager

Automatic data processing has been made much simpler with the use of the TargetLynx Application Manager. Sample lists can be set up to acquire data and then to process the data automatically without analyst intervention.

For screening purposes, a single MRM transition for each compound in addition to its expected retention time is provided in the preconfigured TargetLynx method. After processing, a list of compounds with peak areas above a

pre-defined threshold can be viewed.

Whereas manual processing of this data for each compound would take hours, automatic processing of individual samples can be performed in a few seconds. Numerous reporting formats are available for printing the final results.

Conclusion

The presented screening technique is a useful tool for the identification of toxic compounds within complex biological matrices. It combines a rapid chromatographic run time (15 minutes), with the excellent sensitivity and specificity associated with MRM analysis.

The rapid screening capabilities of the ACQUITY TQD System, mean that monitoring multiple MRM channels is now feasible for much larger panels of compounds.

A starter project is available, which contains everything the user needs to both acquire and process the data. The preconfigured methods currently target a large number of the most commonly identified toxicants. These methods are ready for immediate implementation within the laboratory with minimal user intervention.

For data handling, the TargetLynx Application Manager provides rapid, automatic processing, thus simplifying the task of the laboratory toxicologist and minimising potential delays in reporting results.

All supplied methods are fully customisable and can be easily modified to address the individual customer's needs e.g., additional analytes can be appended to the existing database. Moreover, the screening method can be adapted to provide a full confirmatory technique by specifying additional compound information such as secondary MRM transitions, in addition to ion ratios and their tolerances.

References

1. General Unknown Screening for Drugs in Biological Samples by LC-MS, Water's Application note, Part Number [720001552EN <https://www.waters.com/webassets/cms/library/docs/720001552en.pdf>](https://www.waters.com/webassets/cms/library/docs/720001552en.pdf).
2. ACQUITY UPLC System Brochure, Part Number 720001507EN.
3. ACQUITY TQD Brochure, Part Number [720001767EN <](#)

https://www.waters.com/waters/library.htm?cid=511436&lid=1528852&locale=en_US>.

4. The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).
5. TargetLynx Application Manager, Waters Application note, Part number [720001733EN](#) <
<https://www.waters.com/webassets/cms/library/docs/720001733en.pdf>>.
6. Quantitative Analysis of Multiple Psychotherapeutic Drugs in Human Serum using UPLC-MS/MS. R Watts, E.P. Spencer and M. Wood In Proceedings of the 46th TIAFT Meeting, June 2008, Martinique, French West Indies.

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