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Quantitative toxicology screening for over 1000 compounds in whole blood samples; is there a better way of reporting results?

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Introduction

To help clinical toxicology laboratories reduce the incidence of false positive and false negative reporting a spectral library and database of 1222 clinical and forensic toxicological compounds with both full scan product ion spectra and MRM product ion spectra data has been developed. Each library spectra generated from certified reference material was acquired using a generic approach to sample preparation and LC separation. The MRM Spectrum mode library includes product ion spectra created by combining typically more than 5 precursor-fragment ion transitions for each compound, each precursor-fragment ion transitions has an optimized collision energy resulting in a specific product ion spectra and a high signal intensity. The database also includes structure (as a mol file), RT, CAS number, formula, synonyms, compound class/properties, ChemSpider URL and ID number, InChI and InChIKey. The key advantages of this approach include its simplicity to set up a method and adapt to other needs, high data densities, consistent loop time and a high sampling rate producing reliable quantitation and peak integration without the need to use a predefined a threshold.

Methods

Whole blood was spiked with target compounds and extracted with a QuEChERS method protocol (where possible deuterated internal standards were also included). Chromatographic conditions considered the need to develop a single LC method for a diverse chemical space with a cycle time of 17minutes using a Restek Biphenyl column. The LCMS-8060 triple quadrupole mass spectrometer was set to measure more than 5 precursor-fragment ion transitions for each compound (without a predefined threshold) in both positive and negative ionisation mode.

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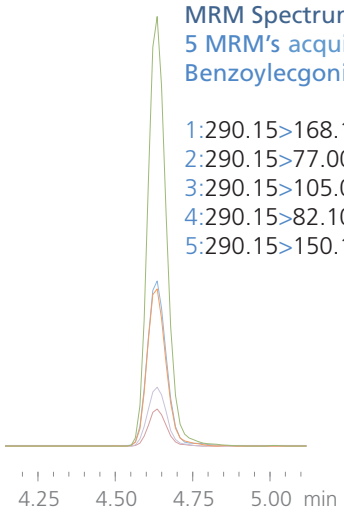
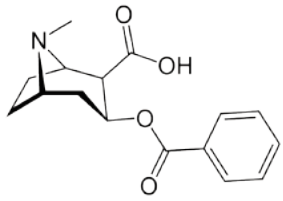
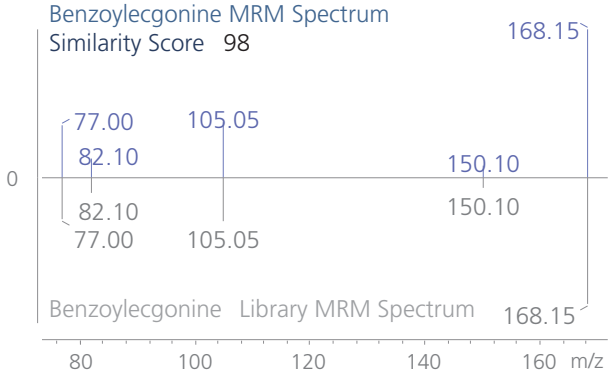


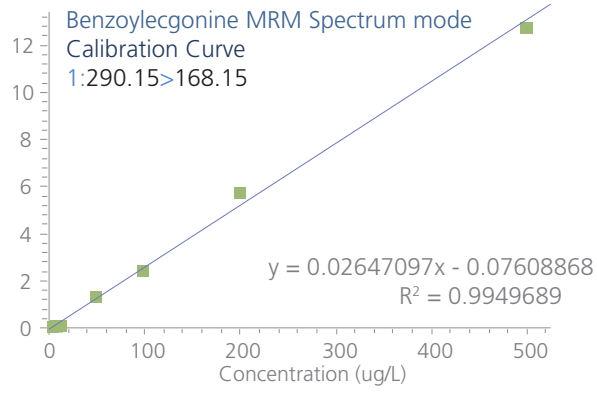
Compound name
Benzoylecgonine
Mass 290.14 [M+H]+
Formula C16H19NO4
CAS 57-62-5



MRM Spectrum mode
Acquires a high number of precursor-fragment ions
The collision energy is optimized for each fragment ion resulting in a highly specific and sensitive library searchable product ion spectrum.



MRM Spectrum mode
Higher specificity
Higher reporting confidence
Library searchable fragment data
In this method the number of precursor-fragment ion transitions monitored was typically greater than 5 MRM's with a target list of 616 compounds (total number of MRM was 3010 with a retention time window of +/-0.5 min per compound; 1msec pause time; 1msec dwell time; Max loop time: 1.14sec).



MRM Spectrum mode
Quantitative data quality
Despite acquiring 3010 transitions in the MRM Spectrum method the quantitative data was near identical to a conventional method monitoring 2 MRM transitions.
MRM Spectrum mode regression analysis;
$y = 0.0264x - 0.0760$; $R^2 = 0.99496$
2 MRM regression analysis
$y = 0.0256x - 0.0027$; $R^2 = 0.99964$

Figure 1. One example of a target compound (in this case benzoylecgonine) acquired by MRM Spectrum mode. In this method a higher number of precursor- fragment ions were monitored to generate a MRM product ion spectrum (for each compound in the screening method up to 6 MRM's were monitored).

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Table 1. Quantitative comparison of the same patient sample measured by a conventional validated 2 MRM method (CHU Limoges) and MRM Spectrum mode using different LC-MS/MS instruments (the 2 MRM data was generated on a LCMS-8050). Both data sets are in close agreement.

Compound	RT	CHU-Limoges	MRM-Spectrum mode
	(min)	Patient sample	RTCHU-Limoges
Morphine	3.32	>500	>500
Benzoylecgonine	4.64	>500	>500
EDDP	7.52	>500	>500
Methadone	8.16	116	127
Ecgonine methylester	1.05	73	72
Hydromorphone	3.48	35	31

Results

The number of precursor-fragment ion transitions monitored in the method was typically greater than 5 MRM's with a target list of 616 compounds (total number of MRM was 3010 with a retention time window of +/-0.5 min per compound; 1msec pause time; 1msec dwell time; Max loop time: 1.14sec).
Despite acquiring 3010 transitions in the MRM Spectrum method the quantitative data was near identical to a conventional method monitoring 2 MRM transitions. The MRM product ion spectrum was successfully used for compound identification by searching a library to increase reporting confidence. As the collision energy was optimized for each fragment ion to generate a product ion spectrum, the library spectrum is highly specific and selective

Patient Data

In developing screening methods for routine clinical pathology laboratories there has been a focus on delivering a rapid, cost-effective analysis that generates near zero false-negative or false-positive results. Current approaches for reporting a positive or negative result have been established and the identification criteria requires at least two MRM transitions with a constant ion ratio (within a pre-defined tolerance) and retention time corresponding to a calibration standard at a comparable concentration. In reducing the incidence of false positive or negative reporting in routine clinical MS/MS assays, a MRM method was developed with a higher number of precursor-fragment ion transitions for each target compound to increase the level of confidence in assay specificity. By combining all MRM transitions into a product ion spectrum, compound identification can be verified and confirmed against a MS/MS reference spectral library with retention time data. Using MRM Spectrum mode can help markedly reduce false reporting without affecting the data quality for optimized quantitation or identification.

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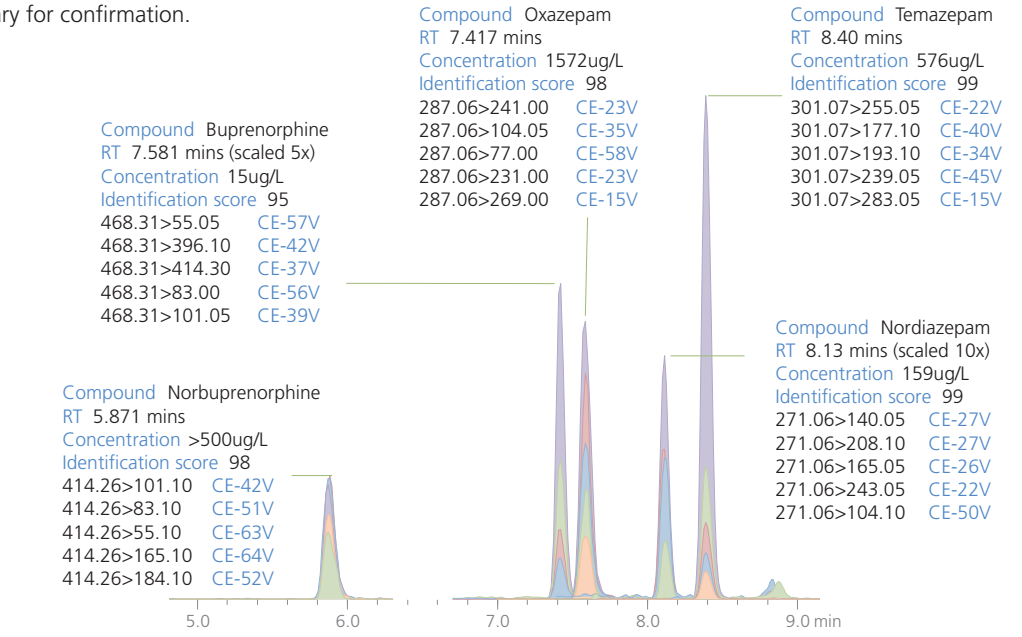
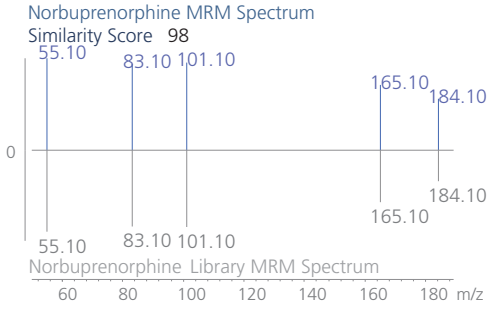
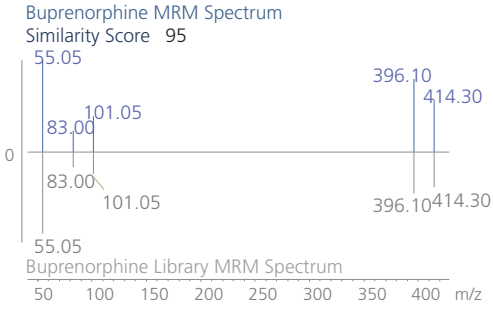
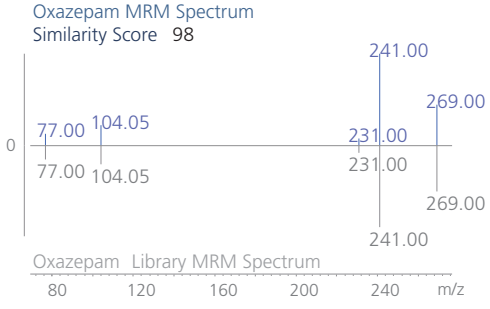
Screening analysis

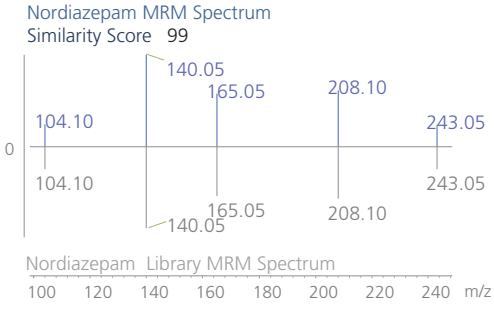
Unknown psychiatric patient sample
Whole blood sample; QuEChERS extraction; Restek Raptor Biphenyl 2.7um 100 x 2.1mm
MRM library for confirmation.











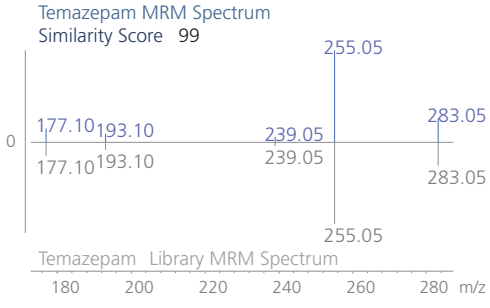


Figure 2. Psychiatric patient sample analysis detected norbuprenorphine, buprenorphine, oxazepam, nordiazepam and temazepam using MRM Spectrum mode. The product ion spectrum can be used for compound identification by searching a library. As the collision energy was optimized for each fragment ion to generate a product ion spectrum, the library spectrum is highly specific and selective.

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Conclusions

Clinical pathology laboratories use highly specific, sensitive methods and technologies working across a multi-disciplinary teams delivering actionable data. However, if a false positive result is reported the downstream impact to unnecessary testing and treatment can be considerable. In this paper, a library of product ion spectra for 1222 compounds has been developed for clinical and forensic toxicology screening to help reduce false positive and false negative reporting. The library enables multi-targeted methods to be developed for routine screening, library identification and quantitation. MRM Spectrum mode results in high data densities and a high data sampling rate across a peak. This approach generates a consistent loop time and sampling rate producing reliable quantitation and peak integration without threshold triggering and creates new opportunities in toxicological screening.

Disclaimer: The Shimadzu LCMS-8060 is intended for Research Use Only (RUO). Not for use in diagnostic procedures. Not available in China.

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