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Toxicological screening for over 1000 compounds in an MRM based acquisition for Library ID in whole blood samples

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Introduction

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.

The scope of the library considers two approaches; a MRM triggered full scan product ion spectra and MRM Spectrum mode. MRM Spectrum mode acquires a high number of fragment ion transitions for each target compound generating a fragmentation spectra which can be used in routine library searching and compound verification using reference library match scores. In this work, MRM Spectrum mode has been applied to analysis of patient samples to quantify and identify targets in whole blood samples extracted using a QuEChERS method.

Methods and Materials

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 a diverse chemical space to create a generic single method approach for clinical and forensic toxicology screening with a cycle time of 17minutes.

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Table 1. LC-MS/MS data acquisition conditions.

Liquid chromatography	
UHPLC	: Nexera LC system
Analytical column	: Restek Raptor Biphenyl 2.7um 100 x 2.1mm
Column temp.	: 50°C
Injection cycle	: 5 µL injection volume
Flow rate	: 0.3 mL/min
Solvent A	: Water + 2mM ammonium formate + 0.002% formic acid
Solvent B	: Methanol + 2mM ammonium formate + 0.002% formic acid
Binary Gradient	:

Time (mins)	%B
1.00	5
2.00	40
10.50	100
13.00	100
13.01	5
17.00	Stop
11-14.2	0.5 mL/min

Mass spectrometry	
LC-MS/MS	: LCMS-8060
Ionisation mode	: Heated ESI
Scan speed	: 15,000 u/sec
Polarity switching time	: 5 msec
MRM Dwell time	: 1 msec
Pause time	: 1 msec
Interface temp.	: 300°C
Heating block	: 400°C
Desolvation line	: 250°C
Heating gas	: 10 L/min
Drying gas	: 10 L/min
Nebulising gas	: 3 L/min
CID gas pressure	: 250kPa
Interface voltage	: 4 kV

Clinical and forensic library

The spectral library contains information on 1222 clinical and forensic toxicological compounds with libraries for both full scan product ion spectra and MRM product ion spectra data. 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.

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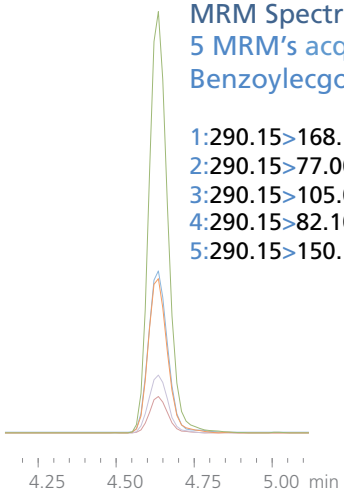
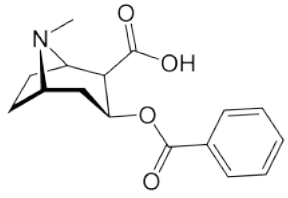
Results

MRM Spectrum mode | Quantitation

Conventional quantitative data acquisition by triple quadrupole LC-MS/MS typically uses 2 MRM per compound; MRM Spectrum mode acquires a higher number of precursor-fragment ion transitions to generate a library searchable product ion spectrum.

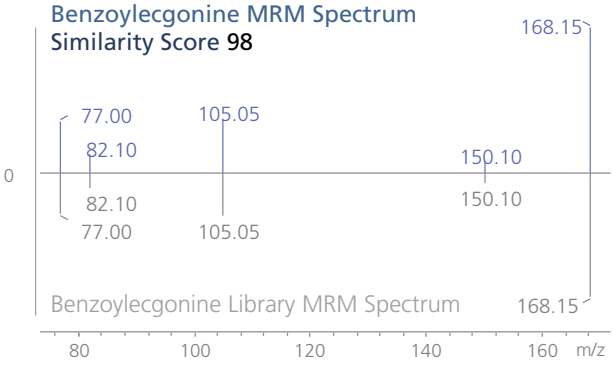


Compound name Benzoylecgonine
Mass 290.14 [M+H]+
Formula C16H19NO4
CAS 57-62-5



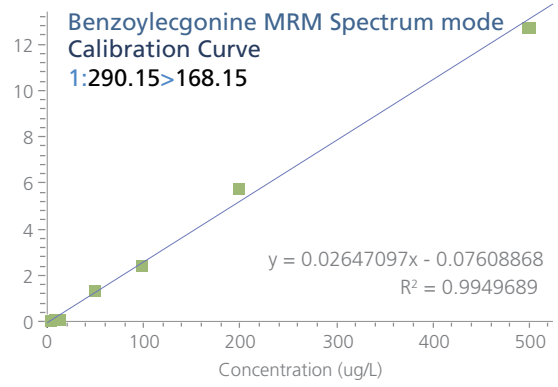
MRM Spectrum mode
Acquires a high number of precursor-fragment ions

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 = 0.02647097x - 0.07608868; R^2 = 0.9949689
2 MRM regression analysis
y = 0.02561410x - 0.002689508; R^2 = 0.9996442

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 2. 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 (min)	CHU-Limoges	MRM-Spectrum mode
		Patient sample	Patient sample
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

MRM Spectrum Mode Results | patient sample

As part of the evaluation, patient sample data was acquired using MRM Spectrum mode to quantify and identify targets.

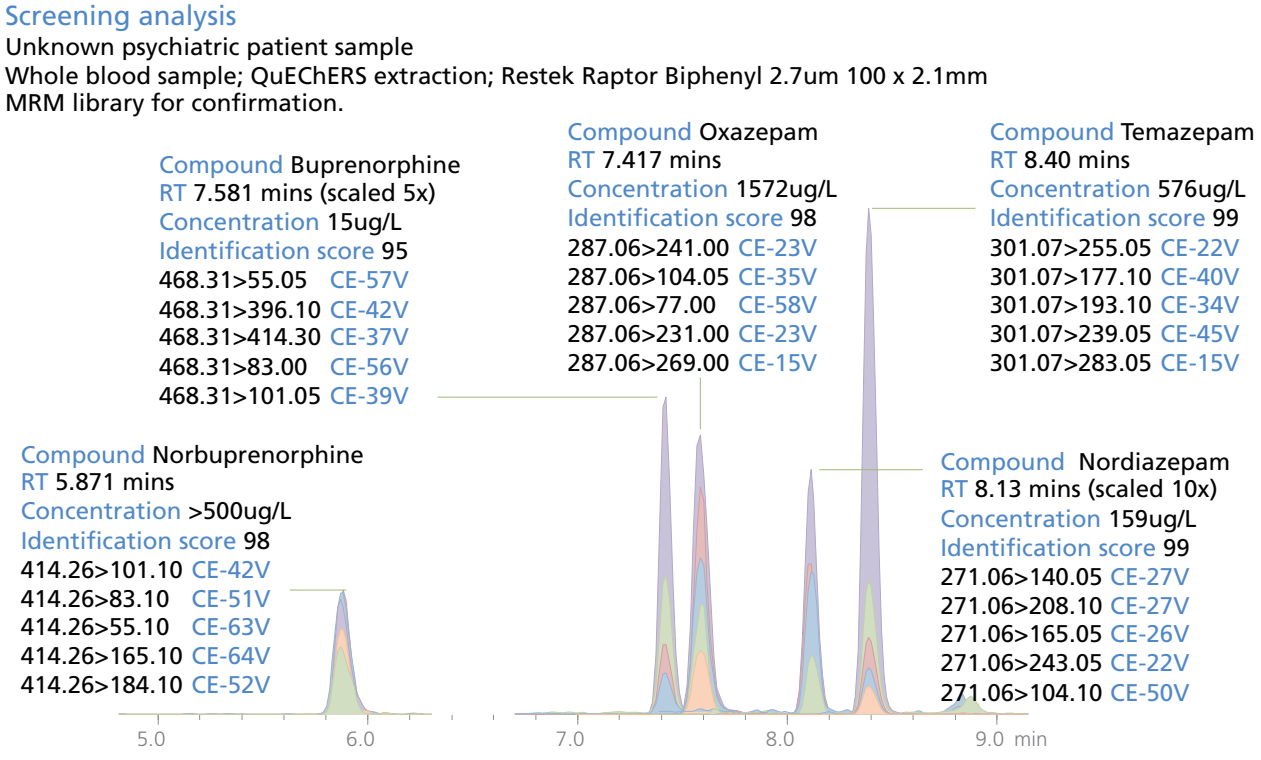

Figure 2. MRM chromatograms are shown for norbuprenorphine, buprenorphine, oxazepam, nordiazepam and temazepam from a psychiatric patient sample.

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Figure 3. 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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MRM Spectrum mode | CAO panel identification

Patient sample data was acquired using MRM Spectrum mode to quantify and identify targets in a CAO panel.

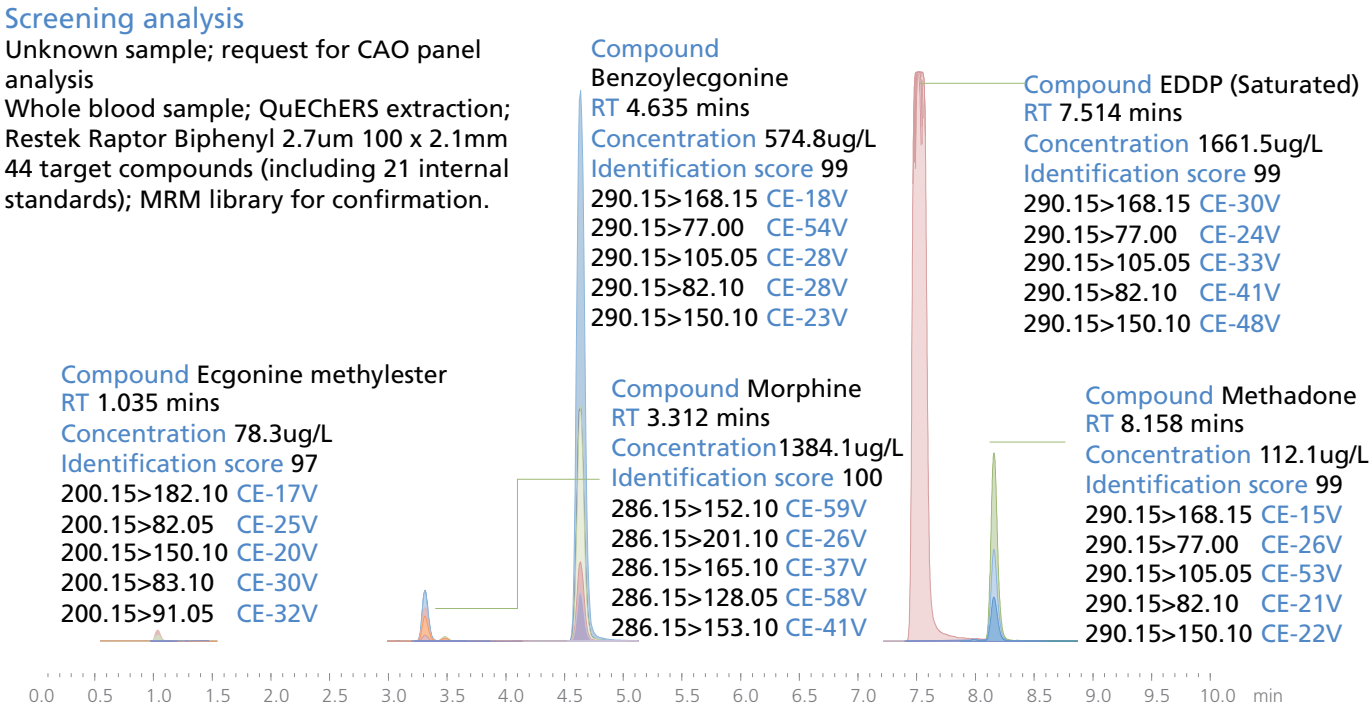

Figure 4. Patient sample analysis for CAO panel of drugs. MRM chromatograms are shown for ecgonine methylester, benzoylecgonine, morphine, EDDP and methadone.

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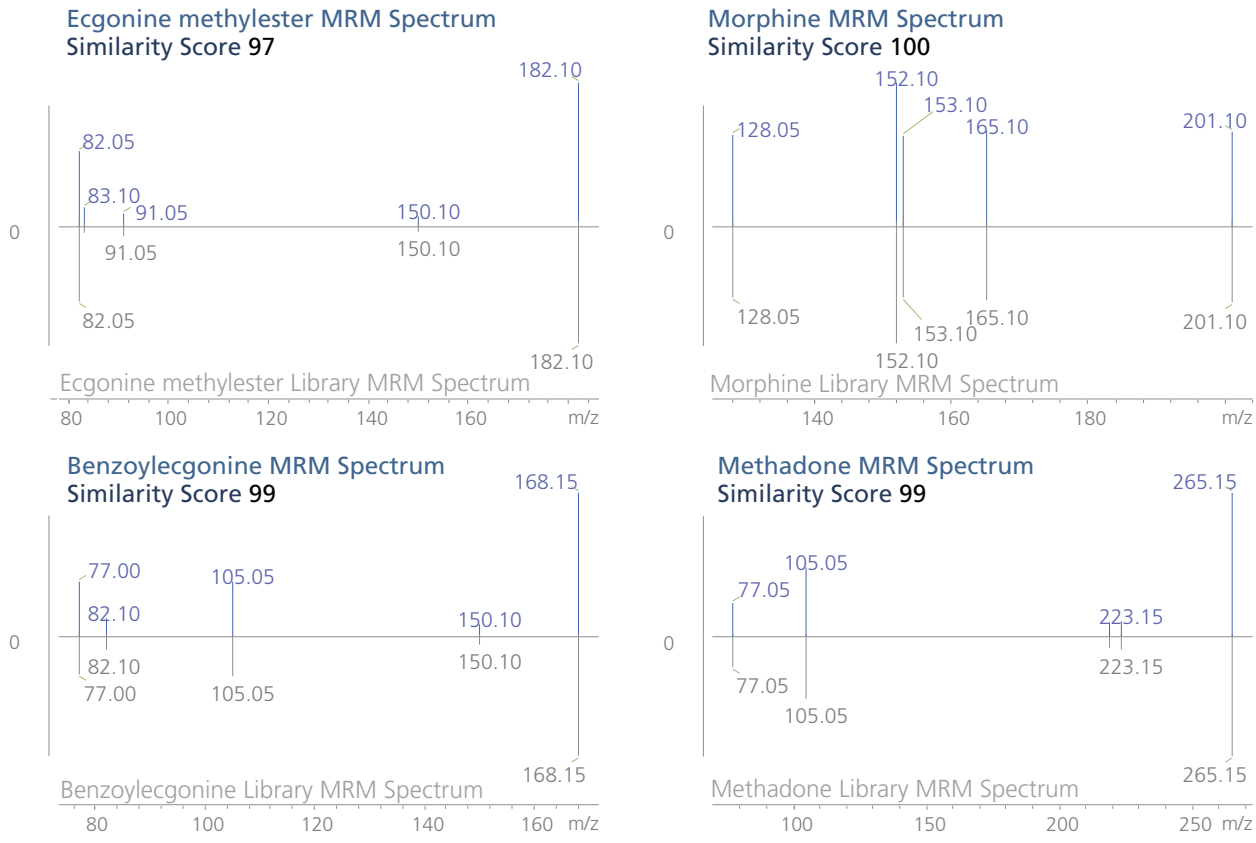

Figure 5. MRM product ion spectra and library similarity scores for ecgonine methylester, benzoylecgonine, morphine and methadone in a patient blood sample.

Conclusions

- 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.

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