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Quantitative clinical toxicological screening comparing Library ID from product ion scan MS/MS to MRM Spectrum mode ID

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Introduction

Forensic toxicological sample measurement is commonly performed in a targeted analysis on selected panels of compounds. When using triple quadrupole platforms for analysis, typically two MRMs are used for compound measurement with a quantifier ion transition and reference ion transition. To help reduce false positive and false negative reporting two alternative approaches have been considered; MRM triggered product ion spectrum and MRM Spectrum mode. MRM Spectrum mode acquires a high number of fragment ion transitions for each target compound generating a fragmentation spectra that could be used in routine library searching and compound verification using reference library match scores.

In this work, we compare different approaches in target quantitation and identification applied to clinical and forensic toxicology.

Methods and Materials

Whole blood was spiked with a panel of 35 benzodiazepines, or 44 CAO compounds (CAO = cocaine, antipsychotics, amphetamines, opiates), Calibration samples and unknown samples were prepared by QuEChERS method with the inclusion of stable isotope standards on preparation.

Chromatographic conditions were optimized for clinical and forensic toxicology screening and considered the need for rapid polarity switching and chromatographic resolution (Figure 2).

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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	: 2 msec
Pause time	: 3 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

Spectral Library >1200 compounds

Each library spectrum was acquired using certified reference materials. MRM triggered product ion spectra registered spectra for three collision energies corresponding to CE 10, 35 and 55V as well as a fourth merged CE spectrum totalling 6084 registered spectra. Optimised MRM transitions were determined for all compounds together with retention time.

In this work, MRM Spectrum mode acquired a library of typically 6 MRM's using certified reference materials acquired by LC. The library included not only MRM transitions for each target compound but also retention time (and relative retention time for each internal standard) and meta data including CAS number, formula, synonyms.

Quantitative clinical toxicological screening comparing Library ID from product ion scan MS/MS to MRM Spectrum mode ID

Results

MRM Spectrum mode

To reduce false negative and false positive reporting a higher number of MRM transitions were used for each target compound to increase the level of confidence in assay specificity. The number of fragment ion transitions monitored for each target compound was dependent upon the chemical structure with typically 6 fragment ions for each compound in this work. MRM Spectrum mode combines conventional MRM quantitation with the generation of a high quality MRM product ion spectrum which can be used in routine library searching and compound verification and identification. A key advantage of using this technique on a fast scanning triple quadrupole mass spectrometer is the capability of library identification without compromising quantitative capability and signal response.

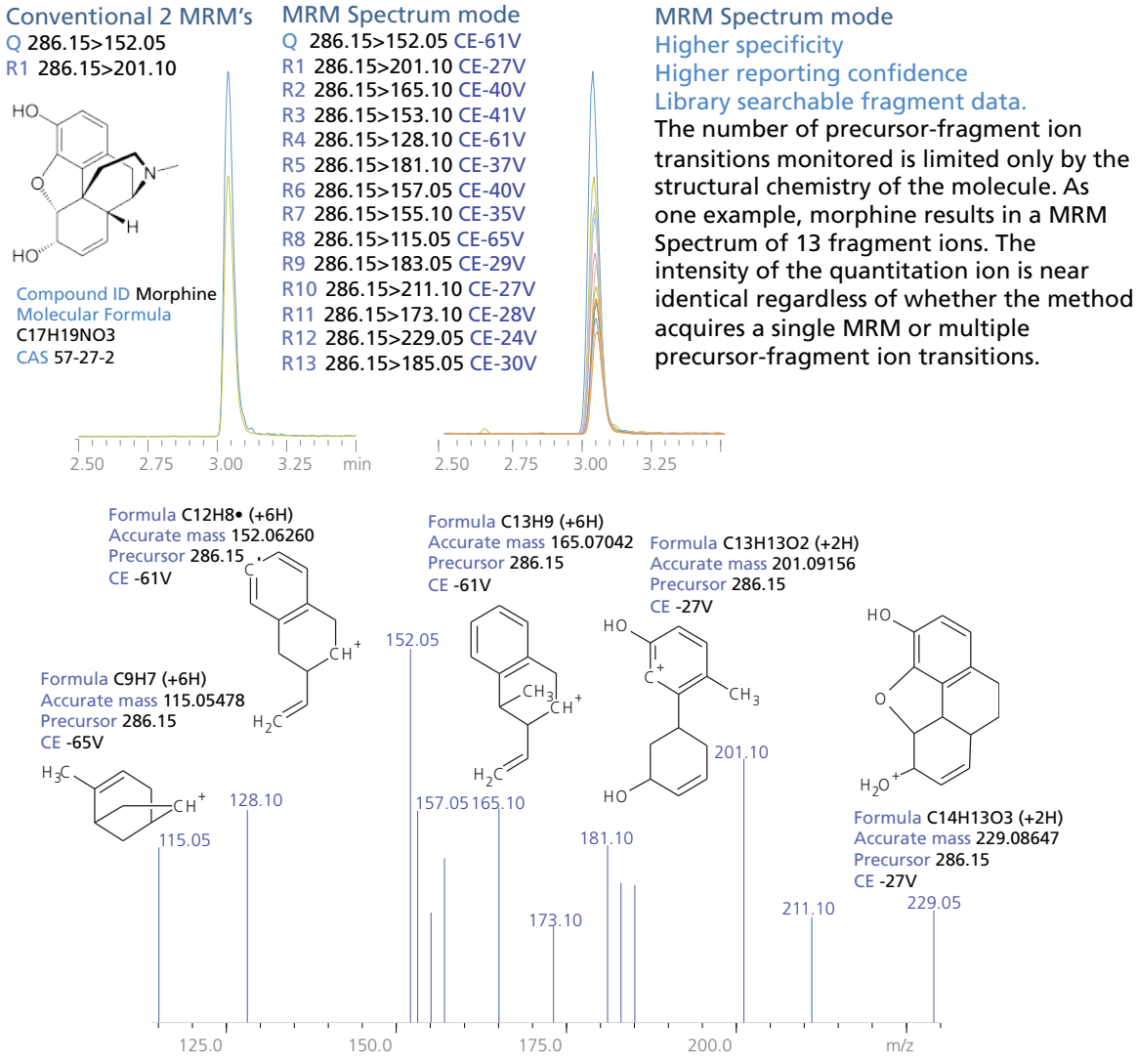

Figure 1. MRM reference spectrum for morphine with putative assigned fragment structures. MRM Spectrum mode combines MRM with the generation of a product ion spectrum. The product ion spectrum can be used for compound identification by searching a library. As the response to each precursor-fragment ion transition has been optimized for a specific collision energy the MRM Spectrum is highly specific and generates strong signal intensities for each fragment ion. (Each precursor-fragment ion transitions structure was assigned using an in house development tool (Structure Analytics) to show commonly described losses and charge migration; the hydrogen deficit is shown in brackets).

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Impact on quantitation

To minimize the possibility of false defect reporting without compromising the accuracy, precision and limits of detection, methods were developed to combine the sensitivity of MRM detection with the identification power of a MRM or full scan product ion spectrum. The methods have the capability of simultaneously using both precursor and product ion information enabling precise, accurate quantitation and library searchable compound identification. To assess the impact of methods designed to increase reporting confidence by library searching on quantitation both product ion spectrum methods were compared to a data generated using a conventional 2MRM method. For each target compound the quantifier ion remains the same but the methods differ in information content and data density.

To test the viability of this approach and to quantify and identify targets in the two test panels, the MRM triggered product ion spectrum acquisition method and MRM Spectrum mode were applied to a series of patient blood samples and compared against a validated LC-MS/MS method using 2 MRM's for each target compound. 44 CAO compounds and 37 benzodiazepines including internal standard compounds were acquired using three different MS/MS methods measured

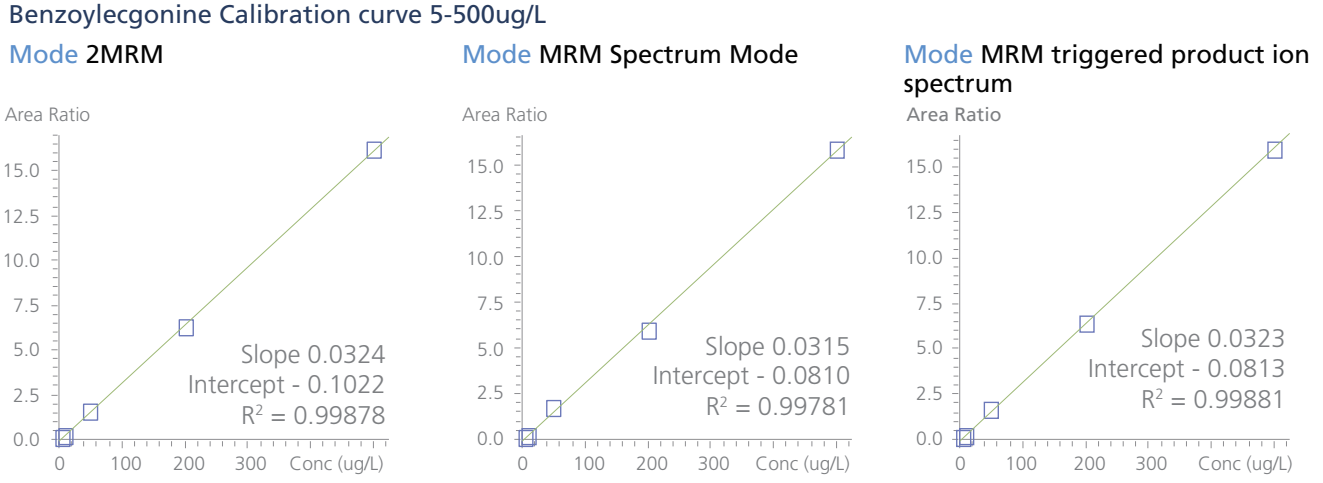

Figure 3. To assess the quantitative impact of both MRM Spectrum mode and a MRM triggered product ion spectrum data acquisition methods, calibration curves were generated over a concentration range of 5-500ug/L spiked into whole blood and extracted with QuEChERS. As one example, the signal response for benzoylecgonine quantifier ion is near identical regardless of the mode of acquisition. (All other compounds in the methods typically achieved R2>0.99, accuracy 85-115% and precision <10%RSD).

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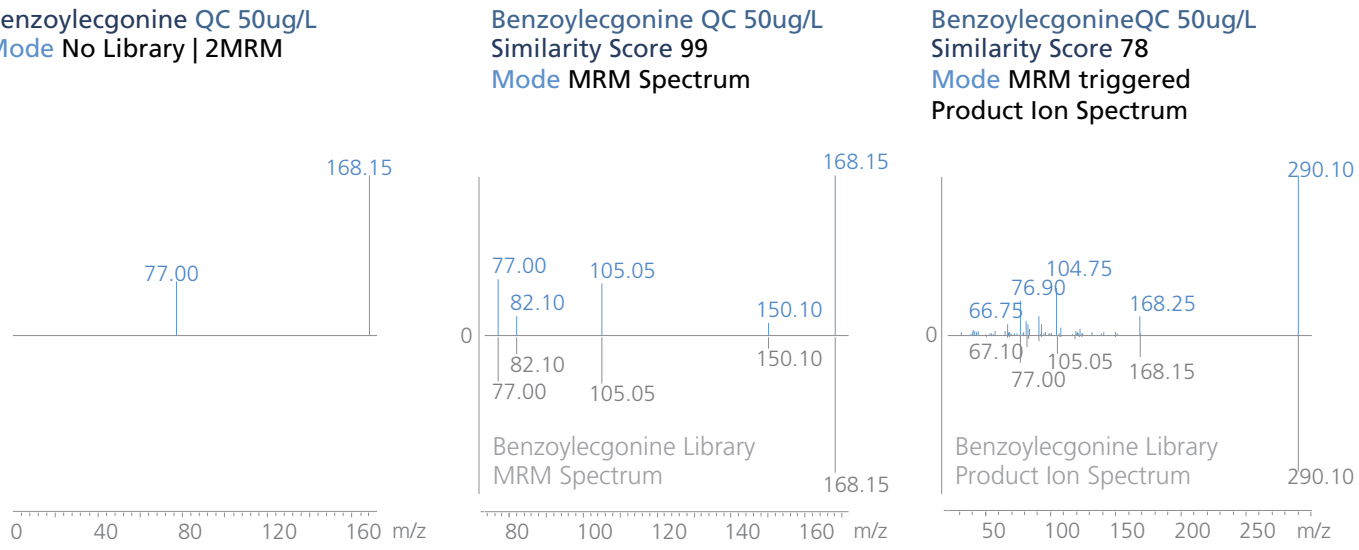

Figure 4. Compared to a conventional 2 MRM data analysis, MRM Spectrum and MRM triggered product ion spectrum data acquisitions deliver library searchable spectra for benzoylecgonine spiked into whole blood at a concentration of 50ug/L.

Product ion spectrum for increased confidence in compound identification

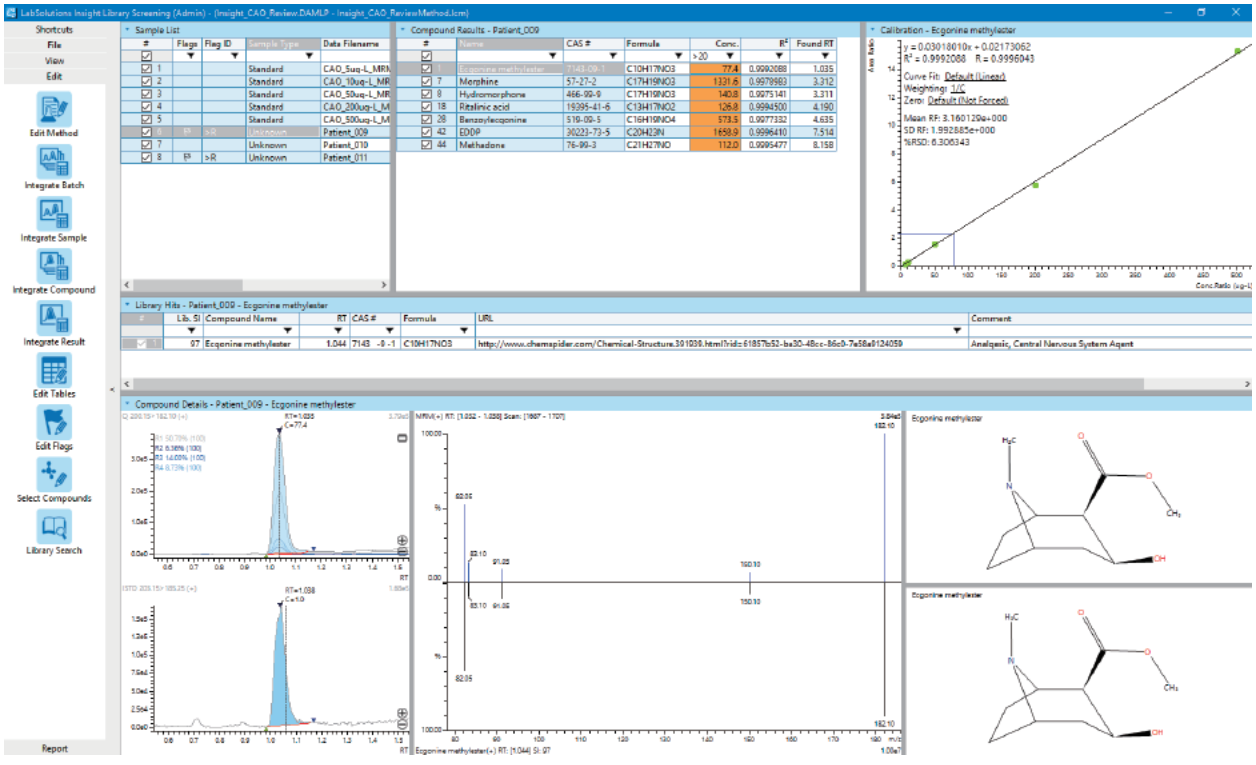

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Figure 5. Using LabSolutions Insight software to review data acquired with unknown patient samples, both MRM triggered product ion spectrum and MRM Spectrum mode deliver the same quantitative data quality compared to a validated conventional 2-3 MRM method.

Compound	Patient Sample – Routine CAO analysis (ug/L)				
	Conventional 2-3 MRM/target	MRM-Spectrum Mode	Library ID# SI	MRM-triggered product ion spectrum	Library ID# SI
	LCMS-8060	LCMS-8060		LCMS-8060	
Benzoylecgonine	>500	>500	99	>500	87
Ecgonine methylester	76.5	77.4	97	80.3	54
EDDP	>500	>500	97	>500	70
Hydromorphone	32.0	31.3	99	39.5	55
Methadone	110.8	112.0	99	111.8	82
Morphine	>500	>500	100	>500	89

Conclusions

- A generic method was developed for clinical toxicology and forensic analysis using a QuEChERS sample preparation method, a single LC analysis and methods for product ion spectrum identification. By combining MRM quantifier ions with either MRM or scanning product ion scan data both MS/MS method result in higher confidence in compound identification as a result of library searching with robust quantitative data. Library identification added increased confidence to compound identification in situations where reference ion ratios were outside method tolerances or if concentrations measured were below or above LLOQ or ULOQ.
- Both MRM triggered product ion spectrum mode and MRM Spectrum mode generate quantitative data in agreement to a validated conventional MRM method.
- MRM triggered product ion spectrum generates highly rich fragment spectra which has been successfully applied to toxicology.
- 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 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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