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Rethinking library identification in quantitative clinical toxicology – transitioning towards MRM Spectrum mode

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Introduction

The majority of clinical decisions are based on laboratory test results. For many laboratories, triple quadrupole MRM methods are used to deliver highly sensitive, selective and robust results for precise quantitation and identification verification. To help transition towards a more effective data review and higher confidence in reporting results we have been rethinking the capability of MRM in compound identification and verification. In this workflow, 6-10 fragment ion transitions were monitored for each target compound as opposed to a conventional approach using 2-3 fragment ions. By acquiring a high number of fragment ion transitions, each target compound had a corresponding fragmentation spectra which 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

Whole blood was spiked with a panel of CAO compounds (cocaine, antipsychotics, amphetamines, opiates). Calibration samples and unknown samples were prepared by QuEChERS method with the inclusion of stable isotope standards on preparation. In this work, MRM Spectrum mode acquired a library of typically 6 or more MRM's per compound using certified reference materials. 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. As a comparison, full scan library spectrum data was also acquired using a MRM triggered product ion spectra for three collision energies corresponding to CE 10, 35 and 55V as well as a fourth merged CE spectrum totalling 6084 registered spectra.

Rethinking library identification in quantitative clinical toxicology – transitioning towards MRM Spectrum mode

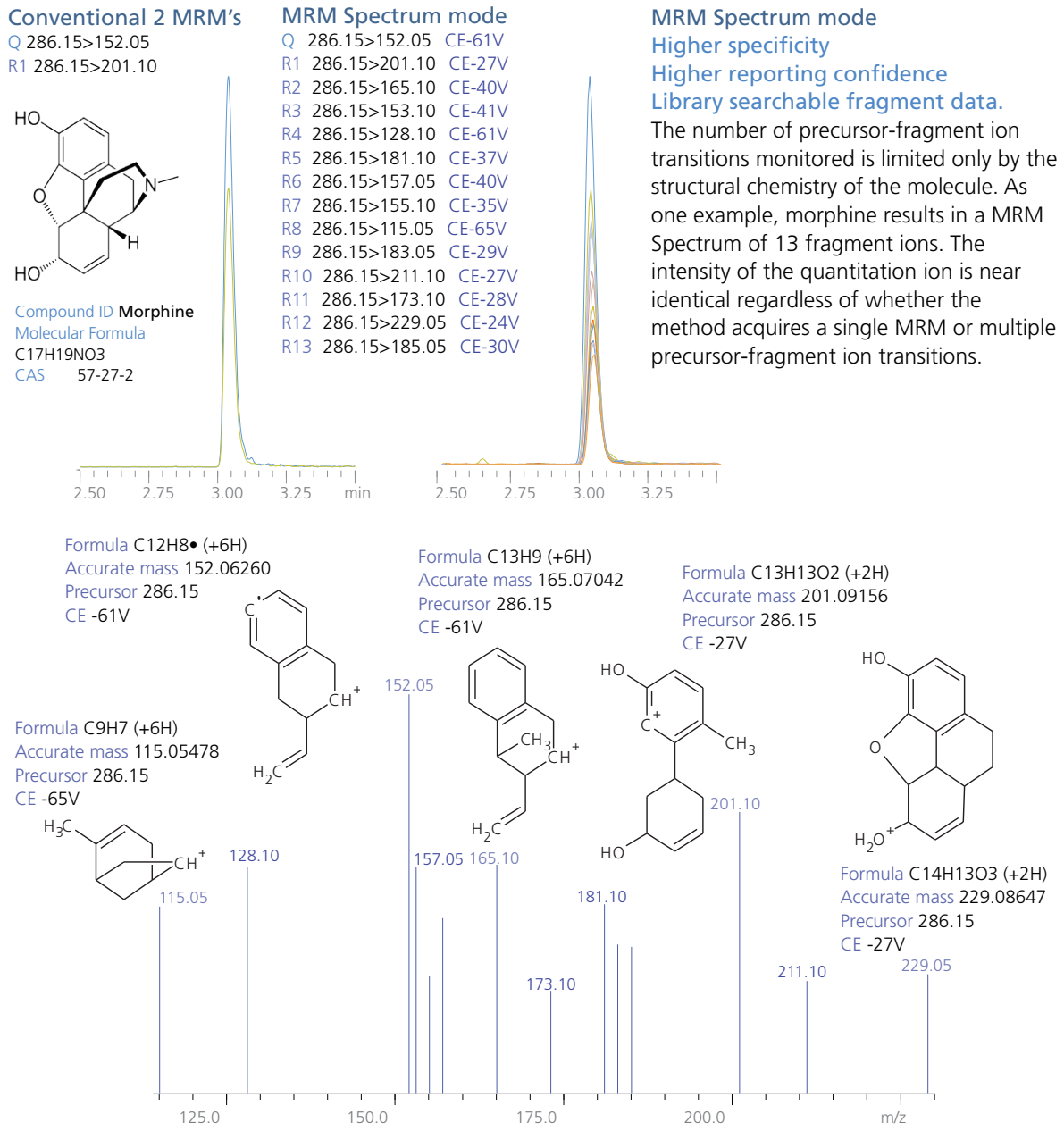

Figure 1. MRM reference spectrum for morphine with putative assigned fragment structures. MRM Spectrum mode combines MRM with the generation of a product ion spectrum. The product ion spectrum can be used for compound identification by searching a library. As the response to each precursor-fragment ion 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).

Rethinking library identification in quantitative clinical toxicology – transitioning towards MRM Spectrum mode

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

Results

To minimize the possibility of false 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 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, 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. CAO compounds including internal standard compounds were acquired using three different MS/MS methods. In patient test samples, the concentration of each target analyte was near identical using the different MS/MS methods with library identification.

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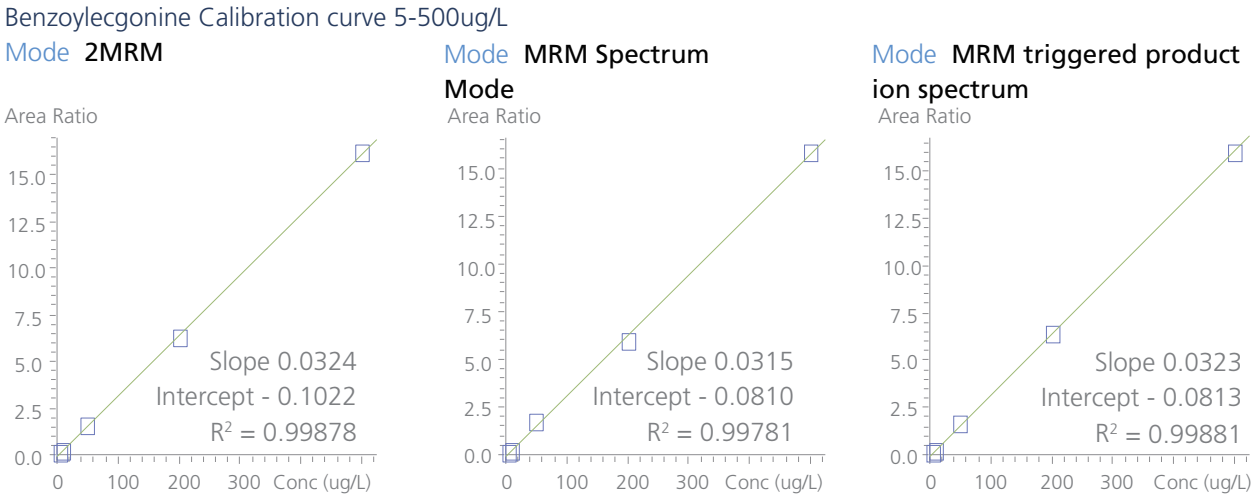

Figure 2. To assess the quantitative impact of both MRM Spectrum mode and a MRM triggered product ion spectrum data acquisition methods, calibration curves were generated over a concentration range of 5-500ug/L spiked into whole blood and extracted with QuEChERS. As one example, the signal response for 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).

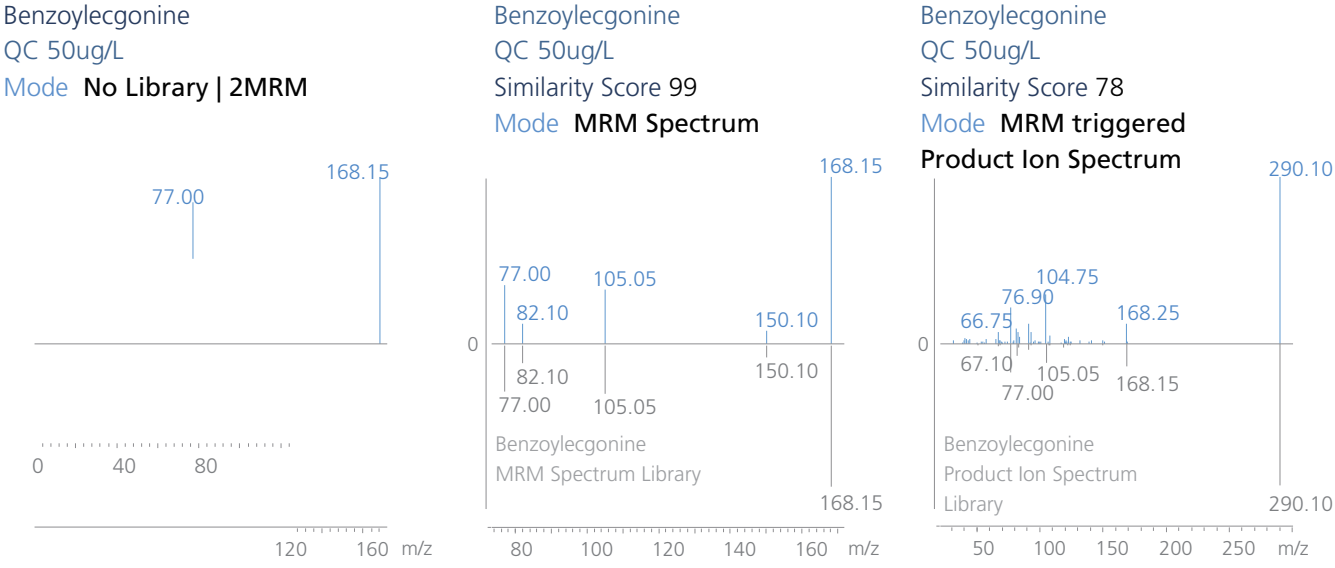

Figure 3. Compared to a conventional 2 MRM data analysis, MRM Spectrum and MRM triggered product ion spectrum data acquisitions deliver library searchable spectra for benzoylecgonine spiked into whole blood at a concentration of 50ug/L.

Rethinking library identification in quantitative clinical toxicology – transitioning towards MRM Spectrum mode

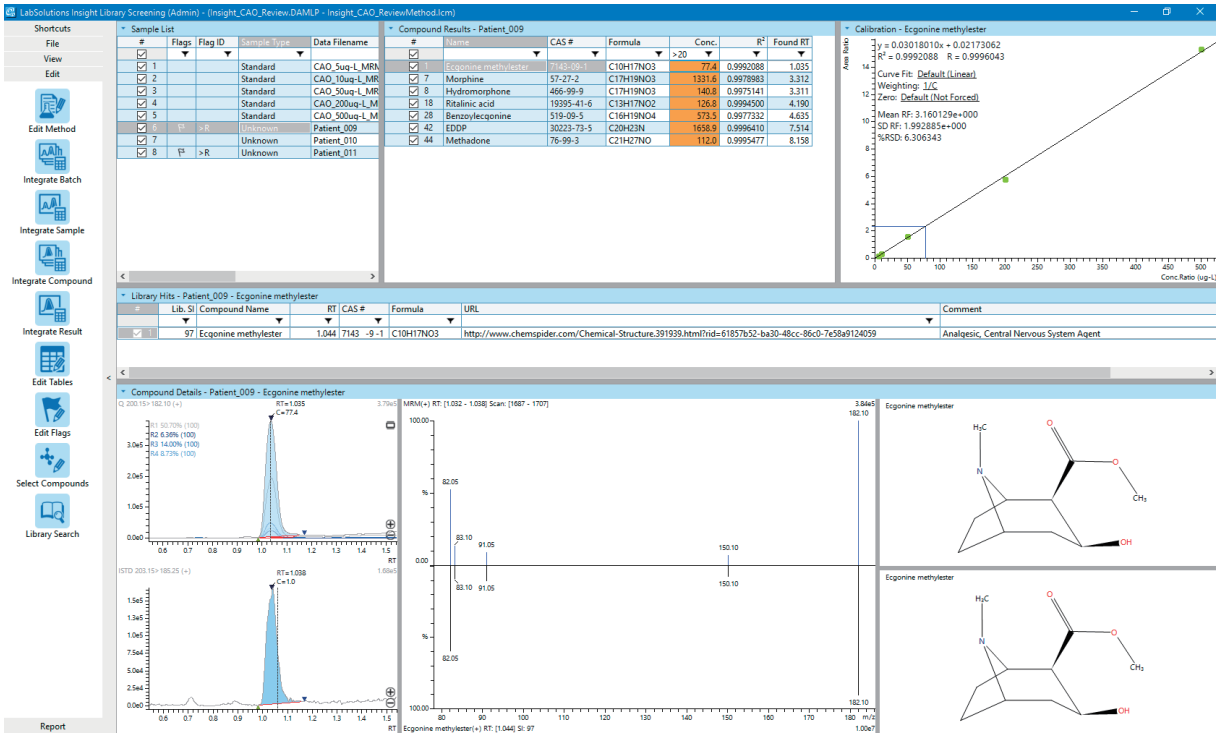

Figure 4. Using LabSolutions Insight software to review data acquired with unknown patient samples, both MRM triggered product ion spectrum and MRM Spectrum mode deliver the same quantitative data quality compared to a validated conventional 2-3 MRM method.

Conclusions

A generic method was developed for clinical toxicology and forensic analysis using a QuEChERS sample preparation method, a single LC analysis and MRM based methods for quantitation and library based identification.

A key advantage of MRM Spectrum mode was the identification power even at trace levels with high data sampling rate across a peak, consistent loop time and sampling rate without threshold triggering.

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