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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,

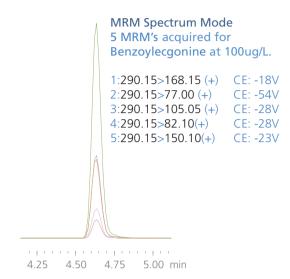
each precursor-fragment ion transitions has an optimized collision energy resulting in a specific product ion spectra and a high signal intensity. The database also includes structure (as a mol file), RT, CAS number, formula, synonyms, compound class/properties, ChemSpider URL and ID number, InChI and 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.

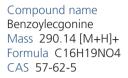
Methods

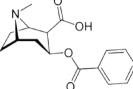
Whole blood was spiked with target compounds and extracted with a QuEChERS method protocol (where possible deuterated internal standards were also included). Chromatographic conditions considered the need to develop a single LC method for a diverse chemical space with a cycle time of 17minutes using 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.



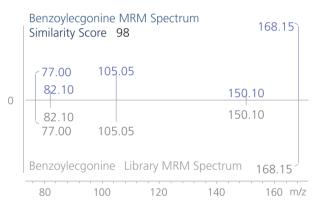






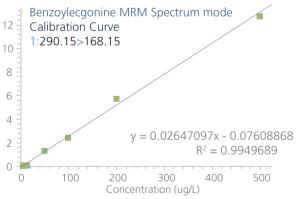
MRM Spectrum mode Acquires a high number of precursor-fragment ions

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).



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.



Screening analysis Unknown psychiatric patient sample Whole blood sample; QuEChERS extraction; Restek Raptor Biphenyl 2.7um 100 x 2.1mm MRM library for confirmation. Compound Oxazepam Compound Temazepam RT 7.417 mins RT 8.40 mins Concentration 576ua/L Concentration 1572ug/L Identification score 98 Identification score 99 287.06>241.00 CE-23V 301.07>255.05 CE-22V Compound Buprenorphine 287.06>104.05 CE-35V 301.07>177.10 CE-40V RT 7.581 mins (scaled 5x) 287 06>77 00 CE-58\/ 301 07>193 10 CF-34V Concentration 15ug/L 287.06>231.00 CE-23V 301.07>239.05 CE-45V Identification score 95 287.06>269.00 301.07>283.05 CE-15V CE-15V 468.31>55.05 CE-57V 468.31>396.10 CF-42V 468.31>414.30 CE-37V 468.31>83.00 CE-56V 468.31>101.05 CF-39V Compound Nordiazepam RT 8.13 mins (scaled 10x) Concentration 159ug/L Compound Norbuprenorphine Identification score 99 RT 5.871 mins 271.06>140.05 CE-27V Concentration >500ug/L 271.06>208.10 CE-27V Identification score 98 271.06>165.05 CE-26V 414.26>101.10 CE-42V 271.06>243.05 CE-22V 414.26>83.10 CE-51V 271.06>104.10 CF-50V 414.26>55.10 CE-63V 414.26>165.10 CE-64V 414.26>184.10 CE-52V 5.0 7.0 9.0 min Norbuprenorphine MRM Spectrum Buprenorphine MRM Spectrum Similarity Score 98 Similarity Score 95 83.10 1₀1.10 55.05 1_{65.10} 396 10 414 30 83.₀00 83.00 184 10 396.10414.30 101.05 165 10 83.10 101.10 Norbuprenorphine Library MRM Spectrum Buprenorphine Library MRM Spectrum 100 120 180 m/z 100 150 200 250 300 400 m/z Oxazepam MRM Spectrum Nordiazepam MRM Spectrum Similarity Score 98 Similarity Score 99 241.00 140.05 208.10 1,65.05 2,69.00 7,7.00 1,04.05 1,04.10 243.05 231.00 231.00 77.00 104.05 104.10 243.05 269.00 208.10 241.00 Oxazepam Library MRM Spectrum Nordiazepam Library MRM Spectrum 120 140 160 180 220 240 m/z Temazepam MRM Spectrum Similarity Score 99 2,55.05 Figure 2. Psychiatric patient sample analysis detected norbuprenorphine, 283.05 1_{77.101}93.10

2,39.05

239.05

240

255.05

283.05

280 m/z

177.10193.10

Temazepam Library MRM Spectrum

220

buprenorphine, oxazepam, nordiazepam and temazepam

used for compound identification by searching a library. As the collision energy was optimized for each fragment ion

highly specific and selective.

to generate a product ion spectrum, the library spectrum is

using MRM Spectrum mode. The product ion spectrum can be



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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