PO-CON1808E

SHIMADZU
Excellence in Science

MRM spectrum mode and Library Searching for Enhanced Reporting Confidence in Forensic Toxicology

ASMS 2018 WP 261

Eishi IMOTO[1], Takeshi ASHIDA[1]
1 Shimadzu Corporation, MS Business Unit, Kyoto, Japan

MRM spectrum mode and Library Searching for Enhanced Reporting Confidence in Forensic Toxicology

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 for precise quantification 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 to clinical and forensic toxicology.

Methods and Pretreatment

Whole blood was spiked with 33 drugs (Hypnotics). Calibration samples were prepared by modified QuEChERS method. In this study, MRM spectrum mode acquired a library of typically 7 or more MRM's per compound using certified reference materials. The library included retention time, CAS number, formula and MRM transitions for each target compound. As a comparison, MRM triggered product ion scan for three collision energies corresponding to CE: 15, 30, 45V as well as a fourth merged CE spectrum was performed for qualitative.

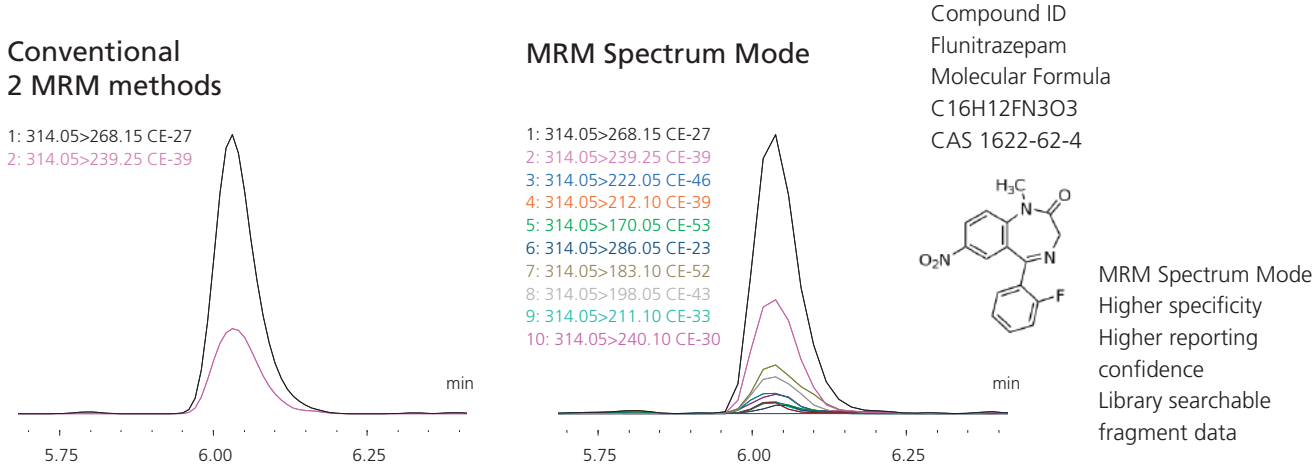


Fig.1 Comparison of conventional MRM and MRM spectrum mode with flunitrazepam

MRM spectrum mode and Library Searching for Enhanced Reporting Confidence in Forensic Toxicology

Table 1. LC and MS conditions

[LC] Nexera™ X2 System

Analytical Column	: Phenomenex Kinetex® XB-C18 (2.1 mmI.D.×100 mmL., 2.6 µm)
Guard Column	: Phenomenex Security Guard Ultra C18 2.1 mmID
Solvent A	: Water + 10 mmol/L ammonium formate + 0.1% formic acid
Solvent B	: Methanol + 10 mmol/L ammonium formate + 0.1% formic acid
Gradient Program	:

Time (min)	%B
0	5
7.5	95
10	95
10.01	5
15	STOP

Flow Rate	: 0.3 mL/min
Column Temp	: 40 °C

[MS] LCMS-8060

Ionization	: ESI (Positive)
Nebulizer Gas	: 3 L/min
Interface temperature	: 300 °C
Desolvation Line	: 250 °C
Heat Block temperature	: 400 °C
Heating Gas	: 15 L/min
Drying Gas	: 10 L/min

MRM spectrum mode and Library Searching for Enhanced Reporting Confidence in Forensic Toxicology

Results

Quantitation

Conventional qualitative data acquisition by triple quadrupole LC-MS/MS which typically uses 2 MRM per compound. MRM product mode acquires a higher number of precursor-fragment ion transitions to generate a library searchable product ion spectrum. For quantitative analysis, these compounds and QuEChERS extraction were diluted at 0.05, 0.1, 0.5, 1.0 ng/mL for calibration points. For accurate quantitative, internal standard was required. Low similarity score was obtained for some compounds, because some drugs have almost same mass which can't separate by nominal mass. Good LC separation was required for the good similarity score.

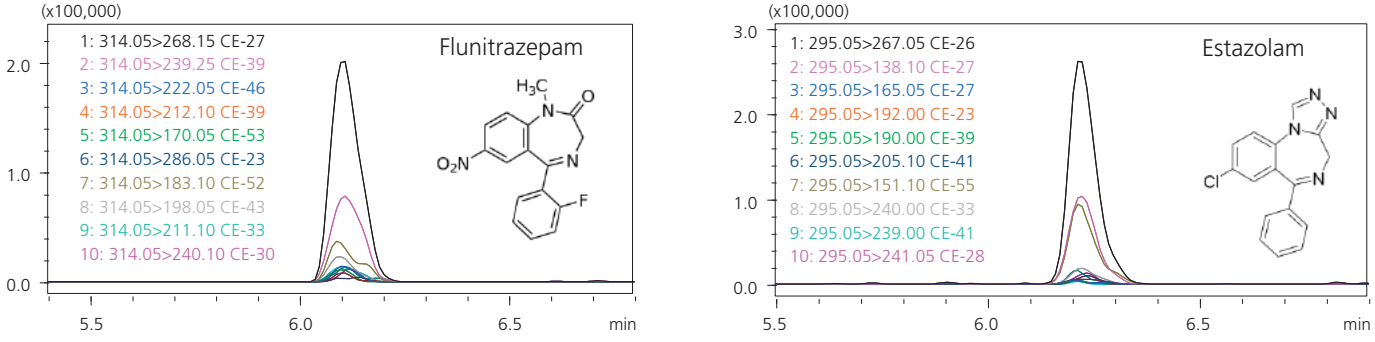


Fig.2 MS chromatogram of flunitrazepam and estazolam post-spiked in whole blood at 1 ng/mL

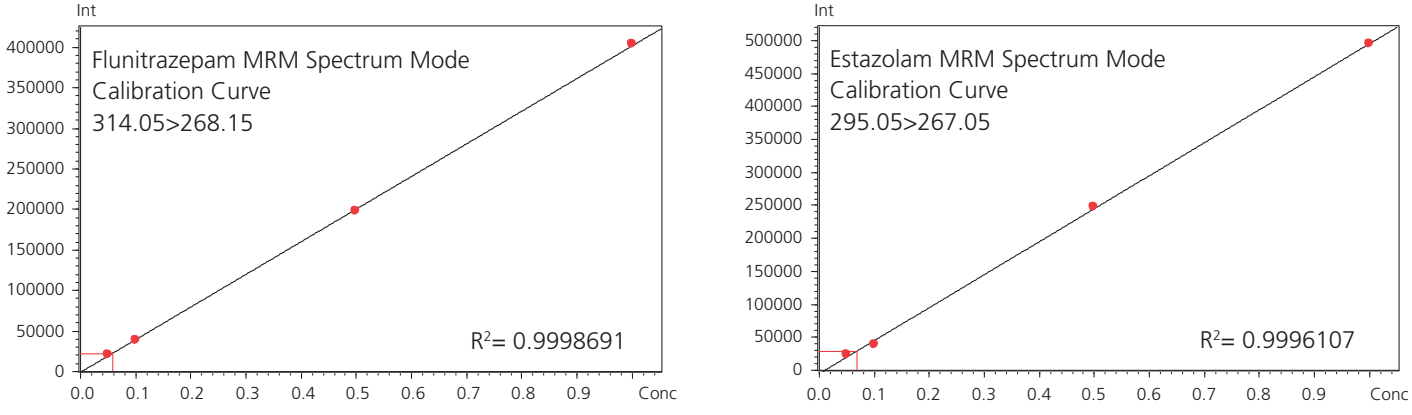


Fig.3 Calibration curve with MRM spectrum mode post-spiked sample (0.05, 0.1, 0.5, 1.0 ng/mL).

MRM spectrum mode and Library Searching for Enhanced Reporting Confidence in Forensic Toxicology

Table 2 Similarity score, retention time, recovery rate, accuracy of typical compounds pre-spiked (2.0 ng/mL) and R^2 for post-spiked calibration curve with MRM spectrum mode.

Compound	Similarity Score	RT (min)	Accuracy (%)	R^2
7-aminoflunitrazepam	97	4.67	107	0.9960
7-aminoclonazepam	88	4.25	106	0.9969
Estazolam	93	6.18	85	0.9996
Zolpidem	96	4.68	78	0.9998
Flunitrazepam	98	6.16	77	0.9998
Bromazepam	65	5.78	115	0.9960
Clotiazepam	93	7.14	79	0.9913
Midazolam	98	5.82	111	0.9950
4-hydroxymidazolam	87	5.51	92	0.9992
Triazolam	76	6.32	87	0.9862
Ethyl loflazepate	77	6.95	89	0.9931
Flurazepam	100	5.46	89	0.9998
Nordiazepam	90	6.71	82	0.9833

Library Identification

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 quantification and library searchable compound identification. To assess the impact of methods designed to increase reporting confidence by library searching on quantification both product ion spectrum methods were compared to a data generated using conventional 2 MRM methods; MRM triggered product ion spectrum and MRM spectrum mode.

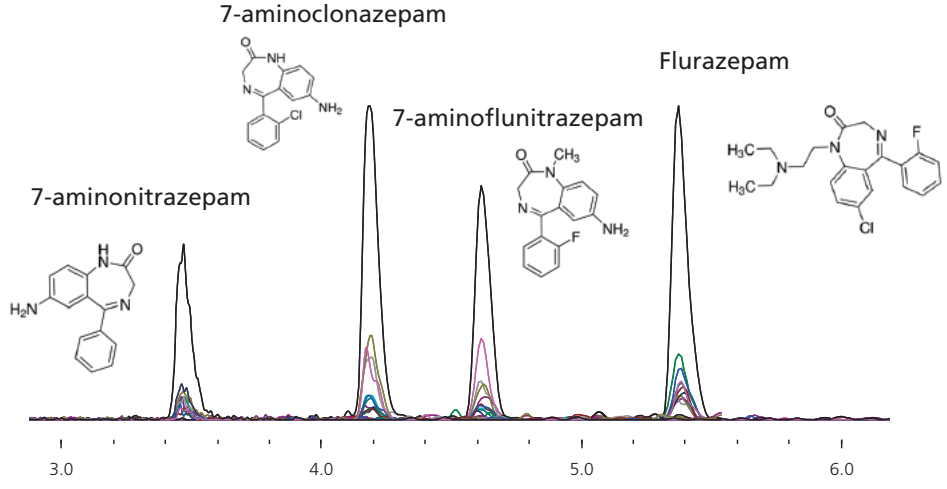


Fig.4 MS chromatogram of 7-aminonitrazepam, 7-aminoclonazepam, 7-aminoflunitrazepam, and flurazepam.

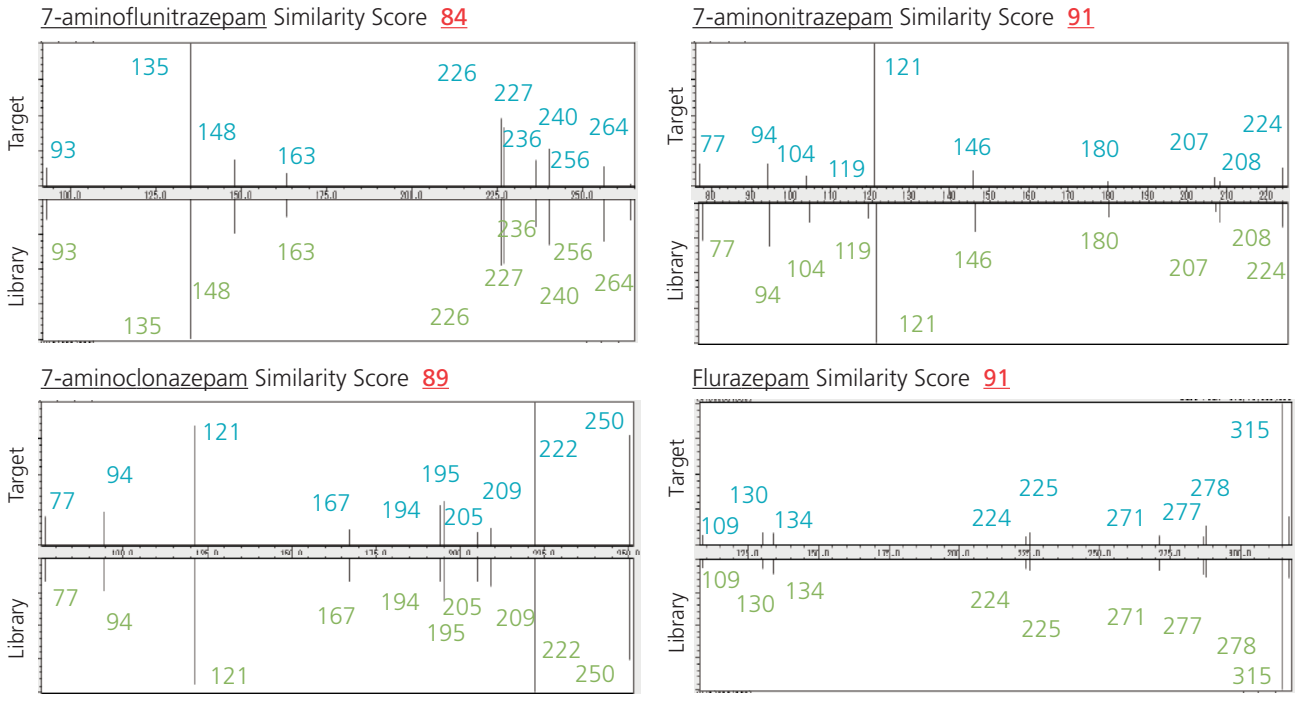
MRM spectrum mode and Library Searching for Enhanced Reporting Confidence in Forensic Toxicology

Table 3 Acquisition parameters of MRM Spectrum Mode and MRM Triggered Product Ion Scan (Threshold was an intensity of 10,000 counts).

Compound	7-amino nitrazepam	7-amino clonazepam	7-amino flunitrazepam	Flurazepam
MS/MS acquisition mode			MRM Spectrum Mode	
Precursor	252.1	286.1	284.1	388.2
Product 1	121.1	222.1	135.1	315.0
Product 2	208.1	194.1	148.1	225.0
Product 3	119.1	209.1	236.2	288.0
Product 4	207.5	205.1	264.1	134.1
Product 5	180.1	167.1	163.2	317.0
Product 6	94.1	121.1	227.1	287.0
Product 7	77.1	250.1	226.1	271.0
Product 8	224.1	94.1	93.1	109.0
Product 9	146.1	77.1	240.1	130.1
Product 10	104.1	195.1	256.1	224.0
MS/MS acquisition mode			MRM Triggered Product Ion Scan	
Precursor	252.1	286.1	284.1	388.2
Product 1	121.1	222.1	135.1	315.0
Product 2	94.1	121.2	226.2	317.0
CE: 15	20 : 262.1	20 : 296.1	20 : 294.1	20 : 398.2
CE: 30	20 : 262.1	20 : 296.1	20 : 294.1	20 : 398.2
CE: 45	20 : 262.1	20 : 296.1	20 : 294.1	20 : 398.2

MRM spectrum mode and Library Searching for Enhanced Reporting Confidence in Forensic Toxicology

MRM Spectrum Mode



MRM Triggered Product Ion Spectrum (Merged Spectrum CE: 15/30/45)

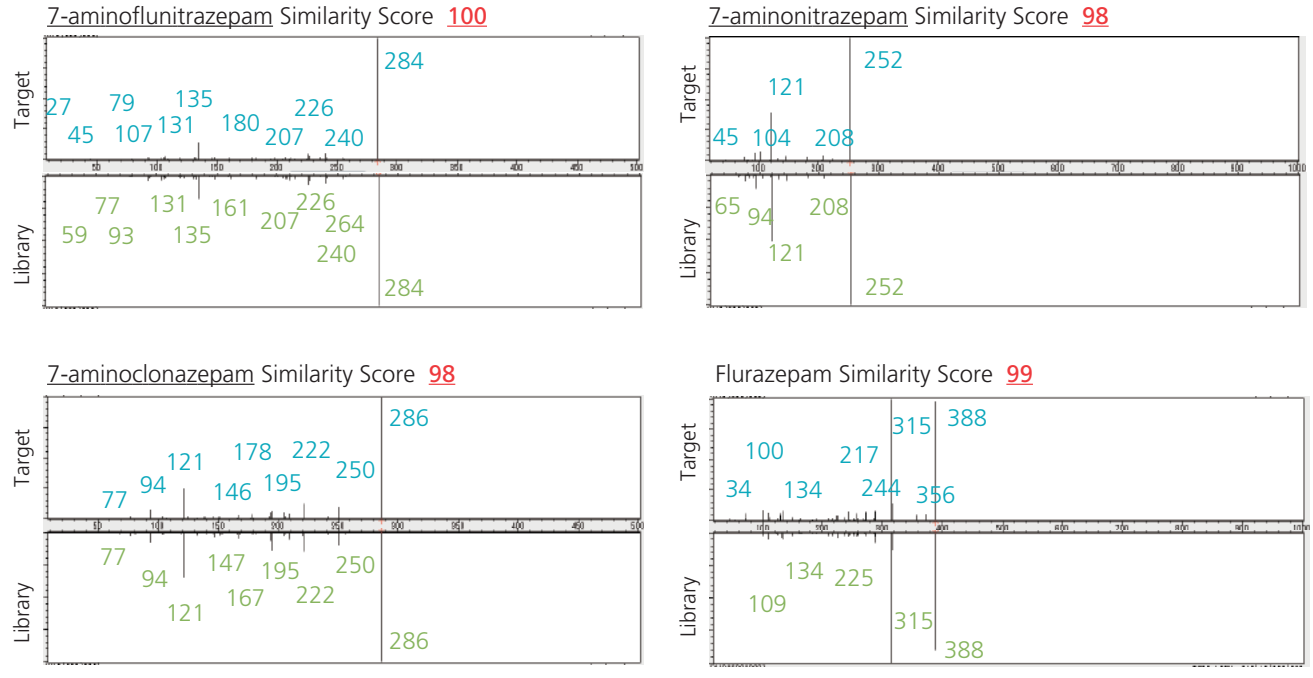


Fig.5 Compared to a conventional MRM triggered product ion scan and MRM spectrum mode, MRM spectrum mode has almost same qualitative ability for these compounds.

MRM spectrum mode and Library Searching for Enhanced Reporting Confidence in Forensic Toxicology

Conclusion

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 quantification and peak integration without threshold triggering and creates new opportunities in toxicological screening.

The product and application are Research Use Only. Not for use in human clinical diagnostics or in vitro diagnostic procedures.

First Edition: June, 2018

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/