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Applikationsbericht

Advances in Data Independent Acquisition Screening for Small Molecules: Using High Resolving Power Multi-Reflecting Time-of-Flight Mass Spectrometry

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Dies ist ein Applikationsbericht, der keinen detaillierten Abschnitt zu Versuchen enthält.

Abstract

Broadband unbiased data acquisition, such as MS^E is a necessity to produce complete sample characterization, facilitating identification of knowns and unknowns. A data independent acquisition (DIA) screening assay for small molecules analysis has been performed. Herein, we demonstrate a further enhancement of DIA specificity, through use of the SELECT SERIES™ MRT, a hybrid quadrupole multi-reflecting time-of-flight mass spectrometer. It provides a unique combination of high resolving power (>200,000 FWHM), and routine part-per-billion (ppb) mass accuracy, independent of acquisition speed.

MS^E precursor and fragment ion ppb accurate mass measurements are acquired routinely. The attained mass accuracy enables more stringent post detection data processing tolerances to be applied, which reduces false detection rates, and simultaneously enhances confidence in small molecule analyte identification. Data were compared with the Waters™ Forensic Toxicology library, based on reference retention time (tr), precursor ion and

fragment ion accurate mass data for over 1900 analytes. Data processing tolerances of t_r (+/-0.35 min) and mass accuracy (+/-2 ppm), diagnostic fragment ion count ≥1 and expected fragment ion tolerance of (+/-0.2 mDa) were applied.

Benefits

Improved analysis efficiency through enhanced identification confidence and reduced false detection rates for the identification of small molecules in non-targeted screening research applications.

- Enhancement of DIA MS^E performance with ppb mass accuracy for small molecule precursor and fragment ions
- Application of more stringent data processing parameters, 2 ppm precursor ion and 0.2 mDa fragment ion tolerance
- Transformative mass measurement resulting from greater mass resolving power affords the opportunity to improve informatics output and meet the analytical challenge of identifying knowns and unknowns where the drug landscape is constantly evolving

Introduction

Forensic toxicology laboratories are frequently required to perform broad screening techniques on complex biological samples to identify drugs of abuse, prescribed agents, and other toxicants. The constant emergence of new psychoactive substances poses a significant analytical challenge. High resolution mass spectrometry ***e.g.,*** Time-of-flight (TOF) analysis, is increasingly used for toxicological screening. Broadband data independent acquisition (DIA) has been previously applied for non-targeted screening of forensic samples. High-resolution mass spectrometry technology (HRMS (20,000 FWHM))has previously been used for the acquisition of unrestricted and unbiased datasets, thus providing a complete profile of the samples, which include precursor and fragment ion information that can be used for non-targeted and targeted workflows.[1–3] DIA was performed using MS^E mode, which is a full scan acquisition method, alternating between a low and high energy to provide information for the intact precursor and fragment ions respectively. The nature of this technique allows for retrospective examination of the data. Comparison with large libraries comprising elemental formulae, reference $t_{r,}$ and high energy fragment ion information, are essential to provide specificity and selectivity in identification,

improving efficiency and reducing false detection rates.

In this study we use the SELECT SERIES MRT (Figure 1) a state-of-the-art hybrid quadrupole Multi Reflecting Time-of-Flight mass spectrometer for the analysis of anonymised authentic human urine samples. Here we demonstrate further enhancements of DIA specificity, through use of a high mass resolving power (>200,000 FWHM).

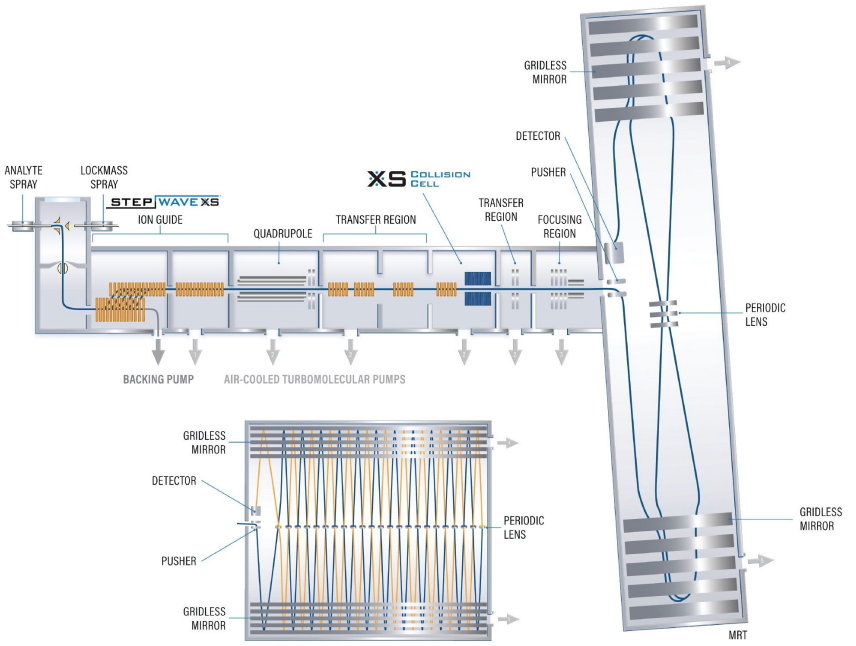

Figure 1. SELECT SERIES MRT instrument schematic.

Experimental

Sample Description

Forensic Toxicology QC System Suitability Test Mix (SST, 10-component mix p/n: 186007361 < https://www.waters.com/nextgen/global/shop/standards--reagents/186007361-forensic-tox-installation-standards-kit.html>)

Authentic human urine samples diluted 1:10 (water)

Library: Forensic Toxicology ES^+ (1975 entries)

LC Conditions

LC system:	ACQUITY UPLC™ I-Class Premier Chromatograph
Column:	ACQUITY UPLC HSS C_{18} (150 mm x 2.1 mm, 1.8 µm) Column
Column temperature:	50 °C
Sample temperature:	6 °C
Injection volume:	5 µL
Flow rate:	0.4 mL/min
Mobile phase A:	5 mM aqueous ammonium formate buffer adjusted to pH 3 with formic acid
Mobile Phase B:	0.1% v/v formic acid in acetonitrile

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
0.0	0.4	87	13	initial
0.5	0.4	87	13	6
10.0	0.4	50	50	6
10.75	0.4	5	95	6
12.25	0.4	5	95	6
12.5	0.4	87	13	6
15.0	0.4	87	13	6

MS Conditions

Acquisition:	ES^+
Capillary voltage:	0.8 kV
Desolvation temperature:	500 °C
Source temperature:	120 °C
Cone voltage:	20V
Collision energy ramp:	15–40 eV
Mass range:	*m/z* 50–2400
MS^E acquisition rate:	10 Hz
Data analysis and visualization:	MassLynx™ v4.2 SCN1026 and waters_connect 3.1.0.243, Tibco Spotfire® 6.0.0 Software (Palo Alto, CA)

Results and Discussion

Data analysis for a series of anonymized authentic human urine samples was performed. Data were compared with the Waters Forensic Toxicology library, based on tr, precursor ion, and fragment ion accurate mass data for 1975 toxicologically relevant analytes, including illicit drugs, pesticides, prescription drugs, and over the counter (OTC) medications.

Prior to screening the authentic human urine samples, the system performance was assessed using an SST mix. Data were processed using a retention tolerance (t_r) of ±0.35 min, precursor ion mass accuracy tolerance of ±2 ppm, and the presence of at least 1 diagnostic fragment ion with a mass tolerance of 0.2 mDa. For the SST mix (250 pg/µL) mass error (RMS) of 522 ppb was obtained and for the dilution series (2.5pg/µL to 500 pg/µl), the mass accuracy RMS errors for the SST mix constituents are shown in Figure 2.

All SST mix analytes were identified, when compared with the library, confirming the stringent mass accuracy data processing parameters could be adopted, providing greater specificity for precursor and fragment ions identification, in turn helping to mitigate false detections.

For complex analyses, the mass resolution that can be achieved using MRT allows matrix interferences and analytes of interest to be distinguished and typically results in ppb mass accuracy. An example of routine ppb mass accuracy performance is shown for the clozapine MS^E fragment ion spectrum (see Figure 3), mass resolution 130000 FWHM is illustrated for the *m/z* 84.08078 fragment.

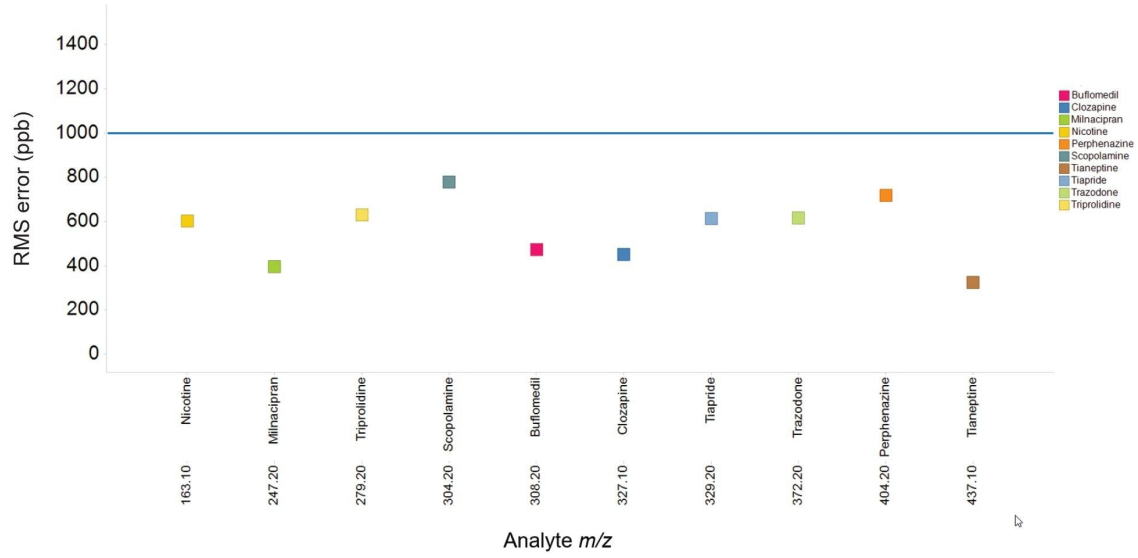

Figure 2. LC-MRT-MS^E ES$^+$ precursor ion mass accuracy ppb (RMS) for SST mix.

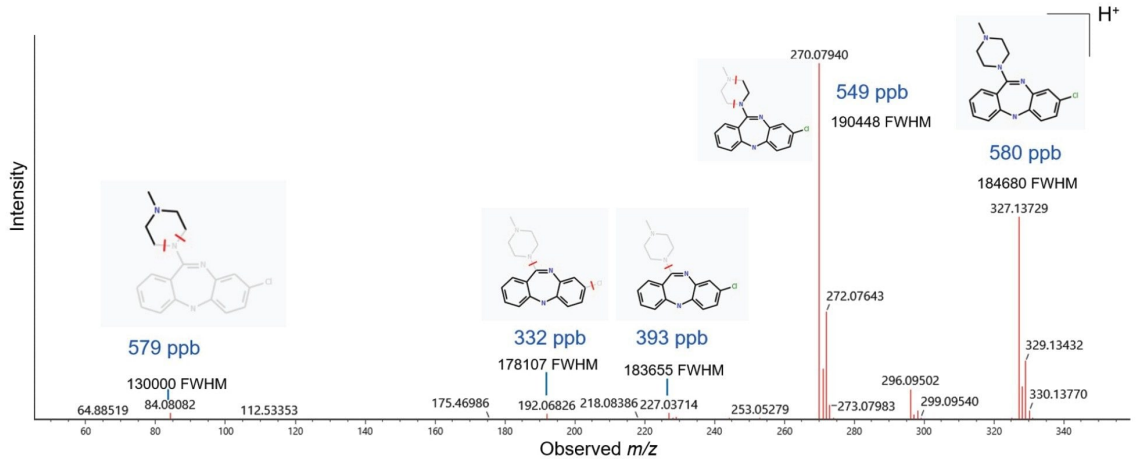

Figure 3. LC-MRT-MS^E ES$^+$ fragment ion spectrum obtained for SST mix constituent clozapine ([M+H]$^+$ m/z 327. 13710).

The same stringent tolerance criteria were also applied when processing the authentic urine samples and were compared with the toxicology library. Identifications made, included illicit drugs and prescribed medications,

together with their metabolites, as well as dietary and endogenous constituents. As an example, in sample “103”, oxycodone has been excluded as a false detection. Methamphetamine has been identified with a mass measurement error of 87 ppb and methadone, -83 ppb (see Figure 4). Drug metabolites have also been identified, as well as compounds resulting from dietary consequence, an overall mass measurement error of 511 ppb (RMS) has been obtained for these compounds, providing confidence that true identifications were made.

In combination with software tools, routine ppb mass accuracy can provide enhanced confidence and a time efficient strategy to determine positive identifications, resulting from recreational drug use. In the case of sample “51”, repeat analyses have confirmed the identification of polydrug use, including illicit, prescription, and OTC drugs. The combination of small molecule drugs identified, is described in Figure 5. The variety of identified drug classes emphasizes the analytical challenge and illustrates why unbiased DIA is required. A total of 14 “drug” compounds, seven drug metabolites, and endogenous urine matrix species were identified. In addition to nicotine, caffeine, and corresponding metabolites.

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Component Summary — RMS Error= 511 ppb

	Component name	Label	Identification status	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	Expected RT (min)	Expected Fragments Found	Expected Fragments Count	Response	Adducts
1	Caffeine		Identified	195.0877	0.074	0.382	2.23	2.08	1	3	56509	+H
2	Chloroquine		Identified	320.1889	0.111	0.346	2.03	1.85	3	3	103397	+H
3	Codeine		Identified	300.1598	0.385	1.284	1.87	1.70	2	2	52950	+H, +K, +Na
4	Codeine, Nor		Identified	286.1441	0.289	1.012	1.73	1.60	1	2	4882	+H
5	Cotinine		Identified	177.1023	0.043	0.240	1.12	1.07	4	4	12723	+H
6	EDDP		Identified	278.1904	0.058	0.207	7.55	7.29	3	3	128601	+H
7	Methadone		Identified	310.2165	-0.026	-0.083	8.69	8.44	4	4	96017	+H
8	Methamphetamine		Identified	150.1277	0.013	0.087	2.70	2.47	2	2	10663	+H
9	Methamphetamine m/z 119.0855		Identified	119.0856	0.032	0.270	2.70	2.47	1	1	1311	+H
10	Morphine		Identified	286.1439	0.104	0.363	1.12	1.10	3	4	16227	+H, +Na
11	Morphine, nor		Identified	272.1282	0.111	0.407	1.11	1.01	4	5	1301	+H
12	Morphine-6-glucuronide		Identified	462.1759	0.054	0.116	0.95	1.00	1	1	127697	+H, +K, +Na
13	Nicotine		Identified	163.1228	-0.162	-0.995	0.76	1.03	4	4	8928	+H
14	Theobromine		Identified	181.0722	0.159	0.876	1.26	1.20	3	3	27517	+H
15	Theophyline/aminophylline		Identified	181.0721	0.052	0.289	1.53	1.46	1	1	84217	+H

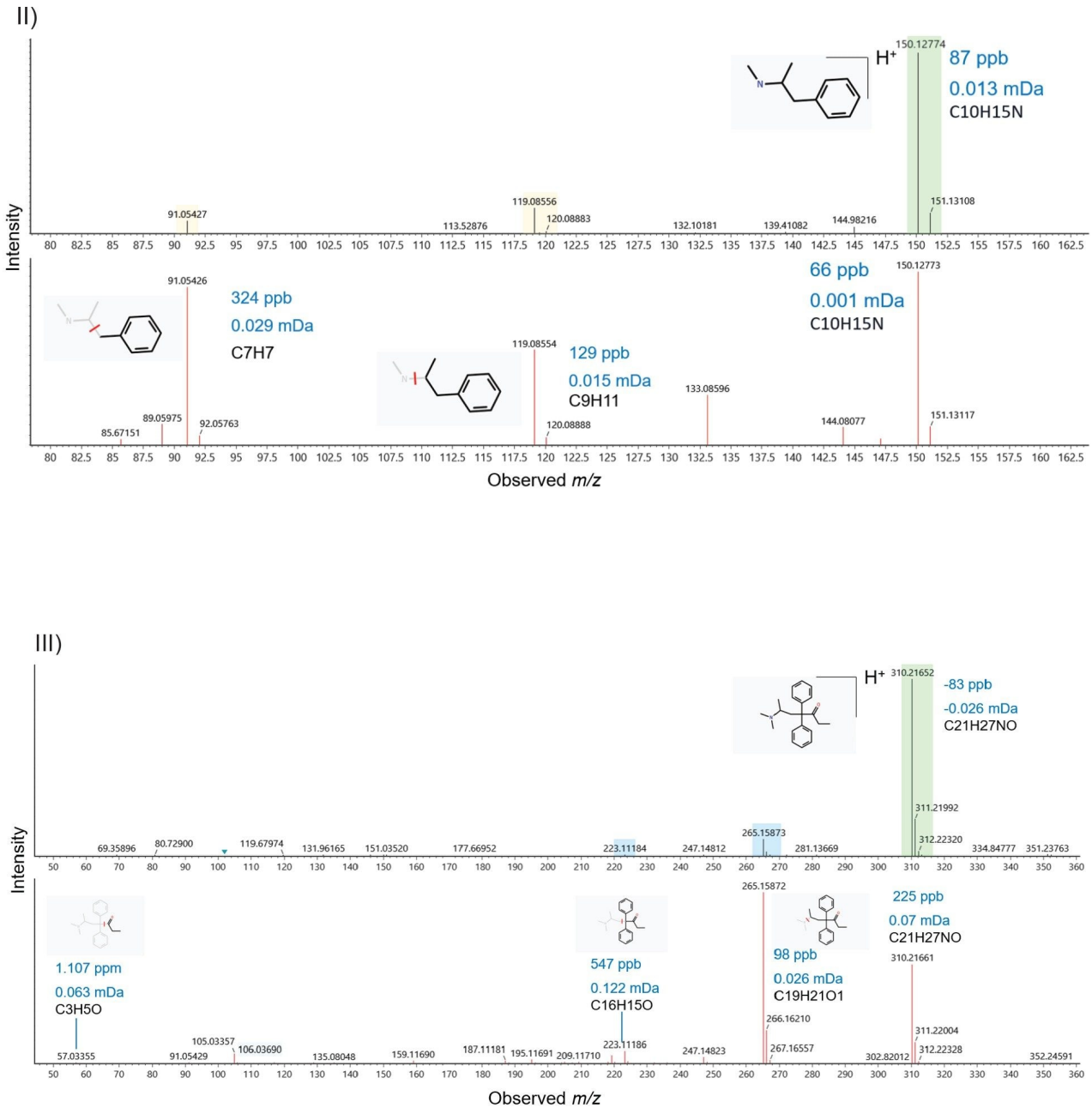

Figure 4. I) Component summary illustrating illicit, prescription, OTC and dietary compounds identified in anonymized human urine "sample 103". II) Methamphetamine enhanced MS^E precursor and fragment ion spectrum. III) Methadone enhanced MS^E precursor and fragment ion spectrum.

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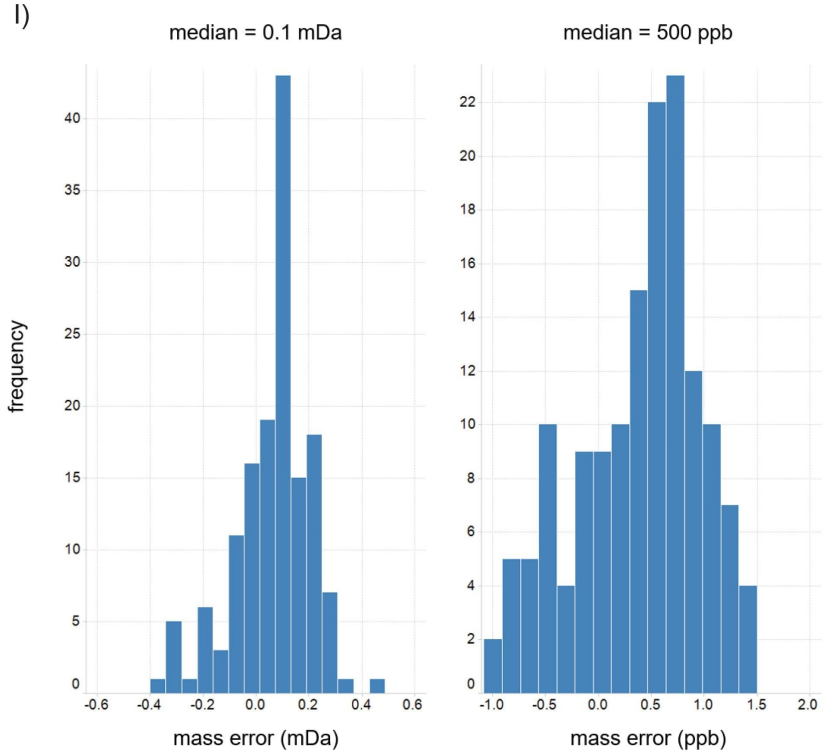
median = 0.1 mDa
median = 500 ppb
frequency
mass error (mDa)
mass error (ppb)

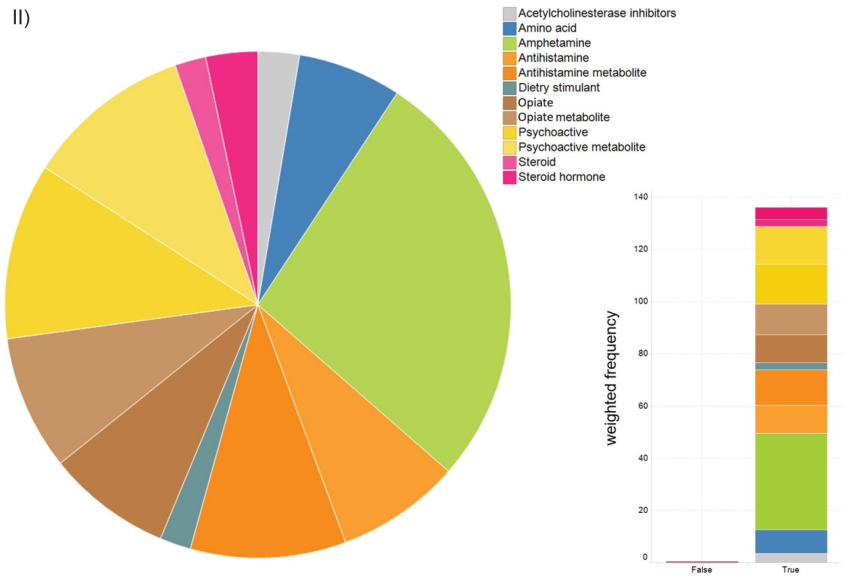

Figure 5. I) Frequency distribution of mass measurement error for analyte identifications determined to be present in "Sample 51". II) Distribution of "drug class" and plot of weighted frequency of observed true/false detections.

During our research, specific investigations were performed, to assess amphetamine (*m/z* 136.11207), methamphetamine (*m/z* 150.12773), pseudoephedrine (*m/z* 166.12264), and MDA (*m/z* 180.10191), which may be susceptible to labile fragmentation because of energy imparted from the ion source/transfer ion optics of mass spectrometers. Figure 4 illustrates the minimal fragmentation of methamphetamine. However, under LCMS analysis conditions, labile fragmentation is observed for amphetamine, although precursor ion, *m/z* 136.11207, forms the base peak ion in the low energy spectrum (Figure 6). Using direct analysis infusion, instrument parameters were optimized to reduce labile fragmentation. At low temperature (100 °C), in the case of amphetamine, a lower source temperature substantially reduces labile fragmentation (inset Figure 6).

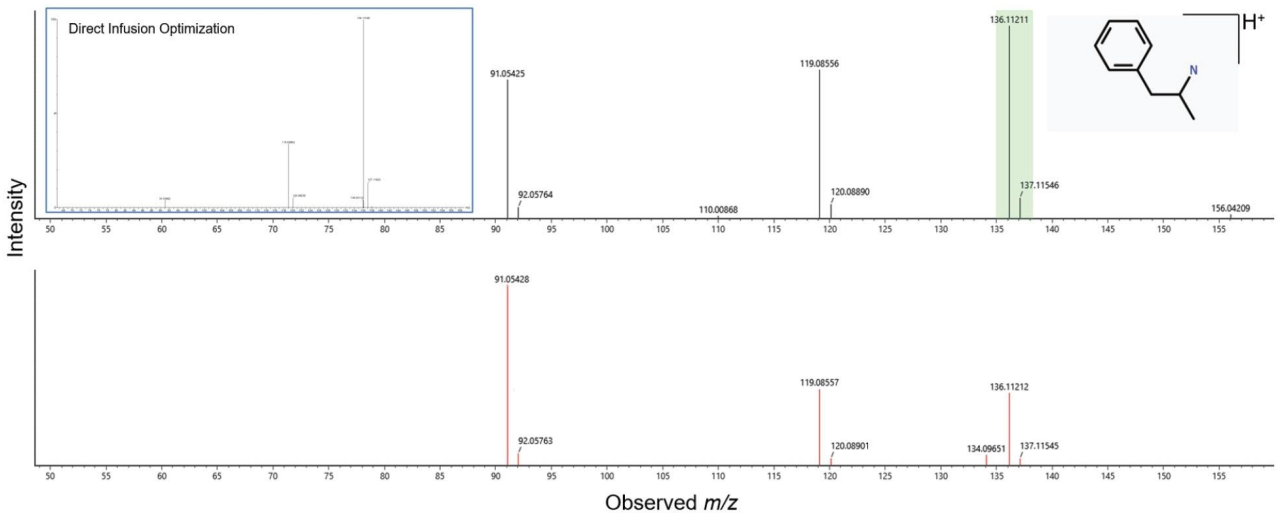

Figure 6. Amphetamine enhanced LCMS^E ES$^+$ precursor and fragment ion spectrum, with, low temperature direct infusion analysis (inset).

Conclusion

A high mass resolving power enhances ion selectivity and subsequently the detection of analytes in complex matrices. The SELECT SERIES MRT instrument's routine ppb mass accuracy performance generates high quality mass spectrometry data, facilitating unequivocal determination of analyte elemental compositions using non-targeted screening workflows. The enhanced mass accuracy specificity can be utilised to improve identification confidence in analytical research involving small drug molecules, in an everchanging drug landscape. Software tools are a key element to fully maximising all available information from the dataset, a symbiotic relationship exists between data quality, and informatics functionality. Stringent data processing tolerances are applied with confidence to improve analysis efficiency. Illicit drugs were identified in all samples using, retention time, precursor ion, and fragment ions with ppb mass accuracy. All samples were also positive for other recreational drug substances or OTC medications.

References

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3. Bonn B, Leandersson C, Fontaine F, Zamora I. Enhanced metabolite identification with MS(E) and a semi-automated software for structural elucidation. *Rapid Commun Mass Spectrom.* 2010 Nov 15;24(21):3127-38.

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