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응용 자료

Enhancing Biotransformation Identification Efficiency Using LC-MS Fine Isotope Structure Produced With Multi Reflecting Time-of-Flight MS

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Abstract

Potentially toxic drug metabolites can be difficult to detect and identify in the presence of complex matrices such as blood and urine. Using multiple analytical approaches to improve detection rates and identification confidence increases cost but more importantly slows, go, no-go decisions. Increasing LC-MS data specificity can help address these bottlenecks.

A UPLC-MS^E (ES+ and ES-) data independent acquisition (DIA) urinary screening assay was previously performed using a SELECT SERIES™ MRT at an acquisition rate of 10 Hz, with a system mass resolving power >200,000 FWHM. Precursor and fragment ion part per billion (ppb) accurate mass measurement facilitated the identification of metabolites of three therapeutic drugs present in a patient's urine sample.

Further confirmatory investigations have been performed using ultra high mass resolution MS in combination with Ultra Performance LC™ (UPLC™). Resolution Enhancement Mode (REM) has a system resolving power >300,000 FWHM and delivers fine isotope structure (FIS) with ppb mass accuracy providing highly specific identification criterion and is a powerful tool to determine elemental composition and facilitate confident

assignment of metabolite identifications.

Benefits

Improved efficiency of drug metabolite workflows through increased mass resolving power, mass measurement accuracy and fine isotope structure at chromatographic timescales.

- Identify unknowns with increased confidence, reduce false detection rates and improve analysis efficiency
- Enhanced analyte detection in complex biological matrices using LC-MS and mass resolving power >300,000 FWHM
- Fine isotope structure distributions with ppb mass accuracy provides additional criteria to enhance identification confidence for small molecule therapeutic drugs and metabolites

Introduction

High resolution mass spectrometry coupled with liquid chromatography is an ideal analytical tool for discovery and identification of drugs and their metabolites in complex biological matrices, aiding drug development workflows. Increasing mass resolution and mass accuracy subsequently increases confidence in compound assignment.

The SELECT SERIES MRT (Figure 1) is a state-of-the-art hybrid quadrupole Multi Reflecting Time-of-Flight Mass Spectrometer (MRT).[1] In MRT mode, where ions experience one pass of the MRT mass analyser, it provides a unique combination of high mass resolving power (>200,000 FWHM), and routine ppb mass accuracy, independent of acquisition speed.[2,3] In Resolution Enhancement Mode (REM), ions experience two passes of the MRT mass analyser, as a result a system mass resolving power of >300,000 FWHM is attained.

Using Ultra Performance LC coupled to a SELECT SERIES MRT (system resolution >200,000 FWHM), a non-targeted urinary screen of a healthy human volunteer was undertaken using ES+/ES- and has been fully described elsewhere.[2,3] Utilizing a metabolite identification workflow naproxen, carbamazepine, acetaminophen therapeutic drugs and metabolites, were identified. At >200,000 FWHM fine isotope structure was attained, providing added confidence when identifying biotransformation products, present in a complex biological matrix. In an extension to this study the samples were analyzed using REM, which provides the opportunity to generate mass spectra with enhanced fine isotope distribution information to facilitate identification of knowns and

unknowns.

In this study we demonstrated the benefit of REM and present identified biotransformation products (Figure 2), including their resolved fine isotope distributions which incorporate ^{2}H, ^{13}C, ^{15}N, ^{18}O, ^{33}S and ^{34}S.

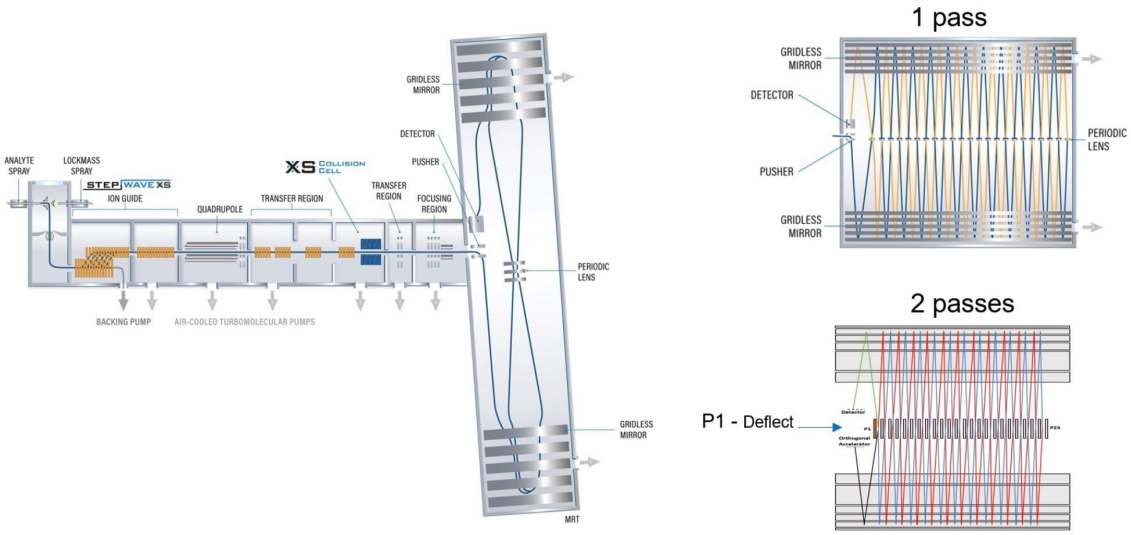


Figure 1. SELECT SERIES MRT instrument schematic illustrating one pass (>200,000 FWHM) and two passes REM mode (>300,000 FWHM).

Experimental

Sample description

Human urine sample diluted 1:10 (H_2O)

Dosage: Carbamazepine (2 x 200 mg tablets), acetaminophen (2 x 500 mg tablets), naproxen (1 x 500 mg tablet).

Sample time points: 0, 2, 4, and 6 hours after medication was administered.

LC Conditions

LC system:	ACQUITY™ UPLC I-Class Premier chromatograph
Column:	ACQUITY UPLC HSS™ T3 C_{18} (100 mm x 2.1 mm, 1.8 µm) Column
Column temperature:	40 °C
Sample temperature:	4 °C
Injection volume:	5 µL
Flow rate:	0.5 mL/min
Mobile phase A:	Water (containing 0.1% formic acid *v/v*)
Mobile phase B:	Acetonitrile (containing 0.1% formic acid *v/v*)

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
0.0	0.5	99	1	initial
1.0	0.5	99	1	6
3.0	0.5	85	15	6
6.0	0.5	50	50	6
9.0	0.5	5	95	6
10.0	0.5	5	95	6
10.1	0.5	99	1	6
12.0	0.5	99	1	6

MS Conditions

Acquisition:	ES+ and ES-

Capillary voltage:	0.8 kV/1 kV
Desolvation temperature:	500 ºC
Source temperature:	120 ºC
Cone voltage:	20 V
Mass range:	m/z 200–600
Acquisition rate:	10 Hz
Acquisition/Processing software:	MassLynx™ v4.2 SCN1026 and waters_connect™ 3.1.0.243

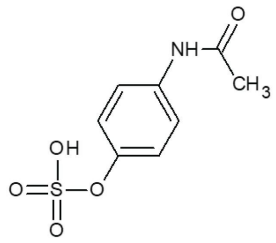
Results and Discussion

High mass resolution enhances ion selectivity of analytes in complex matrices. The number of possible elemental compositions generated with ppb high mass accuracy data is reduced and consequently there is a high degree of confidence in analyte identification. In conjunction with fine isotope structure, enhanced mass accuracy specificity can be utilised to improve identification certainty in research areas involving small molecules, such as metabolite identification.

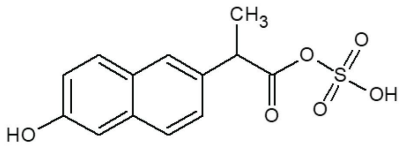
The benefits of utilizing REM mode can be seen in Figure 3, where performing LC-MS (10 Hz) at >300,000 FWHM, for acetaminophen sulfate nine peaks are resolved for the combined A+1 and A+2 fine isotope distributions. Comparatively, six fine isotope peaks are resolved at 200,000 FWHM.

In Figure 4, the FIS is compared determined using 200,000 FWHM and 300,000 FWHM determined for the known acetyl-S-cysteine conjugate of acetaminophen. FIS provides additional identification specificity and in the case of the acetaminophen-acetyl-S-cysteine conjugate, a dual polarity FIS cluster can be generated. At 300,000 FWHM, for the combination of ES+/ES- A+1 and A+2 fine isotope distributions, a total of eighteen isotopic peaks are resolved and can be used to confirm biotransformation identity.

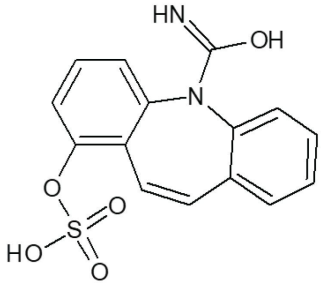
For the human urinary screen performed we have previously reported the identification of desmethyl naproxen sulfate ($[M-H]^-$ *m/z* 295) and carbamazepine hydroxy sulfate metabolite ($[M-H]^-$ *m/z* 331).[2,3] Both metabolites are rarely reported.[4-11] However the combination of ppb mass accuracy for precursor ion and fragments ions, in combination with FIS at 200,000 FWHM facilitated confident identification (see Figure 5). For these chromatographically coeluting (t_r 4.80) biotransformation products, REM data has been obtained (see Figure 6). The benefit of REM is illustrated once more, in Figure 6, for [desmethyl naproxen sulfate–$H]^-$, FIS resolution is enhanced and for [carbamazepine-o-sulfate - $H]^-$ where for A+1 and A+2 combined, ten FIS peaks have been resolved. In the case of carbamazepine-o-sulfate, ppb mass accuracy has been obtained for seven fine isotope distribution peaks. These additional seven identification points add further confidence to the assignment of the detected biotransformation product. For the examples illustrated, sufficient intensity has been obtained to measure the A+1 and A+2 isotopic fine structure. In the case of [carbamazepine-o-sulfate - $H]^-$, the fine isotope peak "d", was observed with a mass measurement error of 1.8 ppm, this is due to a contribution in the resultant centered data from fine isotope peak "c". Utilising REM has also increased the certainty that the three components eluting at 4.66 min, 4.8 min and 5.28 min (Figure 6 (I)) are isomeric biotransformation products. At respective system resolutions the measured fine isotope structure peaks predominantly correspond to the positions of simulated isotopic distributions.[12]



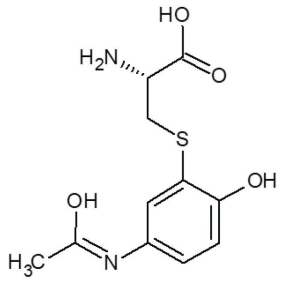
acetaminophen-sulfate



desmethyl naproxen-sulfate

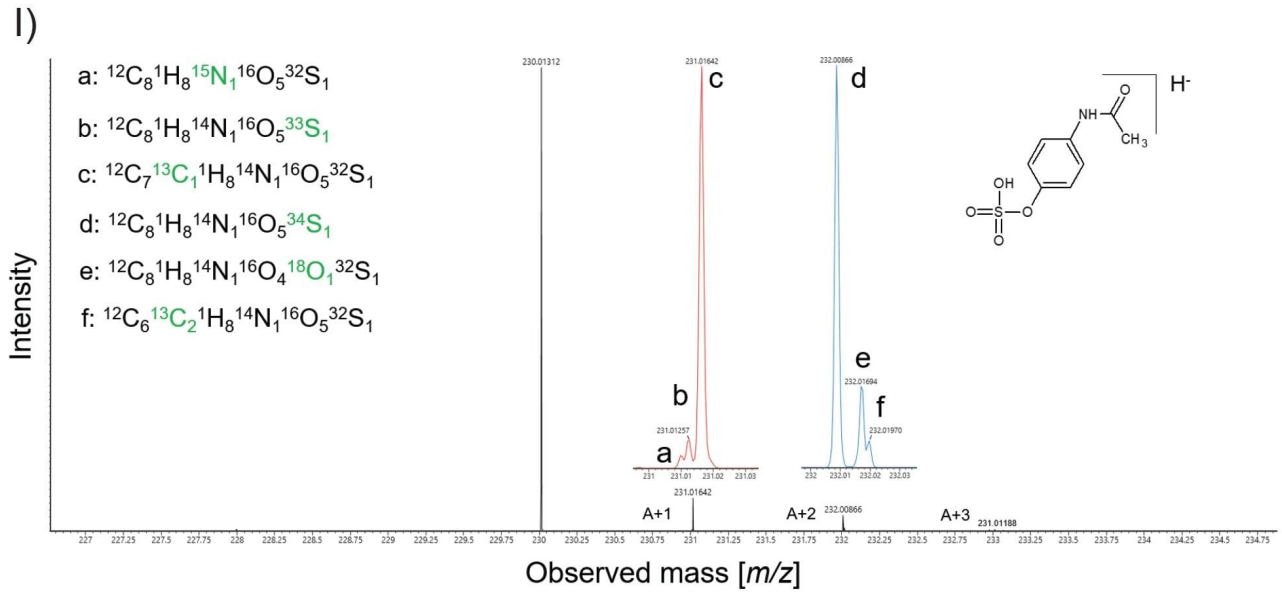


carbamazepine-o-sulphate



acetaminophen-acetyl-S-cysteine conjugate

Figure 2. Example metabolites identified for which fine isotope structure has been obtained using a LC-MS ES+ and ES- (>300,000 FWHM) human urinary screen.


I)
a: $^{12}C_8{}^{1}H_8{}^{15}N_1{}^{16}O_5{}^{32}S_1$
b: $^{12}C_8{}^{1}H_8{}^{14}N_1{}^{16}O_5{}^{33}S_1$
c: $^{12}C_7{}^{13}C_1{}^{1}H_8{}^{14}N_1{}^{16}O_5{}^{32}S_1$
d: $^{12}C_8{}^{1}H_8{}^{14}N_1{}^{16}O_5{}^{34}S_1$
e: $^{12}C_8{}^{1}H_8{}^{14}N_1{}^{16}O_4{}^{18}O_1{}^{32}S_1$
f: $^{12}C_6{}^{13}C_2{}^{1}H_8{}^{14}N_1{}^{16}O_5{}^{32}S_1$
Intensity
230.01312
231.01642
232.00866
231.01257
232.01694
232.01970
231.01188
A+1
A+2
A+3
Observed mass [m/z]

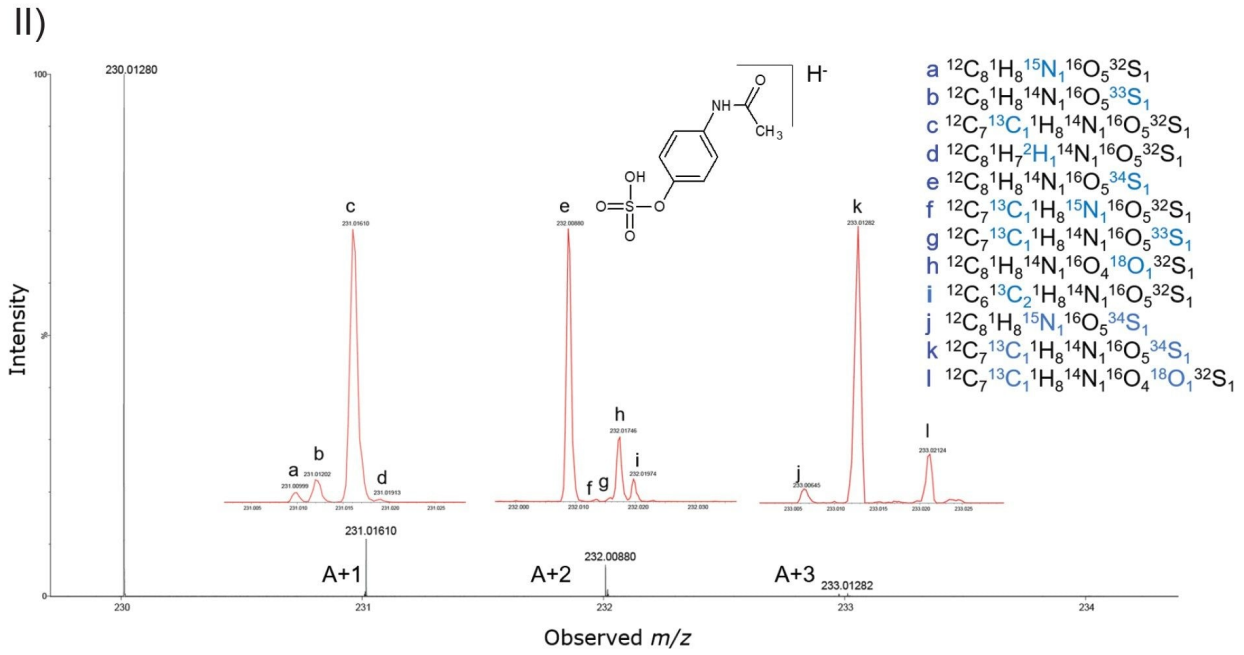
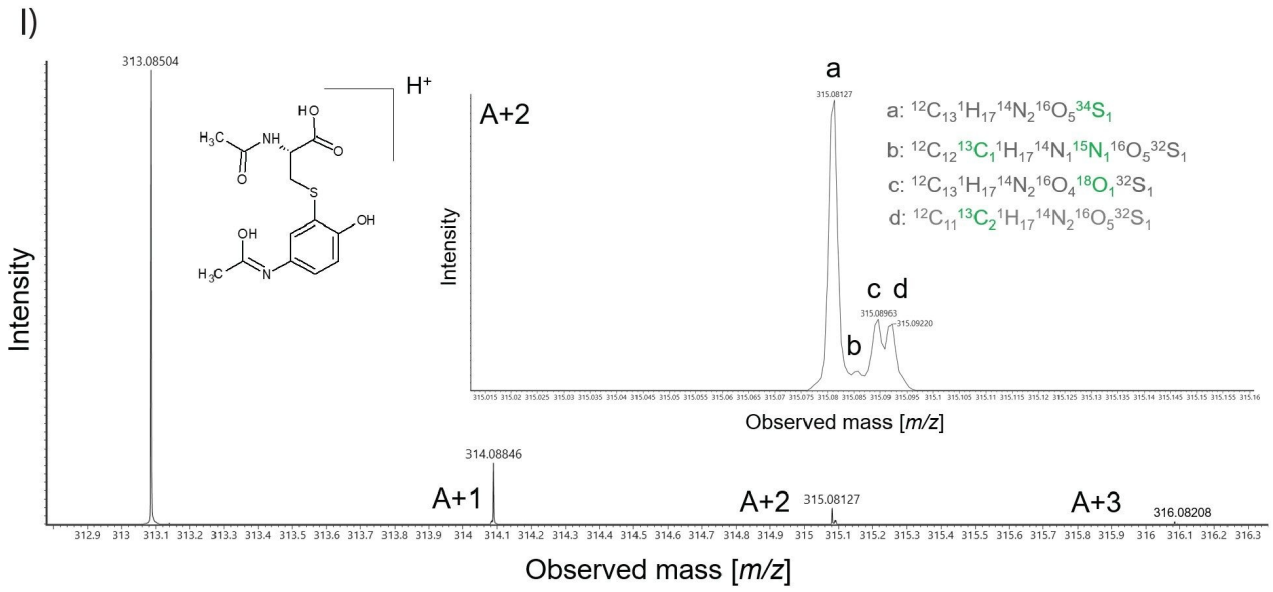
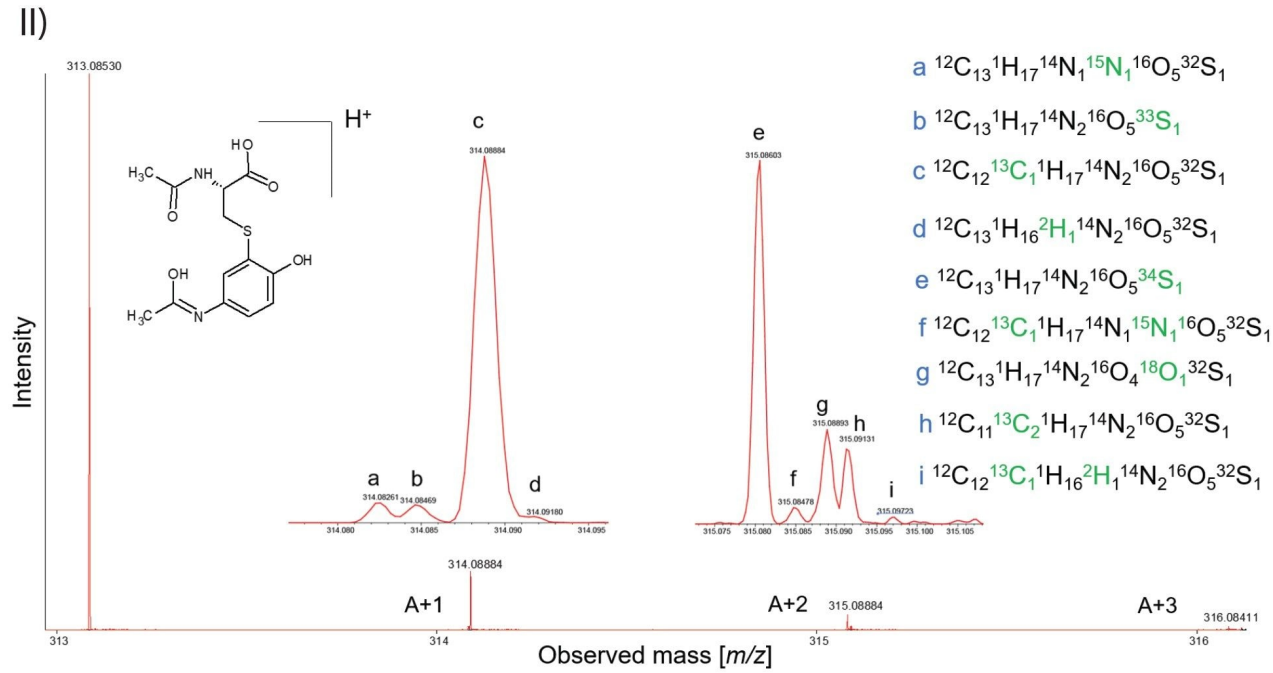


Figure 3. I) 10 Hz UPLC MRT ES- precursor fine isotope spectrum obtained for [acetaminophen sulfate -H]⁻ 200,000 FWHM. II) 10 Hz UPLC MRT ES- precursor fine isotope spectrum obtained for [acetaminophen sulfate -H]⁻ 300,000 FWHM.


I)
313.08504
H+
A+2
a
315.08127
a: $^{12}C_{13}{}^{1}H_{17}{}^{14}N_{2}{}^{16}O_{5}{}^{34}S_{1}$
b: $^{12}C_{12}{}^{13}C_{1}{}^{1}H_{17}{}^{14}N_{1}{}^{15}N_{1}{}^{16}O_{5}{}^{32}S_{1}$
c: $^{12}C_{13}{}^{1}H_{17}{}^{14}N_{2}{}^{16}O_{4}{}^{18}O_{1}{}^{32}S_{1}$
d: $^{12}C_{11}{}^{13}C_{2}{}^{1}H_{17}{}^{14}N_{2}{}^{16}O_{5}{}^{32}S_{1}$
c d
315.08963
315.09220
b
Intensity
Observed mass [m/z]
314.08846
A+1
A+2
315.08127
A+3
316.08208
Intensity
Observed mass [m/z]


II)
313.08530
H+
c
314.08884
e
315.08603
a $^{12}C_{13}{}^{1}H_{17}{}^{14}N_{1}{}^{15}N_{1}{}^{16}O_{5}{}^{32}S_{1}$
b $^{12}C_{13}{}^{1}H_{17}{}^{14}N_{2}{}^{16}O_{5}{}^{33}S_{1}$
c $^{12}C_{12}{}^{13}C_{1}{}^{1}H_{17}{}^{14}N_{2}{}^{16}O_{5}{}^{32}S_{1}$
d $^{12}C_{13}{}^{1}H_{16}{}^{2}H_{1}{}^{14}N_{2}{}^{16}O_{5}{}^{32}S_{1}$
e $^{12}C_{13}{}^{1}H_{17}{}^{14}N_{2}{}^{16}O_{5}{}^{34}S_{1}$
f $^{12}C_{12}{}^{13}C_{1}{}^{1}H_{17}{}^{14}N_{1}{}^{15}N_{1}{}^{16}O_{5}{}^{32}S_{1}$
g $^{12}C_{13}{}^{1}H_{17}{}^{14}N_{2}{}^{16}O_{4}{}^{18}O_{1}{}^{32}S_{1}$
h $^{12}C_{11}{}^{13}C_{2}{}^{1}H_{17}{}^{14}N_{2}{}^{16}O_{5}{}^{32}S_{1}$
i $^{12}C_{12}{}^{13}C_{1}{}^{1}H_{16}{}^{2}H_{1}{}^{14}N_{2}{}^{16}O_{5}{}^{32}S_{1}$
a b
314.08261
314.08469
d
314.09180
g
315.08893
h
315.09131
f
315.08478
i
315.09723
Intensity
314.08884
A+1
A+2
315.08884
A+3
316.08411
Observed mass [m/z]

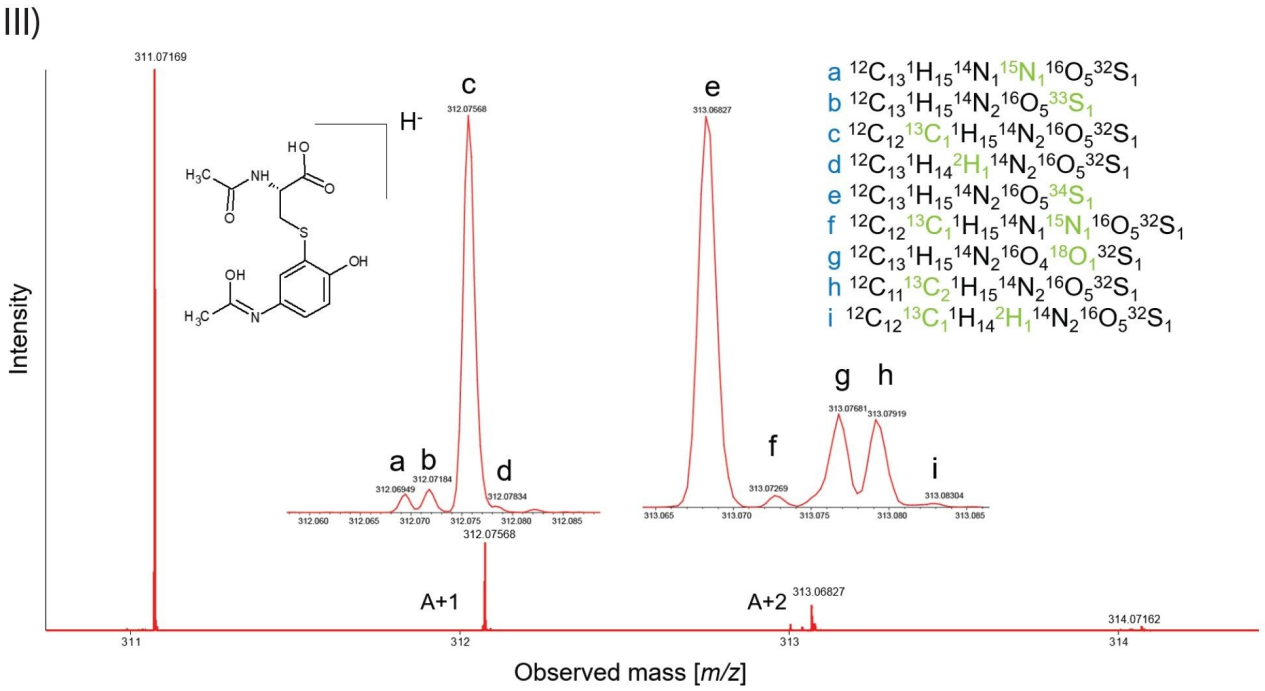
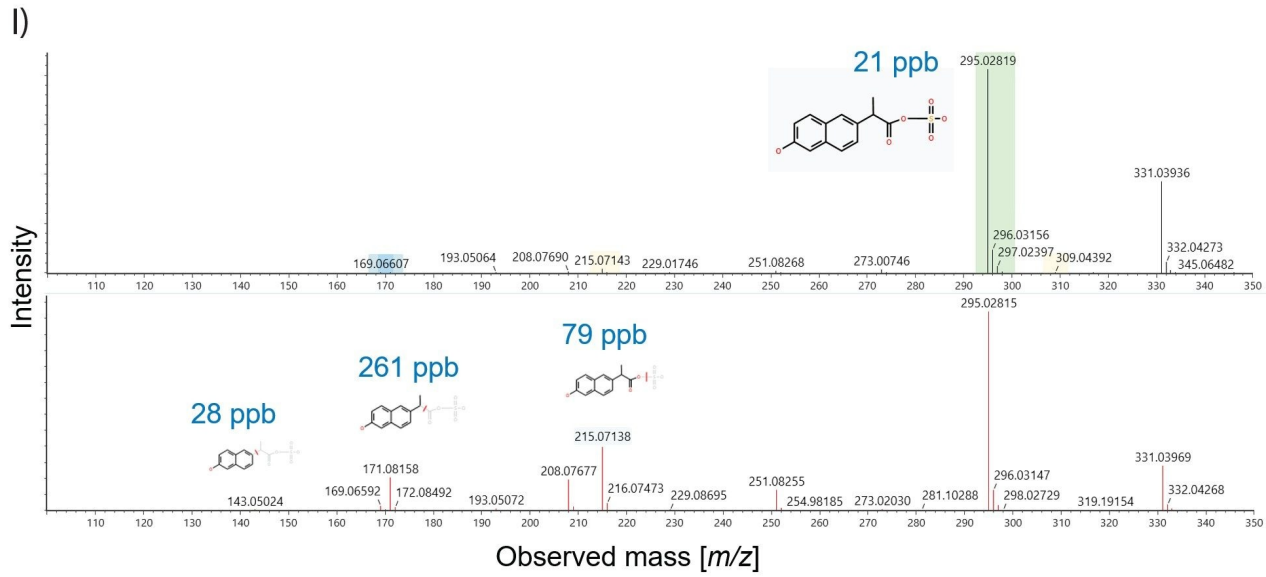
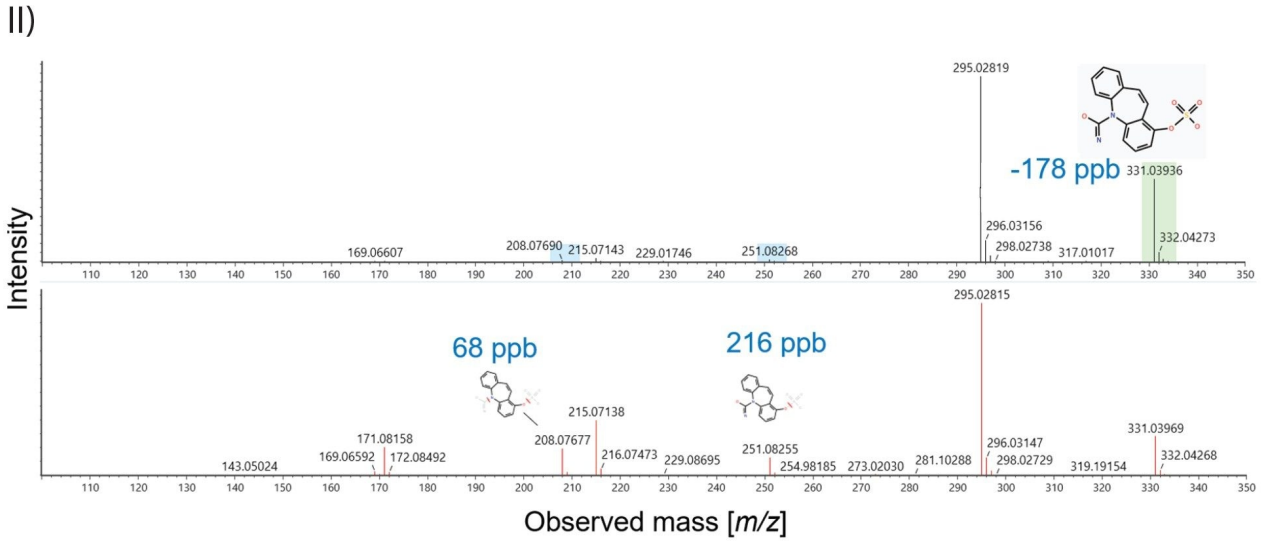


Figure 4. I) 10 Hz UPLC MRT ES+ fine isotope structure observed for acetaminophen-acetyl-S-cysteine conjugate (>200,000 FWHM). II) 10 Hz UPLC MRT ES+ fine isotope structure observed for acetaminophen-acetyl-S-cysteine conjugate (300,000 FWHM). III) 10 Hz UPLC MRT ES- fine isotope structure observed for acetaminophen-acetyl-S-cysteine conjugate (>300,000 FWHM).


I)
Intensity
21 ppb
295.02819
331.03936
296.03156
297.02397
309.04392
332.04273
345.06482
169.06607
193.05064
208.07690
215.07143
229.01746
251.08268
273.00746
295.02815
79 ppb
261 ppb
28 ppb
215.07138
171.08158
169.06592
172.08492
208.07677
216.07473
229.08695
251.08255
143.05024
193.05072
254.98185
273.02030
281.10288
296.03147
298.02729
319.19154
331.03969
332.04268
Observed mass [m/z]


II)
Intensity
295.02819
-178 ppb
331.03936
296.03156
298.02738
317.01017
332.04273
169.06607
208.07690
215.07143
229.01746
251.08268
295.02815
68 ppb
216 ppb
215.07138
171.08158
169.06592
172.08492
208.07677
216.07473
229.08695
251.08255
143.05024
254.98185
273.02030
281.10288
296.03147
298.02729
319.19154
331.03969
332.04268
Observed mass [m/z]

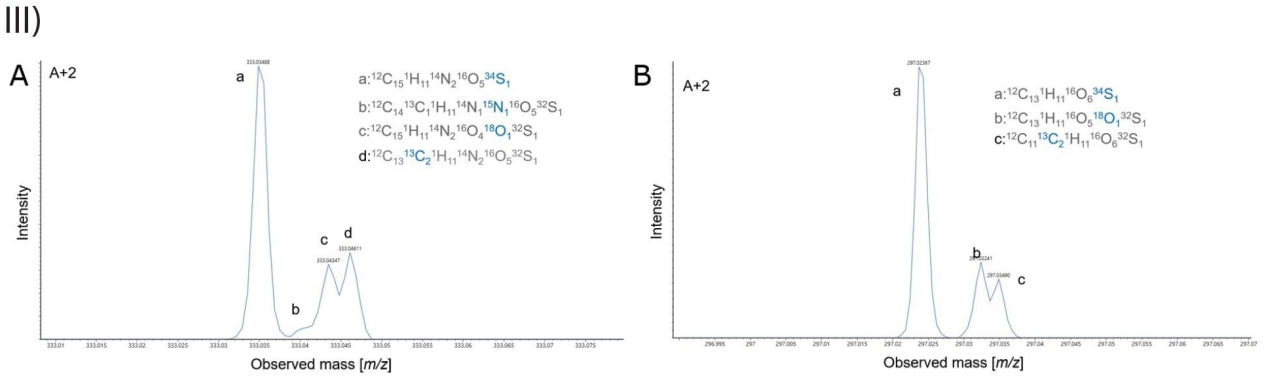
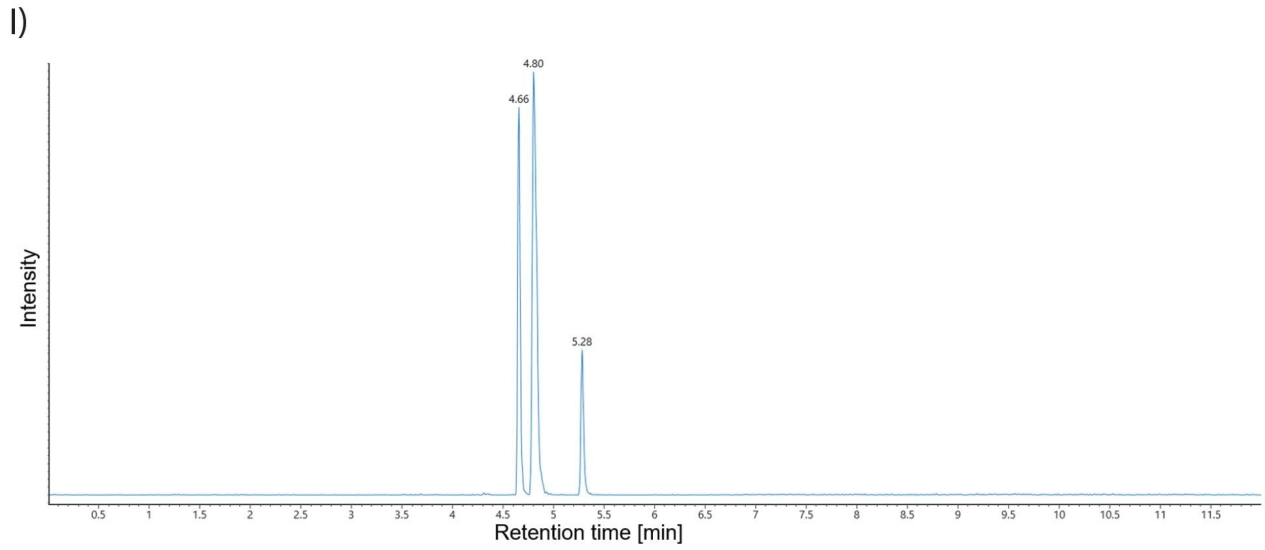
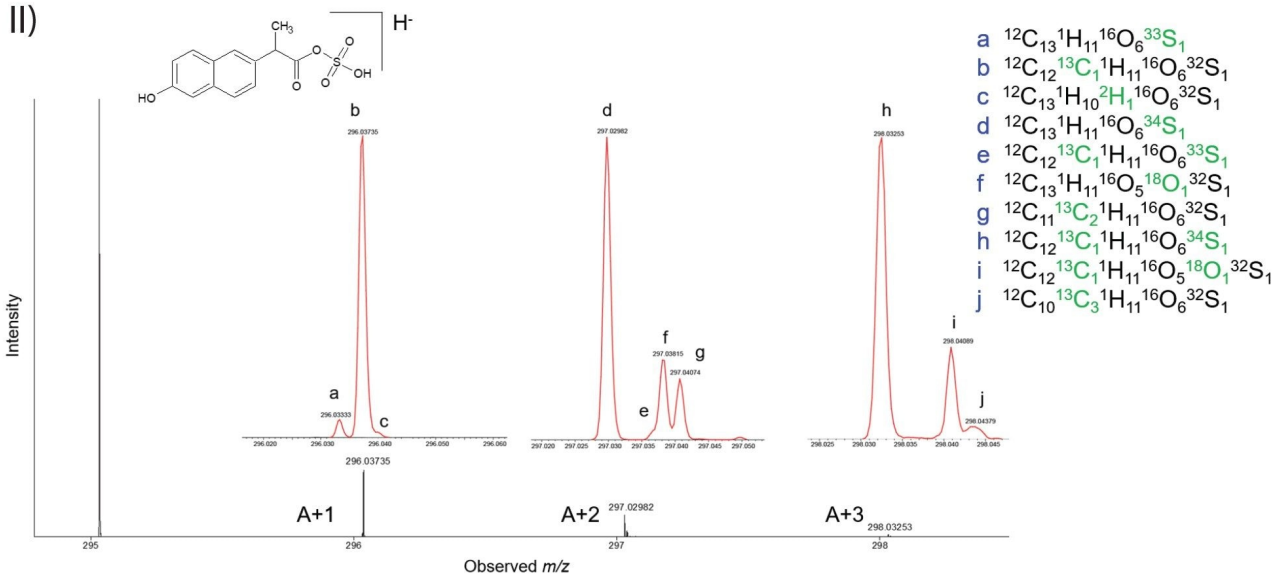
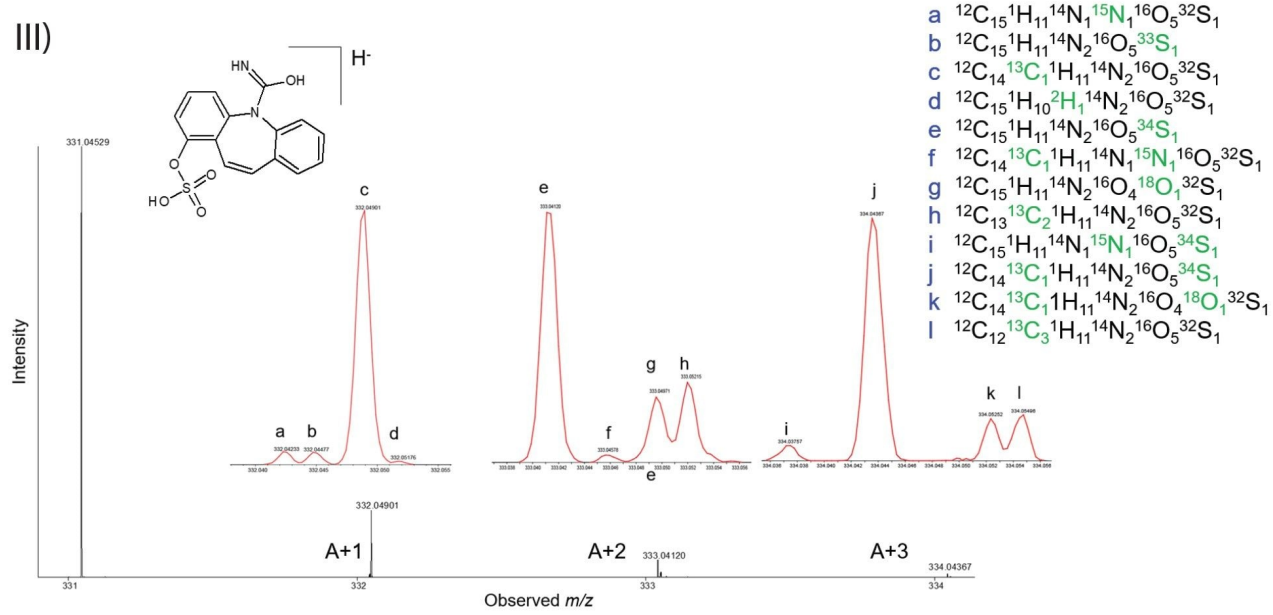


Figure 5. ES- MS^{E} analysis (>200,000 FWHM) precursor and fragment ion spectra obtained for coeluting I) [desmethyl naproxen sulfate – H]$^{-}$ and II) [carbamazepine-o-sulfate - H]$^{-}$. III) (A) carbamazepine-o-sulphate^{-} and (B) desmethyl naproxen sulfate confirmatory A+2 fine isotope structure obtained using 10 Hz UPLC MRT ES-.




II)
H-
HO
OH
a $^{12}C_{13}{}^{1}H_{11}{}^{16}O_{6}{}^{33}S_{1}$
b $^{12}C_{12}{}^{13}C_{1}{}^{1}H_{11}{}^{16}O_{6}{}^{32}S_{1}$
c $^{12}C_{13}{}^{1}H_{10}{}^{2}H_{1}{}^{16}O_{6}{}^{32}S_{1}$
d $^{12}C_{13}{}^{1}H_{11}{}^{16}O_{6}{}^{34}S_{1}$
e $^{12}C_{12}{}^{13}C_{1}{}^{1}H_{11}{}^{16}O_{6}{}^{33}S_{1}$
f $^{12}C_{13}{}^{1}H_{11}{}^{16}O_{5}{}^{18}O_{1}{}^{32}S_{1}$
g $^{12}C_{11}{}^{13}C_{2}{}^{1}H_{11}{}^{16}O_{6}{}^{32}S_{1}$
h $^{12}C_{12}{}^{13}C_{1}{}^{1}H_{11}{}^{16}O_{6}{}^{34}S_{1}$
i $^{12}C_{12}{}^{13}C_{1}{}^{1}H_{11}{}^{16}O_{5}{}^{18}O_{1}{}^{32}S_{1}$
j $^{12}C_{10}{}^{13}C_{3}{}^{1}H_{11}{}^{16}O_{6}{}^{32}S_{1}$
Intensity
296.03735
297.02982
298.03253
A+1
A+2
A+3
295
296
297
298
Observed m/z


III)
H-
HN
OH
HO
331.04529
a $^{12}C_{15}{}^{1}H_{11}{}^{14}N_{1}{}^{15}N_{1}{}^{16}O_{5}{}^{32}S_{1}$
b $^{12}C_{15}{}^{1}H_{11}{}^{14}N_{2}{}^{16}O_{5}{}^{33}S_{1}$
c $^{12}C_{14}{}^{13}C_{1}{}^{1}H_{11}{}^{14}N_{2}{}^{16}O_{5}{}^{32}S_{1}$
d $^{12}C_{15}{}^{1}H_{10}{}^{2}H_{1}{}^{14}N_{2}{}^{16}O_{5}{}^{32}S_{1}$
e $^{12}C_{15}{}^{1}H_{11}{}^{14}N_{2}{}^{16}O_{5}{}^{34}S_{1}$
f $^{12}C_{14}{}^{13}C_{1}{}^{1}H_{11}{}^{14}N_{1}{}^{15}N_{1}{}^{16}O_{5}{}^{32}S_{1}$
g $^{12}C_{15}{}^{1}H_{11}{}^{14}N_{2}{}^{16}O_{4}{}^{18}O_{1}{}^{32}S_{1}$
h $^{12}C_{13}{}^{13}C_{2}{}^{1}H_{11}{}^{14}N_{2}{}^{16}O_{5}{}^{32}S_{1}$
i $^{12}C_{15}{}^{1}H_{11}{}^{14}N_{1}{}^{15}N_{1}{}^{16}O_{5}{}^{34}S_{1}$
j $^{12}C_{14}{}^{13}C_{1}{}^{1}H_{11}{}^{14}N_{2}{}^{16}O_{5}{}^{34}S_{1}$
k $^{12}C_{14}{}^{13}C_{1}1H_{11}{}^{14}N_{2}{}^{16}O_{4}{}^{18}O_{1}{}^{32}S_{1}$
l $^{12}C_{12}{}^{13}C_{3}{}^{1}H_{11}{}^{14}N_{2}{}^{16}O_{5}{}^{32}S_{1}$
Intensity
332.04901
333.04120
334.04367
A+1
A+2
A+3
331
332
333
334
Observed m/z

IV)

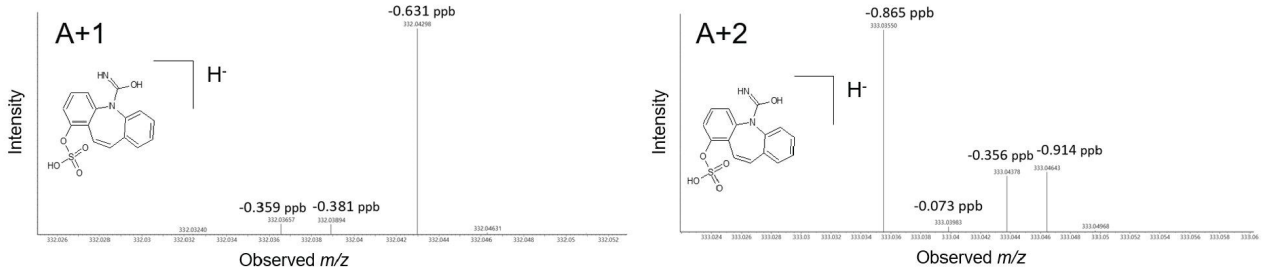


Figure 6. I) 10 Hz UPLC MRT ES+ [carbamazepine-o-sulfate - H]⁻ m/z 331 extracted mass chromatogram. II) ES- fine isotope structure (300,000 FWHM) observed for [desmethyl naproxen sulfate–H]⁻ and III) for [carbamazepine-o-sulfate - H]⁻. IV) A+1 and A+2 fine isotope accurate mass spectra for [carbamazepine-o-sulfate - H]⁻.

Conclusion

Using the SELECT SERIES MRT, routine ppb mass accuracy is attained for precursor and fragment ions when performing complex urinary non-targeted screening analysis, with ES+ and ES- LC-MS at 10 Hz (system resolving power >200,000 FHWM). Utilizing the MRT REM mode the system resolving power is increased to >300,000 facilitating routine ppb mass accuracy of fine structure peak distributions.
For LC-MS acquisition at >200,000 and >300,000 FWHM the measured FIS peaks predominantly correspond to the positions of simulated isotopic distributions. Confidence in therapeutic drug xenobiotics and metabolite identification is enhanced where ppb mass accuracy is attained for mass resolved FIS structure in an LC-MS time frame. This FIS transformative mass measurement affords the opportunity to increase identification criterion options, in turn providing confidence to efficiently identify known and previously unknown biotransformation products.

Increased credence for metabolite identification is illustrated for the detection of phase II metabolite desmethyl naproxen sulfate coeluting with a sulfonated hydroxy carbamazepine, and isomeric biotransformation products.

MRT REM mass spectrometry affords the opportunity to harness multi-faceted benefits including utilising FIS

enhanced specificity in data processing workflows, stable isotope labelling applications, and provide additional identification criterion where fragment ion information is absent for non-labile compounds.

References

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