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Application Notes

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This study demonstrates the benefit of confirmation of putative empirical formulae by interrogation of the fine isotope structure that is provided by high resolving power obtained at chromatographic timescales.

Introduction

High resolution mass spectrometers (HRMS), are prevalent as screening tools for metabolite identification in drug discovery and development, where the constituents of interest are present in complex biological matrices, including urine and blood. Confident identification of both xenobiotic and endogenous analytes is key to the drug discovery and development pipeline, while accurate mass facilitates putative empirical formulae generation. Confidence in an identification is increased when the constituents of the empirical formula for the compound of interest, are confirmed by their presence in the fine isotope structure, which is observed at high resolving power.

Here we present data obtained using a novel acquisition mode on the SELECT SERIES MRT that enables the flight path to be extended such that a second pass of the multi-reflecting time of flight (MRT) analyzer is enabled, which increases the resolving power by >50% and enables >300,000 FWHM. A non-targeted LC-MS screening experiment using the Resolution Enhancement Mode (REM) was undertaken for the analysis of pharmaceutical drug xenobiotics in the urine of a healthy volunteer. This study demonstrates the benefit of confirmation of

putative empirical formulae by interrogation of the fine isotope structure that is provided by high resolving power obtained at chromatographic timescales.

The SELECT SERIES MRT is a hybrid Q-Tof mass spectrometer that employs a multi-reflecting time of flight analyzer.[1] Figure 1 shows the ion path of the MRT operating in a standard single pass mode, the ions enter the analyzer through a orthogonal accelerator and follow a trajectory between lenses P1 and P23 shown in blue, once the ions reach lens P23 they are deflected back in a trajectory towards P1, shown in red, and the detector, resulting in a pathlength of ~47m and a resolving power of >200,000 FWHM, this mode of operation is called multi-reflecting time of flight (MRT) mode. In the MRT mass analyzer ions travel a fixed pathlength from the orthogonal accelerator to the detector, with the observed resolving power, R (being defined as R=pathlength/2x time spread), increasing proportionately with pathlength, this can be achieved by making the analyzer physically longer or by deflecting the ions within the analyzer and enabling them to make multiple passes of the device. The former has practical physical limitations whereas the latter can be achieved by applying a timed pulse to an electrode deflecting the ions within the device.

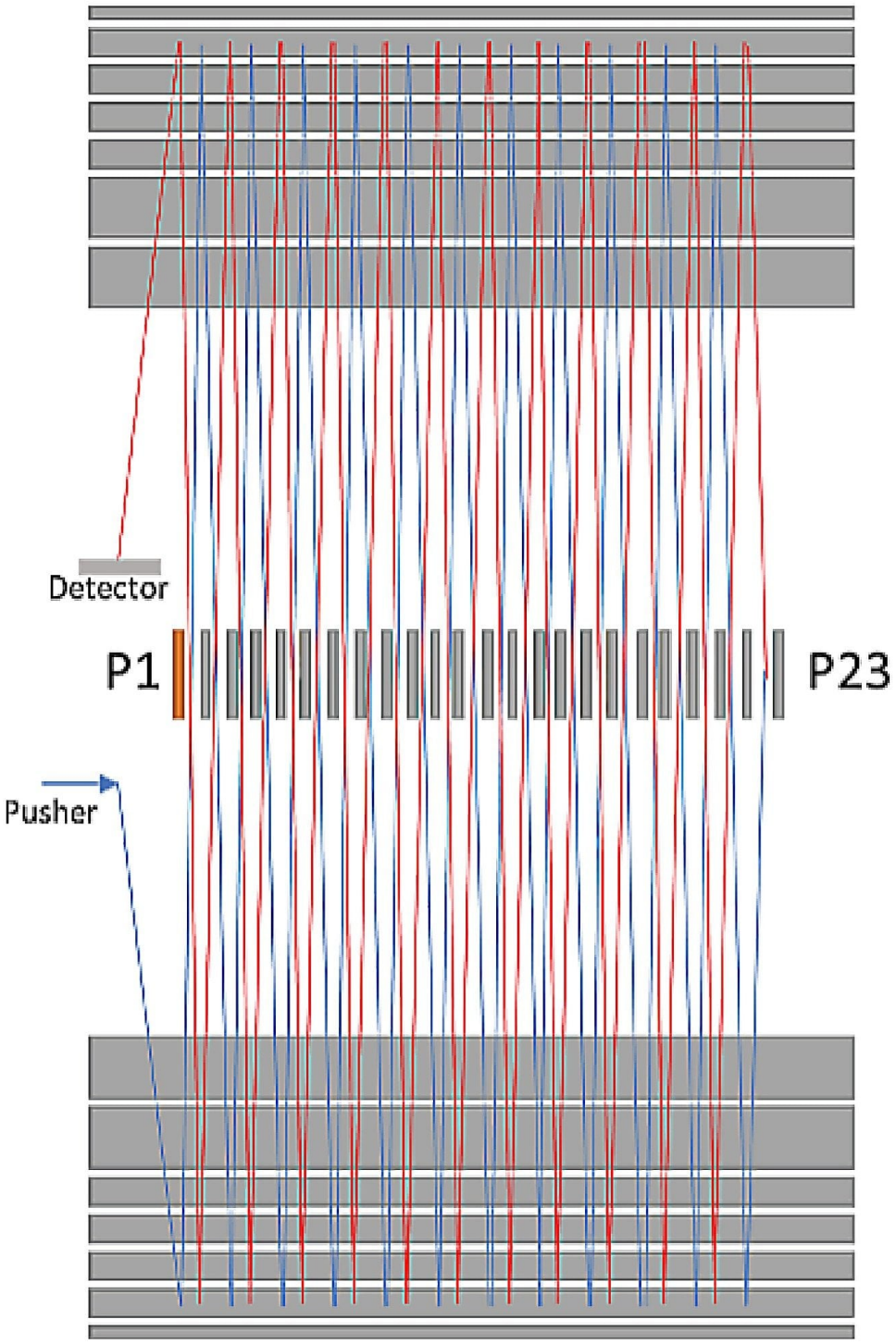

Figure 1. Flight path of MRT operating in a standard single pass (known as MRT) mode.

The analyzer can also be operated with a timed pulse applied to lens P1, deflecting a restricted mass range so as to undergo two passes, increasing the pathlength and hence observed resolving power to >300,000 FWHM. This is shown in Figure 2, the ions enter the analyzer through a orthogonal accelerator and follow a trajectory between lenses P1 and P23 shown in blue (Figure 2A), once the ions reach lens P23 they are deflected back in a trajectory towards P1, shown in red (Figure 2B), a timed pulse is then applied to lens P1 (shown in orange) deflecting the ions back towards P23 (Figure 2C) shown in green, once the ions reach lens P23 they are deflected back again in a trajectory, shown in yellow, towards P1 and finally the detector (Figure 2D), resulting in a pathlength of ~92m and a resolving power of >300,000 FWHM.

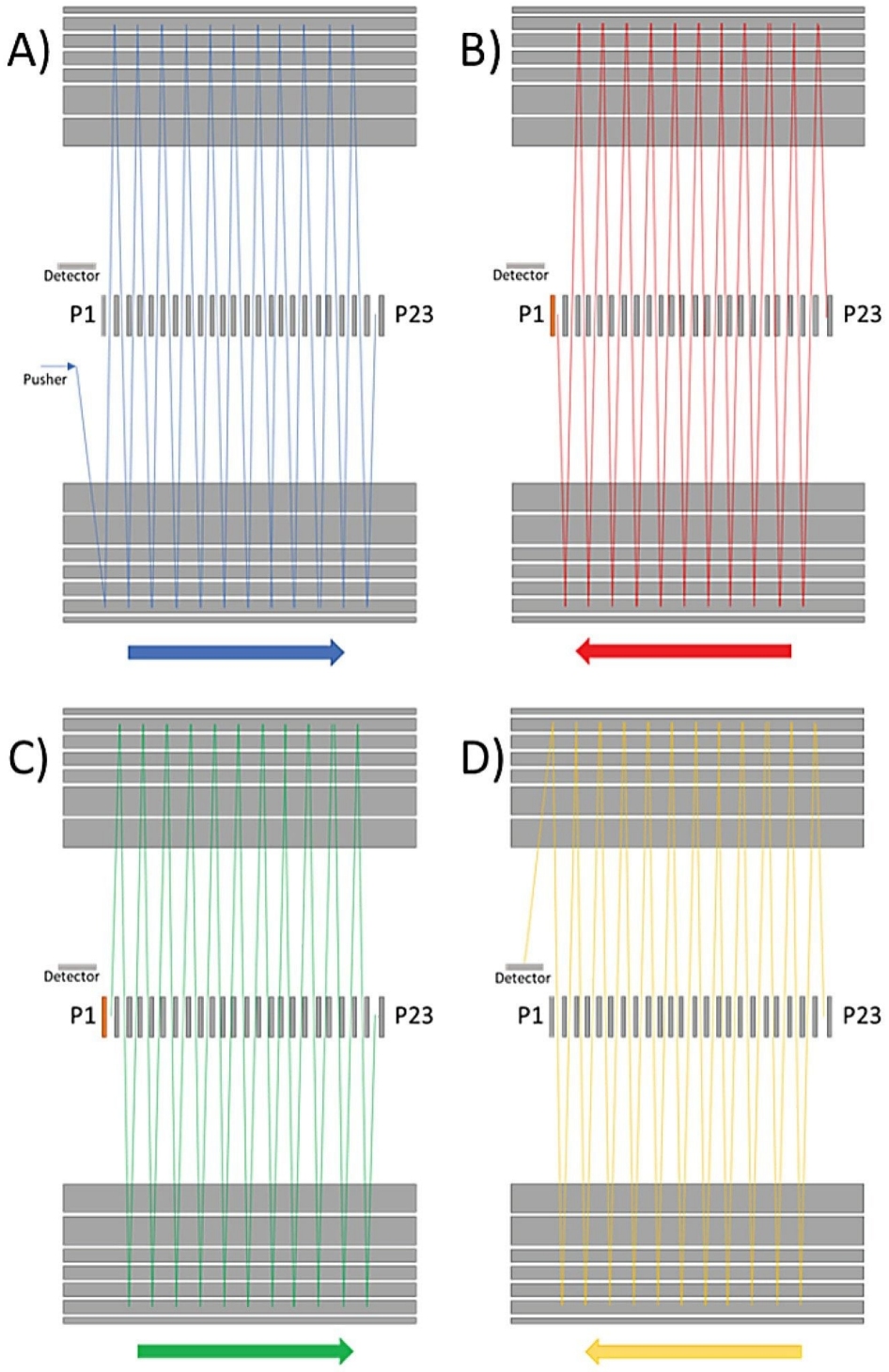

Figure 2. Flight path of MRT operating in Resolution Enhancement Mode (REM)- a

double pass mode,
A: ions pass (shown in blue) from orthogonal accelerator into analyzer and are focused and reflected towards lens P23;
B: ions are deflected by lens P23 back towards lens P1, shown in red;
C: a timed pulse applied to P1 deflects the ions back towards lens P23, shown in green;
D: ions are deflected a second time by lens P23 back towards P1 (pulse off) and onto the detector (shown in yellow).

The transmittable mass range is a function of the selected low *m/z*, with the high *m/z* limit being approximately four times that of the lowest *m/z*, so for a start mass of *m/z* 300 the upper *m/z* would be ~1100 *m/z.*

Another consideration of the described approach to increase pathlength is duty cycle, this can be maintained through a synchronization of the time-of-flight of an ion packet release between the upstream gas cell and the orthogonal accelerator for the *m/z* range of interest. This novel mode of operation is called Resolution Enhancement Mode (REM).

Experimental

Sample Description

Human urine sample diluted:	10:1 (H_2O)
Carbamazepine dosage:	2 x 200 mg tablets
Acetaminophen dosage:	2 x 500 mg tablets
Naproxen dosage:	1 x 500 mg tablet
Sample time points:	2, 4 and 6 hours after medication was administered

LC Conditions

LC system:	Waters 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	6
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

Results and Discussion

Data were acquired in Resolution Enhancement Mode (REM) and the base peak intensity chromatogram for the urine four-hour time point sample is shown in Figure 3A, this is a complex chromatogram with many overlapping features. Extracted mass chromatograms for two co-eluting metabolites at 4.8 minutes, desmethyl naproxen sulfate (*m/z* 295.03, C13H10O6S) and carbamazepine hydroxy sulfate (*m/z* 331.04, C15H10N2O5S), are shown in Figures 3B and 3C respectively.

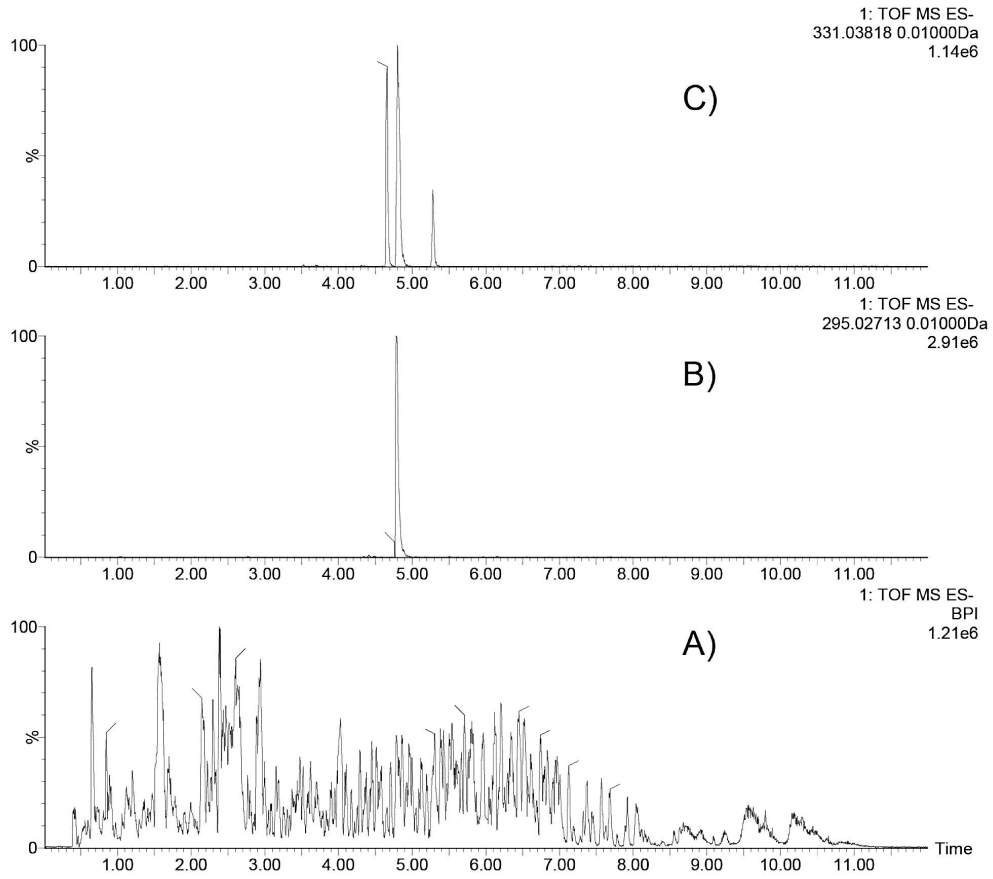

Figure 3A. Base peak intensity chromatogram for the urine four hour time point; 3B. XIC of m/z 295.03, corresponding to desmethyl naproxen sulfate; and 3C: XIC of m/z 331.04, corresponding to carbamazepine hydroxy sulfate.

The corresponding mass spectrum for the chromatographic peak at retention time 4.8 minutes is shown in Figure 4, featuring two metabolites at *m/z* 295.03, corresponding to desmethyl naproxen sulfate and *m/z* 331.04, corresponding to carbamazepine hydroxy sulfate with an example of the representative resolving power observed at *m/z* 331.04 of >300.000 FWHM shown in the inset.

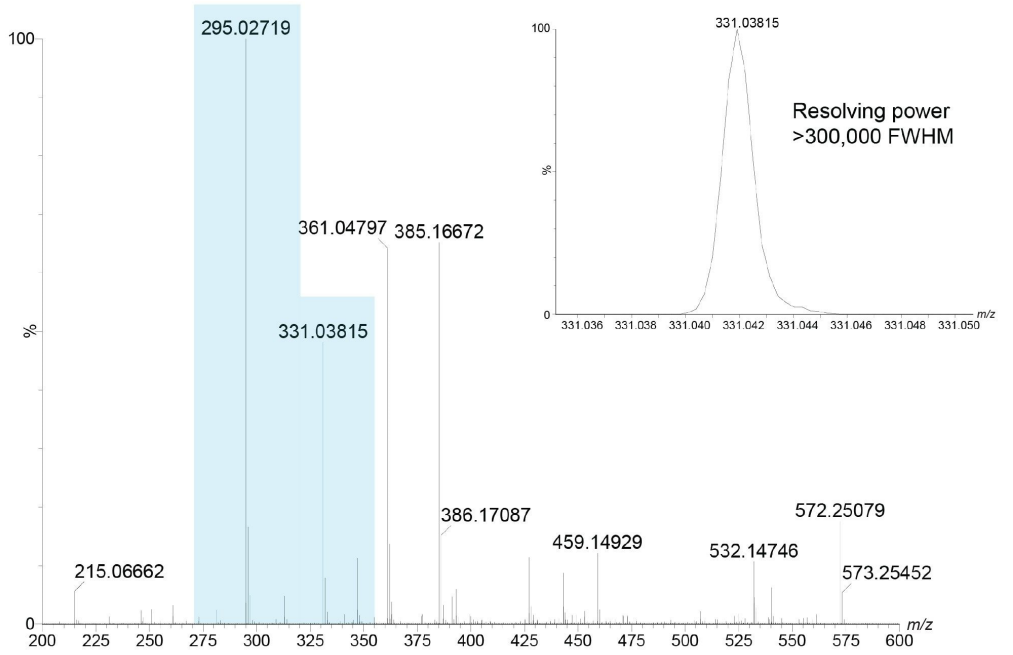

Figure 4. Mass spectrum of chromatographic peak at retention time 4.8 minutes, featuring two metabolites at m/z 295.03,ccorresponding to desmethyl naproxen sulfate and m/z 331.04, corresponding to carbamazepine hydroxy sulfate. Inset: example resolving power observed at m/z 331.04 of >300,000 FWHM.

The observed mass accuracy for the two metabolites was 542 ppb for desmethyl naproxen sulfate and -423 ppb for carbamazepine hydroxy sulfate. Although ppb levels of mass accuracy give confidence in putative empirical formulae, the observation of fine isotope structure significantly increases the confidence in an identification.

The fine isotope structure for desmethyl naproxen sulfate is shown in Figure 5. Confidence in the identification is increased through the confirmation of the presence of oxygen and sulfur in the formula by the presence of the 16O, 18O, 32S, 33S, and 34S isotopes.

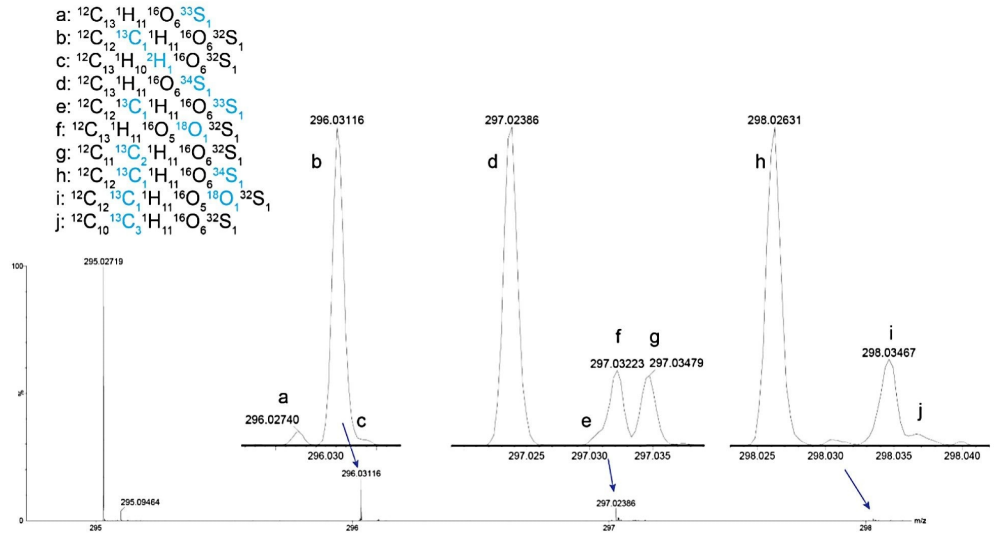

Figure 5. Mass spectrum of desmethyl naproxen sulfate, C13H10O6S, showing the fine isotope structure, with formula confirmatory isotopes for oxygen (16O and 18O) and sulfur (32S, 33S, and 34S).

The fine isotope structure for the second metabolite, carbamazepine hydroxy sulfate, is shown in Figure 6. Confidence in the identification is also increased through the confirmation of the presence of nitrogen, oxygen and sulfur in the formula by the presence of the 14N, 15N, 16O, 18O, 32S, 33S, and 34S isotopes.

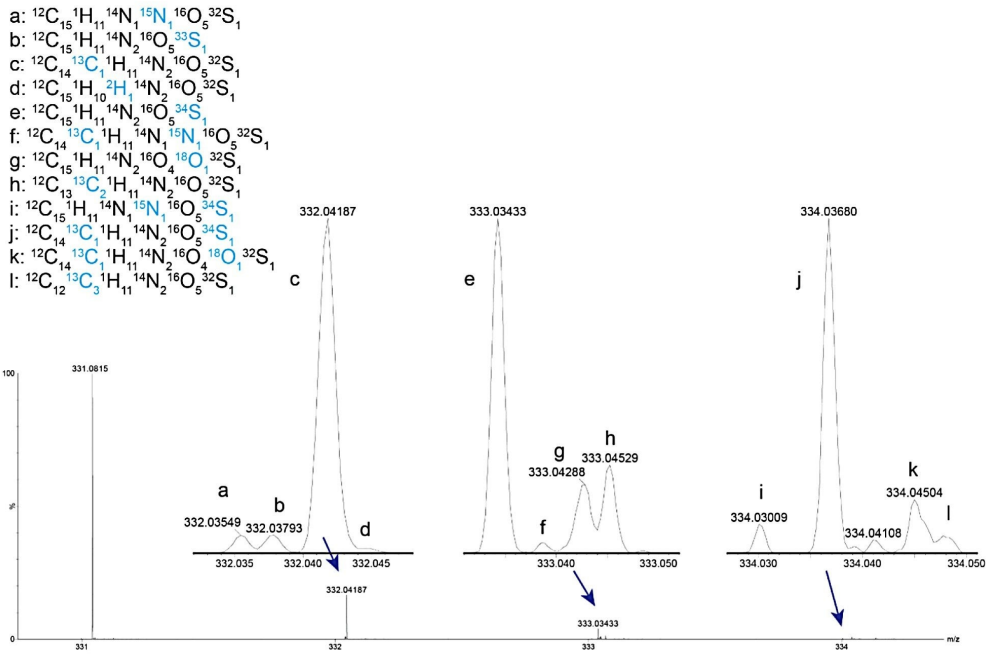

Figure 6. Mass spectrum of carbamazepine hydroxy sulfate, C15H10N2O5S, showing the fine isotope structure, with formula confirmatory isotopes for nitrogen (14N and 15N) oxygen (16O and 18O) and sulfur (32S, 33S, and 34S).

Conclusion

A novel approach to increasing mass resolution on the SELECT SERIES MRT allows an increased confidence in analyte identification. Multiple examples of fine isotope structure being used to corroborate putative empirical formulae as a result of improved resolving power obtained using Resolution Enhancement Mode (REM) at chromatographic timescales.

Featured Products

SELECT SERIES MRT <https://www.waters.com/waters/nav.htm?cid=135082877>

MassLynx MS Software <https://www.waters.com/513662>

720007986, September 2023