Finding and Confirming Identification of Unknown in Sediment Sample by LC/TOF and LC/Trap MS

Application

Forensics, Food Safety, Environmental Protection

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Abstract

This application note describes how the attributes of Time of Flight and Trap mass spectrometry can be combined to provide a highly reliable identification for an unknown compound. Time of Flight mass spectrometry provides a very accurate mass for the parent compound, and Trap mass spectrometry provides fragmentation data; together they greatly diminish the number of candidate compounds, and increase the confidence in a confirmation.

Introduction

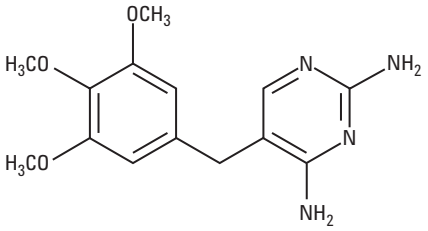
Unknown organic compounds are best identified by spectral data. Trap and Time of Flight (TOF) mass spectrometers can acquire spectral data in parallel fashion and, as such, have high spectral sensitivity. Serial scanning instruments, such as triple quads and single quads, do not display as high a spectral sensitivity in their scan modes as they do chromatographic sensitivity in their selected ion monitoring (SIM) modes.

High spectral sensitivity is preferred for the identification of low level unknowns. Accurate mass measurement with electrospray ionization (ESI) TOF, greatly increases the confidence of an identification because it limits the possible number of candidate compounds. The better the precision and accuracy of the mass measurement, the fewer the number of compounds possible at a given accurate mass.

MS/MS fragmentation data complements accurate mass data. Fragmentation data can often distinguish isomers of the same compound when the accurate mass would be identical. TRAP MS/MS is extremely sensitive in the spectral domain.

References to prior Agilent literature describing TOF principles, techniques, and applications are given [1, 2, 3].

The drug, trimethoprim ($C_{14}H_{18}N_4O_3$, 290.1379 Da), is the example compound chosen here for this technique description.



Experimental

LC Conditions

Mobile phases	A = 10 mM ammonium formate, @ pH 4.5
	B = acetonitrile (ACN)
Gradient:	5% B to 90% B over 7 min, hold 2 min at 90% B, and return to 5% B by 10 min
Flow rate:	0.5 mL/min
Column:	ZORBAX XDB C-18, 2.1 mm × 50 mm, 3.5 µm

MS Conditions

Instrument:	Agilent LC/TOF in positive ion ESI and Agilent LC/Ion TRAP XCT in positive ion ESI, depending on experiment
Nebulizer pressure:	55 psi
V_{cap}	3500 V
Sample injection:	2 to 20 µL depending on sample
Samples:	A 20-drug standard Sediment sample: A methanol/water solution derived from a lake sediment, extracted by the USGS

Results–TOF Screen

From the 20-drug mixture, trimethoprim was chosen for further examination. Its chromatographic and spectral characteristics are shown in Figure 1 (RT = 2.11 min, $(M+H)^+$ = 291.1452 *m/z*).

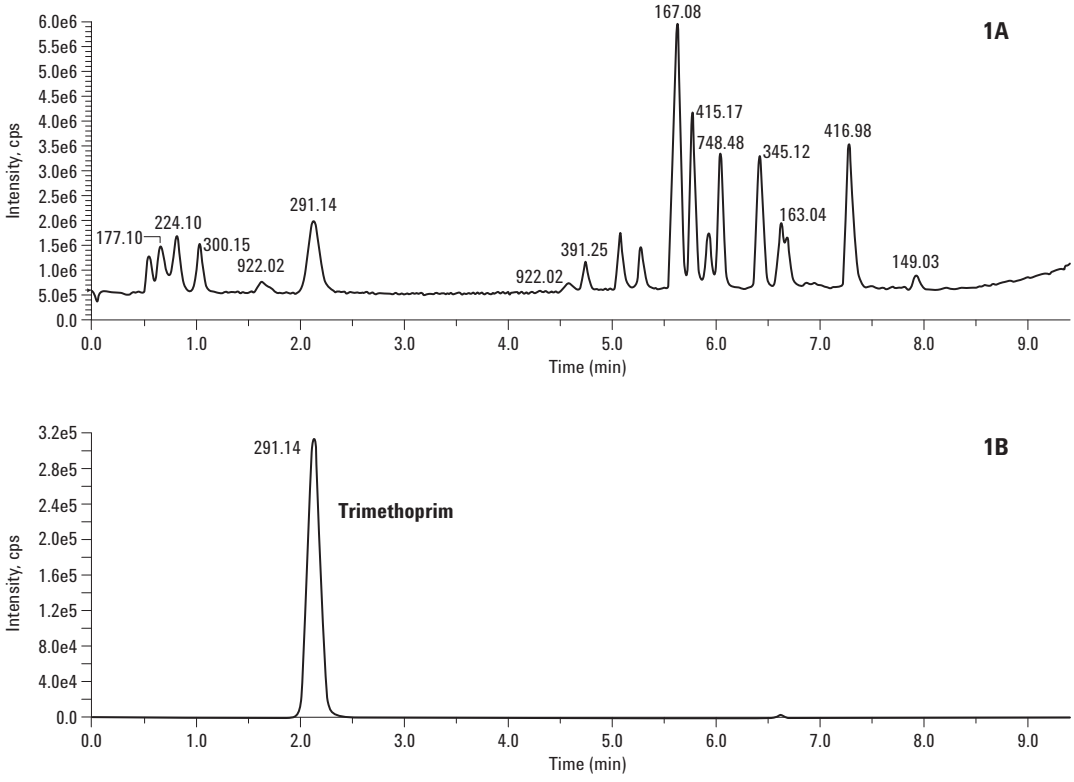


Figure 1. A total ion chromatogram (TIC) of a 20-drug standard (1A), and an extracted ion chromatogram (EIC) 291.12–291.16 *m/z* for trimethoprim (1B). Both were obtained using positive ion ESI TOF/MS.

Sediment Sample

Given the information shown in Figure 1, a search for trimethoprim in the extract of the sediment sample was begun using the ESI TOF/MS.

The TIC and EIC of the sediment extract are shown in Figure 2. If present, trimethoprim is well hidden in the TIC (Figure 2A), but the EIC (291.12–291.16 *m/z)* (Figure 2B) reveals a peak at 2.11 min. While the retention time (RT) and the extracted ion mass are consistent with trimethoprim, the TOF can provide a high resolution, accurate mass spectrum.

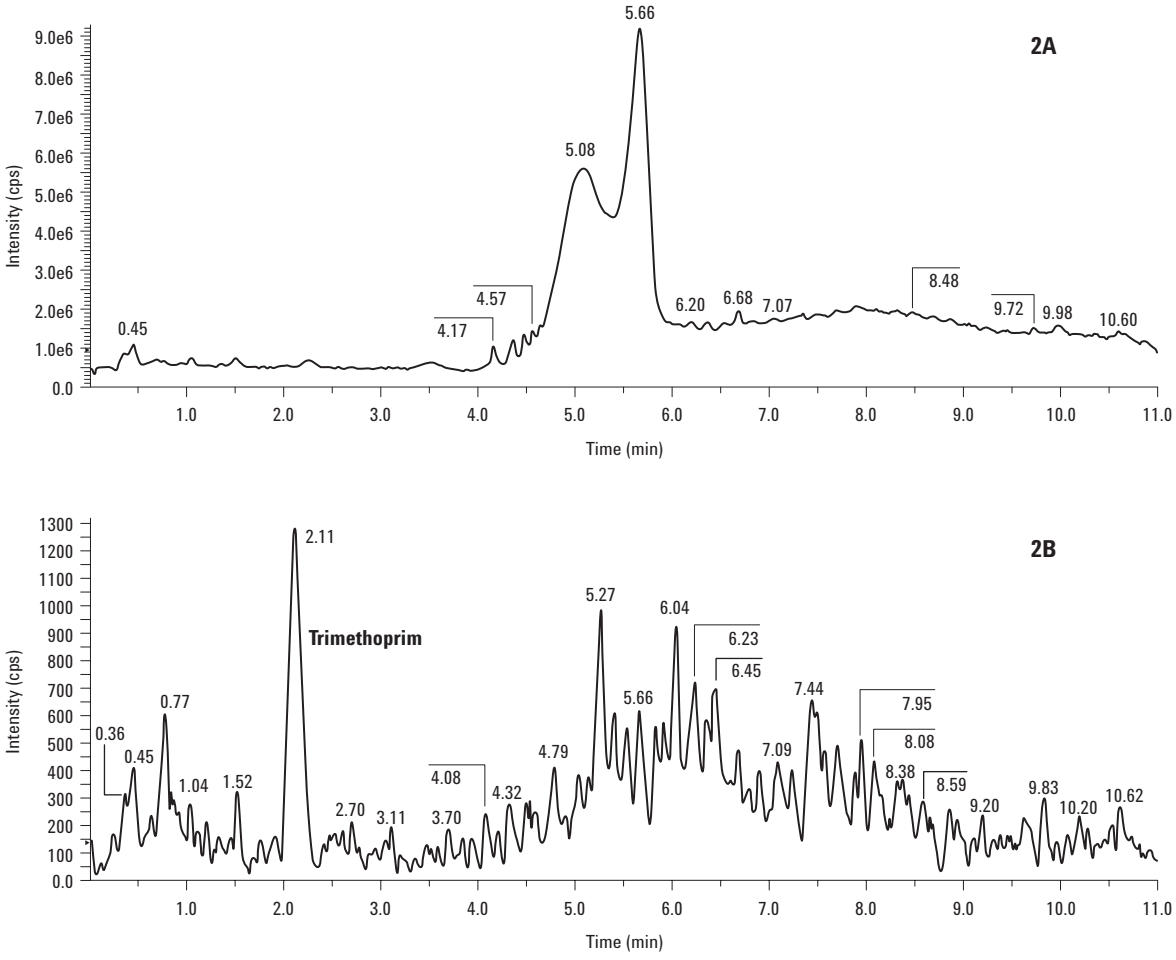


Figure 2. The TIC (2A) and EIC (2B) of the sediment extract. Ion extraction was set for 291.12–291.16 *m/z*.

Spectral background (darker bars) was subtracted from the averaged spectrum (lighter center bar) in Figure 3A. The resultant accurate mass spectrum is displayed in Figure 3B. Even at this low level, the resolution and accurate mass character of the TOF still applies. No adjustment for Lock Mass Threshold is required.

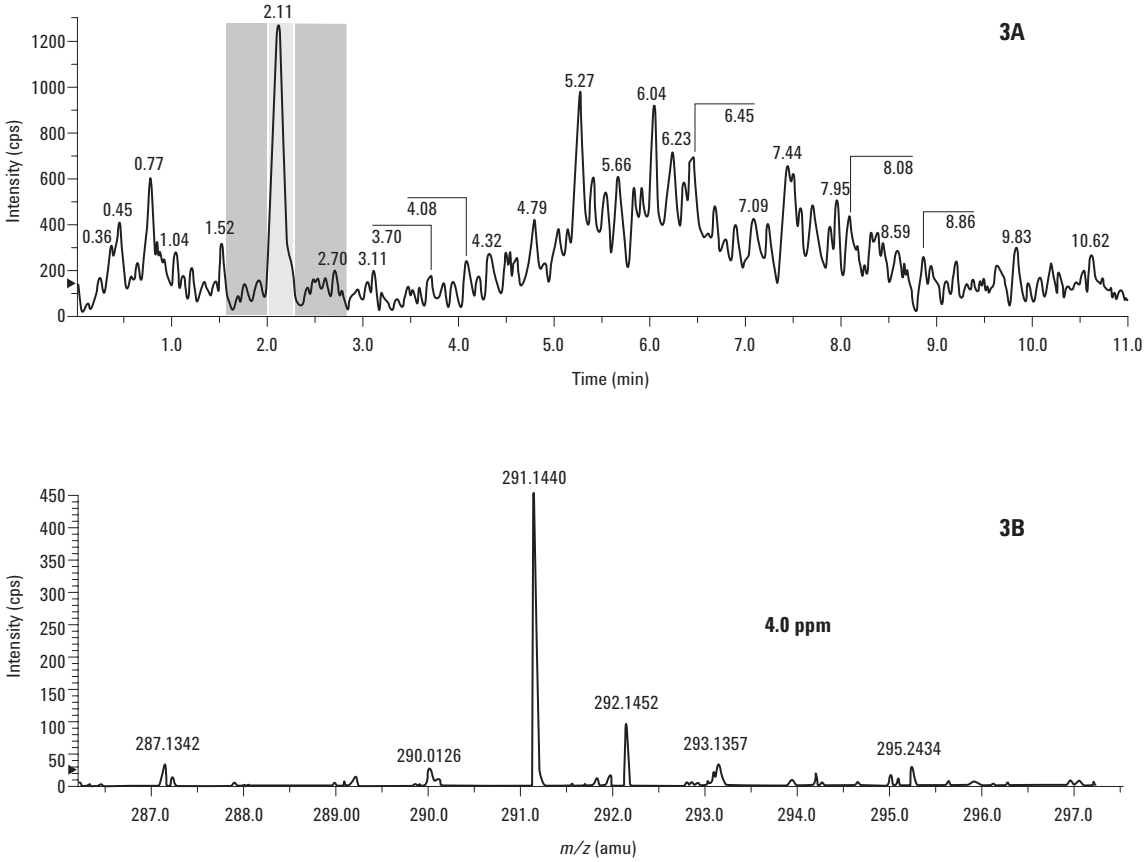


Figure 3. Figure 3A is an EIC for the 291.12–291.16 *m/z* region, and Figure 3B is the TOF for the peak occurring in the 2.016-2.271 min range after subtraction of the indicated adjacent regions. The relative difference between the experimental and calculated mass for trimethoprim is shown as 4.1267 ppm.

Although the RT and the accurate mass are strong indicators for trimethoprim, it is necessary to examine other compounds, which fit this accurate mass measurement [2]. A listing of all theoretically possible compounds, within the set element and composition limits that satisfy the listed mass within 5 ppm, is shown in Table 1.

Table 1. Possible Fits for 291.1440 Da (within 5 ppm)

Formula	Compound	Calculated mass	Error, mDa	Error, ppm
$C_{13}H_{23}O_7$		291.1438300	0.1701800	0.5845210
$C_{12}H_{17}N_7O_2$		291.1438240	0.1755640	0.6030130
$C_9H_{20}N_8OCl$		291.1443120	–0.3116530	–1.0704410
$C_{12}H_{25}N_3OS_2$		291.1433580	0.6423160	2.2061760
$C_{14}H_{27}O_2S_2$		291.1447000	–0.7003800	–2.4056090
$C_{14}H_{19}N_4O_3$	Trimethoprim	291.1451670	–1.1671320	–4.0087720

Six compounds fit within 5 ppm, but based on spectral observations from Figure 3B, the unknown cannot contain chlorine, thereby leaving five candidate compounds.

Results–Trap Confirmation

The next step is Confirmation. Here a fairly standard MS/MS technique can confirm the TOF data.

Using the Agilent Ion TRAP, the positive ion ESI MS data was acquired for the standards. The TIC is shown in Figure 4A, and the EIC, at nominal mass 291 *m/z*, is shown in Figure 4B.

The MS/MS spectra were acquired by the Agilent Ion Trap XCT by isolating and then fragmenting the parent ion at nominal mass 291. The resultant spectrum is shown in Figure 5.

Figure 6 shows extracted MS/MS chromatograms for the trimethoprim standard. Should an unknown show the correct RT and both transitions from nominal mass 291, there is good confirmation for trimethoprim.

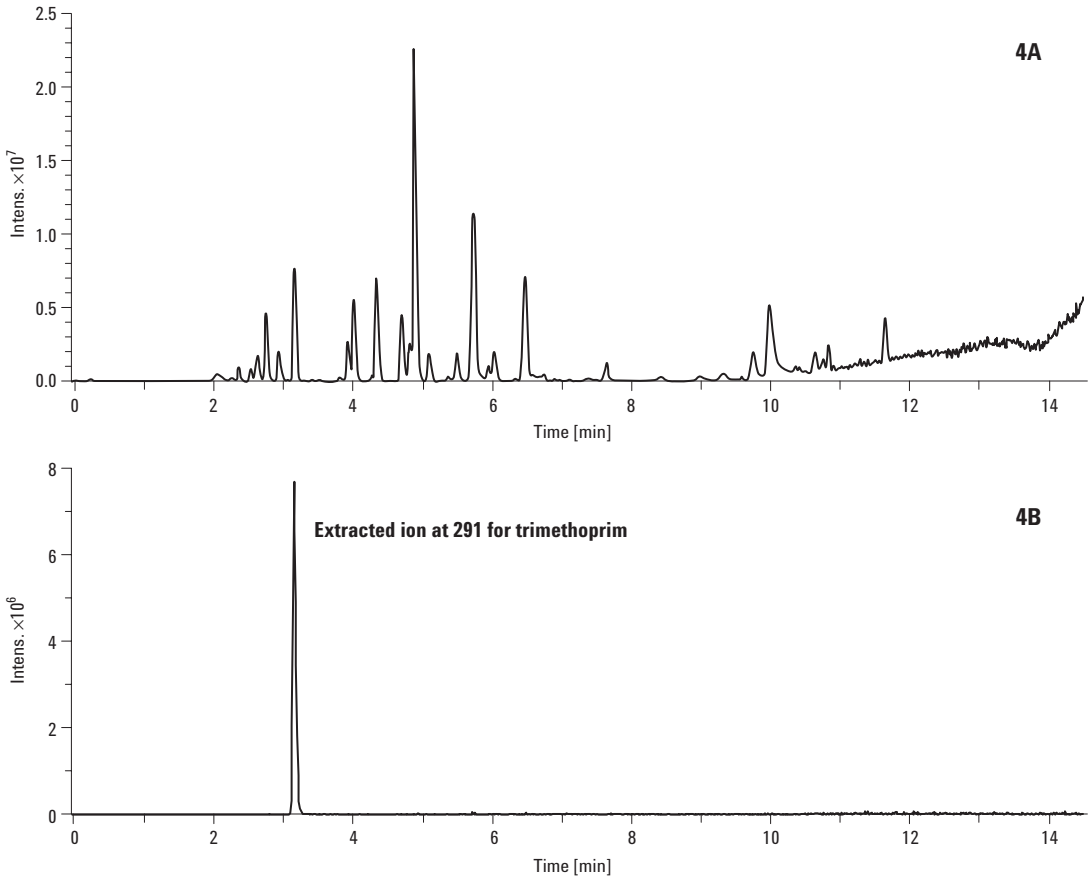


Figure 4. A standard TIC for the reference standard (4A), and an EIC for the target 291 ion (4B), noting the RT.

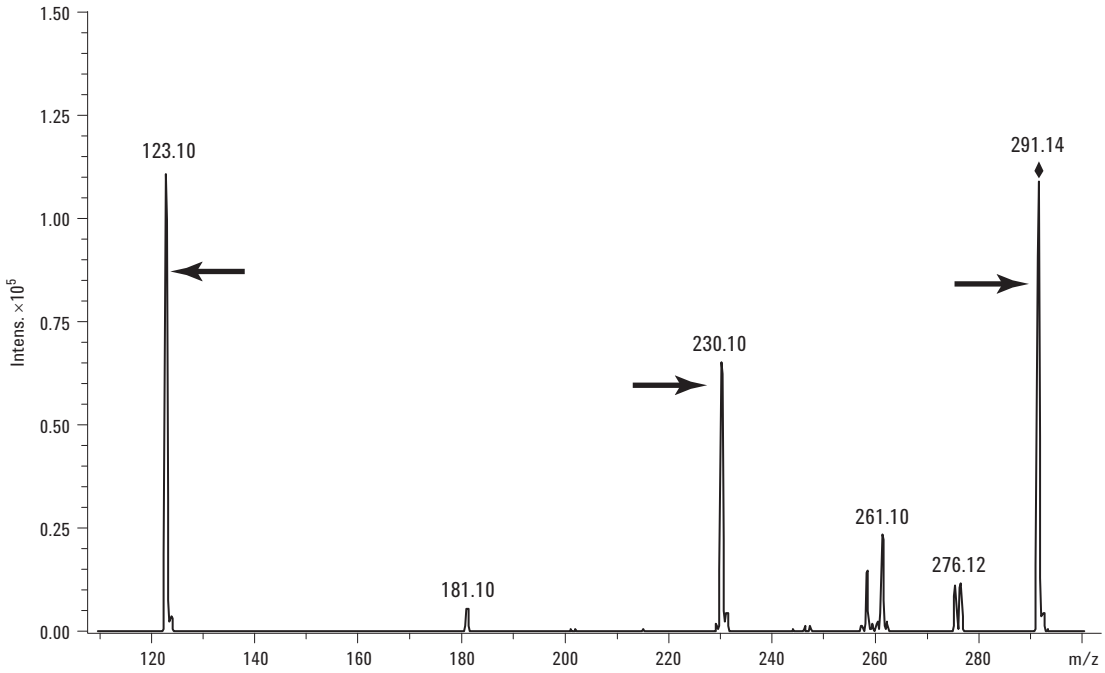


Figure 5. MS/MS spectrum of mass 291 via Ion Trap XCT. Note the indicated parent (291.14) and daughter ions (230.10 and 123.10).

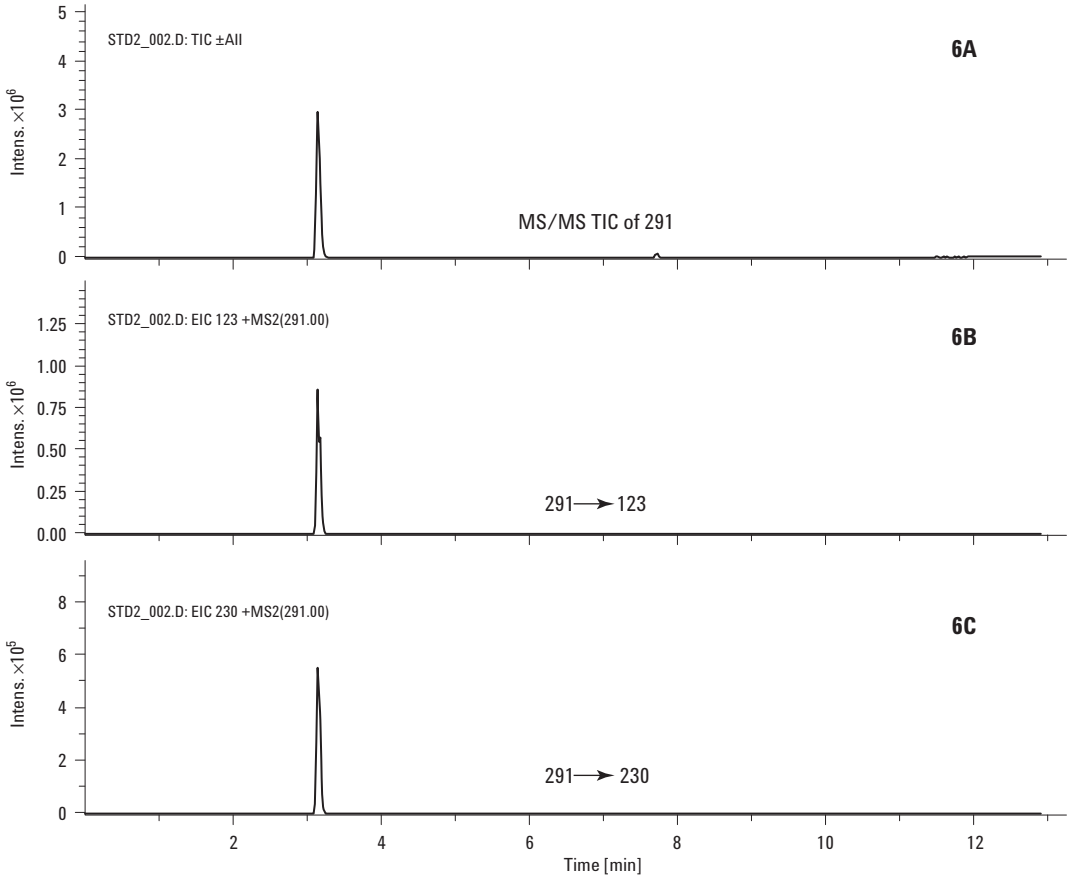


Figure 6. Confirmation of trimethoprim, showing parent and daughter ions at the same RT.

Now let us apply this knowledge to the sediment extract sample. See Figure 7. Figure 7A shows three overlaid chromatograms. The upper most chromatogram is the TIC of all products of 291 *m/z*. The other two indicate the EICs of the trimethoprim-specific transitions of 291→123 *m/z*, and 291→230 *m/z*. Only one compound fits all criteria.

With the Agilent Ion Trap it is not necessary to rely on just chromatographic signals. Even at this low level, MS/MS spectra can be acquired for superior confirmation, as is indicated in Figures 8A and 8B for peaks 1 and 2, respectively. The spectra show that only peak 2 has the critical 291→230 *m/z* ion transition, specific for trimethoprim.

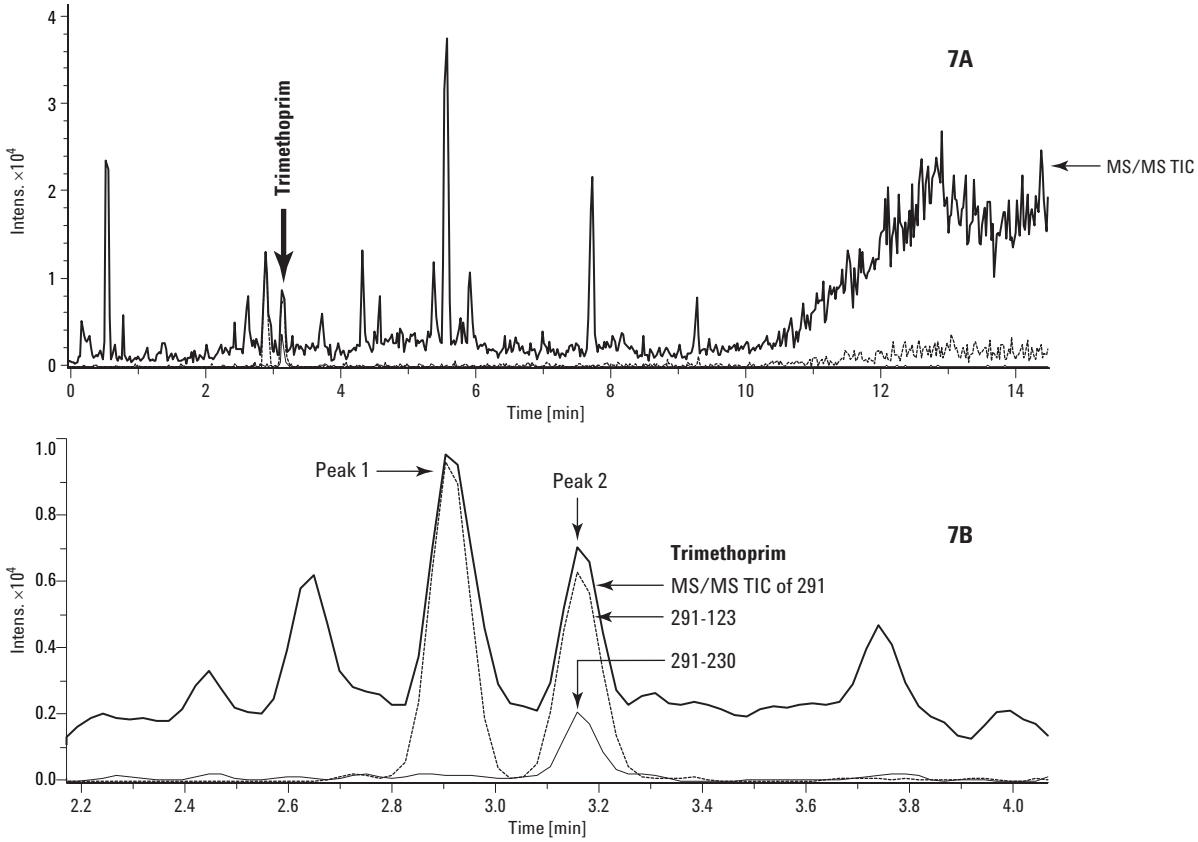


Figure 7. MS/MS chromatograms of the sediment sample for the three diagnostic ions.

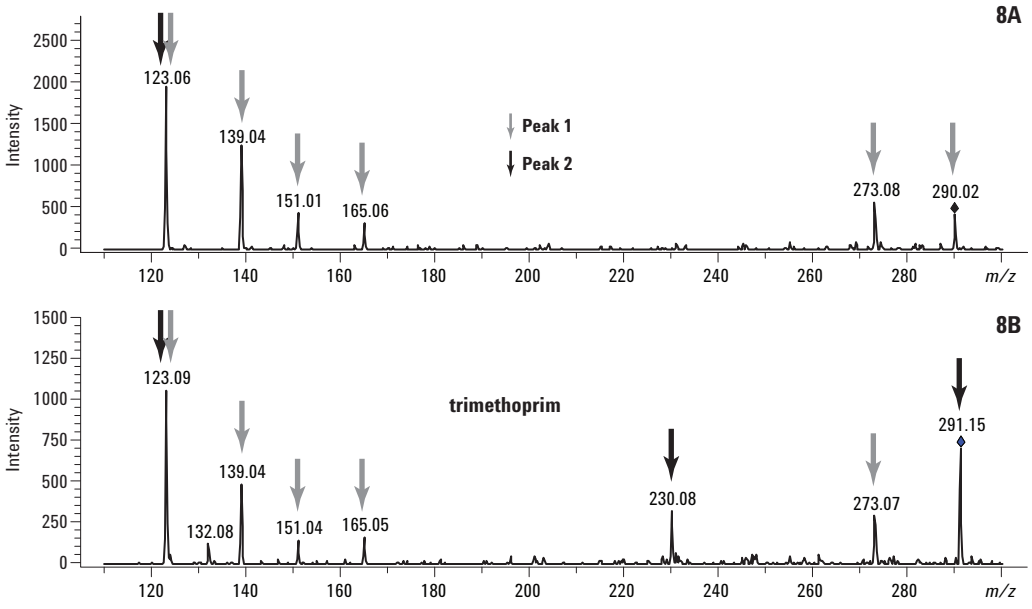


Figure 8. Mass spectra for the two peaks. Only Figure 8B, exhibits the diagnostic 230 ion. Therefore, only the second peak is trimethoprim.

Reasons for believing that PEAK 2 is confirmed as trimethoprim.

- Accurate mass data for trimethoprim, to within 5 ppm, at the RT for the trimethoprim standard.
- MS/MS spectrum containing ions consistent with trimethoprim. Unknown ions in MS/MS spectrum can be explained.
- It is, therefore, most reasonable to believe that PEAK 2 is trimethoprim.

Conclusion

This mode of reasoning to arrive at an identification of an unknown in a complex matrix is now a practical option made possible by the combined use of both Agilent's novel LC/MS TOF and LC/MS Ion TRAP XCT.

References

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2. D. McIntyre, “Automated Empirical Formula Confirmation Using the Agilent LC/MSD TOF,” Agilent Technologies, publication 5989-0625EN, www.agilent.com/chem
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