應用手冊

Rapid Characterization of Impurities in Synthesized Products for the Fine Chemicals Industry

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Abstract

This application note shows the potential of the Waters Atmospheric Solids Analysis Probe (ASAP) for the rapid identification of impurities in synthesized products followed by further characterization using UPLC-TOF/MS.

Introduction

Analytical laboratories studying the products of organic synthesis have to consider many things from confirmation of the final product to identification of impurities. Impurity identification, whether expected or not, is an essential part of the manufacture of fine chemicals, as any impurities could adversely effect the final product. This applies equally to the raw starting materials and the final synthesized product.

For many analytical laboratories, what is required is a rapid verification that the starting materials or synthesized products are the correct materials. At a later stage in the manufacturing process, the detection and identification of impurities becomes more critical. The QC laboratory for the bulk chemical manufacturer is looking to characterize the product for consistency, as well as quality control of reagents and intermediates. The products may have a wide range of chemical properties, placing significant demands on the analytical laboratory on the choice of analytical technique.

This application note introduces a means of obtaining rapid confirmation of the synthesized product, as well as detection of impurities, using the ASAP. This technique allows for rapid, direct analysis of both solid and liquid samples. It is particularly useful for compounds that are non-polar and not normally amenable to analysis by atmospheric pressure ionization techniques.

To fully characterize a sample, the use of additional techniques, such as nuclear magnetic resonance (NMR), infra-red spectroscopy, and LC-MS or GC-MS is required. Here, we show the application of high resolution UPLC-TOF/MS for further characterization of low level impurities.

Experimental

The sample analyzed was octahydroacridine (>97% purity), a compound of great interest as it plays an important role in the preparation of alkaloids, dyes, drugs, and other biologically active compounds.

MS System

Waters LCT Premier XE System

ASAP Conditions

Ionization mode:	ESCi
Sample cone:	30 V
Aperture 1 voltage:	10 V
Source temp.:	120 °C

Desolvation temp.:	50 to 450 °C
Desolvation gas flow:	500 L/hr
Capillary voltage (ESI):	3000 V
Corona current (APCI):	5 μA
Mass range:	*m/z* 50 to 1000
Scan time:	0.50 s
Inter-scan delay:	0.02 s
Lock reference (ESI):	Leucine enkephalin, 200 pg/μL at 3 μL/min

The instrument was operated in the combined electrospray/APCI mode (ESCi). This facilitated the acquisition of both analyte data in APCI mode and reference data (for lock mass correction) in electrospray mode.

The sample was loaded by dipping the melting point capillary from the ASAP into the solid sample; excess removed by blowing with a stream of nitrogen.

UPLC-MS Conditions

UPLC system:	ACQUITY UPLC
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 μm at 35 °C
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Acetonitrile + 0.1% formic acid

Flow rate:	0.6 mL/min
Gradient:	10% B (1 min hold) ⊠ 25% B at 3 min ⊠ 30% B at 4 min
Injection volume:	10 µL
Ionization mode:	ESI+

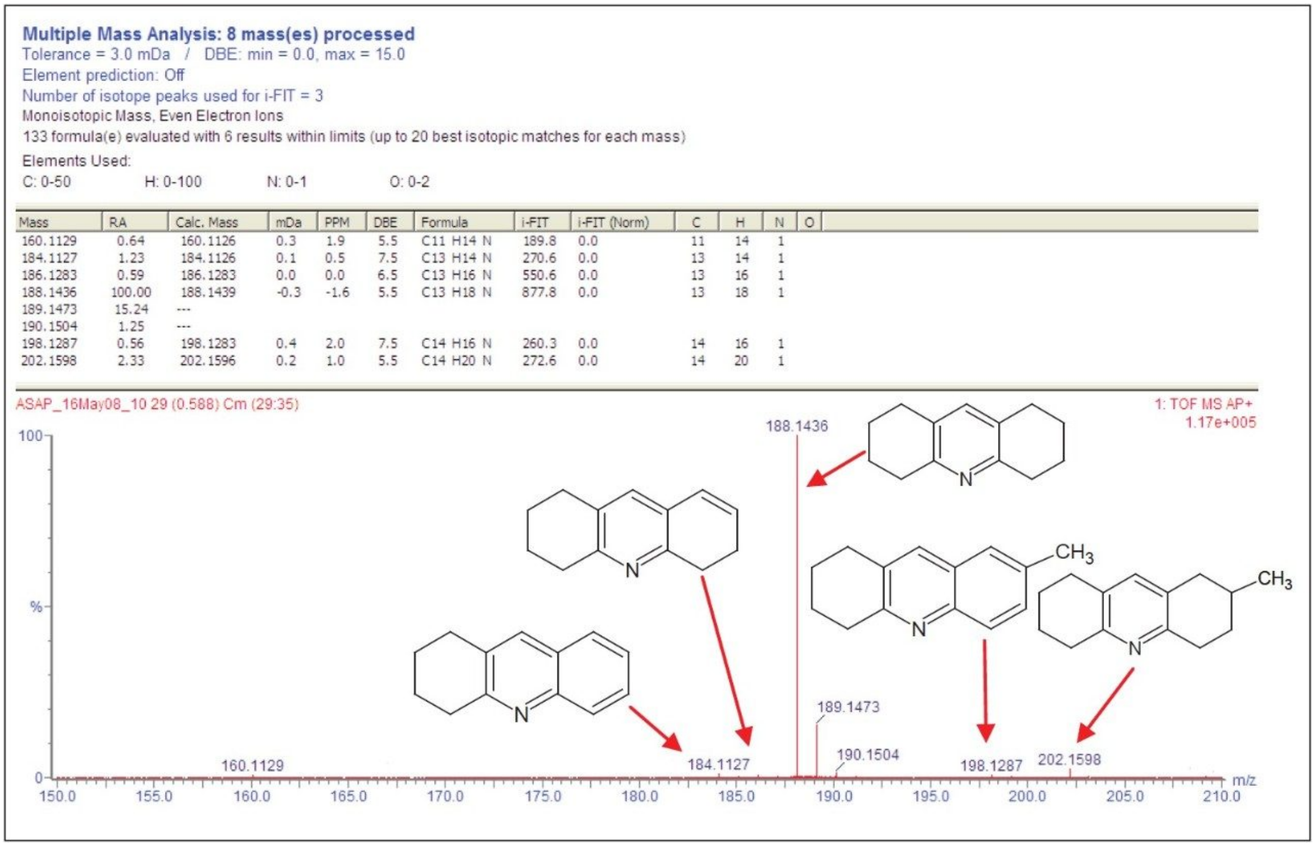
Standard electrospray MS conditions were used for the analysis. Data were acquired with aperture 1 voltages up to 60 V to generate in-source CID fragments for structural elucidation.

The sample was dissolved and diluted in methanol to produce a 6 ng/µL solution.

Results and Discussion

ASAP

The spectrum and elemental composition report from the ASAP analysis of the octahydroacridine solid are shown in Figure 1. The elemental compositions of all ions above 0.5% of the base peak intensity are reported. The spectrum illustrates the expected $[M+H]^+$ ion at *m/z* 188.1436 (-0.3mDa, -1.6ppm) for the major component as well as a number of low intensity peaks. These low intensity peaks could either be fragments or impurities. No elemental compositions are given for the ions at *m/z* 189 and 190, as these are the isotope peaks associated with the major component, but all other masses reported have exact mass measurements of <3 ppm.



Multiple Mass Analysis: 8 mass(es) processed
Tolerance = 3.0 mDa / DBE: min = 0.0, max = 15.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
133 formula(e) evaluated with 6 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 0-50 H: 0-100 N: 0-1 O: 0-2

Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT (Norm)	C	H	N	O
160.1129	0.64	160.1126	0.3	1.9	5.5	C11 H14 N	189.8	0.0	11	14	1	
184.1127	1.23	184.1126	0.1	0.5	7.5	C13 H14 N	270.6	0.0	13	14	1	
186.1283	0.59	186.1283	0.0	0.0	6.5	C13 H16 N	550.6	0.0	13	16	1	
188.1436	100.00	188.1439	-0.3	-1.6	5.5	C13 H18 N	877.8	0.0	13	18	1	
189.1473	15.24	---										
190.1504	1.25	---										
198.1287	0.56	198.1283	0.4	2.0	7.5	C14 H16 N	260.3	0.0	14	16	1	
202.1598	2.33	202.1596	0.2	1.0	5.5	C14 H20 N	272.6	0.0	14	20	1	

Figure 1. ASAP spectrum and elemental composition report for octahydroacridine and potential impurities with postulated structures.

UPLC-TOF/MS

The BPI chromatogram obtained from UPLC-MS analysis of octahydroacridine implies high compound purity, as shown in Figure 2, dominated by the expected compound along with several minor peaks.

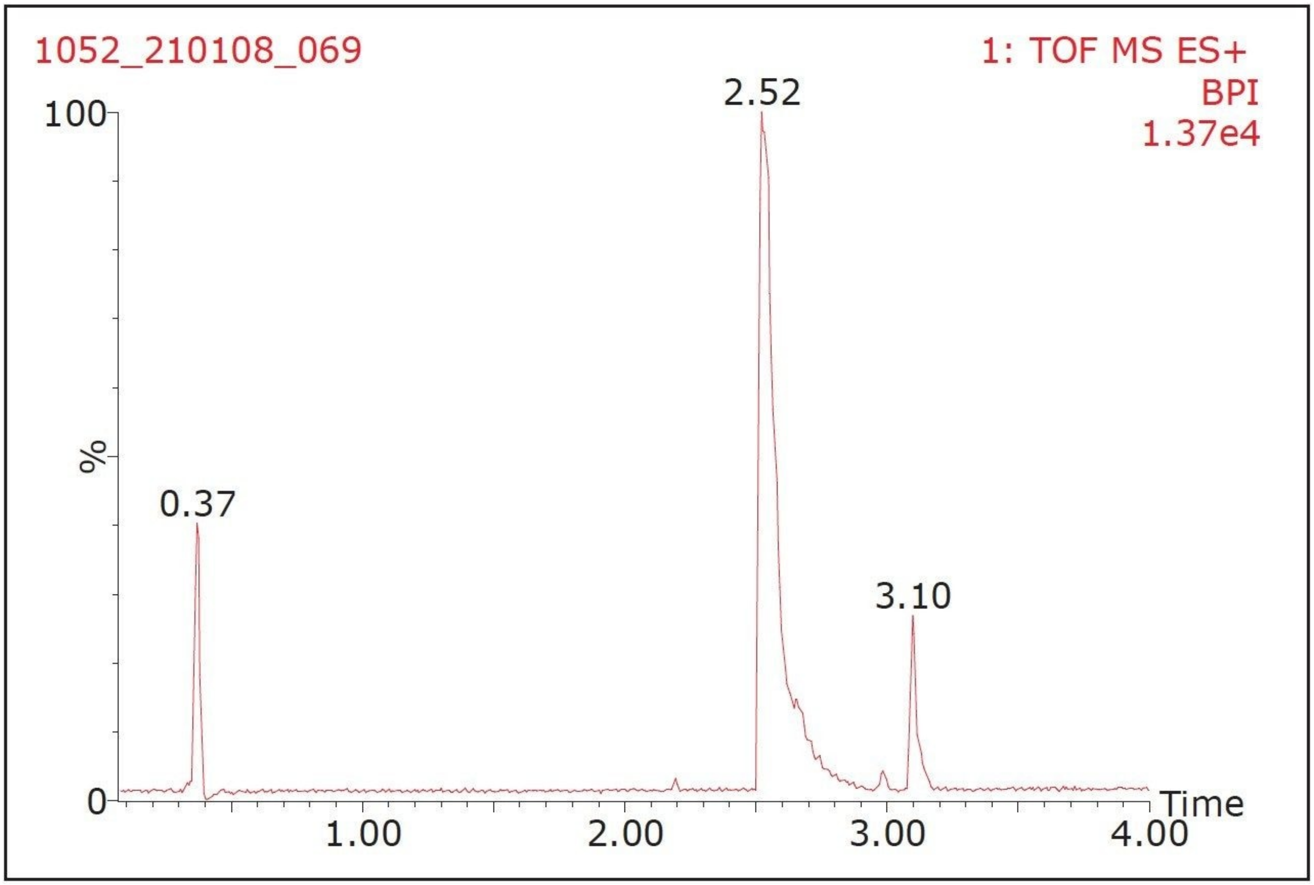


Figure 2. BPI chromatogram from UPLC-TOF/MS analysis of octahydroacridine.

In source, collision induced dissociation (CID) fragments can be generated by increasing the aperture 1 voltage, allowing some structural information about the compound to be determined, as shown in Figure 3. This approach confirms the ions at *m/z* 160 and 186, observed by ASAP, are the result of fragmentation of the major component.

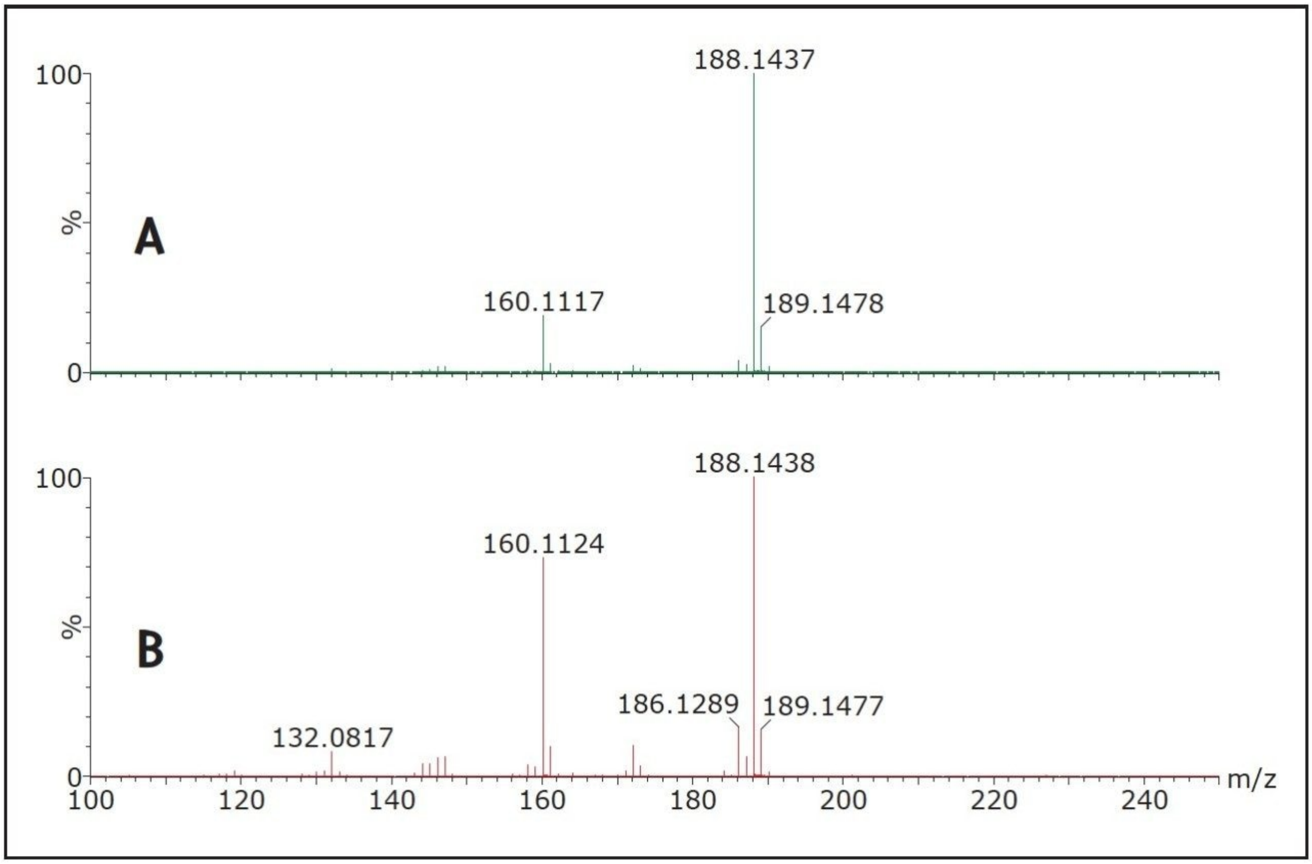
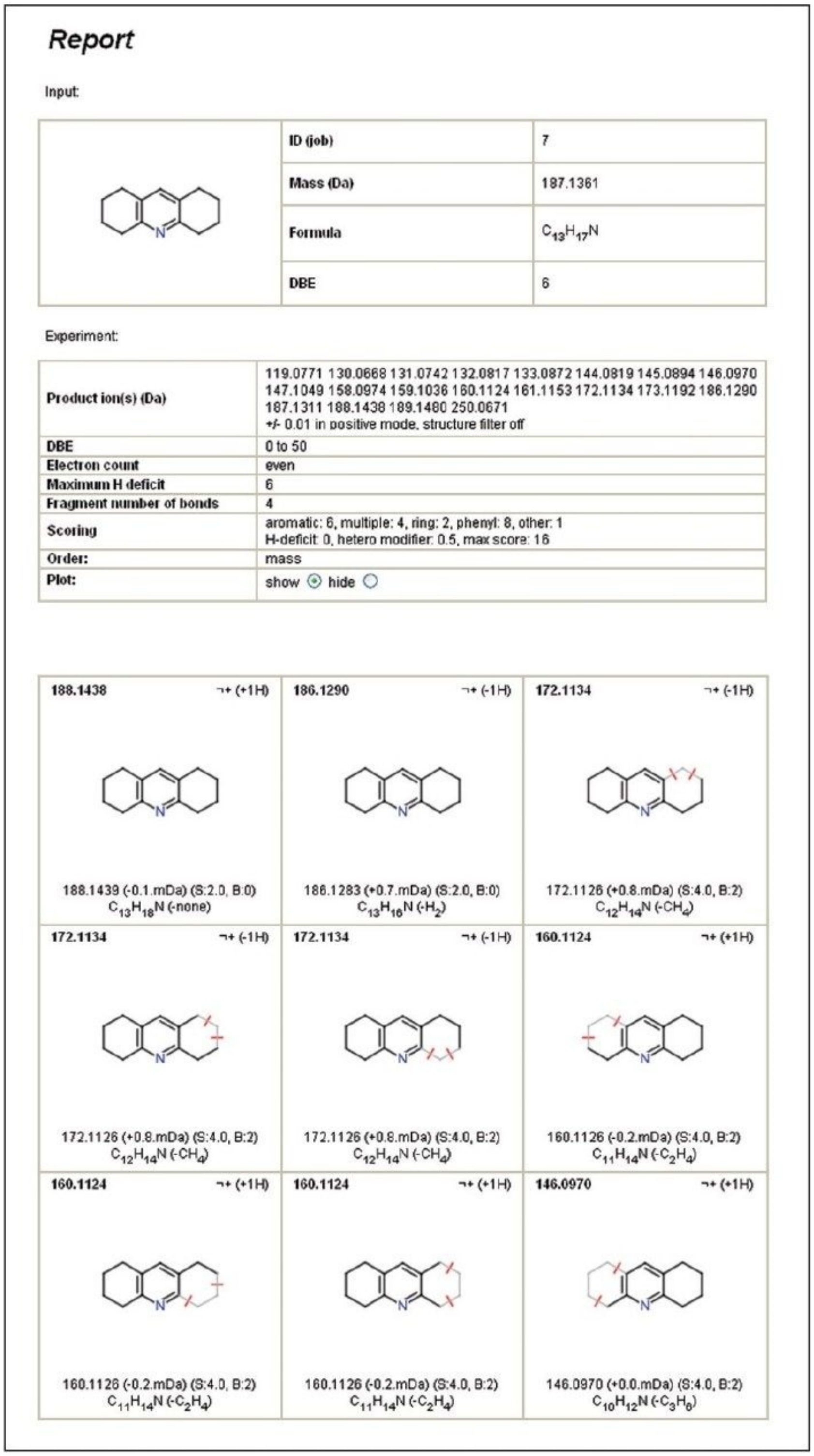


Figure 3. Spectra for octahydroacridine at aperture 1 voltages of 35 V (A) and 60 V (B) showing increasing fragmentation.

The automated structure elucidation tool, MassFragment Software, which uses a systematic bond cleavage and ranking algorithm, was employed to rationalize and identify fragment ion structures from potential impurities. By submitting a postulated precursor structure and a CID high energy spectrum, the MassFragment Software tool was able to generate a report of the possible fragmentation with exact mass confirmation. An extract from the MassFragment Software results from the major component, *m/z* 188, is illustrated in Figure 4.



Report

Input:

ID (job)	7
Mass (Da)	187.1361
Formula	$C_{13}H_{17}N$
DBE	6

Experiment:

Product ion(s) (Da)	119.0771 130.0668 131.0742 132.0817 133.0872 144.0819 145.0894 146.0970 147.1049 158.0974 159.1036 160.1124 161.1153 172.1134 173.1192 186.1290 187.1311 188.1438 189.1480 250.0671 +/- 0.01 in positive mode, structure filter off
DBE	0 to 50
Electron count	even
Maximum H deficit	6
Fragment number of bonds	4
Scoring	aromatic: 6, multiple: 4, ring: 2, phenyl: 8, other: 1 H-deficit: 0, hetero modifier: 0.5, max score: 16
Order:	mass
Plot:	show ● hide ○

188.1438 ¬+ (+1H) 188.1439 (-0.1.mDa) (S:2.0, B:0) $C_{13}H_{18}N$ (-none)	**186.1290** ¬+ (-1H) 186.1283 (+0.7.mDa) (S:2.0, B:0) $C_{13}H_{16}N$ ($-H_2$)	**172.1134** ¬+ (-1H) 172.1126 (+0.8.mDa) (S:4.0, B:2) $C_{12}H_{14}N$ ($-CH_4$)
172.1134 ¬+ (-1H) 172.1126 (+0.8.mDa) (S:4.0, B:2) $C_{12}H_{14}N$ ($-CH_4$)	**172.1134** ¬+ (-1H) 172.1126 (+0.8.mDa) (S:4.0, B:2) $C_{12}H_{14}N$ ($-CH_4$)	**160.1124** ¬+ (+1H) 160.1126 (-0.2.mDa) (S:4.0, B:2) $C_{11}H_{14}N$ ($-C_2H_4$)
160.1124 ¬+ (+1H) 160.1126 (-0.2.mDa) (S:4.0, B:2) $C_{11}H_{14}N$ ($-C_2H_4$)	**160.1124** ¬+ (+1H) 160.1126 (-0.2.mDa) (S:4.0, B:2) $C_{11}H_{14}N$ ($-C_2H_4$)	**146.0970** ¬+ (+1H) 146.0970 (+0.0.mDa) (S:4.0, B:2) $C_{10}H_{12}N$ ($-C_3H_6$)

Figure 4. MassFragment Software report from the CID spectrum of octahydroacridine.

The MassFragment Software report further confirmed the ions observed at *m/z* 160 and 186 are fragments of the major component.

The masses detected by ASAP were extracted as exact mass chromatograms (0.02 Da) and are illustrated in Figure 5. The presence of the potential impurities previously identified by ASAP at *m/z* 184, 186, 198, and 202 were confirmed, along with the fragments at *m/z* 160 and 186 from the major component.

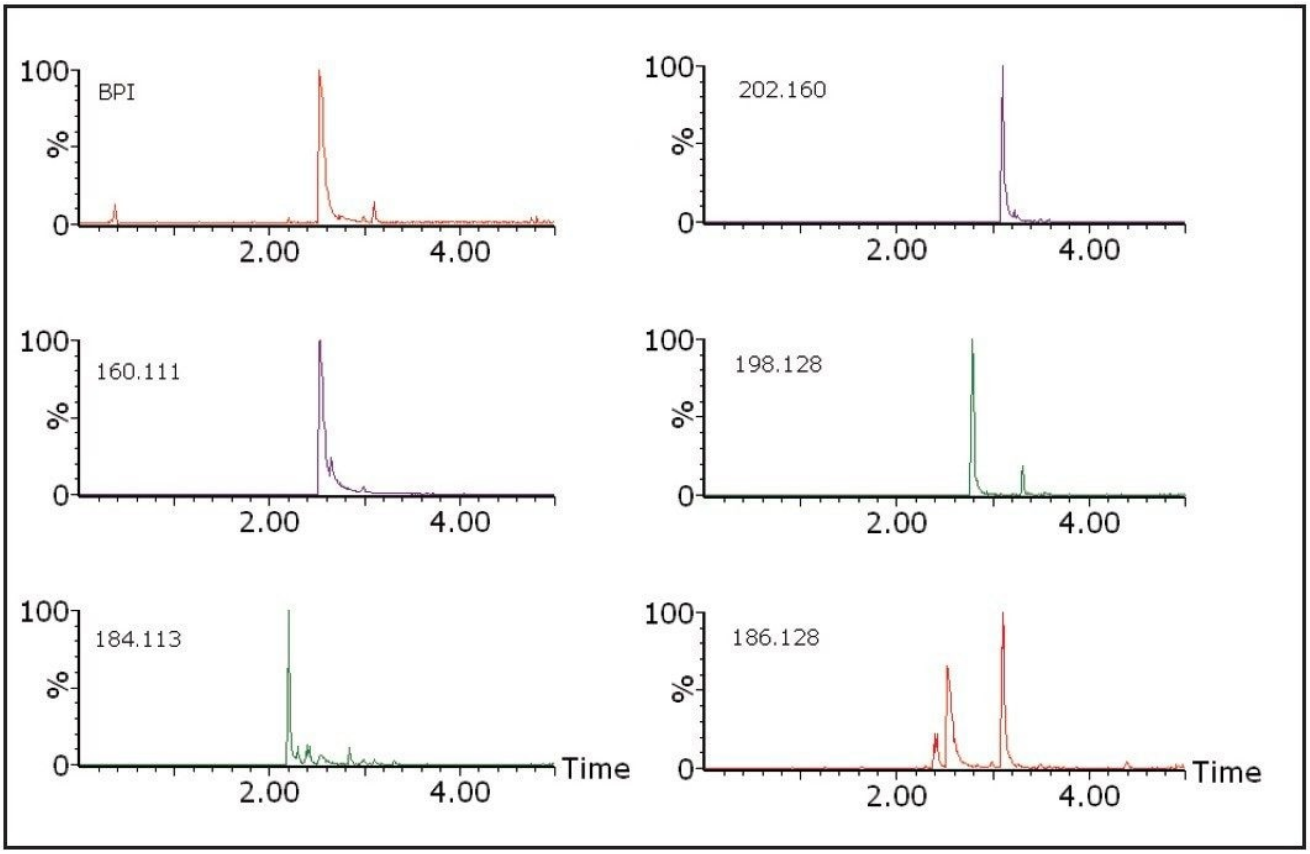


Figure 5. Extracted mass chromatograms of the masses detected by ASAP.

Conclusion

ASAP was used for the rapid analysis of a fine chemical without any sample preparation or chromatographic separation, enabling rapid confirmation of the synthesized product and detection of trace level impurities.

The synthesized product was further characterized by submitting it to analysis by UPLC-MS. Traditionally, this would have been a time-consuming task, requiring the development of chromatographic conditions to separate the impurities from the major product and interpretation of the MS data to give proposed structures.

The high resolution chromatography associated with UPLC enabled rapid separation of the impurities and, when coupled with the sensitivity and exact mass capabilities of the LCT Premier XE System, made this the ideal tool for impurity profiling.

Mass Fragment Software, a software package designed for the interpretation of fragmentation data, enabled rapid automated interpretation of the in-source CID data generated.

For production plants that require rapid confirmation of starting materials or manufactured product, ASAP, in conjunction with the LCT Premier XE System, provides a simple, rapid, high-resolution, cost-effective technique for the study of synthesized organic compounds. This approach combines the advantages of TOF technology – exact mass information, high energy fragment ions, and reliable isotopic patterns with a quick and easy way to introduce the sample – the Atmospheric Solid Analysis Probe.

When further analysis is required for the identification of unexpected impurities, whether as a result of manufacturing, contamination, or degradation, the enhanced resolution associated with UPLC facilitates rapid separation of these impurities from the expected product.

MassFragment Software, a software package specifically designed to facilitate the interpretation of fragmentation data, completes the solution.

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ACQUITY UPLC System <https://www.waters.com/514207>

MassFragment <https://www.waters.com/1000943>

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