Increased Identification of Impurities in Octyl Dimethyl p-Aminobenzoic Acid Using the Agilent 6140 High Throughput LC/MS

Application

Chemical Analysis

Authors

Michael Zumwalt
Agilent Technologies, Inc.
9780 S Meridian Blvd.
Englewood, Colorado 80112-5910
USA

Michael Woodman
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

The 6140 Single Quadrupole LC/MS High Throughput Mass Spectrometer is used to analyze octyl dimethyl para-aminobenzoic acid (OD-PABA) for the presence of impurities. The Agilent 1200 Series Rapid Resolution Liquid Chromatography (RRLC) system is used for the chromatographic separation of the compound from its impurities on a 3.0 mm id C18 column with a 1.8 µm-particle size. The LC/MS interface used in this work is a G1948B electrospray ionization source (ESI) in positive ion mode. While many compounds can be analyzed at the standard scan rate of 5400 amu/sec, one impurity is only clearly seen at the scan speed of 10,000 amu/sec, which is a unique capability of the 6140 system. This impurity is identified as p-dimethylbenzoic acid, a known degradate of octyl-dimethyl-p-aminobenzoic acid (OD-PABA).

Introduction

Para-aminobenzoic acid (PABA) has historically been used as an ultraviolet filter ingredient in sunscreen formulations. As its use can increase the risk of skin cancer, a derivative in the form of OD-PABA is currently and more commonly used. However, as PABA may be formed as a degradate of OD-PABA, it is important to monitor its potential presence in neat standards of OD-PABA. As a commercial product, the purity of OD-PABA is important to manufacturers, not only for the purpose of safety, but for economics as well. In this work we investigate the capability of the Agilent 6140 Single Quadrupole Mass Spectrometer to detect impurities that are seen above 0.1% of the OD-PABA absorbance level in UV.

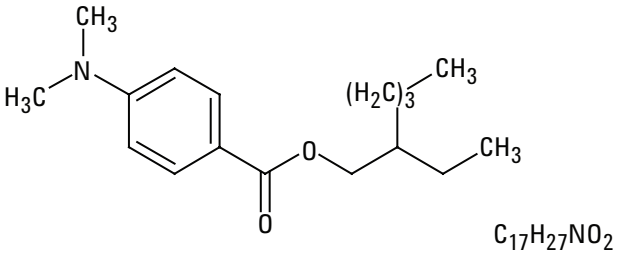


Figure 1. Octyl-dimethyl-p-aminobenzoic acid (OD-PABA).

The structure of the OD-PABA compound analyzed in this work is shown in Figure 1.

Experimental

Sample Preparation

The OD-PABA is obtained at a concentration of 1 mg/mL in methanol. Injection volumes of 5 µL at this concentration are made into the LC/MS system.

LC/MS Method Details

LC Conditions

Agilent 1200 Series binary pump SL, wellplate sampler, thermostatted column compartment

Column:	Agilent ZORBAX SB-C18, 3 × 30 mm, 1.8 µm (p/n 824975-302)
Column temp:	45 °C
Mobile phase:	A = 0.1% formic acid in water B = 0.1% formic acid in acetonitrile
Flow rate:	1.0 mL/min
Injection volumn:	5 µL

Gradient:	Time (min)	%B
	0	25
	7	75

Stop time:	7 min
Post-run time:	2 min

UV Conditions

Sample: 320 nm; Bw, 5 nm; reference off

MS Conditions

Mode:	Positive ESI using the Agilent G1948B ionization source
Nebulizer:	60 psig
Drying gas flow:	12 L/min
Drying gas temp:	350 °C
V_{cap}:	3000 V
MS Scan:	*m/z* 100–450

Cycle times (sec/cycle), 0.09 in Standard Fast Scan mode; 0.04 in Ultra Fast Scan mode

Table 1. Integration Results of Three Significant Peaks Found in OD-PABA Chromatogram of Figure 2

Peak #	Time (min)	Area	Height	Area %
1	0.707	76.4	44.1	0.4
2	5.184	176.6	50	0.925
3	6.005	18847.7	2911.3	98.676

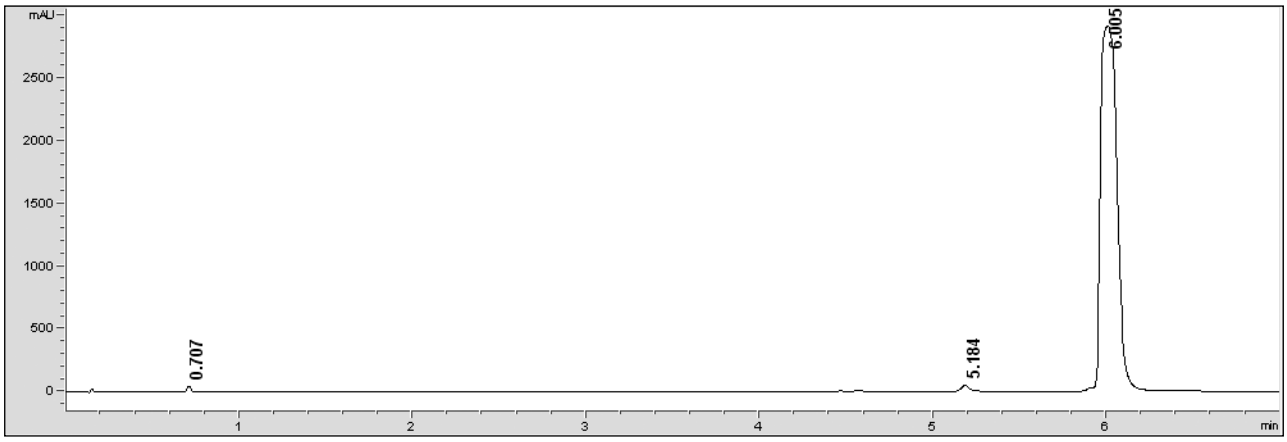


Figure 2. UV chromatogram of OD-PABA at 320 nm absorbance.

Results and Discussion

The UV absorbance of OD-PABA is shown at a retention time of 6.005 minutes in Figure 2. The tabulated integration results for each of the peaks shown are given in Table 1.

The highest data acquisition speed in the Standard Fast Scan mode (5,400 amu/sec) is 0.09 sec/cycle. The total ion chromatogram (TIC) corresponding to this mode is shown in Figure 3A. The Ultra Fast Scan mode (10,000 amu/sec) is only available in the Agilent 6140 mass spectrometer and has a corresponding cycle time of 0.04 sec/cycle. The total ion chromatogram corresponding to the Ultra Fast Scan mode is shown in Figure 3B.

While the Standard Fast Scan is adequate for detecting the peaks at 5.184 and 6.005 minutes in the UV chromatogram, and a few more peaks are detected as well (4.379, 4.478, 4.594, and 5.616 min in the TIC of Figure 3A), the peak at 0.707 minutes in the UV chromatogram is much more easily seen in the Ultra Fast Scan mode of Figure 3B. This is because the earlier eluting peak at 0.707 minutes has a relatively narrower peak width so that the scan speed must be higher to adequately detect signal in such a narrow window of time.

It should be noted that while the faster scan speed in Ultra Fast Scan mode results in the acquisition of more data points across the ion chromatogram, the variation in amount of signal from scan to scan is larger because the amount of time involved with collecting signal is reduced. When less ions are collected during each cycle, the variation in signal from one cycle to the next is larger. As a result, Figure 3B shows more variation of the baseline signal in comparison to Figure 3A.

In Figure 4 an overlay of the two TICs is shown with the region around the 0.707 min peak (0.732 min in the MS) expanded. With more data points acquired in the Ultra Fast Scan mode of the 6140 Single Quadrupole, the impurity peak is more readily seen.

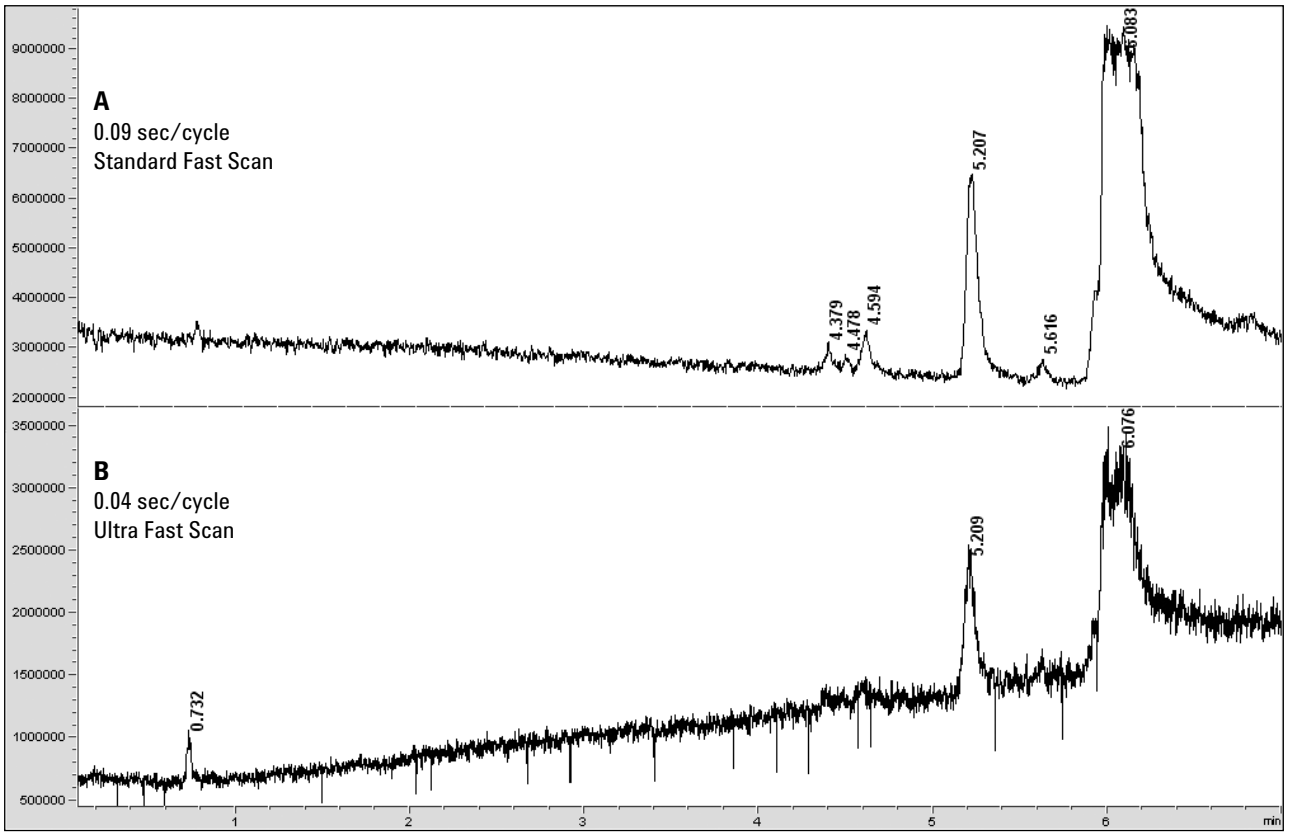


Figure 3. The total ion chromatograms (TICs) corresponding to the highest acquisition speed of the Standard Fast Scan mode (A) and the Ultra Fast Scan mode (B).

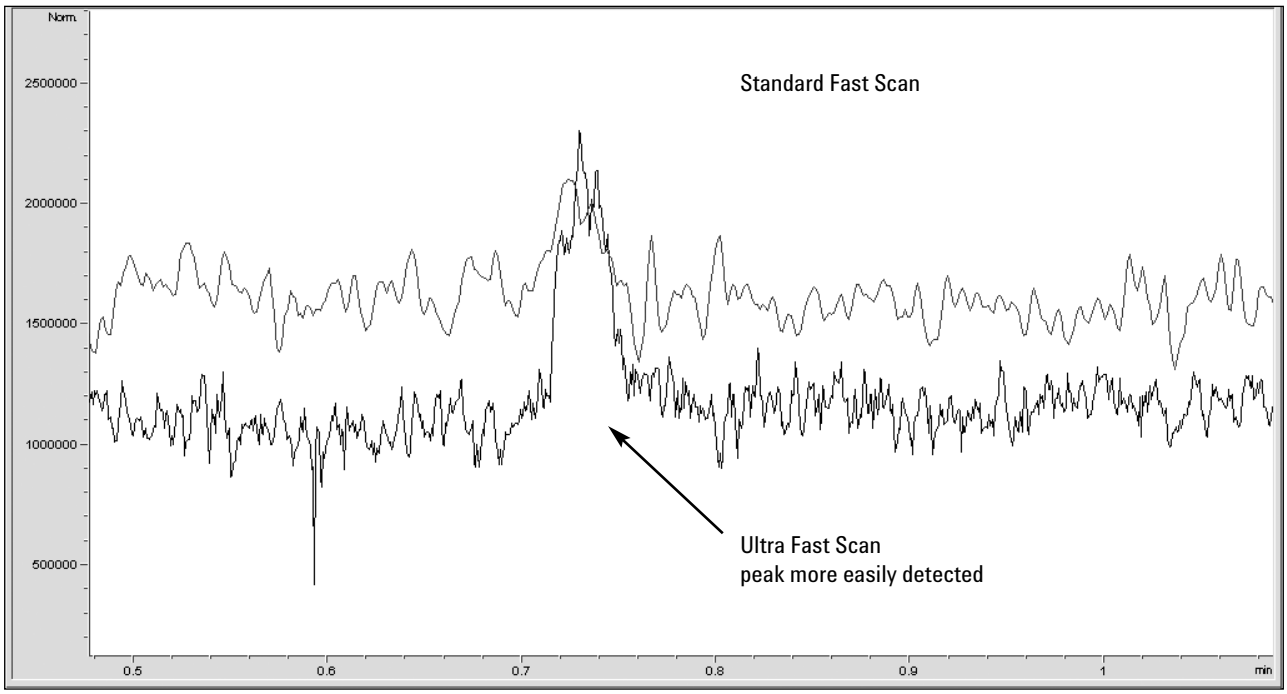


Figure 4. An overlay of the TICs in the expanded region around the peak seen at 0.707 min in the UV chromatogram (Figure 2). The peak is more easily detected in the Ultra Fast Scan mode.

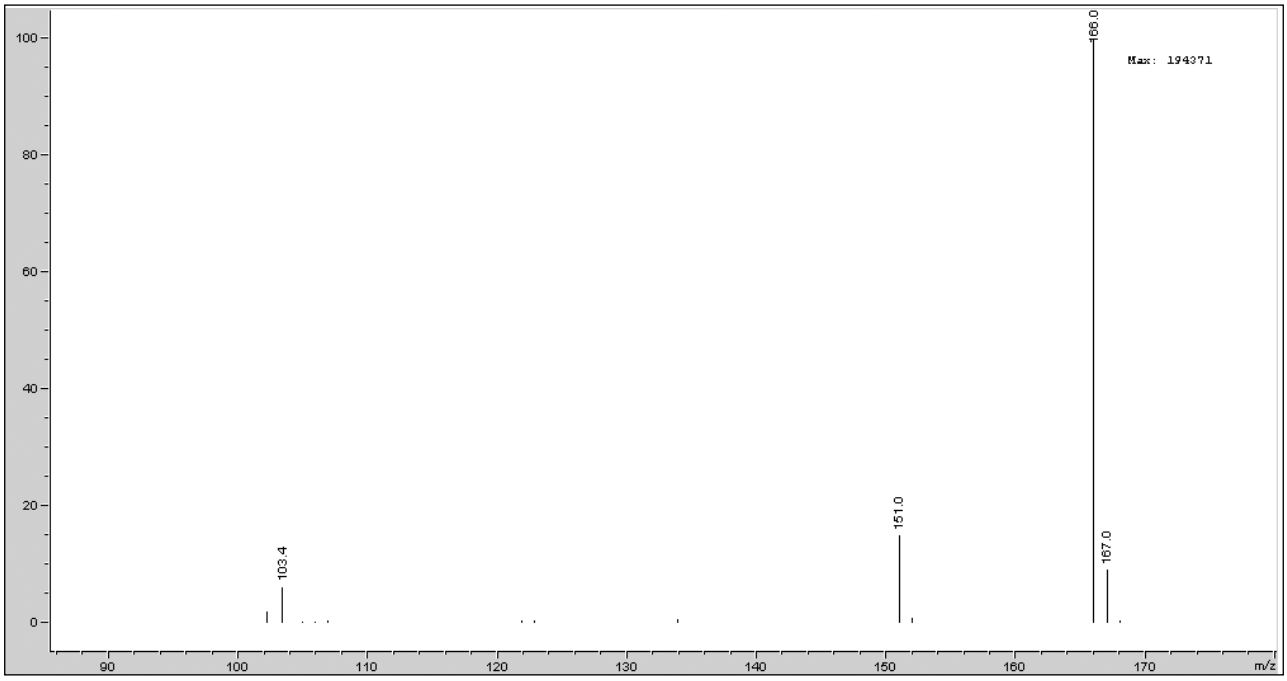
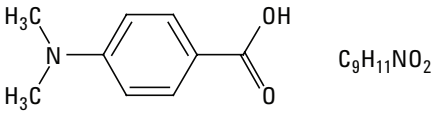


Figure 5. Background subtracted averaged spectrum in Ultra Fast Scan mode of peak at ion chromatogram peak at 0.732 minutes (0.707 minutes in UV).

According to the integration results in Table 1, the peak at 0.707 minutes of the UV chromatogram has a percent relative area of 0.4 % and should be considered an impurity requiring further investigation. A background subtracted spectrum of this



$C_9H_{11}NO_2$

Figure 6. Structure of p-dimethyaminobenzoic acid, which has a protonated ion mass $[M + H]^+$ of 166.0 in positive ion mode using electrospray.

peak is derived from the Ultra Fast Scan mode acquisition and shown in Figure 5.

The *m/z* 166.0 peak clearly dominates the spectrum of Figure 5. A possible structure corresponding to this *m/z* value is shown in Figure 6. This structure corresponds to p-dimethylaminobenzoic acid, which is a known degradate of OD-PABA.

Conclusions

Detection of impurities is enhanced at higher acquisition speeds in mass spectrometry. This work demonstrates the usefulness of the Ultra Fast Scan mode (10,000 amu/sec) in detecting a relatively narrow peak impurity, eluting early (0.707 minutes) in the analysis of the OD-PABA neat standard. The peak, which clearly surpasses the 0.1% area cutoff in the UV chromatogram, is easily detected in the Ultra Fast Scan mode of the Agilent 6140 Single Quadrupole Mass Spectrometer. Upon analysis of the background subtracted averaged spectrum under this peak, an *m/z* 166 ion is clearly observed and believed to be p-dimethylaminobenzoic acid, a known degradate of the OD-PABA compound.

For More Information

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For more details concerning this application, please contact Michael Zumwalt at Agilent Technologies, Inc.

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