Using Software Tools to Improve the Detection of Impurities by LC/MS

Application Note

Christine Miller
Agilent Technologies

Introduction

The analysis of raw materials and finished products for minor impurities presents a challenge in many industries. Liquid chromatography/mass spectrometry (LC/MS) is often the technique of choice because of its broad sample applicability, separating power, and ability to provide structural information. However, without advanced software tools, it is difficult to locate minor impurities in the presence of chemical noise and high-level components. This application brief demonstrates the use of the Agilent 1100 Series LC/MSD SL quadrupole mass spectrometer with ChemStation software for the analysis of minor components in a partially decomposed sample of the bronchodilator salbutamol.

Experimental

The analysis was performed using an Agilent 1100 Series LC/MSD SL. A decomposed stock solution of salbutamol sulfate (10 mg/ml) was diluted in water to 100 pg/µl and injected directly into the LC/MSD. The sample was analyzed in positive electrospray ionization (ESI) mode. Data analysis was performed using the LC/MSD ChemStation software.

Agilent Technologies

Results and Discussion

Figure 1A shows the total ion chromatogram (TIC) for the analysis of the decomposed salbutamol sample. While there were hints of several peaks in the 6.9 to 9.5 minute range, the impurities were largely obscured in the chromatographic baseline. A two-step ChemStation data analysis procedure was used to extract meaningful information from this chromatogram. The first step involved the background subtract (BSB) routine, which subtracts the selected background spectrum from each point in the entire MS data set and saves the results as a separate file so that the original data is always preserved. For this sample, an averaged spectrum from near the apex of the salbutamol peak was background subtracted resulting in greatly enhanced visualization of the impurities (Figure 1B).

Second, the "Overlay base peak chromatogram" (BPC) feature was used to further improve the detection of impurities. The BPC is constructed from the base peak abundance of each scan in the analysis, where the base peak in a spectrum is the ion with the maximum abundance. Creating the BPC of the background-subtracted data for the salbutamol analysis revealed that there were eight or more impurities previously hidden in the chromatographic baseline (Figure 1C).

LC/MS ANALYSIS METHOD

Chromatographic Conditions

Column:	50 x 2.1 mm ZORBAX® SB-C18, 5 µm (p/n 860975-902)
Mobile phase:	A = 0.1% formic acid in water B = 0.1% formic acid in methanol
Gradient:	Start with 4% B at 0.5 min 4% B at 10 min 20% B
Flow rate:	0.250 ml/min
Column temp:	40°C
Injection vol:	0.5 µl
Diode-array detector:	Signal 220, 10 nm

MS Conditions

Source:	ESI positive
Drying gas flow:	10 l/min
Nebulizer:	25 psig
Drying gas temp:	350°C
Vcap:	2000 V
Step size:	0.1
Peak width:	0.1 min
Time filter:	On
Scan:	*m/z* 100–500
Fragmentor:	100 V

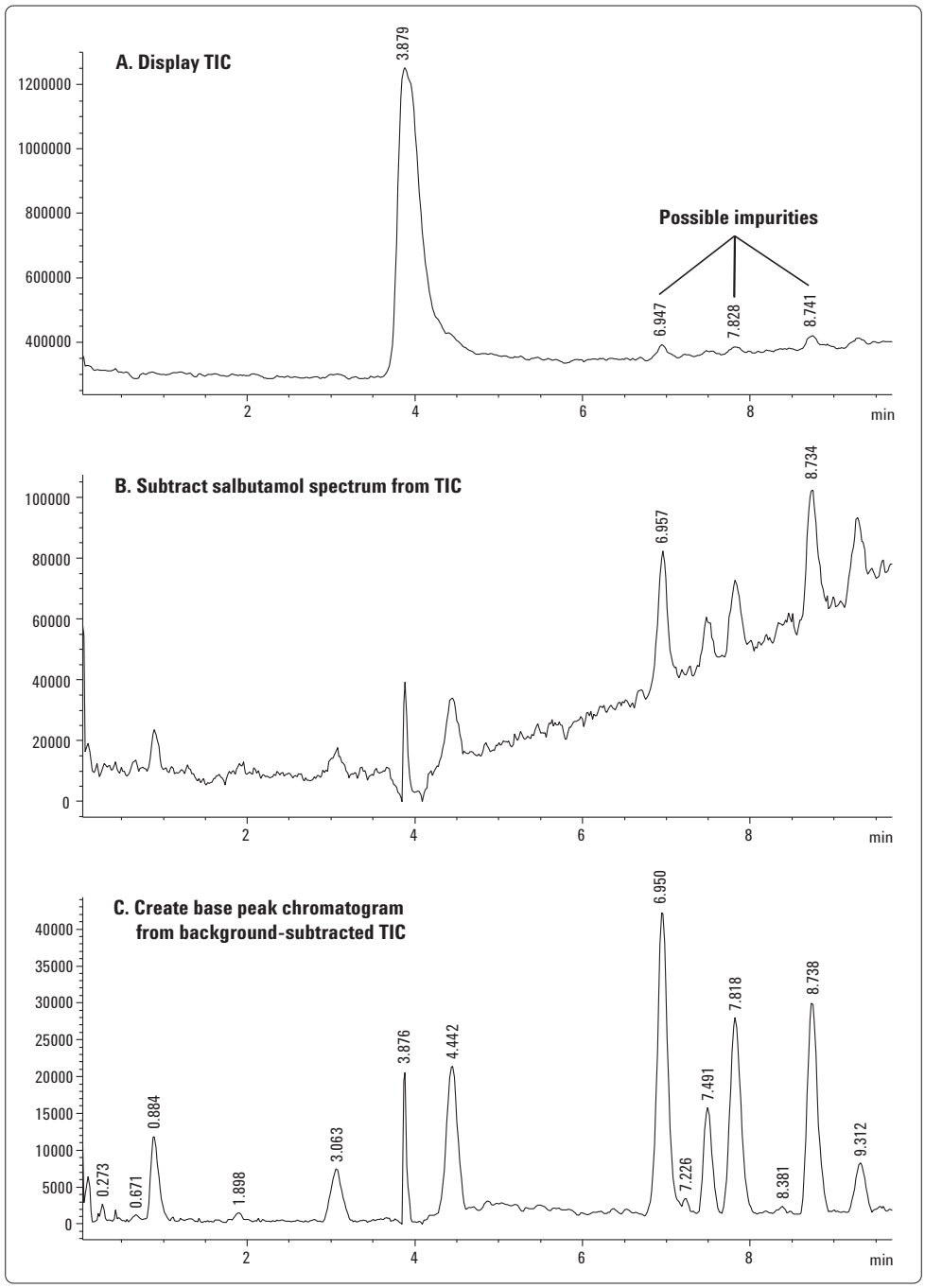

Figure 1. Sequence of steps to extract impurity peaks from total

Figure 2 shows the pseudomolecular ion for each chromatographic peak as well as spectra for some of the impurities. While this analysis was sufficient to satisfy the immediate need, further structural information for the impurities could have been obtained by increasing the LC/MSD fragmentor voltage to generate collision induced dissociation or by using the MS/MS capabilities of an Agilent 1100 Series LC/MSD Trap ion trap mass spectrometer.

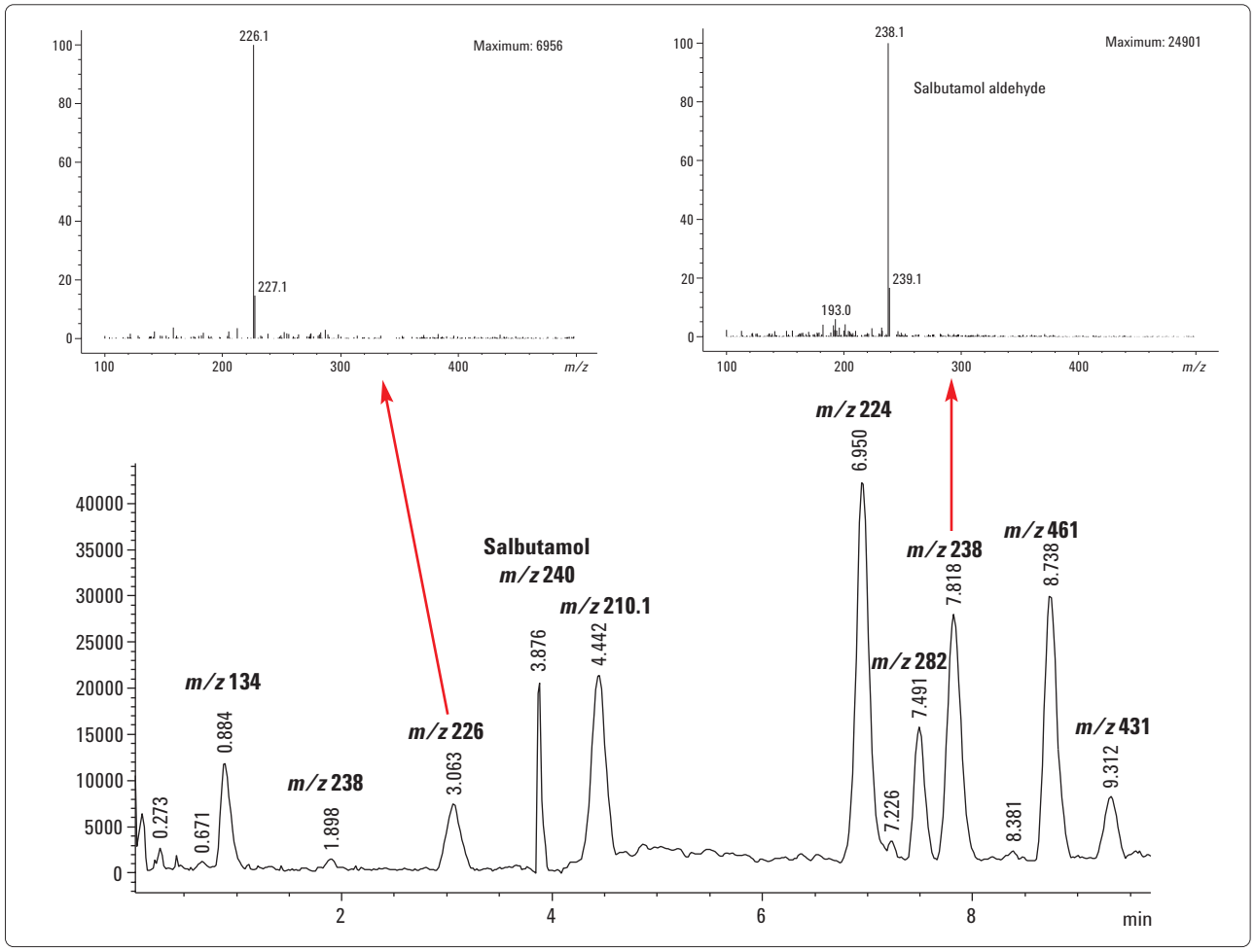

Figure 2. Mass spectral information for salbutamol impurities

Conclusions

This work demonstrates that the LC/MSD ChemStation software is a powerful system for analysis of sample impurities. The ChemStation software provides useful tools for locating minor components previously hidden in the chromatographic baseline.

Author

Christine Miller is an applications chemist at Agilent Technologies in Palo Alto, California U.S.A.

www.agilent.com/chem

Printed in the U.S.A. August 16, 2002
5988-7611EN

Agilent Technologies