application

Agilent Technologies
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Liquid Chromatography | Nonvolatile Buffers

The Effect of Nonvolatile Buffers on the Agilent 1100 Series LC/MSD

Introduction

Nonvolatile buffers are routinely used in HPLC analyses. For LC/MS applications, volatile buffers are generally required. However, there are times when it is necessary to run unmodified LC methods.

The Agilent 1100 Series LC/MSD was designed to be a rugged instrument suitable for self-service mass spectrometry. In order to make the instrument suitable for occasional use by non-MS specialists, it was designed with a low-maintenance, minimal adjustment source.

The HP 1100 Series instrument introduced a new spray geometry. In contrast to previous electrospray sources in which the sample is introduced in-line with the quadrupole axis, the LC/MSD spray is perpendicular to it. See Figure 1. This design reduces the buildup of deposits at critical points in the system. Only the charged ions are directed into the analyzer. Although volatile buffers are still recommended for normal operation, the orthogonal design is much more forgiving of non-volatile buffers for occasional use.

This study looks at the effects of using phosphate buffer in the analysis of theobromine, theophylline, and caffeine.

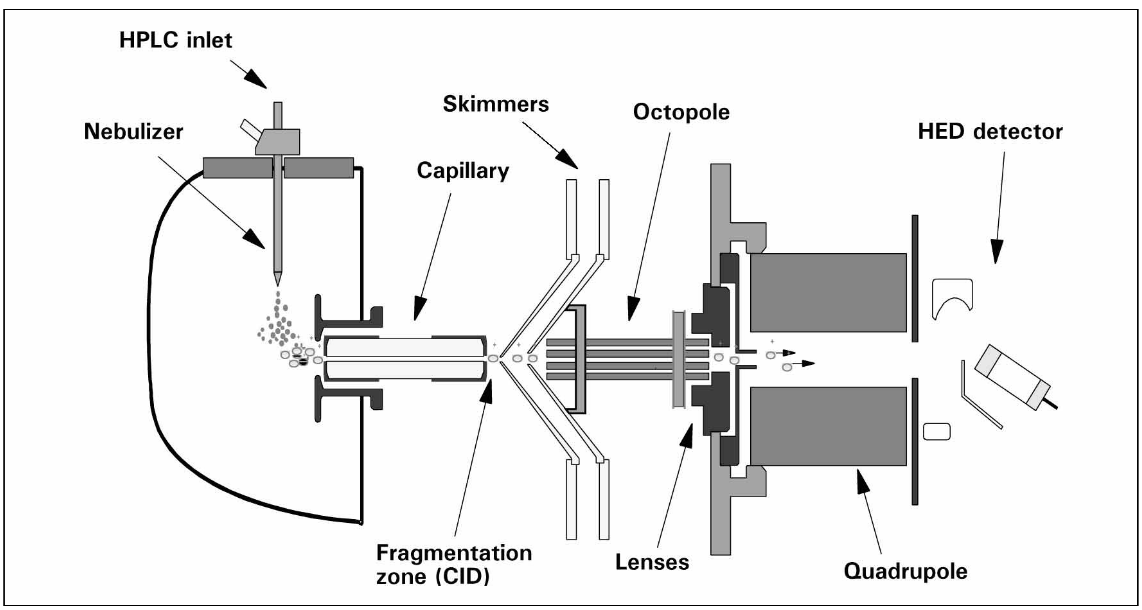

Figure 1. Agilent 1100 Series LC/MSD electrospray interface with orthogonal spray.

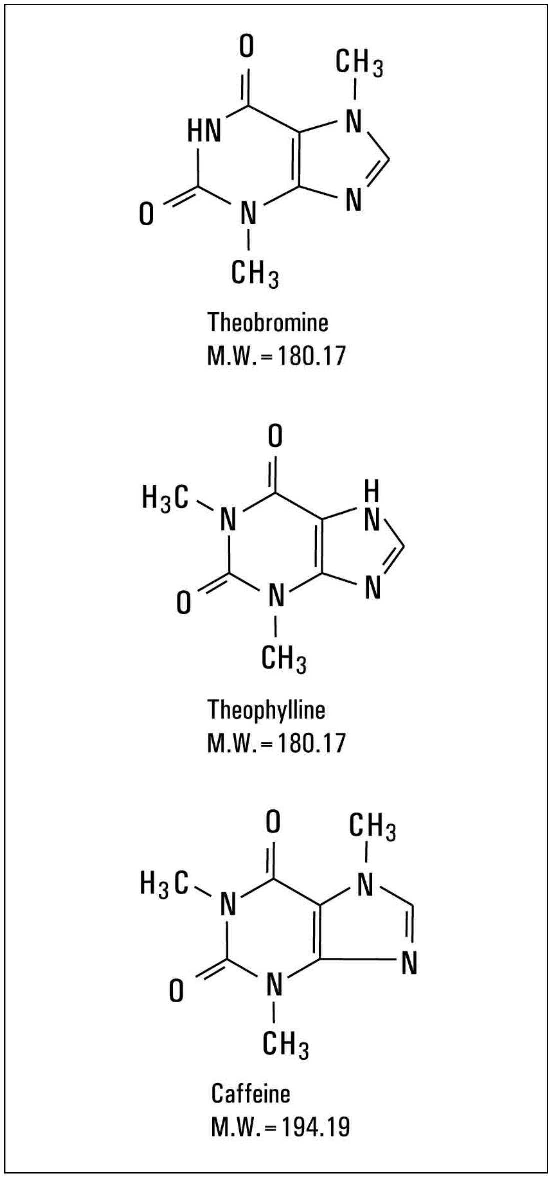

Figure 2. Chemical structure of test compounds.

Chromatographic Conditions	
Column:	2.1 mm x 150 mm Zorbax Eclipse XDB-C18, 5 µm
Mobile phase:	20% methanol in 5 mM ammonium acetate (pH 4.6) or 20% methanol in 5 mM potassium phos phate (pH 4.6 or 2.5)
Flow rate:	0.2 ml/min
Injection vol:	5 µl
MS Conditions	
Source:	ESI
Ion mode:	Positive
Vcap:	3500 V
Nebulizer:	50 psig
Drying gas flow:	120 l/min
Drying gas temp:	350°C
Scan range:	100 - 200 amu

Experimental

The system was composed of an Agilent 1100 Series binary pump, vacuum degasser, autosampler, thermostatted column compartment, diode-array detector, and LC/MSD. The LC/MSD was used with an electrospray ionization (ESI) source. Complete system control and data evaluation were done on the Agilent ChemStation for the LC/MSD.

Theobromine, theophylline, and caffeine were prepared at a concentration of 10 ppm. See Figure 2. Experiments were done using ammonium acetate and potassium phosphate buffers (pH 4.6 and 2.5) in the mobile phase.

Results and discussion

Figure 3 shows a comparison of relative responses for the three target compounds in 5 mM ammonium acetate and potassium phosphate buffers at pH 4.6. The response with the volatile acetate buffer is much higher than with the nonvolatile phosphate buffer. When the pH of the phosphate buffer was changed to 2.5, the response improved. See Figure 4. The response changes with pH because the ionization efficiency is affected. The mass spectra of the peaks show the expected protonated molecular ion for the three compounds. See Figure 5.

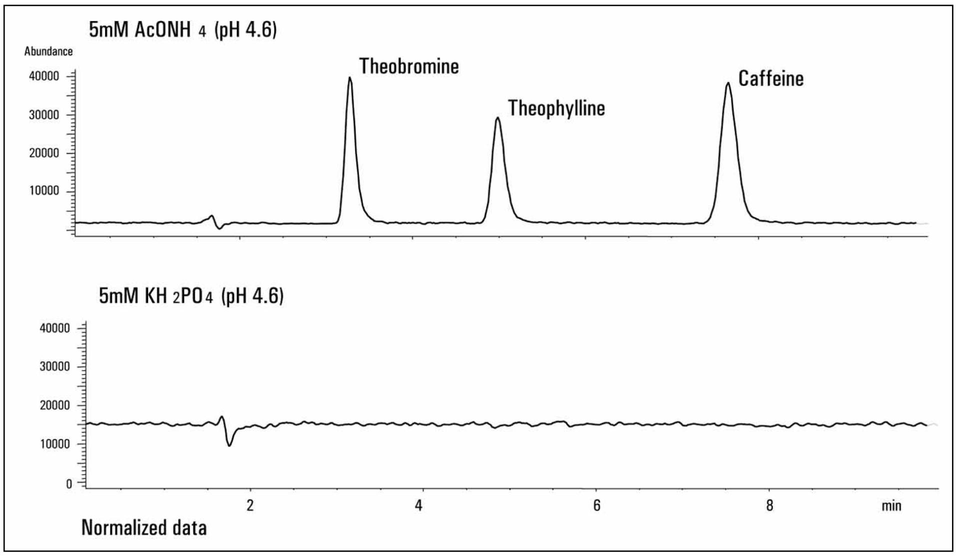

Figure 3. MS Signal (TIC) for ammonium acetate & potassium phosphate buffers (pH 4.6).

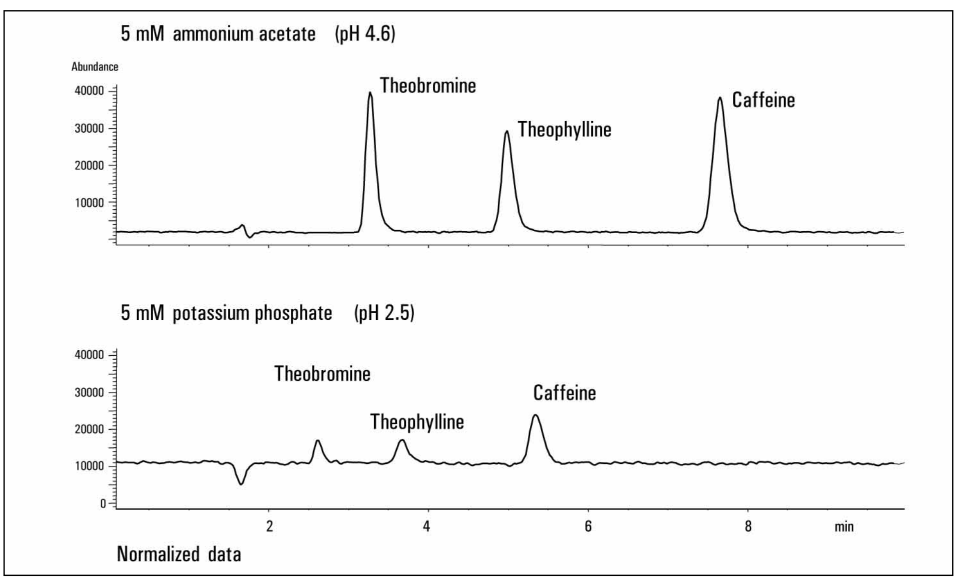

Figure 4. MS signal (TIC) for ammonium acetate (pH 4.6) & potassium phosphate buffers (pH 2.5).

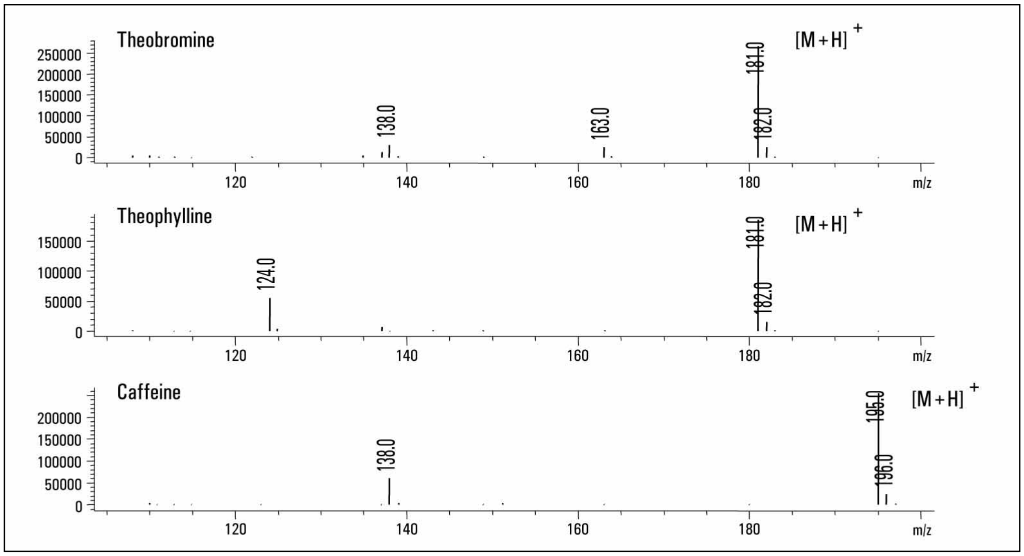

Figure 5. Mass spectra of test compounds.

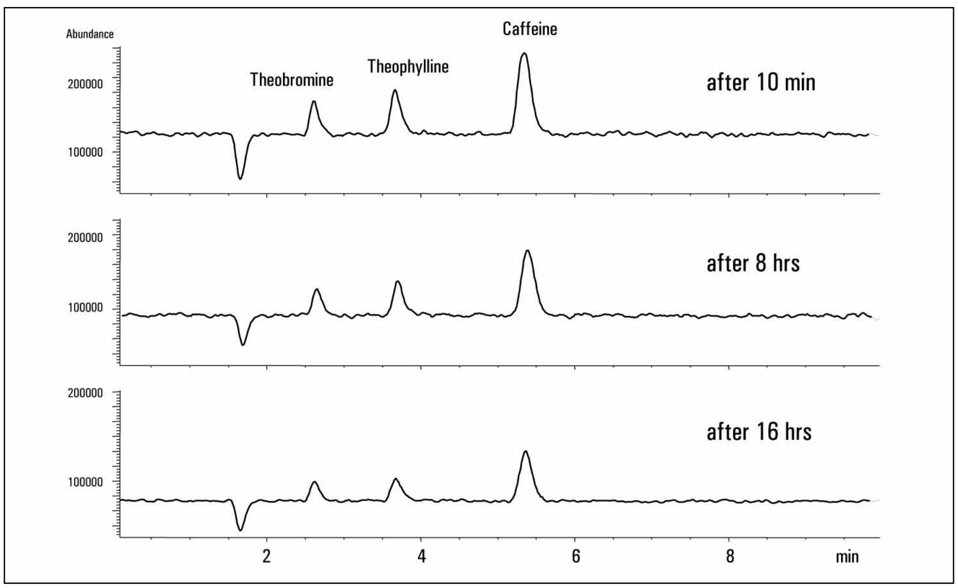

Figure 6. Signal response after repeated injections of phosphate buffer.

The test compounds in phosphate buffer were injected 100 times over 16 hours. The response and spectral integrity at the end of the period were similar to those at the beginning. See Figures 6, 7. The signal does show a drop in response, but this performance may nevertheless be acceptable in some applications. See Figure 8.

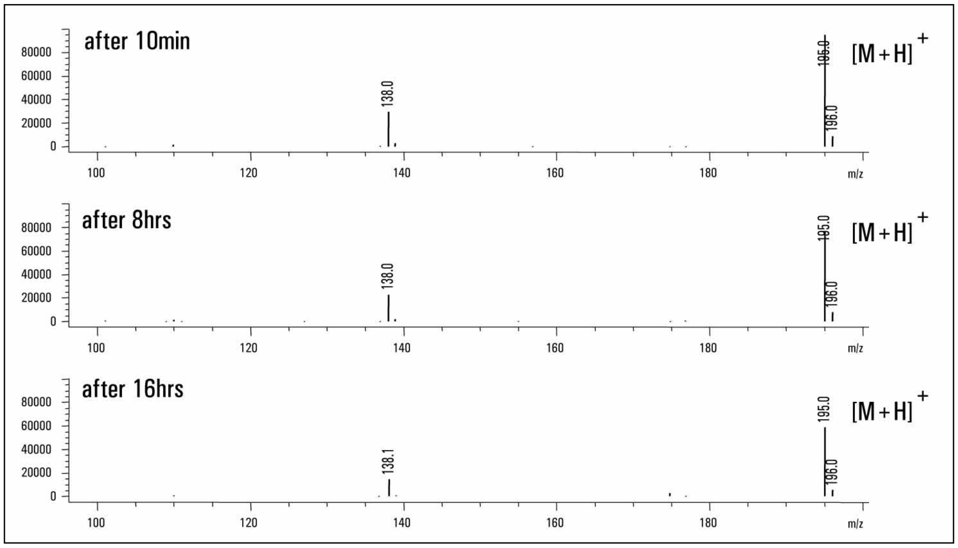

Figure 7. Stability of caffeine spectrum after repeated injections of phosphate buffer.

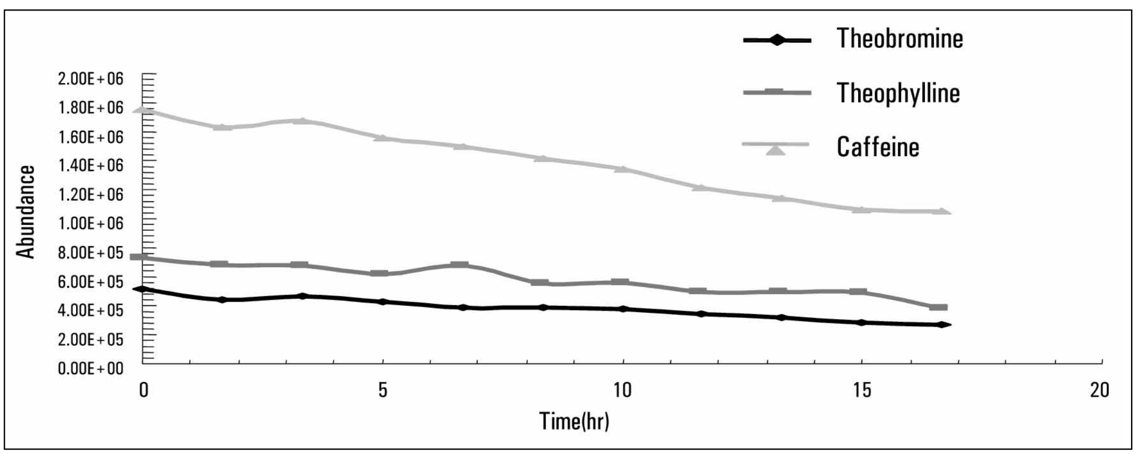

Figure 8. Effect of phosphate buffer on MS response.

Conclusion

It is concluded that the design of the Agilent 1100 Series LC/MSD with orthogonal spray allows the occasional use of some non-volatile buffers. In some cases, slight pH modifications may be made to optimize the signal. For the best long-term performance, however, the method should be modified to use a volatile buffer.

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