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LC/MS Compatible Conditions for the Analysis of Tetracycline Antibiotics

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The use of an oxalic acid mobile phase modifier in conjunction with a Discovery HS F5 stationary phase is shown to provide a suitable system for the separation and mass spectrometric (MS) detection of tetracycline antibiotics.

Due to the chelating nature of tetracyclines, good peak shape is often difficult to obtain using volatile buffers common to LC/MS systems. Phosphate buffers often provide good peak shape; however, the nonvolatile nature of these buffers renders them unsuitable for LC/MS systems. According to Nakazawa, et al., oxalic acid may be used in conjunction with atmospheric pressure chemical ionization (APCI) interfaces when high desolvation temperatures are used. Decomposition of the acid to carbon dioxide and water at these high temperatures prevents clogging of the MS interface. The oxalic acid improves chromatographic response by effectively competing with the analytes for potential chelation sites. Tetracycline, oxytetracycline, chlortetracycline, and doxycycline were separated on a Discovery HS F5 HPLC column using the following conditions that exploit the oxalic acid properties.

Experimental Conditions

Column:	Discovery HS F5, 15 cm × 4.6 mm, 5 µm (Cat. # 567516-U)
Mobile Phase:	5 mM oxalic acid (pH 2.3, unadjusted): acetonitrile, (70:30, v/v)
Temperature:	Ambient
Flow Rate:	1 mL/min
Injection Volume:	10 µL
Detection:	MS, Micromass ZQ Single Quadrupole with an APCI interface operating in positive ion mode
Sample:	10 µg/mL each analyte in 5 mM oxalic acid (pH 2.3, unadjusted)

Results

The structures of the tetracycline antibiotics used in this experiment are presented in Figure 1 and their resolution on Discovery HS F5 is shown in Figure 2. Excellent separation was observed under these conditions along with good MS response. No interface clogging or loss of signal was observed over the course of this investigation.

Conclusions

This application demonstrates the suitability of oxalic acid as a mobile phase modifier for the LC/MS analysis of tetracycline antibiotics using an APCI interface. The ability to use MS detection should improve sensitivity and aid in positively identifying the analytes in complex mixtures. Although the separation could be performed on traditional alkyl phases, the Discovery HS F5 column provided increased retention for the more polar analytes as well as decreased retention for the more nonpolar species in this mixture relative to a C8 phase. These factors resulted in greatly improved separation efficiency.

Reference

(1) H. Nakazawa, et al., "Simultaneous Determination of Residual Tetracyclines in Foods by High-Performance Liquid Chromatography with Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry," *J. Chromatogr. B: Biomed. Sci. Appl.* **732**(1), 55–64 (1999).

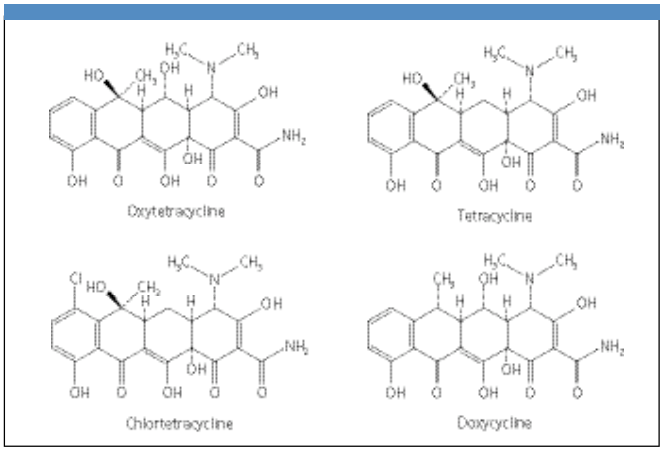

Figure 1: Structures of tetracycline antibiotics oxytetracycline, tetracycline, chlortetracycline, and doxycycline.

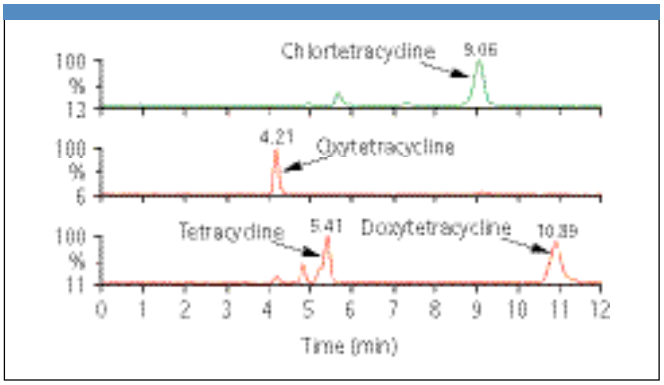

Figure 2: LC/MS response of tetracyclines on Discovery HS F5.

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