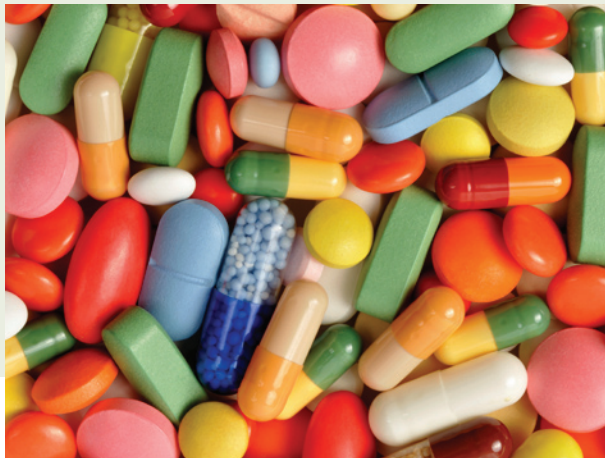
APPLICATION BRIEF

Pharmaceutical

Analysis of Tetracyclines by UHPLC/SQ MS



Tetracyclines are broad-spectrum antibiotics indicated for use against many bacterial infections. Tetracyclines act by interfering with the ability of a bacterium to produce certain vital proteins, thus inhibiting the growth of bacterial organisms in a bacteriostatic manner. These anti-microbial compounds have a common basic structure and are either isolated directly from several species of *Streptomyces* bacteria or produced semi-synthetically.

In addition to treating infections, tetracyclines have been widely applied as feed additives for food-producing animals to prevent disease and increase nutritional output.

Here we demonstrate a quick and robust method for analysis of tetracyclines and their metabolites by UHPLC/SQ MS using the Flexar™ SQ 300 MS as an alternative to UV-based detectors commonly used for this type of analysis.

Experimental Conditions

Target Analytes: Monocycline, tetracycline, chlortetracycline, doxycycline

Sample Preparation Conditions

A standard mixture of four tetracycline class antibiotics was prepared in 50% acetonitrile at a concentration of 1 mg/mL. The stock solution was further diluted to a working concentration of 10 µg/mL in 15% acetonitrile. Reverse phase UHPLC was used to separate them and the mass spectrometer operated in Scan mode for initial analysis.

LOQ and LOD values were determined from data obtained in SIM (Selected Ion Monitoring) mode analysis.

Liquid Chromatography Conditions

Pump Type:	PerkinElmer® Flexar FX-10
Column:	PerkinElmer Brownlee™ C18 column (2.1 mm x 100 mm, 3.0 µm)
Mobile Phase:	A: 100% water containing 0.1% formic acid B: 100% acetonitrile containing 0.1% formic acid
Flow Rate:	2 mL/min
Injection Volume:	1 µL

Gradient:

Time (min)	%A	%B
10	85	15
8	45	55
3	0	100

Mass Spectrometer Conditions

Ionization:	Ultraspray™ ESI – Positive mode
Scan Range:	100-550 m/z
Scan Rate:	1000 u/sec

The [M+H]+ ion of each of the analytes were monitored in three different time periods:

Time Period 1:	(0-3.2 min) SIM ion 458.2 for monocycline; dwell time of 200 ms

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Time Period 2:	(3.2-5.2 min) SIM ion 445.2 for tetracycline; dwell time of 200 ms
Time Period 3:	(5.2-8.2 min) SIM ions 479.2 and 445.2 for chlortetracycline and doxycycline respectively; dwell time of 200 ms each
Capillary Exit Voltage:	18 V

Results

The separation of the four tetracycline compounds was accomplished by reverse phase UHPLC with an 8 min gradient. Each compound in the mixture was unambiguously identified by its mass spectral signature producing [M+H]+ ion as the main ion species. LOQ and LOD values were established as 5 pg and 2 pg respectively. Chromatographic, spectral and SIM mode information for the mixture analysis is shown in Figures 1 through 4.

Conclusions

LC/SQ MS is continuing to be the method of choice for the accurate measurement of analytes across a broad spectrum of industrial applications. The powerful combination of HPLC/UHPLC and the Flexar SQ 300 MS enables users to develop quick, sensitive and robust methodologies for the analysis of a wide variety of compounds. As shown in this application, simultaneously operating in Full Scan and SIM mode, the Flexar SQ 300 MS overcomes the limitations of conventional detectors by providing important molecular weight and structural information, from Full Scan, while still reaching the highest sensitivity in SIM mode.

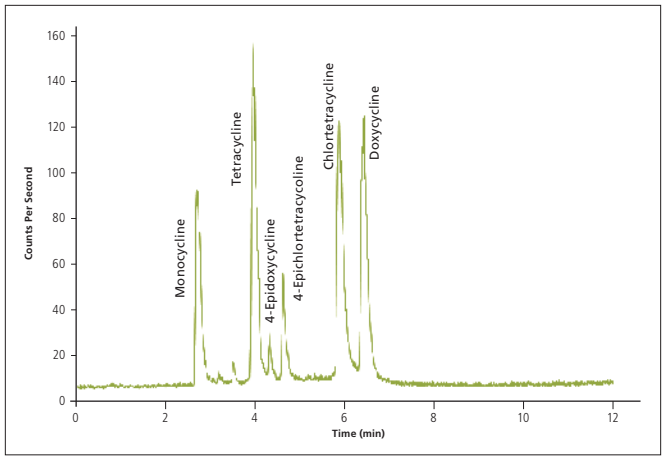

Figure 1. Chromatogram of tetracyclines by UHPLC/SQ MS in Full Scan mode. 4-epidoxycycline and 4-epichlortetracycoline are degradation products of chlortetracycline and doxycycline respectively.

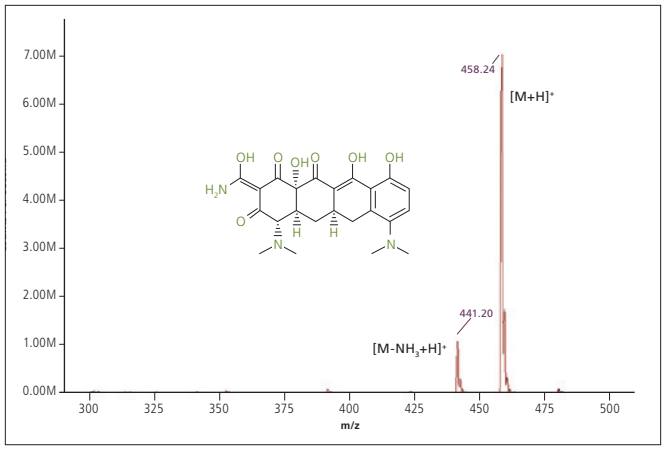

Figure 2. Mass spectrum of monocycline.

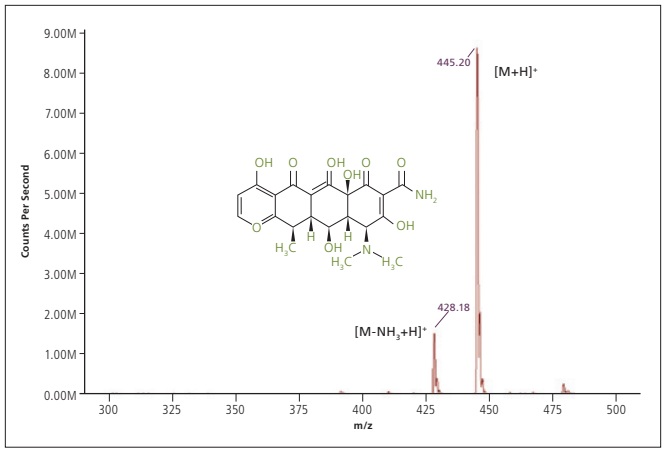

Figure 3. Mass spectrum of doxycycline.

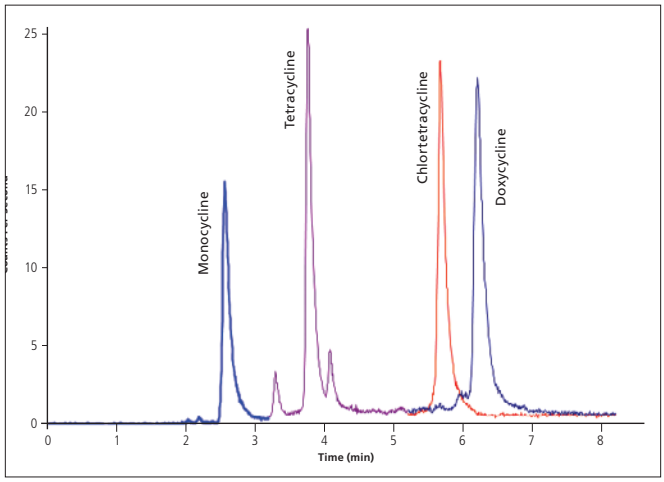

Figure 4. Overlaid chromatograms obtained in SIM mode of the 4 target compounds. 50 pg of each was injected on the column.

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