

Application Note

Tetracyclines in Honey

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief describes the methods to determine the presence of tetracyclines in honey.

Introduction

Tetracyclines (TCs), an antibiotic, is not permitted in apiculture. This method can monitors the presence of TC's in honey.

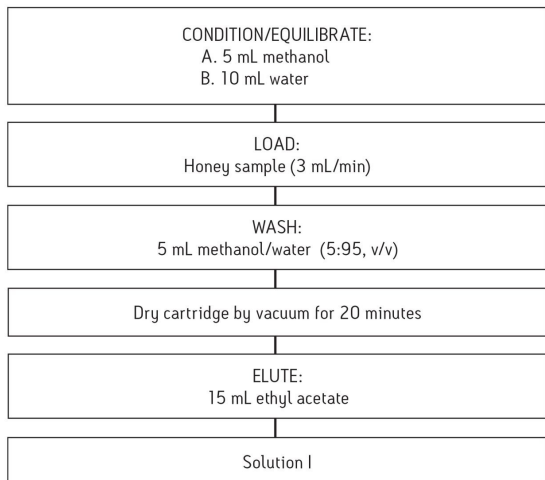
Experimental

Pre-treatment

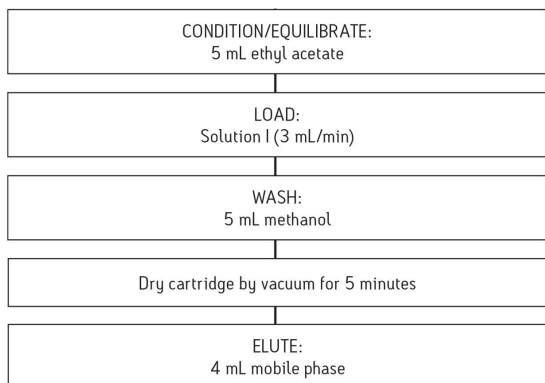
1. Add 30 mL EDTA-McIlvaines buffer to 6 g sample, mix thoroughly for 1 minute.
 2. Centrifuge at 3000 rpm for 5 minutes and collect supernatant for SPE.
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SPE Procedure

Cartridge I: Oasis® HLB 6 cc/500 mg



Cartridge II: Sep-Pak® Accell™ CM 3 cc/500 mg



Solutions

Mcllvaines Buffer

1. Thoroughly mix 1000 mL 0.1 M citric acid with 625 mL 0.1 M disodium hydrogen phosphate dihydrate.
2. Adjust with sodium hydroxide or hydrochloric acid to pH 4 ± 0.05 , if necessary.

EDTA-Mcllvaines Buffer

1. Add 60.5 g disodium EDTA to 1625 mL Mcllvaines Buffer and mix thoroughly.

LC Conditions

Column:	SunFire C ₈ , 3.5 µm, 2.1 x 150 mm
Mobile phase:	Acetonitrile:methanol: 0.4% formic acid (18:4:78)
Flow rate:	0.2 mL/min
Injection volume:	20 µL
Column temp.:	25 °C

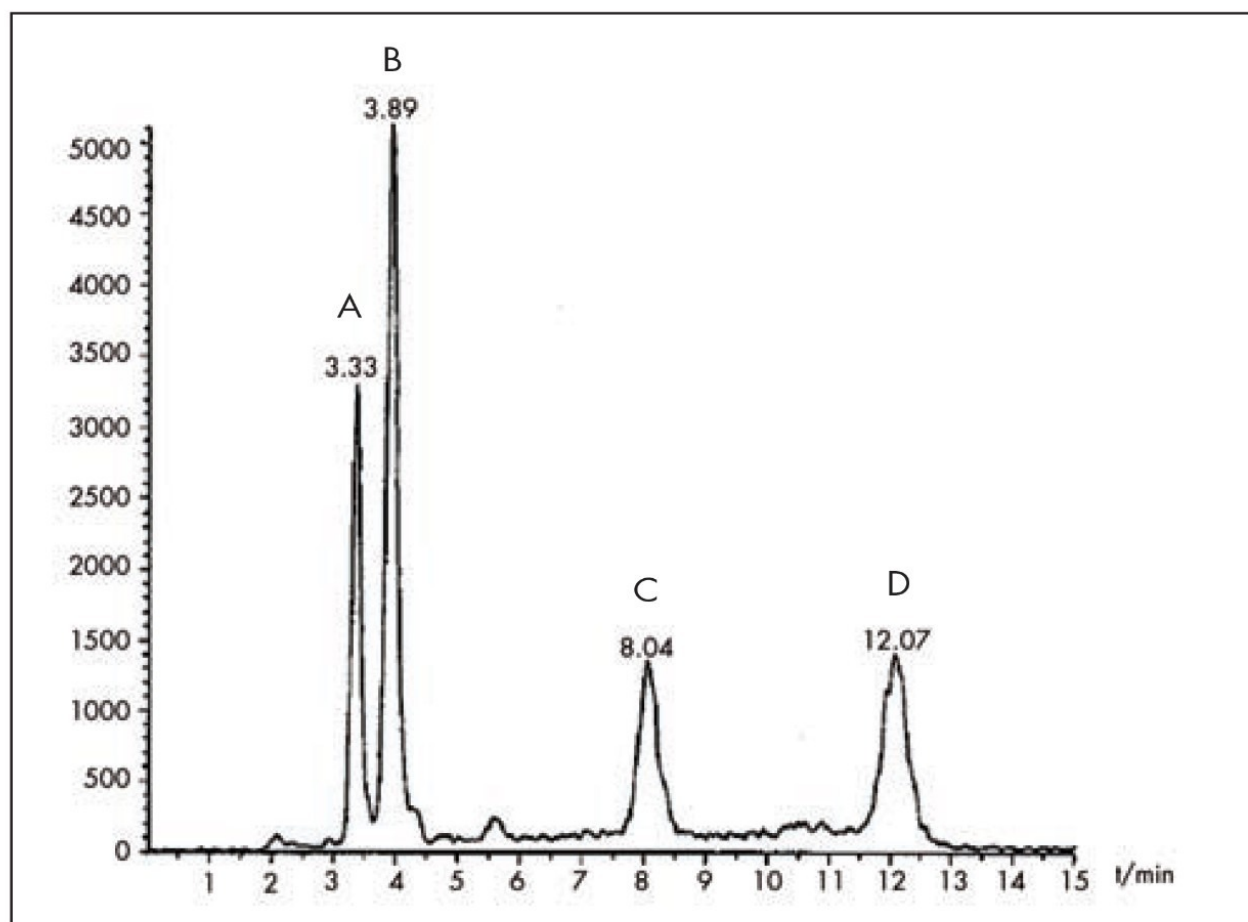
MS Conditions

MS System:	Waters Quadrupole MS
Ionization mode:	Positive electrospray (ESI+)
	Multiple reaction monitoring

Analyte	MRM for Quantification	MRM for Quantification
Oxytetracycline	461 → 426	461 → 426
	461 → 443	
	461 → 381	
Tetracycline	445 → 410	445 → 410
	445 → 154	
	445 → 428	
Chlortetracycline	479 → 444	479 → 444
	479 → 154	
	479 → 462	
Doxycycline	445 → 428	445 → 428
	445 → 410	
	445 → 154	

MRM method parameters.

Results and Discussion



Chromatogram of A. oxytetracycline, B. tetracycline, C. chlortetracycline and D. doxycycline standards.

Analyte	Concentration (mg/kg)	Average Recovery
A. Oxytetracycline	0.002	88.0
	0.010	95.3
	0.100	95.8
	0.050	93.6
B. Tetracycline	0.002	81.9
	0.010	82.6
	0.050	84.5
	0.100	89.3
C. Chlortetracycline	0.002	87.2
	0.010	86.0
	0.050	86.6
	0.100	90.8
D. Doxycycline	0.002	85.2
	0.010	85.3
	0.050	86.8
	0.100	87.9

References

1. China GB/T 18932.23 - 2003.

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