

SPESolid Phase
Extraction

Application Note

World Leaders in Sample Preparation

SPE214**ISOLATION OF TETRACYCLINES FROM HONEY**

Spe-ed™ Cartridge	Cat. No. 12006- Octadecyl C18/18%, 500mg/6mL. Process with <i>Spe-ed</i> Mate 10 or 30.
Sample Preparation	Dissolve 25gms. Of honey in 50mL of 0.1 M Na ₂ EDTA Mcilvan buffer (pH 4.0). Filter sample.
Cartridge Conditioning	10mL saturated aqueous N _{a2} EDTA. DO NOT ALLOW CARTRIDGE TO RUN DRY!
Sample Addition	Aspirate prepared sample completely through cartridge.
Cartridge wash	20mL water. 0.4mL ethyl acetate. Air-dry cartridge for 5 minutes.
Analyte Elution	50mL of ethyl acetate / 10% methanol
For Hp TLC and TLC Analysis	Take 40mL of the ethyl acetate / 10% methanol eluate, and concentrate under vacuum at 30° C; Dissolve residue in 0.1mL of methanol.
Mino, CTC, DC, MTC and OTC by Hp TLC	Pre-develop silica gel Hp TLC plates (E. Merck, 5641) with saturated aqueous N _{a2} EDTA. Air dry at room temperature for 1 hour, and activate at 130° C for 2 hours before use. Apply 5uL of sample (residue dissolved in methanol.) Develop plate in chloroform / methanol/ 5% aqueous N _{a2} EDTA (65:20:5). Spray developed plate with 0.2M aqueous Magnesium chloride, and then air dry at room temperature. The fluorescent spots of T C's produced with Mg ²⁺ are observed under UV (360nm)

Applied
Separations930 Hamilton Street · Allentown, PA 18101
610-770-0900 · 610-740-5520 (fax)
www.appliedseparations.com

World Leaders in Sample Preparation

**OTC, TC, DMCTC
and CTC by TLC**

RP-8 TLC plates (E. Merck 15424).
Apply 5 μ L of sample (residue dissolved in methanol).
Develop plate with methanol / acetonitrile / 0.5 M aqueous oxalic acid (1:1:4) pH 3.0.
Spray developed plate with 0.2 M aqueous magnesium chloride, and then air dry at room temperature. Spray again with 10% TEA in methanol.

HPLC Analysis: For quantification of tetracycline detected in TLC screens, use the following procedure for additional clean up of the C18 elute.

Spe-ed cartridges

Cat. No. 2316- COOH carboxylic Acid,
500mg /6mL. Process with *Spe-ed Mate* 10
or 30

Cartridge conditioning

10% methanol in ethyl acetate.

Sample Addition

Aspirate 5mL of 10% methanol in ethyl acetate (elute from C18 cartridge) completely through cartridge.

Sample Elution

10mL of mobile phase methanol/
acetonitrile / 0.01 M aqueous oxalic acid
(2:3:16).

HPLC Analytical conditions**Detection**

UV 350nm.

Column

C8, 3 μ m, 75cm x 4.6mm.

Mobile Phase

Methanol / acetonitrile/ 0.01 M aqueous oxalic acid (2:3:16) pH 3.0.

1mL/minute

100 μ L injection volume.

Note: *Since sample matrix interferences and concentrations may vary from sample to sample, it may be necessary to adjust the wash and elution solvent/solution strength and/or volume to optimize isolation.*