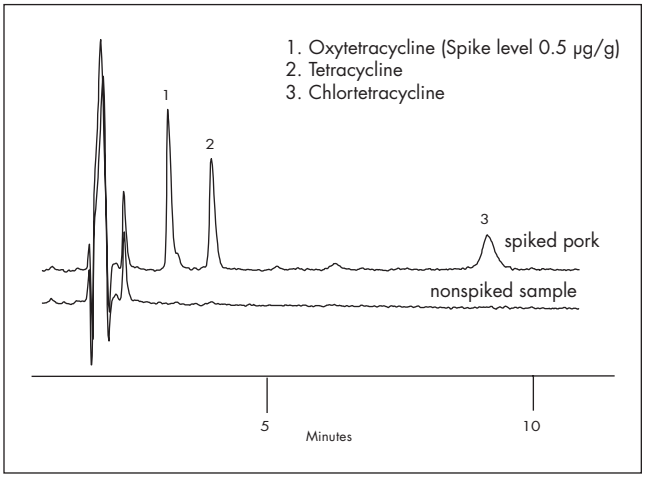
## Pretreatment

1. Add 20 mL McIlvaines Buffer-EDTA to 5 g homogenized sample in a 50 mL centrifuge tube and mix thoroughly.
2. Centrifuge at 3000 rpm for 10 minutes.
3. Collect supernatant.
4. Repeat steps 1 and 2 with the residue. Collect supernatant.
5. Combine both supernatant portions for SPE analysis.




## SPE Procedure

Oasis® HLB 6 cc/200 mg

CONDITION/EQUILIBRATE:
A. 3 mL methanol
B. 2 mL water

↓

LOAD:
40 mL sample (4 mL/min)

↓

WASH:
2 mL 5% methanol in water

↓

ELUTE:
3 mL methanol

↓

Evaporate to 0.2 mL

↓

Reconstitute with 1 mL 50 mM oxalic acid

## Solutions

### McIlvaines 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-McIlvaines Buffer

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

## LC Conditions

| | |
|---|---|
| Instrument: | Waters Alliance® HPLC 2695 System |
| Column: | Nova-Pak® $C_8$, 3.9 x 150 mm, 4 µm |
| Flow rate: | 0.8 mL/min |
| Mobile phase: | Acetonitrile: 50 mM oxalic acid (1:4) |
| Injection volume: | 60 µL |
| Detector: | 2487 Dual Wavelength Detector |
| UV wavelength: | 365 nm |

## Ordering Information

| Description | Part Number |
|---|---|
| Oasis HLB, 6 cc/200 mg, 60 µm, 30/box | WAT106202 |
| Nova-Pak $C_8$, 3.9 x 150 mm, 4 µm | WAT035876 |
| LCMS Certified Vials | 600000751CV |

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