Pretreatment

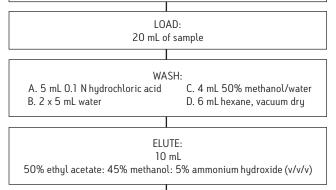
- 1. Weigh 1 g sample into a 30 mL centrifuge tube.
- 2. Add 50 μ L TMPD* solution (1 mg/mL).
- Add standard solutions (Malachite Green MG, Leucomalachite Green LMG, 0.1 µg/mL) and internal standard, leave to stand for 10 minutes.
- Add 10 mL McIlvaines Buffer (pH 2.6) /methanol (50:50, v/v) solution; homogenize for 45 seconds.
- 5. Centrifuge at 5000 rpm for 20 minutes, transfer supernatant into centrifuge tube.
- Repeat steps 4 and 5, combine the two portions of supernatant. A 20 mL aliquot will be used for SPE.

*TMPD= N, N, N', M'- Tetramethyl-1,4-phenylenediamine dihydrochloride

SPE Procedure

Oasis® MCX 6cc/150 mg

CONDITION/EQUILIBRATE: A. 5 mL methanol B. 5 mL water



Dry eluant at 50 °C under nitrogen

Reconstitute with 50% acetonitrile (100 μ L)

Solutions

McIlvaines Buffer (pH 2.6) :

- 0.1 M citric acid monohydrate (A) Dissolve citric acid monohydrate (10.5 g) in water (500 mL).
- O.2 M disodium hydrogen phosphate dihydrate (B) -Dissolve disodium hydrogen phosphate dihydrate (14.2 g) in water (500 mL).
- 3. Mix A (445.5 mL) and B (54.5 mL).

McIlvaines Buffer (pH 2.6): methanol (50:50 v/v):

Mix McIlvaines Buffer (pH 2.6) (250 mL) with methanol (250 mL).

LC Conditions

Instrument:	Waters Alliance [®] HPLC 2695 System			
Column:	SunFire [™] C ₁₈ , 4.6 x 150 mm, 5 µm			
	Self-packed F	bO ₂ oxidi	oxidising column:	
	4.6×20 mm, (PbO ₂ : diatomaceous earth = 3:1); Both ends are fitted with 2 µm stainless steel frit: This column is connected between the analytical columns and the detector			
Flow rate:	2 mL/min			
Mobile phase:	A. 125 mM ammonium acetate, adjust to pH 4.5 with formic acidB. acetonitrile			
Gradient:	Time (min)	A%	В%	
	0	45	55	
	7	10	90	
	10	10	90	
Injection volume:	50 µL			
Detector:	2487 Dual Wavelength UV Detector			
UV wavelength:	619 nm			

Ordering Information

Description	Part Number
Oasis MCX, 6 cc/150 mg, 30 µm, 30/box	186000256
SunFire C ₁₈ , 4.6 x 150 mm, 5 μm	18600255
Qsert [™] Vials	186001126

Ref: Jointly developed by Waters China applications team and Beijing CIQ

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