

Pretreatment

1. Weigh 1 g sample into a 30 mL centrifuge tube.
2. Add 50 μ L TMPD* solution (1 mg/mL).
3. Add standard solutions (Malachite Green MG, Leucomalachite Green LMG, 0.1 μ g/mL) and internal standard, leave to stand for 10 minutes.
4. 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.
6. 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	C. 5 mL McIlvaines Buffer (pH 2.6)
LOAD: 20 mL of sample	
WASH:	
A. 5 mL 0.1 N hydrochloric acid B. 2 x 5 mL water	C. 4 mL 50% methanol/water D. 6 mL hexane, vacuum dry
ELUTE: 10 mL 50% ethyl acetate: 45% methanol: 5% ammonium hydroxide (v/v/v)	
Dry eluant at 50 °C under nitrogen	
Reconstitute with 50% acetonitrile (100 μ L)	

Solutions

McIlvaines Buffer (pH 2.6) :

1. 0.1 M citric acid monohydrate (A) - Dissolve citric acid monohydrate (10.5 g) in water (500 mL).
2. 0.2 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 μ m Self-packed PbO ₂ oxidising column: 4.6 x 20 mm, (PbO ₂ : diatomaceous earth = 3:1); Both ends are fitted with 2 μ m stainless steel frits. 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 acid B. acetonitrile		
Gradient:	Time (min)	A%	B%
	0	45	55
	7	10	90
	10	10	90
Injection volume:	50 μ L		
Detector:	2487 Dual Wavelength UV Detector		
UV wavelength:	619 nm		

Ordering Information

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