

## Pretreatment

1. Weigh 1 g sample into a 30 mL centrifuge tube.
2. Add 50 µL TMPD\* solution (1 mg/mL).
3. Add standard solutions Malachite Green (MG), Leucomalachite Green (LMG), 0.1 µg/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<sup>®</sup> MCX 3 cc/60 mg

CONDITION/EQUILIBRATE:	
A. 2 mL methanol	C. 2 mL McIlvaines Buffer (pH 2.6)
B. 2 mL water	
LOAD:	
20 mL of sample	
WASH:	
A. 2 mL 0.1N HCl	C. 2 mL 50% methanol/water
B. 2 x 2.5 mL water	D. 3 mL hexane, vacuum dry
ELUTE:	
5 mL	
50% ethyl acetate: 45% methanol: 5% ammonium hydroxide (v/v/v)	
Dry eluant at 50 °C under nitrogen	
Reconstitute with 50% acetonitrile (100 µL)	

## 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).

## UPLC Conditions

Instrument:	Waters ACQUITY UPLC <sup>®</sup> System		
Column:	ACQUITY UPLC BEH C <sub>18</sub> , 2.1 x 50 mm 1.7 µm		
Flow rate:	0.4 mL/min		
Mobile phase:	A. 0.1% formic acid in water B. 0.1% formic acid in acetonitrile		
Gradient:	Time (min)	A%	B%
	0	40	60
	3	5	95
	5	40	60

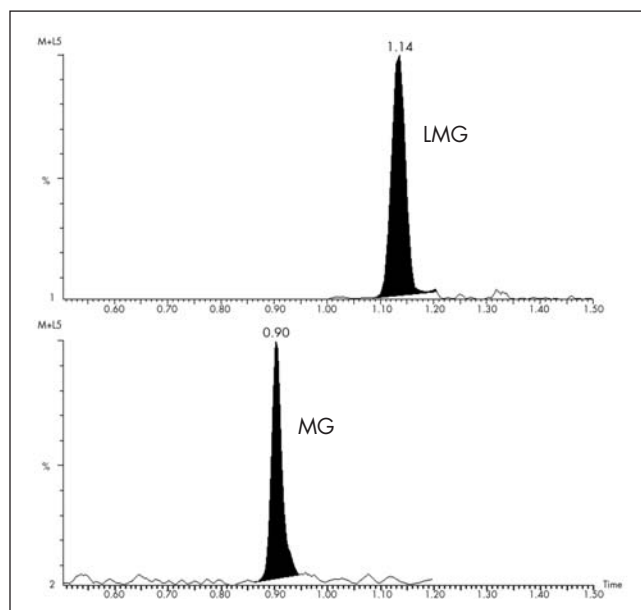
## MS Conditions

Instrument:	Waters Quattro Premier™
Ionization Mode:	Positive electrospray (ESI <sup>+</sup> ) Multiple reaction monitoring

Analyte	MRM Transition
LMG	331.2 → 239.1
	331.2 → 316.2
MG	329.2 → 208.1
	329.2 → 313.1

MRM method parameters.

## Results



The LOD of LMG and MG are 0.02 ppb and 0.01 ppb, respectively. The recoveries of LMG and MG in fish is between 50 - 80%.

## Ordering Information

Description	Part Number
Oasis MCX, 3 cc/60 mg, 30 $\mu$ m, 100/box	186000254
ACQUITY UPLC BEH C <sub>18</sub> , 2.1 x 50 mm, 1.7 $\mu$ m	186002350
ACQUITY UPLC BEH C <sub>18</sub> , 2.1 x 50 mm, 1.7 $\mu$ m, 3/pk	176000863
Qsert <sup>™</sup> Vials	186001126