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アプリケーションノート

Malachite Green in Fish (UPLC-MS/MS)

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application describes methods to determine malachite green in fish using UPLC-MS/MS.

Introduction

Malachite Green (MG) is an effective and inexpensive fungicide used in aquaculture. During metabolism MG reduces to Leucomalachite Green (LMG), which has been shown to accumlate in fatty fish tissues. Both MG and LMG have demonstrated putative carcinogenic activity, and thus, have been banned for use in aquaculture by both the United States Food and Drug Administration (US FDA) and European Union (EU).

Experimental

Pre-treatment

1. Weigh 1 g sample into a 30 mL centrifuge tube.

2. Add 50 µL TMPD* solution (1 mg/mL).

3. Add standard solutions MG, LMG, 0.1 µg/mL and internal standard, leave to stand for 10 minutes.

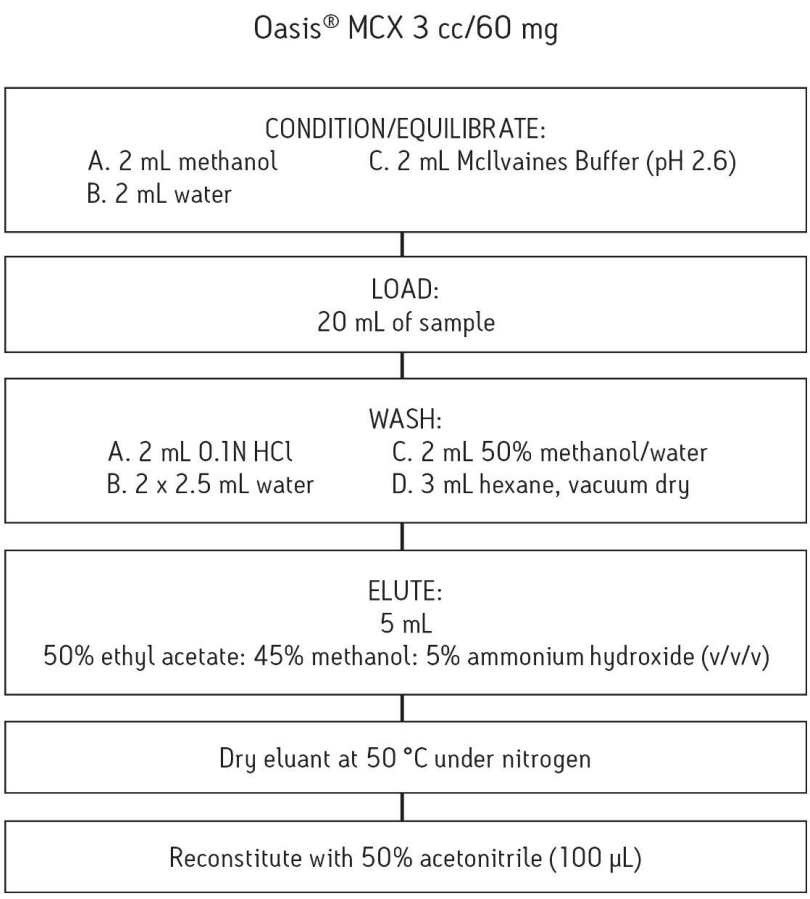
4. Add 10 mL McIlvaines Buffer (pH 2.6) /methanol (50:50 v/v) solution; homogenize for 45 seconds.

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



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

System:	ACQUITY UPLC
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm
Flow rate:	0.4 mL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol

Gradient

Time (min)	%A	%B
0	40	60
3	5	95
5	40	60

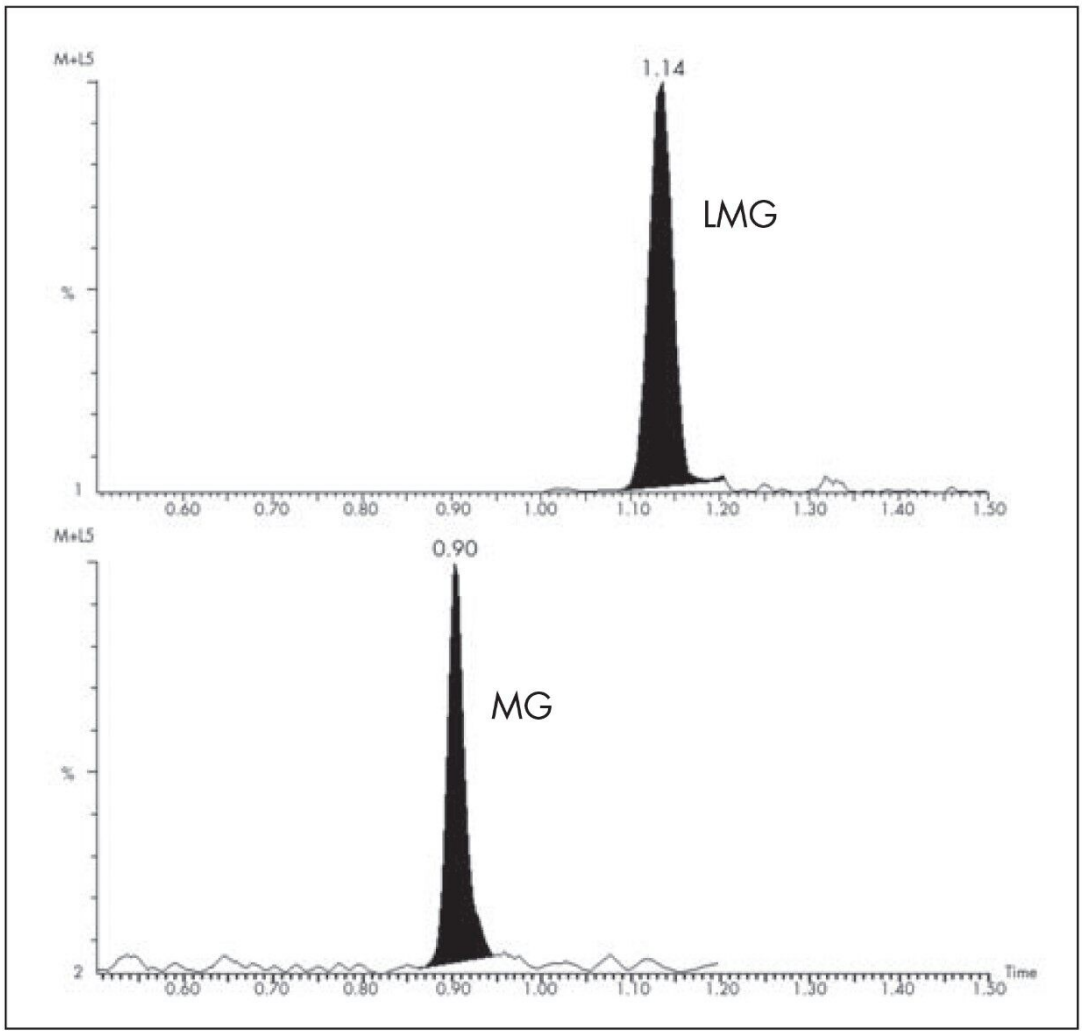
MS Conditions

MS System:	Waters Quattro Premier
Ionization mode:	Positive electrospray (ESI^+)
	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 and Discussion



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%.

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ACQUITY UPLC System <https://www.waters.com/514207>

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