

Note d'application

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 accumulate 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

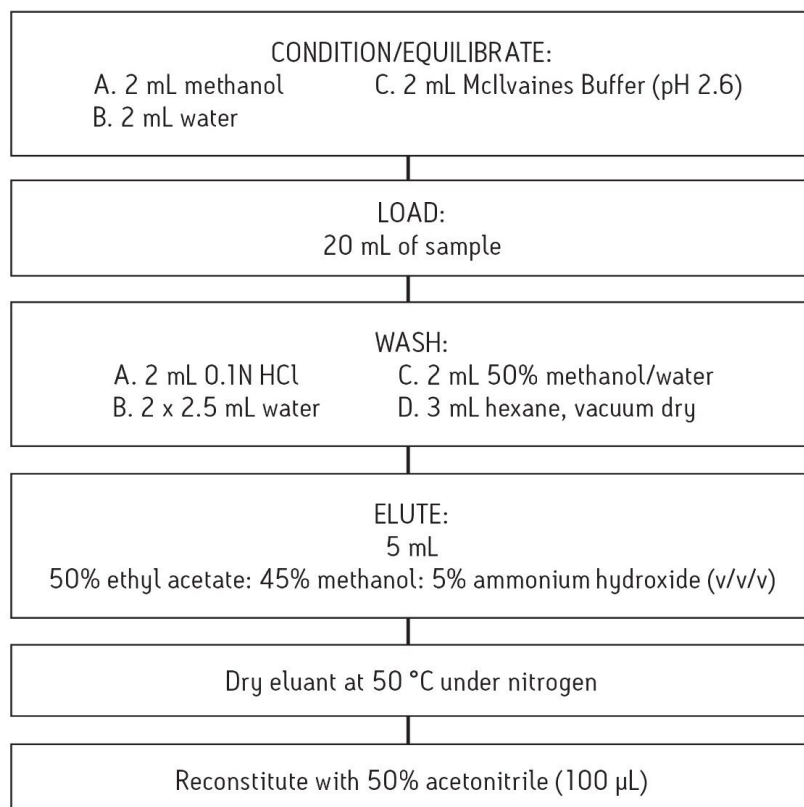
Pre-treatment

1. Weigh 1 g sample into a 30 mL centrifuge tube.
2. Add 50 μ L TMPD* solution (1 mg/mL).
3. Add standard solutions MG, 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 3 cc/60 mg



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 C₁₈, 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

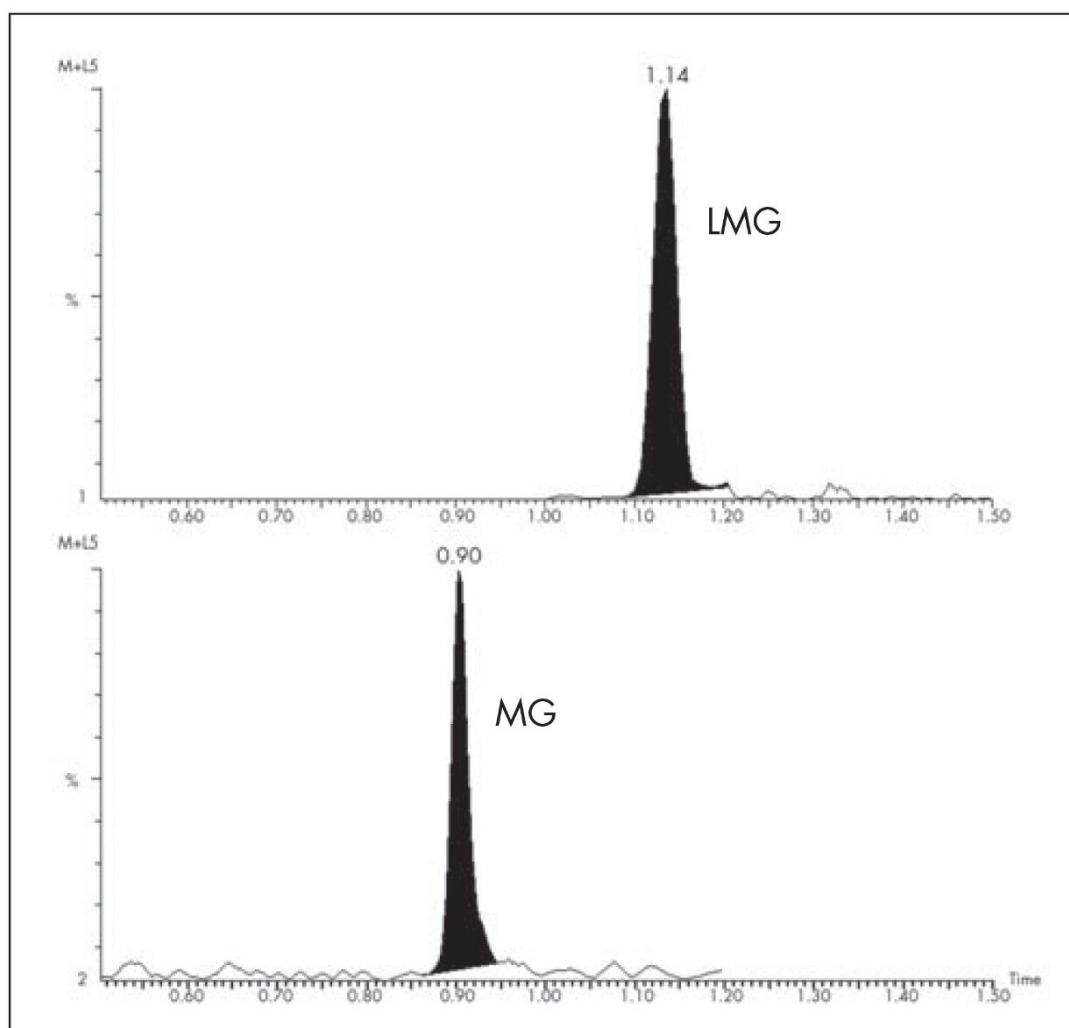
Ionization mode: Positive electrospray (ESI⁺)

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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