Waters™

Note d'application

Malachite Green in Fish (HPLC/UV)

Waters Corporation

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

Abstract

This application brief describes how to determine malachite green in fish by HPLC/UV.

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).

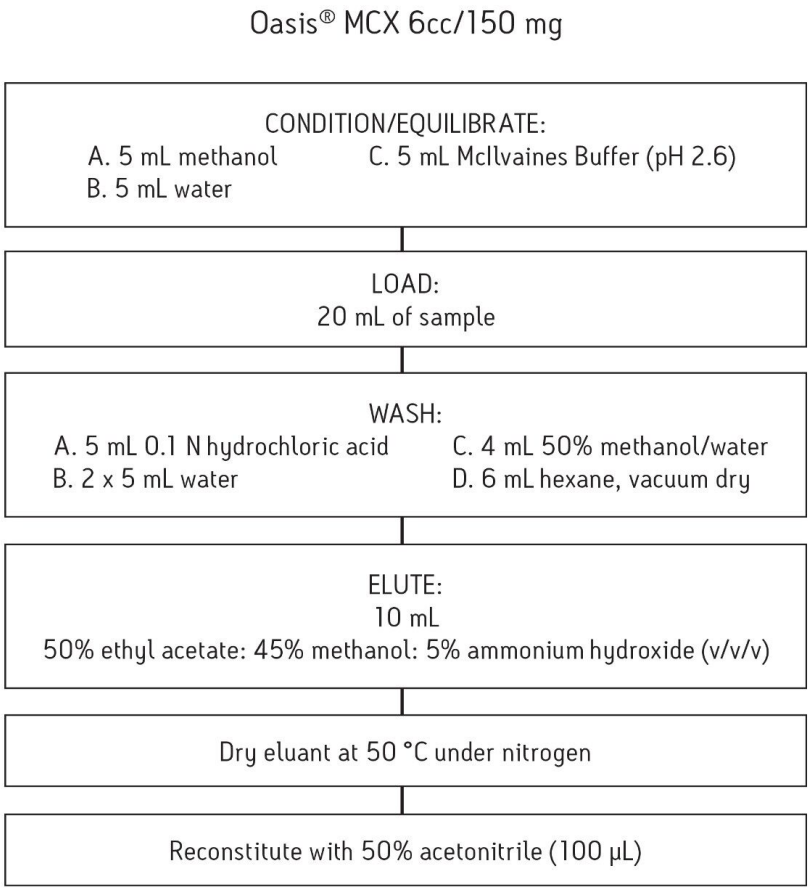
Experimental

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 (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:	Alliance HPLC 2695
Column:	SunFire C_{18}, 5 µm, 4.6 x 150 mm Self-packed PbO_2 oxidising column: 4.6 x 20 mm, (PbO_2: diatomaceous earth = 3:1); Both ends are fitted with 2 µm 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
Mobile phase B:	acetonitrile
Detector:	2487 Dual Wavelength UV Detector
UV wavelength:	619 nm

Gradient

Time (min)	%A	%B
0	45	55
7	10	90
10	10	90

Featured Products

Alliance HPLC System <https://www.waters.com/534293>

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