MACHEREY-NAGEL
Chromatography application note

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Determination of triphenylmethane dyes from aquaculture samples

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Abstract

This application note describes the determination of triphenylmethane dyes from aquaculture samples using dispersive SPE (QuEChERS, dSPE) for sample clean-up. The eluates from dSPE cleaning are finally analyzed by HPLC-MS/MS.

Introduction

Motivated by the various potential health benefits the human feeding behavior changes more and more and leads to an increasing demand for aquatic products. Farming aquatic species is getting an important way for producing big amounts of aquatic products all over the world because of overexploiting the world's fish stock. A big challenge of farming aquatic species is the control of infectious diseases. Triphenylmethane dyes (TPM) are organic compounds originally used as textile and paper dyes and have been found effective as antibacterial, antifungal, and antiparasitic agents in fisheries. They accumulate in fish and metabolize to the equivalent, colorless leuco-forms, which are also known as mutagenic [1]. For protecting human health triphenylmethane dyes have been banned in many countries according to the recommendations of national and/or international related agencies. The EU has implemented a minimum required performance limit (MRPL) for the sum of Malachite Green and Leucomalachite Green of 2 µg/kg [2].

QuEChERS ("Quick, Easy, Cheap, Effective, Rugged and Safe") methodology is known as a common sample preparation technique in modern analysis of pesticides in food. Using this special SPE methodology allows a quick and cost-efficient determination of different analytes in strongly matrix-contaminated samples by GC-MS and LC-MS [3].

In this application note a QuEChERS method for the determination of triphenylmethane dyes from aquaculture samples was developed. The identification and quantification of Malachite Green, Crystal Violet and its metabolites were finally carried out by ESI mass spectrometry on a NUCLEODUR® π^2 column.

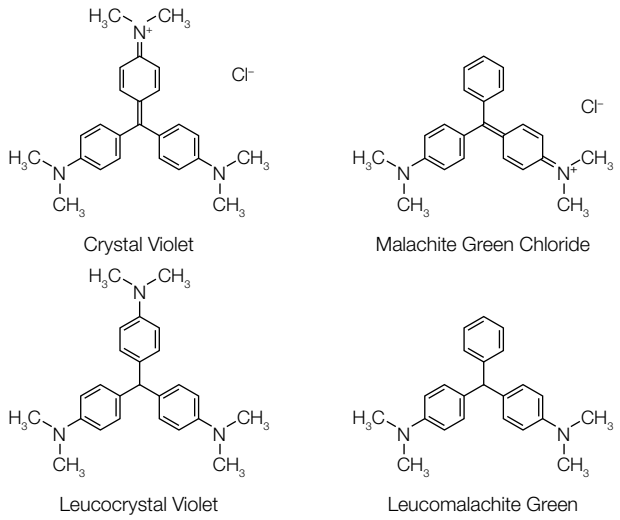

Figure 1: Compounds of interest.

Dispersive solid phase extraction (dSPE)

Extraction

- Weigh out 5 g of homogenized sample into a 50 mL brown centrifuge tube
- Add 5 µL of internal standard solution (β = 1 µg/mL Malachite Green-d_5 and Leucomalachite Green-d_5 each in acetonitrile)
- Add 25 µL of standard solution (β = 200 ng/mL Malachite Green, Leucomalachite Green, Leucocrystal Violet, Crystal Violet each in acetonitrile) for determining recovery rate
- Add 5 mL water and shake
- Add 10 mL 1 % acetic acid in acetonitrile and shake
- Add the CHROMABOND® QuEChERS extraction Mix II (REF 730971)
- Shake vigorously for 30 sec and cool down the mixture in an ice bath
- Centrifuge the mixture at 4500 rpm, for 5 min at 4 °C

Clean-up

- Put 5 mL acetonitrile supernatant in a 15 mL brown centrifuge tube
- Add the CHROMABOND® QuEChERS clean-up Mix III (REF 730972), (for samples with high fat content add 1 mL hexane)
- Shake vigorously for 30 sec
- Centrifuge the mixture at 4500 rpm, for 5 min at 4 °C
- Dilute the extract 1:1 with an aqueous solution of 5 mmol/L ammonium acetate + 1 mL/L formic acid and filter through a syringe filter (CHROMAFIL® Xtra PET-20/13, REF 729208)

Triphenylmethane dyes from aquaculture samples

Subsequent analysis: HPLC-MS / MS

Chromatographic conditions

Column:

EC 100/3 NUCLEODUR® π^2, 3 µm (REF 760636.30)

Eluent A:

5 mmol/L ammonium acetate + 0.1 % formic acid in water

Eluent B:

acetonitrile

Gradient:

in 10 min from 20 % to 85 % B, hold for 5.0 min, in 1.0 min to 20 % B, hold 20 % B for 5.0 min

Flow rate:

0.4 mL/min

Temperature:

25 °C

Injection volume:

5 µL

MS conditions

LCMS 8050 (Shimadzu), ion source ESI, positive ionization mode, scan type MRM, interface heater on, interface current 4000 V, interface temperature 300 °C, DL temperature 250 °C, nebulizing gas flow 3.00 L/min, heating gas on, heating gas flow 10.00 L/min, heat block 400 °C, drying gas on, drying gas flow 10.00 L/min

MRM transitions

Analyte	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
Crystal Violet	372.2	356.3	251.1
Leucocrystal Violet	373.9	358.0	238.2
Leucomalachite Green	330.9	316.2	239.1
Leucomalachite Green-d_5	335.9	321.1	243.1
Malachite Green	328.9	313.1	207.9
Malachite Green-d_5	333.9	318.3	213.1

Table 1: MRM transitions for the analysis of triphenylmethane dyes.

Chromatograms

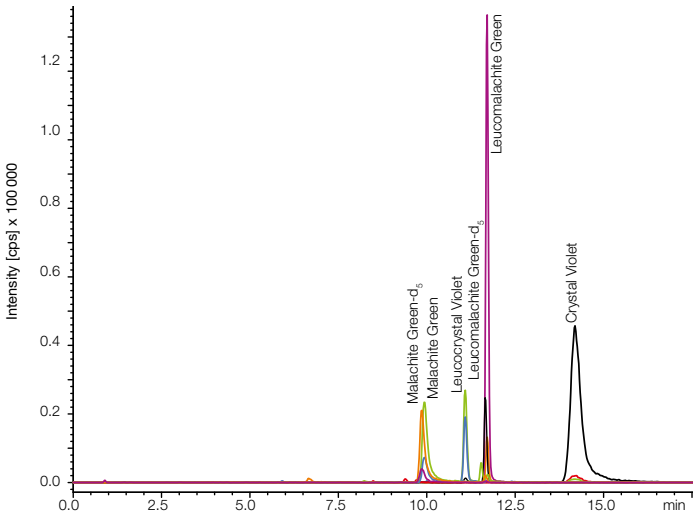

Figure 2: Chromatogram of brown trout sample spiked with 1.0 µg/kg sample for each analyte.

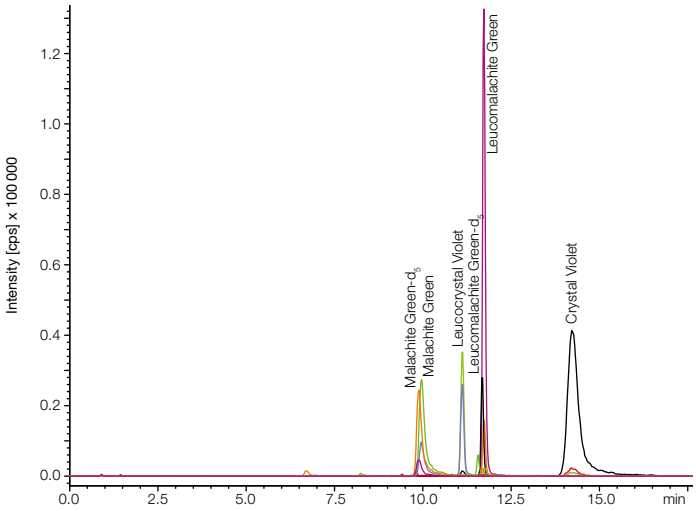

Figure 3: Chromatogram of shrimp sample spiked with 1.0 µg/kg sample for each analyte.

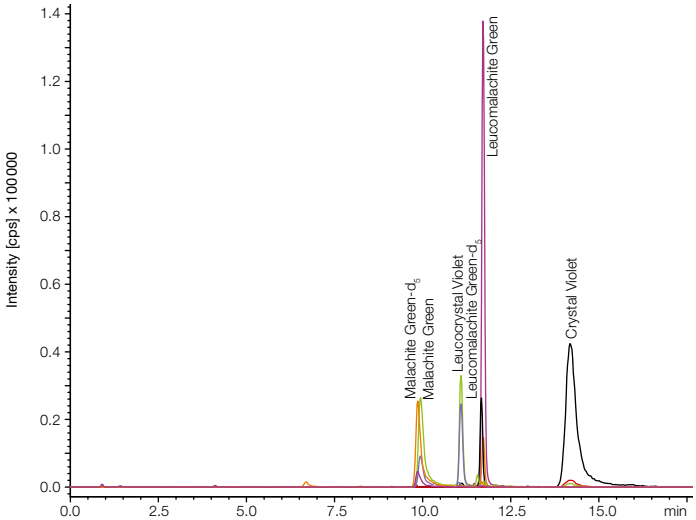

Figure 4: Chromatogram of tuna sample spiked with 1.0 µg/kg sample for each analyte.

Triphenylmethane dyes from aquaculture samples

Calibration curve

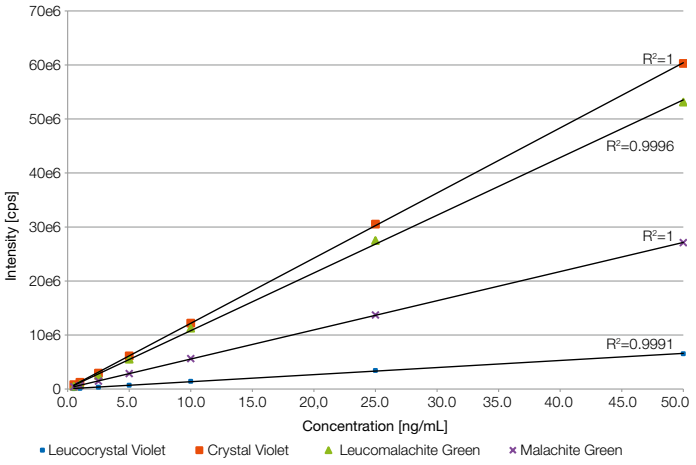

Figure 5: Calibration curves in concentration range between 0.5 ng/mL and 50 ng/mL for each analyte.

Recovery rates

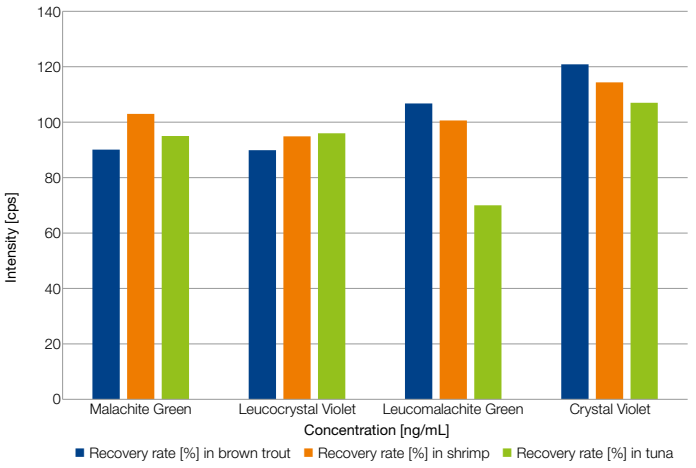

Figure 6: Recovery rates for presented dispersive solid phase extraction method.

Conclusion

The results show that the determination of triphenylmethane dyes from aquaculture samples could be carried out successfully with all the tested products. By using QuEChERS methodology it was possible to recover more than 70 % of TPM dyes from different matrices as shown in figure 6. The use of brown centrifuge tubes protects the analytes from light-induced degradation and leads to better sensitivities and recovery rates. Figure 2, 3 and 4 show chromatograms of QuEChERS extracts from different matrices with high signal response. The calibration curves in concentration range between 0.5 ng/mL and 50 ng/mL for each analyte are presented in figure 5. The identification and quantification of TPM dyes in the cleaned sample extracts were carried out by ESI mass spectrometry on an EC 100/3 mm, 3.0 µm NUCLEODUR® π^2 column. The chromatographic results of the extraction of aquatic products spiked with 1 µg/kg presented in figure 2, 3 and 4 show less matrix interferences signals.

In summary the presented application describes a quick and convenient method for the determination of TPM dyes from aquatic samples.

References

[1] BfR Expert Opinion No. 007/2008, 24th August 2007.

[2] European Commission Decision 2002/657/EC of 12th August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, as amended by Decision 2003/181/EC (4), (Official Journal of the European Communities L 221, 17th August 2002, p. 8–36).

[3] M. Anastassiades, S. J. Lehotay, D. Stajnbaher, F. J. Schenck, J. AOAC Int. 86 (2003), 412–431.

Additional information

The following applications regarding “Triphenylmethane dyes from aquaculture samples” and further applications can be found on our online application database at *www.mn-net.com/apps*

SPE: MN Appl. No. 306560 (QuEChERS trout)
MN Appl. No. 306570 (QuEChERS shrimp)
MN Appl. No. 306580 (QuEChERS tuna)

HPLC: MN Appl. No. 128430

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 760636.30, EC 100/3 NUCLEODUR® π^2, 3 µm

REF 730971, CHROMABOND® QuEChERS extraction Mix II

REF 730972, CHROMABOND® QuEChERS clean-up Mix III

REF 729208, CHROMAFIL® Xtra PET-20/13

REF 702293, Screw neck vials N 9, 1.5 mL

REF 702107, N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red

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