

DETERMINATION OF MALACHITE GREEN AND LEUCOMALACHITE GREEN BY HPLC WITH POST-COLUMN OXIDATION



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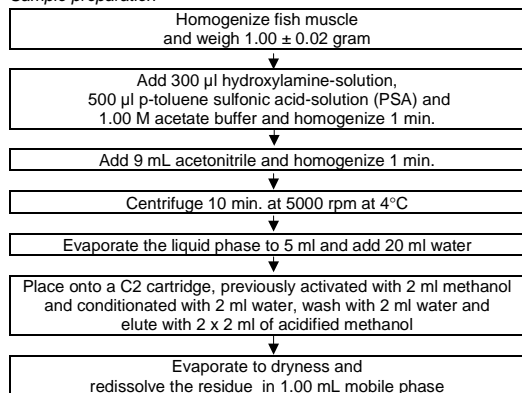
Introduction

Malachite green is an organic dye used worldwide as a fungicide and ectoparasiticide in fish farming. The substance has never been authorised as a veterinary medicine in the European Union, but although it is not approved for use in aquaculture, its low cost, ready availability and high effectiveness make prohibited use likely.

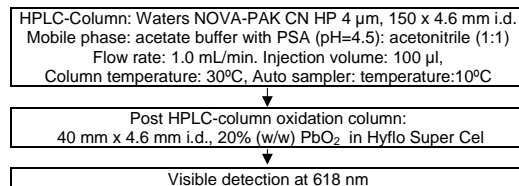
In the present study, the method of Plakas et al. [1] was modified by reducing the use of chemicals and the time spent for sample clean-up. The lifetime of the post column reactor was increased by using column-switch.

Experimental

Sample preparation



HPLC-analysis



Method Development

On-line postcolumn oxidation

The lifetime of the post column reactor was increased by using column-switch. The column-switch option made it possible to switch the on-line post column reactor on and off. In that way only the leucomalachite green peak passed through the column and thus the column was spared from other oxidizable coextractives. Moreover this prevented the possibility for further oxidation of the malachite green peak. The post column reactor was rinsed with mobile phase at 1 ml/min. at least 10 minutes before a new injection and it was kept in mobile phase.

Optimisation of the concentration of PbO₂ in the mixture was found to be important, as too high concentration cause extra peaks (derivatives of malachite green) on the chromatogram.

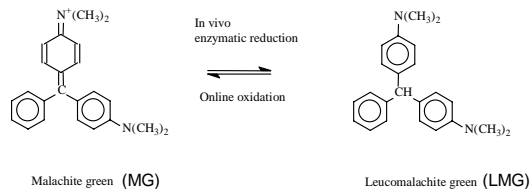


Figure 1. Malachite green and the metabolite leucomalachite green

Choice of solid phase extraction column

Figure 2 shows the results of a SPE-study on fortified muscle of trout at 100 µg/kg leucomalachite green and 100 µg/kg malachite green. Four different types of reversed phase SPEs were eluted with 4 fractions of 2 mL of acidified methanol. The analytes were retained strongly by C18-SPE, but both C8-SPE and C2-SPE were suitable for sample clean up. The C2-SPE was selected.

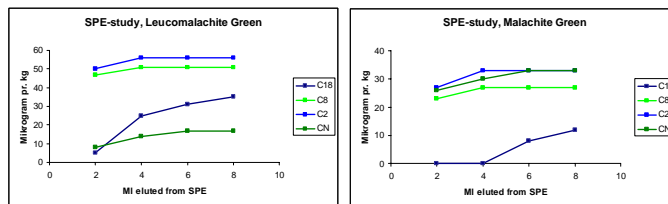


Figure 2. SPE-study

Results

25 samples of fish were analysed at the NRL-laboratory in Mørkhøj in 2002. 20 samples of fish analysed according to the EU residue monitoring programme were found to be compliant. One sample of five non-Danish fish was found to be non-compliant. The non-compliant sample was analysed in two series and confirmation was done by co-chromatography. Quantification was made on basis of duplicate analyses in the second series. The results are presented in table 1.

The analytical limits CC_α and CC_β are presented in table 2.

In figure 3 chromatograms of a standard-solution (1 ng/ml), a fortified sample (4 µg/kg LMG and 1.5 µg/kg MG) and the positive sample are shown.

Table 1. Three determinations of a positive sample corrected for recovery

	LMG µg/kg	MG µg/kg	LMG Recovery	MG Recovery
26-11-2002	517	53	65%	72%
04-12-2002	398 514	27 37	67% 67%	70% 70%
Mean	476	39		
Std. Derivation	68	13		
% CV	14%	33%		

Table 2. Decision limit CC_α and detection capability CC_β

Analytes	CC _α µg/kg	CC _β µg/kg
LMG	0,7	1,1
MG	0,6	0,9

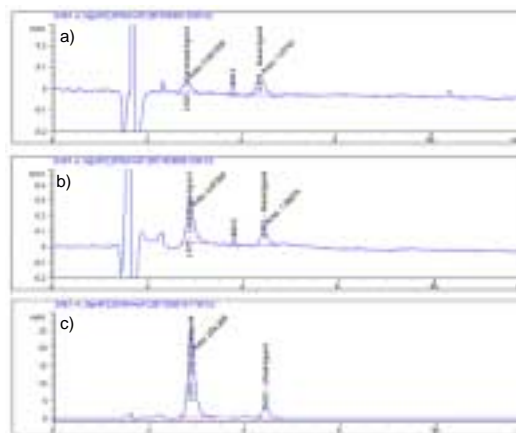


Figure 3. Chromatogram of a) Standard in solution at 1 ng/ml, b) Blank trout fortified at 4 µg/kg LMG and 1.5 µg/kg MG, c) Positive sample.

Conclusion

A fast and specific method for determination of malachite green and the metabolite leucomalachite green in fish is implemented.

References

[1] Plakas, Said, Stehly and Roybal (1995), J. AOAC International 78 No.6, 1388-1394