Quality control and food safety

Trout Malachite Green

Even though malachite green (MG) is banned as a veterinary pharmaceutical for animals used for human consumption, authorities regularly find residues of this toxic compound or its metabolites during routine checks of fish farms. Scientists from GERSTEL, TeLA and Agilent Technologies have succeeded in improving detection limits and in automating sample preparation for the determination of MG and its metabolite leucomalachite green (LMG) in fish products using automated SPE coupled with LC/Iontrap-MS.

The triphenyl methane dye Malachite green (MG) is highly efficient in battling fungi, bacteria and various single cell parasites. MG, however, is under suspicion for being a human carcinogen and for causing damage to genetic material if it reaches the human organism through consumption of contaminated foods.

Malachite green (MG) is traditionally administered as a fungicide in aquaculture, either as treatment or to prevent infections. Once inside the fish organism, MG is metabolized and reduced to leucomalachite green (LMG) which accumulates in fatty tissue. Fish that are contaminated with MG or LMG should not be consumed since they pose a health risk. In 2003, the EU Commission set threshold value of 2 µg/kg as the upper concentration limit for MG and LMG.

GERSTEL MultiPurpose Sampler MPS with SPE

Sample preparation

A fish filet sample was homogenized with a water/acetonitrile mixture, extracted, centrifuged and the supernatant collected. The extraction procedure was repeated twice. The extracts were subsequently combined and concentrated before being taken up in a mixture of water and ethanol. Sample clean-up was performed using automated SPE in a GERSTEL MultiPurpose Sampler (MPS).

LC/MS Method

The MPS was integrated in an Agilent 1100 LC/MS Iontrap System, consisting of a binary pump, a thermostated column compartment, a Diode Array Detector and an XCT+ Iontrap-MS. The LC/IT-MS was used in Electron Spray Ionization (ESI), positive ion mode. The injection volume used for all determinations was 5 µL. The separation was performed on a Zorbax SB-C18 column (50 x 2.1 mm, 1.8 µm) with a flow rate of 0.6 mL/min in gradient mode (Eluate A: 0.1 % formic acid, eluate B: acetonitrile). The column was kept at 50 °C. The complete

system control, including sample preparation, sample introduction, LC/MS analysis and data handling, was performed using the GERSTEL MAESTRO Software integrated with the Agilent Technologies ChemStation Software (Rev. A10.03).

Results and Discussion

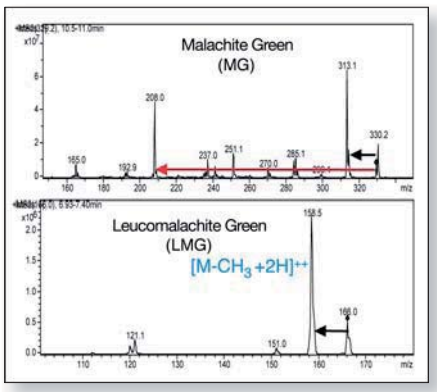
Malachite green (MG) and its metabolite leucomalachite green (LMG) are easily ionized using Electron Spray Ionization (ESI) in positive ion mode. MG differs from LMG in that it forms a doubly charged ion (m/z 166) in addition to the single charged molecular ion $[M+H]^+$. This is due to the non-planar sterical organization of the central carbon in the leuco form. In MS^2 mode, the MG-precursor ion forms a product ion (m/z 313), while the doubly charged LMG precursor also forms a doubly charged fragment. The transition can be used for highly sensitive determination of LMG. Using these transitions, limits of determination of 0.5 µg/kg for MG and 0.05 µg/kg for LMG can be achieved.

Automated SPE directly coupled with the LC/MS system provides recoveries as high as 90 % and excellent reproducibility for the SPE step. Additionally, automated SPE reduces the time required for sample preparation by 50 % compared with the manual procedure.

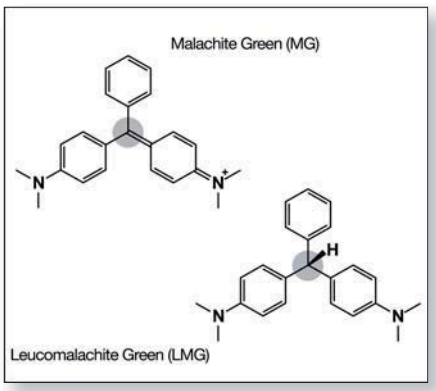
Conclusion

The described automated SPE/LC/IT-MS system enables automated sample cleaning and sample preparation followed directly by injection and analysis of the generated extracts. The sample preparation method is easily adapted to individual requirements by selecting the desired steps from a simple menu by mouse-click. The entire method including sample introduction, LC/MS analysis and data handling steps is performed using one integrated method and one sequence table from within the Agilent Technologies ChemStation Software.

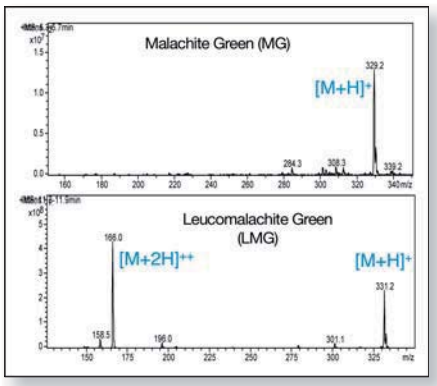
The sample clean-up steps ensure the removal of interfering matrix residue leading to significantly better signal to noise ratios and improved detection limits for MG and LMG in the MS system. The method is rugged and stable. RSDs range from 3.4 % to 5.3 % while recoveries are in the range from 89.5 % to 90.3 %.



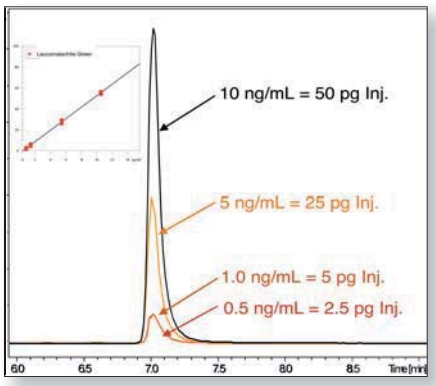
Mass spectra of malachite green (MG) and leucomalachite green (LMG)



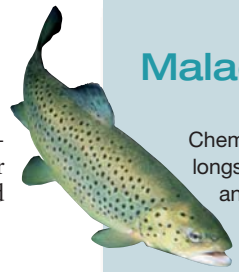
Chemical structure of malachite green and leucomalachite green



MS^2 spectra of MG and LMG



Calibration curve for leucomalachite green



Malachite green

Chemically, malachite green belongs to the group triphenyl methanes and is mainly used as a synthetic colorant, for example in lacquers.

Malachite green (MG) is also a highly effective disinfectant, capable of fighting various parasites, such as fungi, germs and single cell organisms that attack fish and fish roe. For this reason, MG is often used in fish aquaria, especially against white dot disease caused by the ichthyophthirius multifiliis parasite.

Malachite green is suspected of being a human carcinogen and of causing damage to human genetic material. To avoid any health risk to consumers, MG has been banned from use in animals destined for human consumption within the European Union (EU).

In the German state of Baden-Wuerttemberg a total of 336 samples were analyzed for triphenyl-methane compounds in 2005. Samples were taken from salt and fresh water fish as well as from trout roe. Forty four trout samples and one catfish sample were found to contain leucomalachite green (LMG), the main metabolite of MG. The concentrations found ranged from 2 to over 100 µg/kg. One trout sample was found to also contain MG at 1.5 µg/kg. The large number of tests and "positives" resulted from testing all basins in three fish-producing companies after random tests had revealed traces of MG. All cases where samples were found to contain residues of LMG were subsequently officially pursued.

(Source: Monitoring of food products, consumer products, cosmetics and animal feed. Annual report, Ministry of food and agriculture, Baden-Württemberg, Mail box 10 34 44, 70029 Stuttgart, Germany).

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