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Improving MS Detection of Malachite Green in Fish Products using Automated SPE coupled with an LC/Ion Trap MS system

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Introduction

Malachite green (MG) is a triphenyl methane dye that is highly efficient in battling fungi, bacteria and various single cell parasites. MG is traditionally used in aquaculture to treat and prevent fungal infections. MG, which is structurally related to known carcinogenic triphenylmethane dyes, is metabolized to leucomalachite green (LMG) and deposited in the fatty tissue of the fish. MG is under suspicion of being a human carcinogen and for causing damage to the human genetic material. Consumption of fish that is contaminated with MG is assumed to pose a significant health risk to humans. In 2003 the European Commission set the MRPL (minimum required performance limit) for MG and LMG to 2 µg/kg. Even though malachite green is banned as a veterinary pharmaceutical for animals used for human consumption, the authorities regularly find residues of this toxic compound or its metabolites during routine checks of fish farms. The work presented in this application note describes the configuration of a combined fully automated Solid Phase Extraction (SPE)

system coupled with LC/Ion Trap MS. Method parameters are shown that provide improved detection limits for the two compounds coupled with automated sample preparation for highest laboratory productivity.

EXPERIMENTAL

Sample Preparation. A fish filet sample was homogenized and extracted with an acetonitrile/water mixture. Following centrifugation, the supernatant was collected. The extraction process was repeated twice. Collected extracts were evaporated to dryness and resolved in a methanol/water mixture. The clean-up step was performed automatically, using a GERSTEL SPE system (Figure 1).

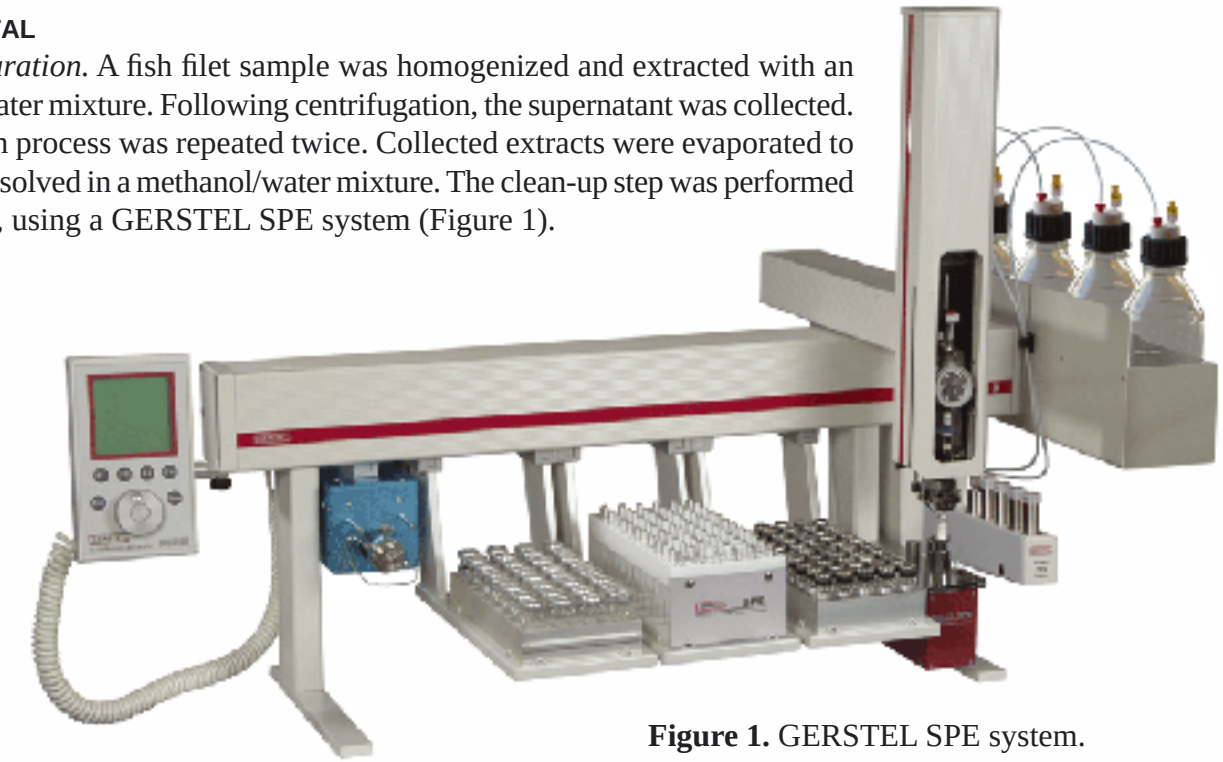

Figure 1. GERSTEL SPE system.

The user-defined SPE clean-up method is shown in Figure 2.

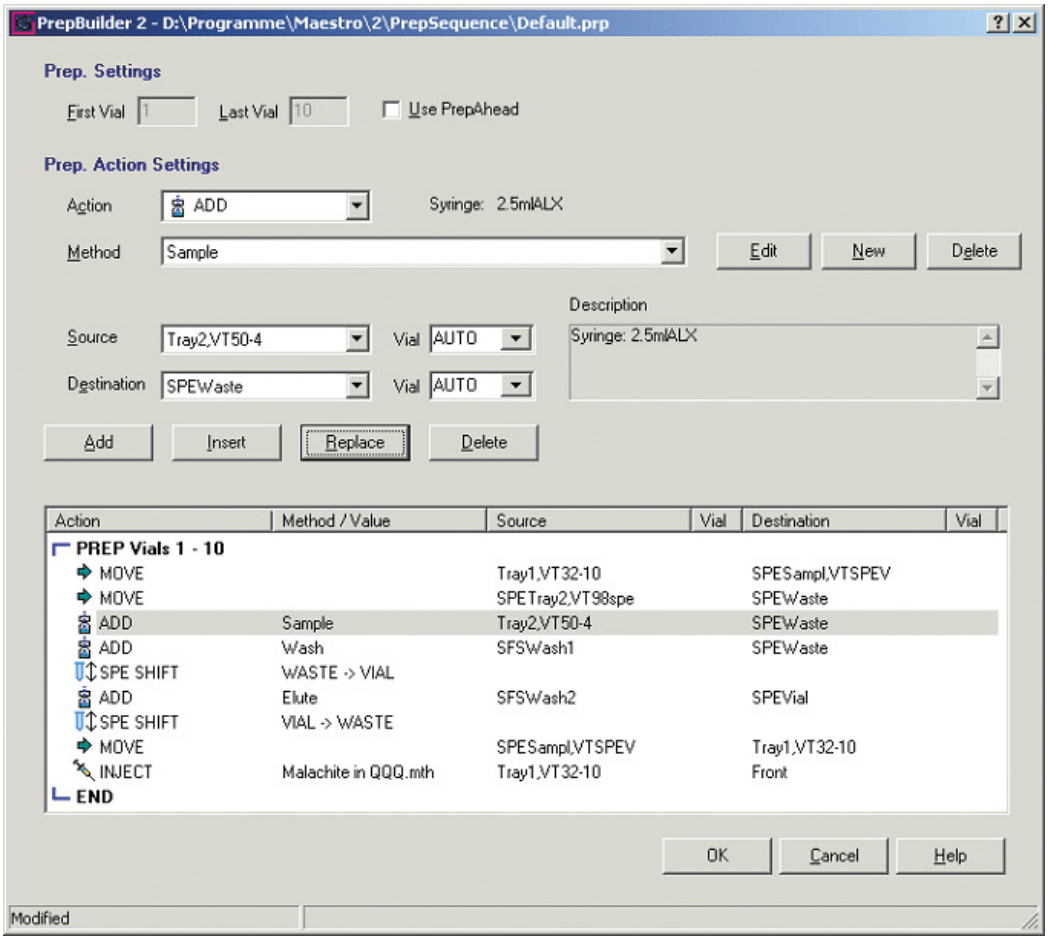

Figure 2. Method steps for SPE clean-up.

LC/MS Method. The GERSTEL SPE system was integrated in an Agilent 1100 LC/MSD Ion Trap system, consisting of a binary pump, thermostatted column compartment, diode array detector and an Agilent XCT+ Ion Trap MS. The LC/ITMS was used in electron spray ionization (ESI), positive ion mode. The injection volume used for all analyses was 5 µL. Chromatographic separations were performed on a Zorbax SB-C18 (50 x 2.1mm, 1.8µm), using a flow rate of 0.6 mL/min in gradient mode (Eluent A: 0.1% formic acid, Eluent B: acetonitrile).The column temperature was set to 50°C. Complete system control including sample preparation, LC/MS analysis and data evaluation was performed using GERSTEL MAESTRO software integrated with the Agilent ChemStation (Rev.A10.03).

Results and Discussion

Malachite green, as well as its main metabolite leuco malachite green, are easily ionized using electrospray ionization in positive ion mode. In contrast to MG itself, the metabolite forms a doubly charged ion (m/z 166) in addition to the singly charged molecular ion $[M-H]^+$ (Figure3).

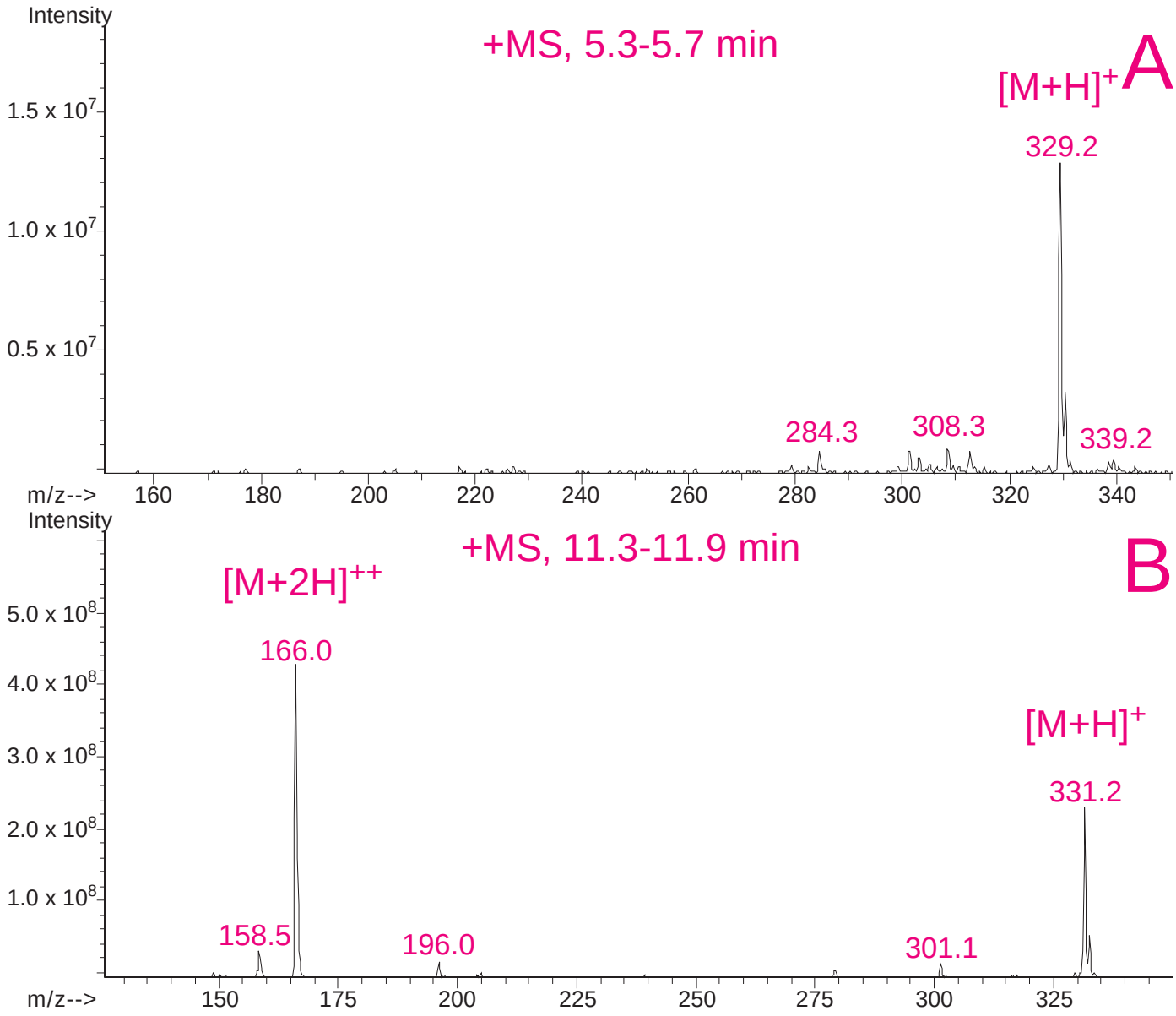

Figure 3. Mass spectra of malachite green (A) and leuco malachite green (B).

This is due to the non-planar sterical structure of the central carbon in the leuco form in contrast to the central carbon in malachite green (Figure 4). In MS^2 mode of the iontrap the malachite green precursor ion forms a product ion (m/z 313), while the doubly charged leuco malachite green precursor forms a fragment, which is also doubly charged (Figure 5). This transition is highly selective and can be used for extremely sensitive determination of leuco malachite green.

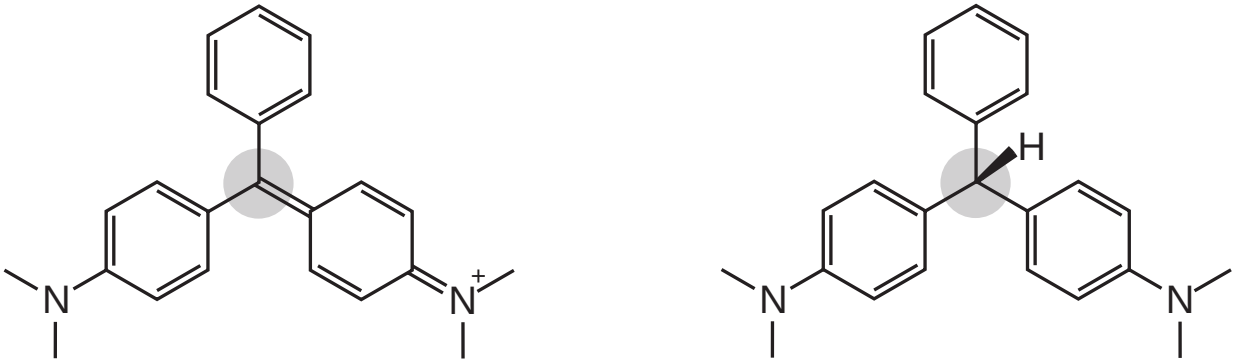

Figure 4. Chemical structures of malachite green (left) and leuco malachite green (right).

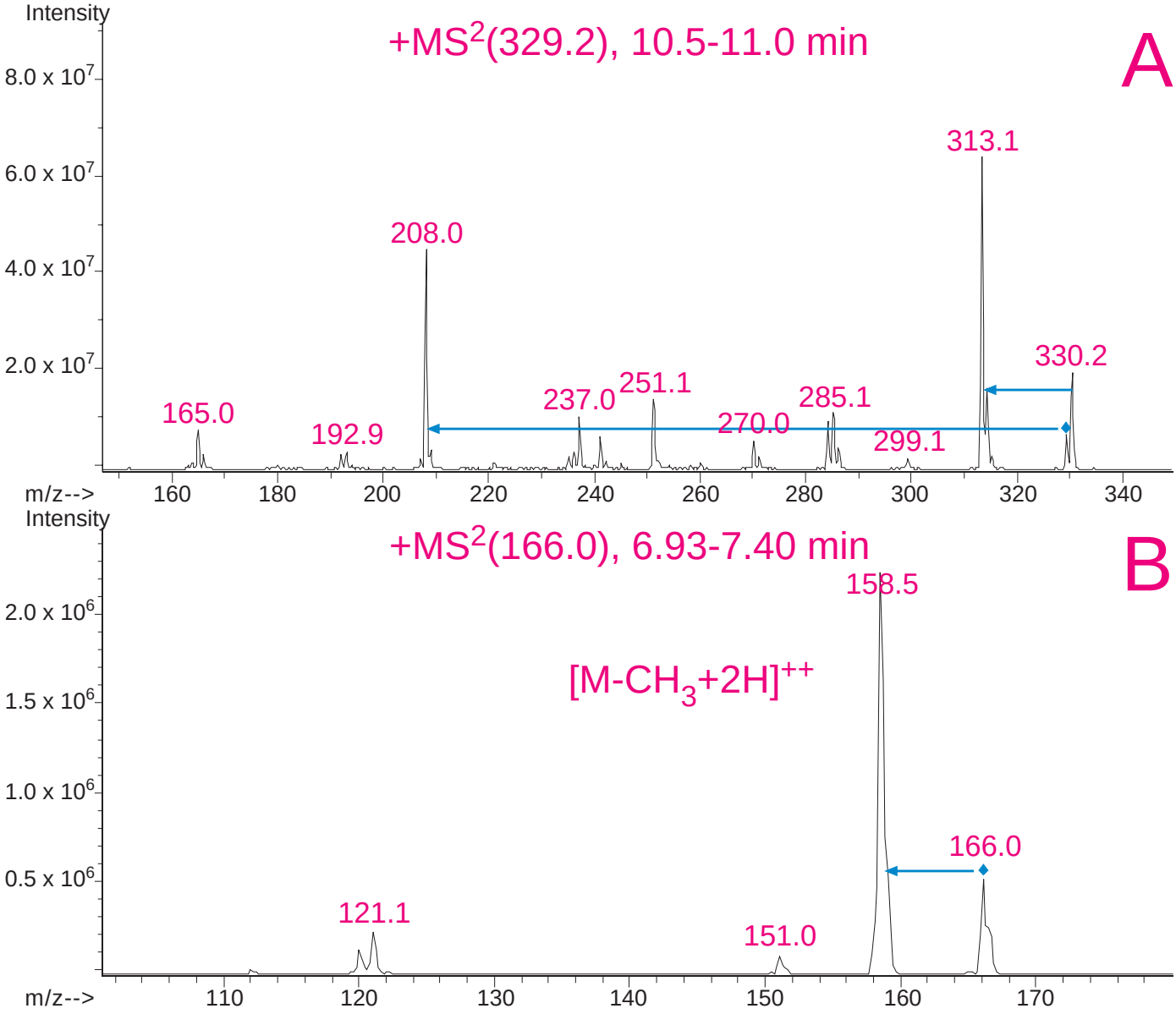

Figure 5. MS^2 Spectra of malachite green (A) and leuco malachite green (B).

Using these two transitions, it was possible to reach limits of determination of 0.5 µg/kg for malachite green and 0.05 µg/kg for leuco malachite green (Figure 6).

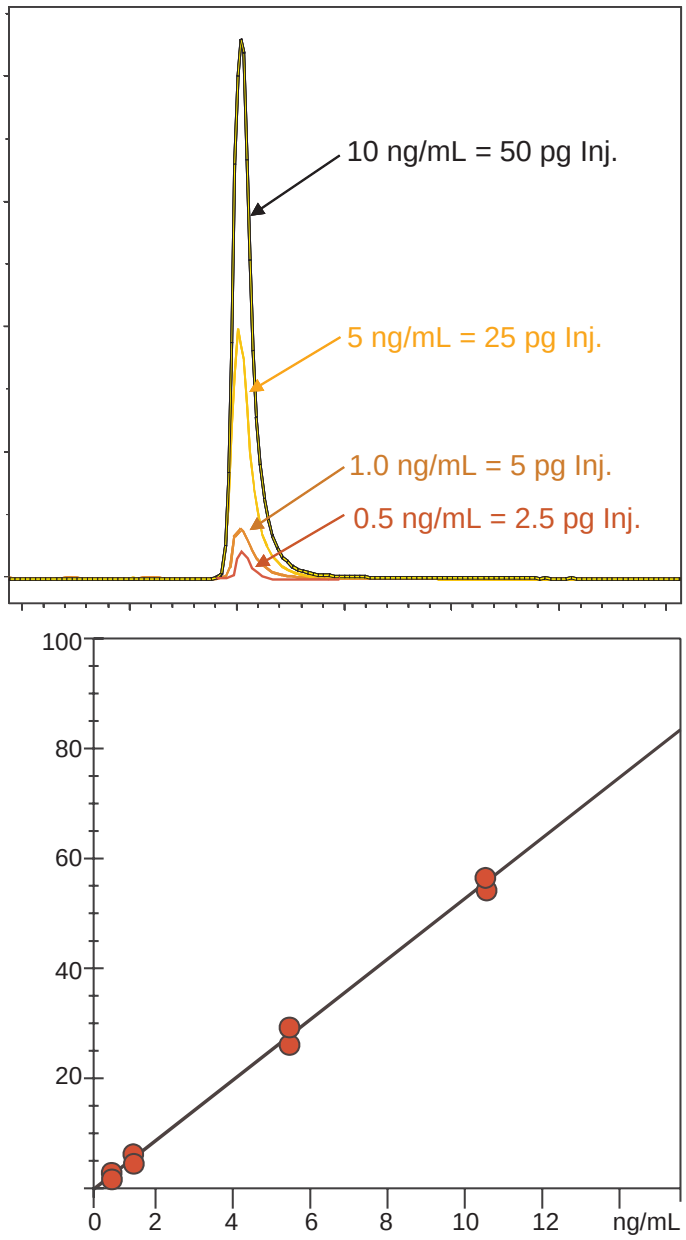

Figure 6. Calibration curve for leuco malachite green.

Automated Solid Phase Extraction (SPE) directly coupled to the LC/MS system provided a combination of high recovery rates, in excess of 90%, and excellent reproducibility of the sample preparation step. Moreover, the time required for sample preparation was reduced by approximately 50% compared with manual preparation using SPE cartridges, enabling a significant increase in throughput and laboratory productivity.

Conclusions

The described combined automated SPE/LC/ITMS system enables automated clean-up of extractions of homogenized fish fillet samples immediately followed by analysis of the cleaned extract. A user defined sample preparation and introduction method was set up by mouse-click from a software menu and automatically executed in the Agilent ChemStation environment. The clean-up step was found to reduce interferences from residual matrix, resulting in significant improvements in both MS response and limits of determination. The method was found to be rugged and stable (RSDs 3.4-5.3 %), providing good extraction recoveries (89.5-90.3 %). The entire method including sample introduction, LC/MS analysis and data handling was performed using one integrated method and one sequence table from within the Agilent Technologies ChemStation software. In future, other compounds of interest in food safety analysis, such as pesticides and nitrofurans, will be investigated using the described sample preparation method.

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