Closed-loop Stripping Analysis of Synthetic Musk Compounds From Fish Tissues With Measurement by Gas Chromatography-Mass Spectrometry With Selected-Ion Monitoring

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Abstract

Synthetic musk compounds have been found in surface water, fish tissues, and human breast milk. Current techniques for determining these compounds in fish tissues require tedious sample cleanup procedures. A simple method for screening for these compounds in fish tissues has been developed: closed-loop stripping of saponified fish tissues in a 1-L vessel. Due to their high vapor pressures, synthetic musks are stripped from the matrix onto a solid sorbent (abselut® NEXUS) using a Wheaton purge and trap vessel. We analyzed two homogenized samples of whole fish tissues with spiked synthetic musk compounds using closed-loop stripping analysis (CLSA) and pressurized liquid extraction (PLE). The analytes were not recovered in high yields but the extraction yield was sufficiently reproducible for at least semi-quantitative purposes (screening).

Introduction

Previous reports in the literature have shown that Galaxolide, Tonalide, musk xylene, and musk ketone, amongst others in this class of personal care products, are major micro-pollutants in most samples from surface water, human breast milk, fish, and human adipose tissues (1-7). Sonication, liquid-liquid, Soxhlet, and pressurized fluid extraction (PLE) have been used to extract synthetic musk compounds from fish tissues (8). These extraction techniques are expensive and nonselective. The process of isolating analytes of interest from lipid and cholesterol interferences are time consuming.

We compared the use of PLE and CLSA for the extraction of synthetic musk compounds from fish tissues using GC/MS in the selected-ion monitoring mode as the detection method. CLSA isolates the musks from lipids, thereby eliminating some cleanup steps.

1 Fish Collection and Preparation

Carp (*Cyprinus carpio*) with an average mass of 2.12 kilograms were collected by net from a lake in the southwestern United States. After cleaning, each fish was homogenized using a Hobart meat processor. About half of the total homogenate was packed in a wide-mouth amber jar and sealed with paraffin tape before it was refrigerated at -80 ^{o}C pending extraction. To avoid sample carryover, the stainless steel components of the Hobart meat processor were washed sequentially with hot tap water using Iso-clean® soap, DI-water with 10% acetic acid, and DI-water after each fish was processed.

2 Closed-loop Stripping of Musk Compounds From Fish Tissues

A 25-µL volume of a mixture of three surrogate standards (2,6-dinitrotoluene, pentachloronitrobenzene, and 2,2'-dinitrobiphenyl) was added to 100 g of homogenized fish tissues in a 1-L Wheaton purge and trap vessel. Five hundred mL of DI-water was added to the vessel, followed by the addition of 50 mL of 1 N sodium hydroxide solution and a magnetic stirrer bar. The 1-L vessel was placed on the top of a magnetic stirrer/hot plate (Fig. 1).

The fish sample was stirred and a pH of 12.75 was recorded using a pH meter. Heat was supplied by a recirculating water bath that provided flow through the water jacket surrounding the purge and trap vessel. A constant temperature of 50 ^{o}C was maintained inside the vessel.

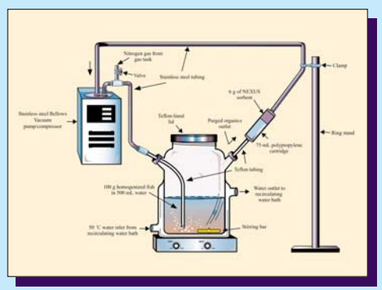

Fig. 1 Closed-loop stripping analysis apparatus.

Ultra-pure nitrogen gas at a flow of 4 L min^{-1} (maximum capacity of the pump) was used for purging the saponified fish tissue, while six grams of solid-phase adsorbent material (abselut® NEXUS, from Varian, Harbor City, CA, USA) (4) was used for trapping organic compounds that were being stripped from the solution. The nitrogen gas was recycled for 24 h. The sorbent cartridge was desorbed with polar and non-polar solvent, concentrated, and analyzed by GC/MS in the selected-ion monitoring mode.

3 Pressurized Liquid Extraction

An average of 2.3 g of fish tissue was weighed and transferred into a smooth surface mortar. Twenty-five µL of the solution of surrogate standards was added to the fish sample in the mortar. Eight samples from the same homogenized fish tissue were also spiked with the surrogate standards mixture and 100 µL of musk standards. This solution contained 15 musk compounds (see Table 1), each at a concentration of 20 µg/mL. Each fish sample was homogenized with diatomaceous earth hydromatrix and selectively extracted with polar and non-polar solvent, using PLE system. Each PLE extract was concentrated to 300 µL using a Turbo Vap II solvent evaporator at 35 ^{o}C under a gentle stream of nitrogen, and solvent exchanged to methylene chloride. The volume was then concentrated to 1 mL for gel permeation chromatography (GPC).

4 Extract Cleanup

Residual lipids from all fish PLE extracts were removed by gel permeation chromatography. This procedure has been detailed elsewhere (8). A subsequent cleanup was carried out by eluting the extract through 1 g of 3-aminopropyl derivatized silica solid-phase in a 6-mL polypropylene cartridge (Strata NH_2, from Phenomenex, Torrance, CA, USA). The extracts were concentrated and analyzed by GC/MS in the selected-ion monitoring mode.

5 GC/MS-SIM Analysis

Extracts from CLSA and PLE were individually reconstituted in toluene and concentrated to 90 µL, after which 10 µL of the internal standard, naphthalene-d_8 (100 µg/mL concentration), was added. Samples were analyzed using GC/MS in the selected-ion monitoring mode. An HP-5 MS capillary column was used to achieve baseline separation under the following GC conditions: Initial oven temperature was set at 90 ^{o}C, 0 min hold, ramped at 10 ^{o}C/min to 300 ^{o}C, and 10 min hold. EI at 70 eV, 3.94 scans/s were recorded (Figures 2 and 3).

6 Results and Discussion

The percentage spike recoveries for 15 musk compounds obtained from a 2.3 kg carp ranged from 88 (± 40) to 110 (± 2) for pressurized liquid extraction and from 11 (± 1) to 32 (± 3) for CLSA spiked samples (Table 1). However, the musk metabolites with low vapor pressures were not sufficiently recovered for the CLSA samples. For the PLE samples, the percent recoveries for 4-amino musk xylene, 2-amino musk xylene, and amino musk ketone were 107 (± 6), 92 (± 6), and 104 (± 8), respectively, while the percent recoveries of CLSA samples were 7 (± 5), 6 (± 4), and 2 (± 2), respectively.

Table 1. Percent spike recoveries of musk compounds in PLE and CLSA of fish tissues.

Analytes	Henry's Law Constants*	PLE % spike recovery (%RSD)**	CLSA % spike recovery (%RSD)***
Musk xylene	7.73 x 10^{-6}	93(5)	14(3)
Musk ketone	1.90 x 10^{-6}	101(6)	11(1)
Musk ambrette	7.05 x 10^{-4}	97(10)	15(5)
Musk tibetene	9.96 x 10^{-4}	102(4)	13(3)
Musk moskene	1.54 x 10^{-4}	94(5)	18(6)
Versalide®	9.96 x 10^{-3}	97(1)	32(2)
Galaxolide®	7.56 x 10^{-4}	96(3)	23(1)
Phantolide®	7.73 x 10^{-3}	110(2)	28(2)
Cashmeran®	1.42 x 10^{-1}	88(40)	29(3)
Celestolide®	7.05 x 10^{-3}	96(3)	32(3)
Traseolide®	1.94 x 10^{-2}	106(5)	29(2)
Tonalide®	1.09 x 10^{-2}	101(4)	32(2)
4- Amino musk xylene	3.79 x 10^{-7}	107(6)	7(5)
2- Amino musk xylene	3.79 x 10^{-7}	92(6)	6(4)
Amino musk ketone	9.30 x 10^{-8}	104(8)	2(2)

* Estimated values derived by EPA and SRC EPI-Suite in Ref. [9].
** Percent recovery of musk compounds from PLE of 2.44 g homogenized fish tissues (n=3)
*** Percent recovery of musk compounds from CLSA of saponified 100 g homogenized fish tissues in salted DI water (n=3)

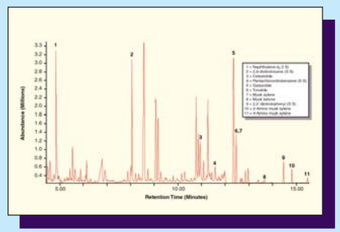

Fig. 2 CLSA-GC-MS chromatogram for synthetic musk compounds in unspiked 100 g fish sample (*Cyprinus carpio*).

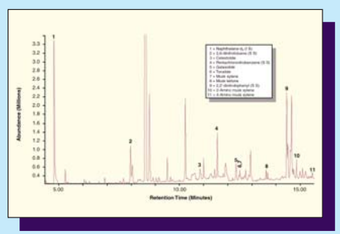

Fig. 3 PLE-GC-MS chromatogram for synthetic musk compounds in unspiked 2.09 g fish sample (*Cyprinus carpio*).

Conclusion

Closed-loop technology, when coupled to a high efficiency solid-phase adsorbent such as abselut® NEXUS, may be employed to economically screen biota for organic contaminants that have sufficiently high vapor pressures.

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