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Analytical Methods

Synthetic musk in seafood products from south Europe using a quick, easy, cheap, effective, rugged and safe extraction method

M. Saraiva [a], J. Cavalheiro [b], L. Lanceleur [b], M. Monperrus [b,*]

[a] National Health Institute Doctor Ricardo Jorge, (INSA), Av. Padre Cruz, 1649-016 Lisbon, Portugal
[b] Laboratoire de Chimie Analytique Bio-inorganique et Environnement (LCABIE), Institut Pluridisciplinaire de Recherche sur l'Environnement et les Matériaux (IPREM), CNRS UMR 5254, Université de Pau et des Pays de l'Adour, Hélioparc Pau Pyrénées, 2, av. P. Angot, 64053 Pau Cedex 9, France

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ABSTRACT

This study aims at developing a method for the determination of 9 synthetic musk compounds in seafood products by combining the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method and determination by gas chromatography mass spectrometry (GC–EI–MS). Method detection limits (MDL) ranging between 0.001 and 1.94 ng g^{-1} were obtained. The linearity is higher than 0.9899 in the range MDL – 100 ng g^{-1} with precision below 18% and recoveries between 46% and 120% were obtained. The method was applied to quantify musk compounds in seafood products from the European southwest coast (oysters, mussels, salmon organs, glass eels). Galaxolide and Tonalide exhibited the highest concentration levels ranging between MDL – 96.4 ng g^{-1} and MDL – 6.85 ng g^{-1}, respectively. Contamination levels observed for the two nitro musks (musk xylene and musk ketone) are significantly lower ranging between MDL – 0.6 ng g^{-1} and MDL – 0.09 ng g^{-1}, respectively. Analysis of different organs of salmons showed higher concentrations in liver and gonad than in muscle tissues.

1. Introduction

Synthetic musk compounds are mainly components of personal care products and household cleaners. Production has increased steadily in recent years with a worldwide production volume today of about 6000 tons per year (Mottaleb, Osemwengie, Islam, & Sovocool, 2012). Due to recent concerns about the toxicity of nitro-musks on humans and the environment, their use has been phased out, but the use of polycyclic musks in particular HHCB (Galaxolide) and AHTN (Tonalide) has continued to increase (Reiner & Kannan, 2011; Rimkus, 1999; Wu, Liu, & Ding, 2012)

Several studies have identified the entry of these compounds in the influent of wastewater treatment plants and their release into the effluent due to their incomplete removal by treatment processes (Reiner & Kannan, 2011). Because of their ubiquity and highly lipophilic properties, they have also been identified in aquatic organisms such as marine mammals, fish and mussels (Lee, Lee, & Oh, 2010) and their toxic effects such as endocrine disruption have been of great concern over the last decade, as well as their not well-known degradation products (Schreurs et al., 2004).

According to the European Union Risk Assessment Report, the lowest observed effect concentration (LOEC) for galaxolide in aquatic organisms is 0.182 mg L^{-1} (EU, 2008). Several studies report HHCB concentrations present in different organisms between non-detected–52.6 μg kg^{-1} of fresh weight in Danish trouts (Duedahl-Olesen, Cederberg, Pedersen, & Højgård, 2005), 42–230 μg kg^{-1} of lipid weight in fish from alpine Switzerland lakes (Schmid, Kohler, Gujer, Zennegg, & Lanfranchi, 2007), 234–970 μg kg^{-1} of fresh weight in bluegill from Texas, USA (Mottaleb et al., 2009), 89–102 μg kg^{-1} in Portuguese marine mussels (Picot Groz et al., 2014) and 16–367 μg kg^{-1} of dry weight in different seafood species from Tarragona, Spain (Trabalón et al., 2015).

Most of analytical methods developed to determine musk fragrances in fish tissue used various extraction techniques such as Soxhlet, micro-wave assisted extraction (MAE), focused ultrasound-solid liquid extraction (FUSLE), and pressurised liquid extraction (PLE) usually followed by a clean-up step (silica gel, fluorisil and/or gel permeation chromatography (GPC)) prior to analysis with GC MS (Vallecillos, Pocurull, & Borrull, 2015a, 2015b). However, there is still a challenge in developing more routine and environment-friendly methodologies. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) sample preparation procedure was introduced in 2003 by Anastassiades, Lehotay, Stajnbaher, and Schenck (2003) and was generally used for the analysis of pesticides residues in food matrices. These methods involve an extraction step followed by a clean-up step which is unconditionally necessary before the GC analysis, particularly for

* Corresponding author.
E-mail address: mathilde.monperrus@univ-pau.fr (M. Monperrus).

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biological tissues, because the extracts generally contain lipids and other higher molecular weight compounds which may cause matrix effects (Kallenborn et al., 1999). The QuEChERS procedure is based on dispersive solid phase extraction (d-SPE) which ensures larger and more reproducible recoveries of the analytes with acidic or basic properties, does not require SPE apparatus such as cartridges, vacuum pumps, drying out and solvent evaporation steps. d-SPE uses less sorbent, smaller amounts of sample and less equipment and provides better interaction with the extract for the clean-up step. It is therefore quicker and cheaper when compared to traditional SPE and its main limitation is that it can only be used when the SPE sorbent removes matrix components and not the analytes (Wilkowska & Biziuk, 2011). The first typical QuEChERS applications were the analysis of pesticides in fruit and vegetable matrices (Anastassiades et al., 2003). This procedure was then applied to the analysis of other molecules in biological tissues, such as triclosan and methyltriclosan (Gonzalo-Lumbreras, Sanz-Landaluze, & Cámara, 2014), persistent organic pollutants (Norli, Christiansen, & Deribe, 2011), pyrethroid pesticides (Jia et al., 2012), endocrine disrupters (Munaretto et al., 2013), brominated flame retardants (Kalachova, Cajka, Sandy, Hajslova, & Pulkrabova, 2013; Sapozhnikova & Lehotay, 2013) and volatile compounds (Yamamoto et al., 2014). In this work, a QuEChERS procedure was optimized and validated in order to determine synthetic musk compounds in biological tissues. The method was applied to investigate the occurrence of these compounds in seafood samples (oysters, mussels, glass eels, salmons) from the European southwest coast.

2. Materials and methods

2.1. Reagents and materials

The solid standards of synthetic fragrances, 1,3,4,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(γ)-2-benzopyran (galaxolide or HHCB, 25% diethylphtalate), 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (tonalide or AHTN, 99%), 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone (cashmeran or DPMI, 99%), 4-acetyl-1,1-dimethyl-6-tert-butylindane (celestolide or ADBI, 99%), 6-acetyl-1,1,2,3,3,5-hexamethylindane (phantolide or AHMI, 99%) were obtained for LGC Standards (Barcelona, Spain). The 1,1,3,3,5 pentamethyl-4,6-dinitroindane (musk moskene or MM) was purchased as 10 mg L^{-1} solution in cyclohexane from the same company. The 4-aceto-3,5-dimethyl-2,6-dinitrotert-butylbenzene (musk ketone or MK) was purchased the Dr. Ehrenstorfer GmbH (Augsburg, Germany) with 99% and 98% purity, respectively. 2,4,6-trinitro-1,3-dimethyl-5-tert-butylbenzene (musk xylene or MX) was obtained from Sigma–Aldrich (St. Louis, USA) as 100 mg L^{-1} in acetonitrile. The internal standard, [$^2H_{15}$]-musk xylene, was also supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany) as 100 mg L^{-1} in acetone.

Acetonitrile and ethyl acetate, used to prepare stock solutions and as organic solvents in the QuEChERS procedure were of analytical grade and were supplied by Sigma Aldrich (Lyon, France). Chloroform, methanol, sulfuric acid, orthophosphoric acid and vanillin used for the lipid content analysis were of analytical grade and were supplied by Sigma Aldrich (Lyon, France).

The QuEChERS extract tubes were obtained from Agilent Technologies (Massy, France) and contained the citrate buffer salt mixture (4 g of $MgSO_4$, 1 g of NaCl, 0.5 g disodium citrate sesquihydrate and 1 g of trisodium citrate dihydrate). Two dispersive SPE tubes for the clean-up step containing either 900 mg of $MgSO_4$, 150 mg of octadecyl C18 and 150 mg of primary secondary amine (PSA) exchange or 900 mg of $MgSO_4$ and 150 mg PSA were obtained from Agilent Technologies (Massy, France).

2.2. Sample collection

Twelve different biological samples were collected in 7 different locations along the European southern coast. Table 1 shows the different organisms that have been collected (oysters, mussels, salmon and glass eels) with the sampling date and their origin. Sampling sites are representative of the different aquatic environments of the southern Europe as they include near-shore environments (La Rochelle, France; San Vicente de la Barquera, Spain), coastal lagoons (Arcachon, France; Aveiro, Portugal) and estuaries (Urdabai, Spain; Sado, Portugal; Adour, France) (Fig. 1). The separation of flesh and shell of oysters and mussels samples was made after freezing the samples. Glass eels were frozen as well as the salmon's tissues (liver, muscle and gonads). Frozen samples were then lyophilized, grinded, sieved and homogenized. The glass amber bottles foreseen for the sample storage were previously decontaminated using nitric acid (65% from Merck) purified with a sub boiling distillation system (Milestone, SubPUR) and ultra pure MQ water (Millipore element).

2.3. Sample preparation

The optimized QuEChERS procedure required the weight of 0.2 g of lyophilized sample (balance AG 204 from Mettler Toledo) into an extraction tube, as well as 1 mL of acetonitrile and 5 μL of internal standard ([$^2H_{15}$]-MX) at 1 μg L^{-1}. The tube was manually shaken for one minute and then placed in an ultrasonic bath (Model 8510 from Branson) for 10 min. Subsequently, the following salt mixture was added: 170 mg of sodium citrate, 80 mg of hydrogenated disodium citrate, 670 mg of magnesium sulfate

Table 1
Description of the samples origin, date and site of collection and the site description.

Sample name	Sample type	Sampling date	Sampling site	Site description
LR_WO	Wild oysters	April-2013	La Rochelle, France	Near-shore
LR_CO	Cultivated oysters	April-2013	La Rochelle, France	Near-shore
SVB_CO	Cultivated oysters	May-2013	San Vicente de la Barquera, Spain	Near-shore
AB_CO	Cultivated oysters	April-2013	Arcachon Bay, France	Coastal lagoon
AB_WO	Wild oysters	April-2013	Arcachon Bay, France	Coastal lagoon
AB_WM	Wild mussels	April-2013	Arcachon Bay, France	Coastal lagoon
AVR_CO	Cultivated oysters	January-2014	Aveiro, Portugal	Coastal lagoon
URD_WO	Wild oysters	May-2013	Urdaibai, Spain	Estuary
SADO_WO	Wild oysters	April-2013	Sado, Portugal	Estuary
NIV_SM	Salmon muscle	October-2011	Nivelle, France	Estuary
NIV_SL	Salmon liver	October-2011	Nivelle, France	Estuary
NIV_SG	Salmon gonads	October-2011	Nivelle, France	Estuary
ADR_GE	Glass eels	March-2014	Adour, France	Estuary