Journal of Chromatography A, 1339 (2014) 13–25

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Development of a multi-preservative method based on solid-phase microextraction–gas chromatography–tandem mass spectrometry for cosmetic analysis☆

Gerardo Alvarez-Rivera, Marlene Vila, Marta Lores, Carmen Garcia-Jares, Maria Llompart*

Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Chemistry, Campus Vida, University of Santiago de Compostela, E-15782 Santiago de Compostela, Spain

ARTICLE INFO

Article history:
Received 20 December 2013
Received in revised form 23 February 2014
Accepted 24 February 2014
Available online 5 March 2014

Keywords:
Preservatives
Antioxidants, Cosmetics
Solid-phase microextraction
Personal care products
GC–MS/MS

ABSTRACT

A simple methodology based on solid-phase microextraction (SPME) followed by gas chromatography–tandem mass spectrometry (GC–MS/MS) has been developed for the simultaneous analysis of different classes of preservatives including benzoates, bronidox, 2-phenoxyethanol, parabens, BHA, BHT and triclosan in cosmetic products. *In situ* acetylation and subsequent organic modifier addition have been successfully implemented in the SPME process as an effective extractive strategy for matrix effect compensation and chromatographic performance improvement. Main factors affecting SPME procedure such as fiber coating, sampling mode, extraction temperature and salt addition (NaCl) were evaluated by means of a $3 \times 2^{3-1}$ factorial experimental design. The optimal experimental conditions were established as follows: direct solid-phase microextraction (SPME) at 40 °C and addition of NaCl (20%, w/v), using a DVB/CAR/PDMS fiber coating. Due to the complexity of the studied matrices, method performance was evaluated in a representative variety of both rinse-off and leave-on samples, demonstrating to have a broad linear range ($R^2 > 0.9964$). In general, quantitative recoveries (>85% in most cases) and satisfactory precision (RSD < 13% for most of compounds) were obtained, with limits of detection (LODs) well below the maximum authorized concentrations established by the European legislation. One of the most important achievements of this work was the use of external calibration with cosmetic-matched standards to accurately quantify the target analytes. The validated methodology was successfully applied to the analysis of different types of cosmetic formulations including body milks, moisturizing creams, deodorants, sunscreen, bath gel, dental cream and make-up products amongst others, demonstrating to be a reliable multi-preservative methododology for routine control.

1. Introduction

Preservatives are essential ingredients widely added in daily used cosmetics and personal care products (PCPs) such as toothpastes, shampoos, creams, deodorants, etc., with the primary purpose of preventing spoilage, whether from microbial growth or undesirable oxidative processes. The esters of benzoic acid (benzoates) and *p*-hydroxybenzoic acid (parabens), 2,4,4-trichloro-2′-hydroxydiphenyl ether (triclosan, TCS), 2-phenoxyethanol, bromine-containing preservatives as 5-bromo-5-nitro-1,3-dioxane (bronidox), as well as the antioxidants 2-*tert*-butyl-4-methoxyphenol (BHA) and 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT) are frequently used preservatives in cosmetic formulations.

Despite their protective effect, the widespread use of these products has led to a social concern about the unintended harmful effects that some of these ingredients could have on consumer's health. Although benzoates were determined to be safe in the present practices of use in cosmetics [1], other ingredients such as parabens have been reported to have estrogenic/antiandrogenic-like properties [2,3]. In addition, a potential relationship between breast cancer and prolonged dermal exposure to paraben-containing products is suggested [4]. BHA and TCS may also modulate and disrupt the endocrine system [5,6]; whereas in the case of bronidox, the formation of carcinogenic nitrosamines is to

☆ "Presented at the XIII Scientific Meeting of the Spanish Society of Chromatography and Related Techniques (SECyTA2013), 8–11 October 2013, Puerto de la Cruz, Tenerife, Canary Islands, Spain.".

* Corresponding author. Tel.: +34 881814225; fax: +34 881814468.
E-mail address: maria.llompart@usc.es (M. Llompart).

http://dx.doi.org/10.1016/j.chroma.2014.02.075
0021-9673/

be expected when co-formulated with products containing amines or amino derivatives [7].

In the EU context, these ingredients are subjected to several restrictions according to the EU Cosmetics Regulation [8], where limitations, requirements, label warnings, and the maxima permissible concentrations are indicated. As regards the above mentioned preservatives, the European legislation has established maxima allowed concentrations ranging from 0.1% (w/w), for bronidox, to 1% (w/w) for 2-phenoxyethanol. The maximum allowed limit for each individual paraben, as acid, has been set at 0.4% (w/w), and 0.8% (w/w) for mixtures of esters, while the maximum allowed level for TCS is 0.3% (w/w).

Therefore, to allow authorities to control the content of preservatives in the wide variety of marketed personal care products, effective and convenient methodologies are required. Methods for preservatives analysis in cosmetic samples are mainly focused on parabens determination, whereas analytical methods for the analysis of more than one class of preservatives are still a field under development. Liquid chromatography (LC) [9–11], capillary electrophoresis (CE) [12–14], capillary zone electrophoresis (CZE) [15,16] and micellar electrokinetic chromatography (MERKC) [17–20] have been widely used. Flow injection analysis (FIA) has also been employed enhancing sample throughput [21]. Although in less extent, gas chromatography (GC) coupled to mass spectrometry detectors has been applied [22]. Nevertheless, using GC approach, a derivatization step previous to phenolic preservatives analysis is highly recommendable to improve chromatographic performance [23,24]. Acetylation is an advantageous derivatization procedure, offering a high efficient derivatization process using low-cost reagents, compared to silylation agents. This derivatization strategy, using acetic anhydride and pyridine has been firstly applied by the authors, to the determination of multi-class preservatives in cosmetics previous to GC–MS analysis [25,26]. A variation of this reaction, employing acetic anhydride and sodium hydrogen phosphate, has also been performed in aqueous samples for the determination of phenolic preservatives such as parabens and triclosan in water [27].

However, the chromatographic analysis of cosmetic samples becomes a challenging task without a good sample pretreatment. Common sample preparation strategies involving several steps are frequently tedious and time-consuming, and the use of hazardous solvents is usually required. Moreover, the possible presence of interferences that could distort the results is not rejectable. In an attempt to overcome these problems, advanced extraction techniques such as supercritical fluid extraction (SFE) [24], solid-phase extraction (SPE) [28], pressurized liquid extraction (PLE) [26] and matrix solid-phase dispersion (MSPD) [25,29], have been recently applied for the determination of preservatives and other different additives in cosmetics.

Solid-phase microextration (SPME) is one of the most attractive extraction techniques due to its simplicity and high extraction efficiency. SPME integrates sampling, extraction, concentration and sample introduction into a single uninterrupted process, which makes this procedure a valuable alternative analytical technique to more traditional procedures, reducing the laboratory generated waste and time for sample preparation [30]. This technique has been applied for the determination of parabens and some antioxidants in cosmetic samples [31,32]. In these papers, the optimization of variables affecting the SPME procedure was carried out using the one-factor-at-a-time approach. By contrast with the factorial design method, this classical approach has the following drawbacks: (i) it requires more runs for the same precision in effect estimation; (ii) it cannot estimate interactions effects; (iii) the conclusions from its analysis are not general; (iv) it can miss optimal settings of factors [33]. Thus, using an experimental design approach, the optimization of a SPME procedure for the analysis of bronidox in rinse-off cosmetics was reported [34].

This work aims to optimize, validate and put into practise a simple methodology based on solid-phase microextraction followed by gas chromatography–tandem mass spectrometry (SPME–GC–MS/MS) for the simultaneous analysis of different classes of preservatives in a representative and wide variety of rinse-off and leave-on cosmetics. *In situ* acetylation was used for target compounds derivatization using acetic anhydride and sodium hydrogen phosphate. Major efforts were focused on avoiding matrix effects, an essential requirement to make feasible the analysis of cosmetic ingredients by external calibration, considering the broad diversity of cosmetic products. After that, factorial design was selected to simultaneously evaluate the main experimental factors affecting SPME. It is important to highlight that this is the first time MS/MS has been applied to the detection of target preservatives in personal care products (except for some parabens [22]), which is expected to increase the selectivity of the determinations in such complex matrices.

2. Experimental

2.1. Reagents and materials

Methylbenzoate (MeBz; 99%), ethylbenzoate (EtBz; 99%), butylbenzoate (BuBz) and phenylbenzoate (PhBz; 99%) were supplied by ChemService (West Chester, USA). 2-phenoxyethanol (Phox; 99%) was obtained from Fluka Chemie GmbH (Steinheim, Germany). Bronidox (≥99.0%) was acquired from Fluka (Sigma–Aldrich Chemie GmbH, Buchs, Switzerland). Methylparaben (MeP; 99%), ethylparaben (EtP; 99%), propylparaben (PrP; 99%), butylparaben (BuP; 99%), benzylparaben (BzP; 99%), butylated hydroxyanisole (BHA; ≥98.5%), butylated hydroxytoluene (BHT; 99%), and triclosan (TCS; ≥97.0%) were purchased from Aldrich (Milwaukee, WI, USA). Isopropylparaben (*i*-PrP; ≥99%) and isobutylparaben (*i*-BuP; ≥97%) were acquired from TCI Europe (Belgium).

As isotopically labeled internal standard (IS), deuterated methyl-4-hydroxybenzoate-2,3,5,6-d_4 (MeP-d_4; 98 atom% D), propyl 4-hydroxybenzoate-2,3,5,6-d_4 (PrP-d_4; 98 atom% D), and triclosan-d_3 (2,4-dichlorophenoxy-d_3) (TCS-d_3, 97 atom% D), was obtained from C/D/N Isotopes (Quebec, Canada), whereas 2,6-Di(tert-butyl-d_9)-4-methyl(phenol-3,5,O-d_3) (BHT-d_{21}, 98 atom% D) were purchased from Sigma–Aldrich.

Acetone, ethyl acetate, and acetic anhydride were provided by Merck (Darmstadt, Germany). Sodium chloride (99.7%) was supplied by Prolabo (VWR, Fontenay-sous-Bois, France). Sodium hydrogenphosphate heptahydrate was acquired from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). All solvents and reagents were of analytical grade.

Individual stock solutions of each compound were prepared in acetone. Further dilutions and mixtures in the same solvent were prepared by convenient dilution of the stock solution to spike cosmetic samples (when needed). Derivatized standards in ethyl acetate were prepared by adding 100 μL of acetic anhydride containing 2.5% of pyridine to 1 mL of the standard solution. The mixture was then maintained at 80 °C for 10 min, and then allowed to cool down before GC analysis [25]. Stock and working solutions were stored in a freezer at −20 °C protected from light. For daily evaluation of the GC equipment, a derivatized solution of 0.5 μg mL^{-1} of the target compound in ethyl acetate was also prepared to direct injection into the chromatograph.

Commercially available 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane–divinylbenzene (PDMS/DVB), 85 μm polyacrylate (PA), 75 μm carboxen–polydimethylsiloxane