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Determination of synthetic phenolic antioxidants and their metabolites in water samples by downscaled solid-phase extraction, silylation and gas chromatography–mass spectrometry

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ABSTRACT

The development and performance evaluation of an analytical method dedicated to the comprehensive determination of the most relevant antioxidants and their metabolites in aqueous environmental samples is presented. This was achieved by a miniaturised solid-phase extraction (SPE) with 10 mg Oasis HLB cartridges, which allow to achieve a concentration factor of 200, reducing organic solvent wastes (1 mL of ethyl acetate suffices for complete elution) and SPE costs and eliminating the need for solvent evaporation that otherwise compromises the recoveries of butylated hydroxytoluene (BHT) and 2,6-di-*tert*-butylcyclohexa-2,5-diene-1,4-dione (BHT-Q). Analytes were then determined by gas chromatography–mass spectrometry (GC–MS) after derivatisation with N-methyl-N-(*tert*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) in a single run. BHT-d_7 and *n*-propyl-paraben-d_4 (PrP-d_4) were used as surrogate internal standards. These surrogates allowed obtaining relative recoveries in the 80–110% range for all analytes even with complex wastewater samples and LODs at the 2–44 ng L^{-1} level taking into account blank issues often associated to antioxidants analysis. The method was applied to sewage and river waters, showing that the seven analytes could be detected in raw wastewater. BHT and BHT-Q were the most concentrated species in that type of sample (in the 275–871 ng L^{-1} range). On the other hand two metabolites of BHT, 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (BHT-CHO) and 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (BHT-COOH) appeared to be the most ubiquitous species, being found in all samples in the 10–150 ng L^{-1} concentration range.

1. Introduction

Antioxidants are substances which prolong the shelflife of foodstuffs by protecting them against deterioration caused by oxidation, such as fat rancidity and colour changes. Since natural antioxidants are usually of poor stability, manufacturers prefer to use synthetic antioxidants. Many synthetic compounds are active as antioxidants, but only a few are used because of very strict safety regulations. The most frequently used are the synthetic phenolic antioxidants (SPA). FDA [1] and EU [2] have established the permitted food phenolic antioxidants and amounts of their allowable usage. SPAs currently permitted for use in food are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ), propyl gallate, octyl gallate and dodecyl gallate, usually at concentrations up to 100–200 μg g^{-1} of SPAs in oils or fats, either singly or in combination. The use of SPAs is not restricted to foodstuffs. Thus, they are permitted in many types of packaging materials, in adhesives that come in contact with food and also in cosmetics, personal care products and pharmaceuticals. Among the SPAs, BHA and BHT are the most used antioxidants.

The results of scientific studies about the consumption of these additives are controversial since several studies have shown a potential link between BHA, BHT and cancer [3,4], while other studies have shown no link [5,6], and even a protective effect [7]. Nevertheless, their degradation products should be evaluated since they may pose an environmental or human health risk [8].

Studies on the metabolism of BHT have revealed that there are two main metabolic processes [9]; that is, oxidation of the alkyl substituent and oxidation of the aromatic ring system. 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (BHT-COOH) is a major metabolite formed by oxidation of the alkyl substituent and may be generated via the corresponding alcohol (BHT-OH) and aldehyde (BHT-CHO). Moreover, oxidation of the π-system of BHT leads, amongst others,

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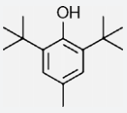
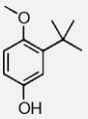
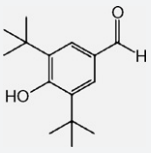
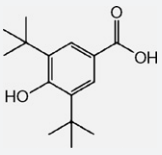
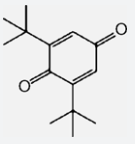
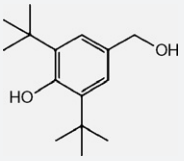
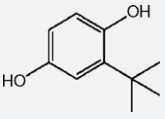
Table 1
Analyte abbreviations, structures and other relevant data.

Abbreviation	IUPAC name	CAS	Formula	Estructura	Monisotopic MW	Log K_{ow}[a]	pK_a[a]	P_v (Torr)[a]
BHT	2,6-di-*tert*-Butyl-4-methylphenol	128-37-0	$C_{15}H_{24}O$		220.18	5.319 ± 0.235	12.75 ± 0.4	0.00624
BHA	2-*tert*-Butyl-4-methoxyphenol	25013-16-5	$C_{11}H_{16}O_2$		180.12	2.998 ± 0.235	11.82 ± 0.18	4.46E−3
BHT-CHO	3,5-di-*tert*-Butyl-4-hydroxybenzaldehyde	1620-98-0	$C_{15}H_{22}O_2$		234.16	4.769 ± 0.279	8.33 ± 0.40	1.28E−3
BHT-COOH	3,5-di-*tert*-butyl-4-Hydroxybenzoic acid	1421-49-4	$C_{15}H_{22}O_3$		250.17	4.796 ± 0.253	4.77 ± 0.10	3.28E−5
BHT-Q	2,6-di-*tert*-Butylcyclohexa-2,5-diene-1,4-dione	719-22-2	$C_{14}H_{20}O_2$		220.15	3.902 ± 0.381	–	2.81E−3
BHT-OH	2,6-di-*tert*-Butyl-4-(hydroxymethyl)phenol	88-26-6	$C_{15}H_{24}O_2$		236.18	3.675 ± 0.251	12.00 ± 0.40	3.37E−4
TBHQ	2-*tert*-Butylbenzene-1,4-diol	1948-33-0	$C_{10}H_{14}O_2$		166.10	2.333 ± 0.225	10.78 ± 0.18	1.12E−3

[a] Software calculated value, from SciFinder Scholar Database 2006: http://www.cas.org/products/sfacad/.

to 2,6-di-*tert*-butylcyclohexa-2,5-diene-1,4-dione (BHT-Q). On the other hand, the degradation of BHA produces TBHQ.

Most of the methods described in the literature for the quantitative analysis of antioxidants or antioxidant mixtures have been developed for the analysis of foodstuffs and food packaging [10]. In these cases, liquid chromatography with UV detection was the most common determination technique following the extraction by liquid–liquid extraction (LLE) or solid-phase extraction (SPE) of the sample. However, those methods are not applicable for trace analysis in environmental matrices because they do not offer the necessary selectivity and sensitivity. Hence, the establishment of sensitive and selective analytical methods to monitor the widespread in the environment of antioxidants and their degenerative products is a real need.

In aqueous environmental samples (i.e. wastewater samples and river water) most of the data of occurrence of these analytes have been obtained in multi-residue studies which evaluated the presence of one or two antioxidants together with a wide range of other organic compounds such as pharmaceuticals, phthalates, phenols, etc. In those methods, LLE [11], solid-phase microextraction (SPME) [12] and mainly SPE [13,14] have been used as pre-concentration techniques followed by GC–MS determination. However, studies dedicated to the development of analytical methods and subsequently the occurrence of SPAs and their metabolites in the aqueous environment are very scarce. The only exceptions are the works of Fries and Püttman, who studied BHT together with its metabolite BHT-CHO in river, ground and wastewater samples of Germany, where these pollutants were typically detected in the 10–2000 ng L^{-1} range, depending on the sample nature [15,16].

It is then necessary to develop analytical methods that allow the determination of antioxidants and a broader range of metabolites in the aqueous environment. Therefore, the goal of this work was the development and performance evaluation of a method that allows the determination of the three main synthetic phenolic antioxidant (i.e. BHT, BHA and TBHQ) together with their four most relevant metabolites (BHT-CHO, BHT-COOH, BHT-OH and BHT-Q; TBHQ is also a metabolite of BHA) in water by GC–MS combined to SPE for the enrichment of samples. Moreover, critical aspects associated