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Short communication

Determination of antioxidants by solid-phase extraction method in aqueous food simulants

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Abstract

An analytical method for the determination of specific migration levels of phenolic antioxidants and one phosphite antioxidant in the aqueous food simulants established by European legislation has been developed. This method involves solid-phase extraction (SPE) of the antioxidants with silica C_{18} cartridge and their determination by liquid chromatography (LC) with diode-array detection. The achieved results in the studies of elution volume determination, breakthrough volume and accuracy are showed. Recoveries in the range of 78–104% and a relative standard deviation between 2.0 and 7.7% have been achieved.

Keywords: Antioxidants; Food packaging; LC–UV; Specific migration; SPE

1. Introduction

Hindered phenols, primary antioxidants, are added to polyolefins to manage the oxidation reaction in the polymer for long-term protection and organophosphites, secondary antioxidants are short-term antioxidants designed to provide protection during processing or fabrication to finished product [1,2]. In this way, phosphites help to preserve the primary antioxidants [3] during processing step.

If the polyolefins are used in packaging food, these compounds or their degradation products could migrate from plastics into foodstuffs during the processing or storage. So, European legislation imposes specific migration limits upon individual substances with the potential to migrate from plastics into foodstuffs according to their individual toxicity [4,5]. Migration test can be carried out using the simulants laid out in the Council Directive 82/711/EEC: distilled water (simulant A) for aqueous foods, acetic acid 3% (w/v) (simulant B) for acidic foods, ethanol 10% (v/v) (simulant C) for alcoholic foods and rectified olive oil or other fatty food simulants (simulant D) for fatty foods [6].

The aim of this work is to develop an analytical method with suitable detection limits and good repeatability and reproducibility for the determination of hindered phenols and organophosphite antioxidants at the concentration range required by the legislation. This method will be allow to know the specific migration levels of more usual commercial antioxidants used in low-density polyethylene (LDPE) and polypropylene (PP).

The studied antioxidants are the phenolic antioxidants, Ethanox 330; Irganox 1010; Irganox 1076; BHT (butylated cresol); tertbutylhidroxyanisole (BHA); the phosphite antioxidant Irgafos 168 and its degradation product DBP.

In a previous work [7], an analytical method for the liquid–liquid extraction (LLE) of the same antioxidants from the simulant A using hexane as extraction solvent has been performed, but at this time the interest is to develop a solid-phase extraction (SPE) method because this technique offers some advantages compared with LLE as lower processing time, a substantial solvent saving and its possible automatization [8]. On the other hand, the interest is to extend the

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applicability of the method to the three aqueous food simulants A–C.

As far as known, there are no published SPE methods for preconcentration of studied antioxidants, so sample preparation for phenolic compounds analysis from aqueous matrix using SPE packed with different sorbents has been used. Jahr [9] employed a SPE column cartridge packed with styrene-divinylbencene-copolymer for the determination of phenols in water after their acetylation. Rodríguez et al. [10] evaluated two SPE procedures for the preconcentration of chlorophenols in drinking water using graphitized carbon black cartridges (after derivatisation of the sample) or styrene-divinylbencene. In this way, non polar reversed-phase sorbents with silica base were the first materials tested in SPE of phenols in water and good results were achieved if a derivatisation step previous SPE is introduced [11,12]. For example, Bao et al. [13] developed a method for the analysis of phenol, alkylphenols, halogenated phenols and nitrophenols in aqueous samples by extraction with C_{18} disk achieving recoveries higher than 80% for most of the phenolic compounds. Considering that the methods of the different sorbents include a derivatisation step and *n*-alkylsilicas sorbents are widespread used because of its good reproducibility in retention, rapid equilibrium with mobile phases and very few irreversible adsorption of solutes [8], in this work SPE packed with C_{18} sorbent for the determination of phenolic antioxidants was tested.

2. Materials and methods

2.1. Reagents and solvents

Methanol and tetrahydrofuran LC-gradient grade were supplied by Merck (Darmstadt, Germany). Water was purified on a Milli-RO system (Millipore, Bedford, MA, USA).

The studied antioxidants were obtained from the following sources: butylated hydroxyanisole (BHA, mixed isomers 2[3]-t-butyl-4-hydroxyanisole; 2[3]-*t*-butylhydroquinone monomethyl ether, minimun 90%-3-isomer/9%-2-isomer) CAS No. [25013-16-5]; 2,6 ditertbutyl-*p*-cresol (BHT, 99%) CAS No. [128-37-0] and 1,3,5-trimethyl-2,4,6-tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)benzene (Ethanox 330, 99%) CAS No. [1709-70-2] from Sigma–Aldrich (Steinheim, Germany). 2,4-Bis (1,1-dimethylethyl)-phenol (DBP, ≥98%) CAS N° [96-76-4] from Fluka (Buchs, Switzerland). Tris(2,4-ditert-butylphenyl)phosphite (Irgafos 168) CAS N° [31570-04-4]; pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate (Irganox 1010) CAS N° [6683-19-8] and octadecyl-3-(3,5-di-tertbutyl-4-hydroxyphenyl)-propionate (Irganox 1076) CAS [2082-79-3] from Ciba (Basel, Switzerland).

Individual stock standard solutions of each antioxidant (1000 mg L^{-1}) were prepared in acetonitrile for BHA, DBP, BHT and Irganox 1010, in a mixture of methanol:tetrahydrofuran (75:25) for Ethanox 330 and in tetrahydrofuran for Irganox 1076 and Irgafos 168.

According to Garde et al. [14–15] fully oxidized Irgafos 168 is obtained after 24 h of dissolution in tetrahydrofuran (THF). Therefore, this antioxidant is detected as its oxidized product because THF is employed in stock standard solution preparation.

Stock standard solutions containing all the compounds were prepared from individual standard solution (1000 mg L^{-1}) by dilution with acetonitrile.

2.2. Instrumentation and chromatographic conditions

The chromatographic experiments were carried out following the method developed in a previous work [7]. A Waters 2695 equipment (Waters, Milford, MA, USA) with a gradient pump and automatic injector was used. The seven analytes were completely separated using a stainless steel column 3.9 mm × 150 mm packed with Nova Pack C_{18}60 Å, 4 μm particle size (Waters). The detection system was a model 996 UV photodiode array (Waters). The chromatographic conditions are summarized in Table 1. The signal acquired from detector was recorded by a personal computer operated under the Millenium^{32} software V. 3.20 (Waters).

Each compound was identified by comparison of its retention time with corresponding peak in the standard solution and its UV spectrum. Quantification was carried out using a calibration plot of external standard (five points between 2.5 and 20 mg L^{-1}).

2.3. Solid-phase extraction

The used silica C_{18} cartridges Sep-Pack Plus 360 mg were purchased from Waters (Milford, MA, USA). A vacuum system Büchi (Flawil, Switzerland) V-500 equipped with pressure controller Büchi B-721 was used to force the sample through the cartridge.

As pretreatment step, the samples of simulant (A, B or C) fortified with the antioxidants were modified by adding acetic acid and/or ethanol until concentration of 3% (v/v) at acetic acid and 10% (v/v) at ethanol for all samples.

Before extraction, silica C_{18} cartridges were conditioned first with 4 mL of methanol and then with 4 mL of distilled water. The spiked samples were percolated at a flow rate of

Table 1
Elution gradient conditions for LC analysis

Time (min)	Methanol (%)	Water (%)	Curve
0	20	80	
5	60	40	Linear
9	100	0	Linear
17	100	0	Linear
18	20	80	Convex
20	20	80	Linear

Flow = 1 mL min^{-1}, wavelength = 276 nm, column oven temperature = 30 °C, injection volume = 20 μL.

approximately 1–2 mL min^{-1}, the vacuum system was adjusted at 970 mbar or less in order to maintain this flow rate. Elution of the retained antioxidants was carried out (without vacuum) with methanol and THF. Both solvents were passed sequentially and collected together.

3. Results and discussion

According to Rodríguez et al. [16] derivatisation reactions of phenols can be used to improve efficiency of SPE procedures by enhancing reversed-phase interactions. Phenol acetylation with acetic anhydride in presence of carbonate [13] or hydrogencarbonate [11,17] is one of the most studied derivatisation procedures. Therefore, in our first assays, the acetylation of antioxidants previous to its SPE with silica C_{18} was proved. The aqueous sample spiked with antioxidants was acidified with acetic acid until 3% (p/v) (simulant B), then the phenolic antioxidants were derivatisated with acetic anhydride in presence of sodium carbonate. Sample was passed through the column C_{18}. The studied antioxidants were eluted with methanol, solvent used to desorb retained phenols in silica sorbents [16] and THF because all the considered antioxidants show high solubility in it. The obtained recoveries were not good enough (69–88%) and the variability was too much large. The reason of these low recoveries may be that the derivatisation reaction was unfinished. Some compounds have high molecular mass and this may have a negative effect in the derivatisation reaction. On the other hand, this method only could be applied to the simulants A and B because poorer recoveries were achieved for the simulant C (11–69%). So that, a different procedure without derivatisation was carried out, the sample spiked with antioxidants (simulant A, B or C) is modified by adding acetic acid and/or ethanol until a final concentration 3% at acetic acid and 10% at ethanol and this modified sample is passed through the silica C_{18} cartridge. Finally, the retained antioxidants are eluted with methanol and tetrahydrofuran.

3.1. Determination of elution volume

A volume of 100 mL of water Milli-RO spiked with antioxidants until 0.5 mg L^{-1} with acetic acid until 3% and ethanol until 10% were employed for all analysis.

Antioxidants elution was carried out passing two solvents, methanol and THF through the column. The elution volume of the two solvents, methanol and tetrahydrofuran was modified between 2 and 10 mL to determine the minimum volume necessary that guarantee the complete elution of all antioxidants. Equal volumes of methanol and THF were employed in each test. In this way, 10 mL of methanol and 10 mL of THF were used in the first assay and 2 mL of methanol and 2 mL of THF in the last.

The plot of Fig. 1 shows that recovery results are approximately constant (between 70 and 100 %) when the total volume is higher or equal than 8 mL (4 mL of methanol + 4 mL of THF). The individual effect of methanol and THF in the recovery of the analytes was studied injecting their eluents separately. A minimum methanol elution volume of 4 mL achieves the best recoveries for most antioxidants (Fig. 2). When the total volume is lower than 8 mL the achieved recoveries with methanol decrease from a range of 55–99% until 29–89% (Fig. 2) while the recoveries with THF (Fig. 3) increase from a range between 0 and 20% until 0–46% (Fig. 3), because this last solvent is eluting analytes that insufficient methanol was not able to recover.

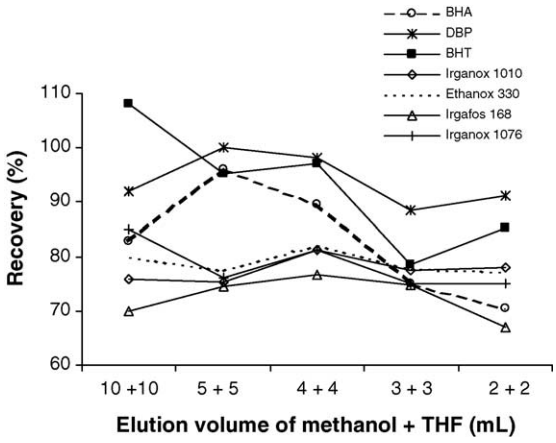

Fig. 1. Achieved recoveries for the total elution volume assays by SPE C_{18} from samples of 100 mL with 10% ethanol and 3% acetic acid spiked until 0.5 mg L^{-1} for each antioxidant.

On the other hand, it can be seen that there are compounds that are better eluted with methanol than with THF, as BHA and Irganox 1010. Both antioxidants are not recovered with THF (Fig. 3) even when only 2 or 3 mL of methanol are used in the first elution and significant amounts of BHA and Irganox 1010 remain in the SPE cartridge (Fig. 2).

Irganox 1076 was the best eluted analyte by THF, as can be seen in Fig. 3 where 2 mL of THF recovers 46% of this antioxidant when 2 mL of methanol only desorbs 29% of Irganox 1076 (Fig. 2).

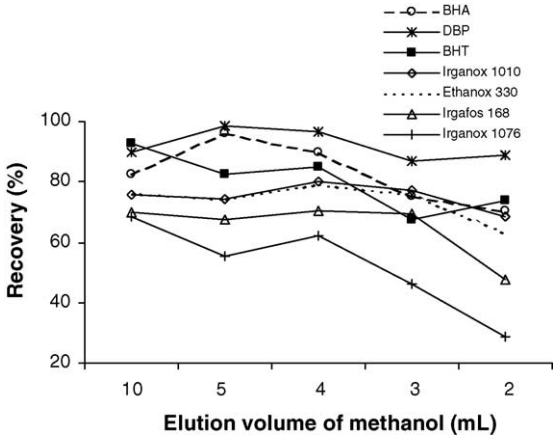

Fig. 2. Achieved recoveries with methanol in the elution volume assays by SPE C_{18}.

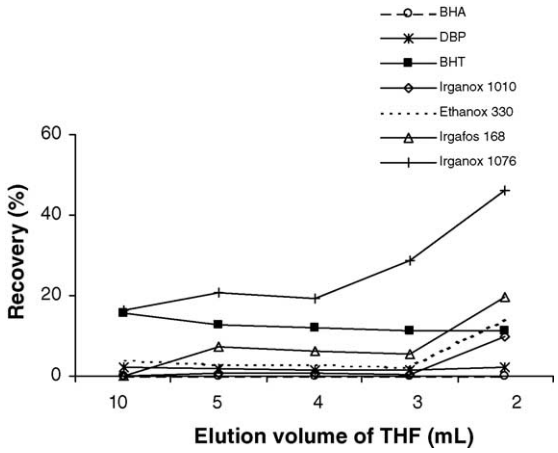

Fig. 3. Achieved recoveries with THF in the elution volume assays by SPE C_{18}.

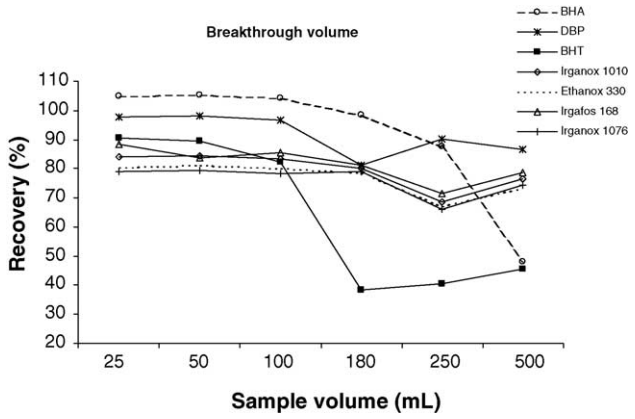

Fig. 4. Achieved recoveries for the breakthrough volume assay by SPE C_{18} from samples with 10% ethanol and 3% acetic acid spiked with 0.05 mg of each antioxidant.

So, the methanol volume was fixed in 5 mL in order to guarantee the total elution of BHA and Irganox 1010 and to complete the elution of all antioxidants the volume elution was increased until 8 mL of total solvent with 3 mL of THF.

A new assay with the final volumes of elution, 5 mL of methanol and 3 mL of THF was performed. After their pass through the column independently, they were collected together and mixed by shaking. Then, the final solution was analysed by LC. The obtained recoveries were similar to the maximum achieved recoveries for the previous assays with higher elution volume, (4 + 4), (5 + 5) and (10 + 10) mL.

3.2. Determination of breakthrough volume

An assay for the determination of breakthrough volume was performed according to the procedure described by Henion [8]. It consists of preconcentrating samples of increasing volumes, each containing the same amount of analytes. As the sample volume increases, the analyte concentration decreases. While breakthrough does not occur, the amount preconcentrated remains constant but when breakthrough occurs, the amount extracted is reduced.

The assay was performed for samples spiked with 0.05 mg of each antioxidant, acetic acid until 3% and ethanol until 10%. The sample volume was modified to determine the breakthrough volume of each compound from 25 until 500 mL. The achieved results are showed in the Fig. 4. The obtained recoveries for the assays between 25 and 180 mL are in the range of 78–105%, they can be considered constant for all antioxidants except for BHT whose recovery decreases for the 180 mL assay until 47%. For the other antioxidants, recovery decreases in the 250 and 500 mL assays and it keeps constant for DBP. According to this, 100 mL can be fixed as a suitable sample volume for SPE by C_{18} for all considered antioxidants, because breakthrough does not occur at this value.

3.3. Repeatability

The repeatability assay of the developed SPE C_{18}–LC–UV analytical method was performed for samples of 100 mL spiked with 0.5 mg L^{-1} of each antioxidant, acetic acid until 3% and ethanol until 10%. In the Table 2, the achieved recoveries for the seven antioxidants are showed, they are between 78 and 104%, with a relative standard deviation between 2.0 and 7.7%. These achieved recoveries are better than the obtained results in previous studies, where extraction of antioxidants in the simulant A by liquid–liquid extraction (LLE) using hexane as solvent was employed. The LLE–LC–UV recoveries were between 66 and 88%, with a relative standard deviation in the range 3.4–13% [7]. Therefore, SPE C_{18}–LC–UV method is allowed to obtain higher recoveries with better precision than LLE method.

Table 2
Repeatability, detection and quantification limits of the SPE C_{18}–LC–UV analytical method ($n = 7$) for an aqueous sample of 100 mL spiked until 0.5 mg L^{-1}, 3% acetic acid and 10% ethanol

$n = 7$	Recovery (%)	R.S.D.	xDL (mg L^{-1})	xQL (mg L^{-1})
BHA	104	2.0	0.052	0.17
DBP	97	3.0	0.038	0.13
BHT	82	6.2	0.045	0.15
Irganox 1010	83	6.6	0.048	0.16
Ethanox 330	80	6.6	0.034	0.15
Irgafos 168	86	7.7	0.071	0.24
Irganox 1076	78	6.5	0.060	0.20

The obtained values of detection and quantification limits [18] are satisfactory included in the range of 0.034–0.071 and 0.13–0.24 mg L^{-1}, respectively (Table 2).

The developed SPE C_{18}–LC–UV method without acetylation allows obtaining recoveries similar the achieved for SPE of derivatisated phenols from aqueous samples. So, Bao et al. [13] obtained recoveries in the range 75–139% with R.S.D. between 3 and 9% for alkylphenols and chlorophenols and lower values for phenol and nitrophenol using SPE C_{18} of the acetyladed compounds. Rodríguez et al. [16] achieved recoveries in the range 78.3–100.2% with R.S.D. between 4.4 and 13.0% when they use carbon cartridges and recoveries in the range 84.6–104.0% with R.S.D. between 6.2 and 11.1% when they use styrene-divinylbenzene cartridges for SPE of chlorophenols from tap water samples.

4. Conclusions

- The achieved recoveries with SPE C_{18} are better than the obtained results in previous studies, where extraction of antioxidants in the simulant A was performed by liquid–liquid extraction (LLE). The LLE results obtained were between 66 and 88%, with a relative standard deviation in the range 3.4–13% [7] in front of the achieved with the SPE method, from 78 to 104%, with a relative standard deviation between 2.0 and 7.7%.
- The quantification limits of this analytical method SPE–LC–UV are smaller than 0.24 mg L^{-1} for all studied antioxidants in 100 mL of sample. These values are quite lower than the SML established by Directive 2002/72/CE for Irganox 1076 and for BHT (classified as cresol).
- The method is valid for the three aqueous food simulants established by the legislation (A–C) with the condition of modifying the sample until 3% at acetic acid and 10% at ethanol before SPE.
- SPE C_{18} allows achieving high recoveries with good precision for all phenolic antioxidants from aqueous food simulants without acetylation step previous SPE.

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References

[1] F. Chen-Yu Wang, J. Chromatogr. A 891 (2000) 325.
[2] M. Thilén, R. Shishoo, J. Appl. Polym. Sci. 76 (2000) 938.
[3] D.R. Stevenson, D.L. Stein, in: S. Al-Malaika, A. Golovoy, C.A. Wilkie (Eds.), Specialty Polymer Additives, Blackwell Science, Oxford, 2001, p. 43.
[4] R. Ashby, in: R. Ashby, I. Cooper, S. Harvey, P. Tice (Eds.), Food Packaging Migration and Legislation, Pira International, Leatherhead, Surrey, UK, 1997, p. 17.
[5] Directive 2002/72/EEC, L 39 (6 August 2002) Brussels, p. 2.
[6] Directive of the Council 82/711/EEC, L 297 (18 October 1982) Brussels, p. 26.
[7] M.S. Dopico-García, J.M. López-Vilariño, M.V. González-Rodríguez, J. Chromatogr. A 1018 (2003) 53.
[8] M.C. Hennion, in: Barceló (Ed.), Sample Handling and Trace Analysis of Pollutants Techniques, Applications and Quality Assurance, Elsevier, Ámsterdam, 2000, p. 4.
[9] D. Jahr, Chromatographia 47 (1998) 49.
[10] I. Rodríguez, M.C. Mejuto, M.H. Bollaín, R. Cela, J. Chromatogr. A 786 (1997) 285.
[11] T.J. Boyd, J. Chromatogr. A 662 (1994) 281.
[12] V. Janda, H. Van Langenhove, J. Chromatogr. 472 (1989) 327.
[13] M.L. Bao, F. Pantani, K. Barbieri, D. Burrini, O. Griffini, Chromatographia 42 (1996) 227.
[14] J.A. Garde, R. Catalá, R. Gavara, J. Food Prot. 61 (1998) 1000.
[15] J.A. Garde, R. Catalá, R. Gavara, R.J. Hernandez, Food Addit. Contam. 18 (2001) 750.
[16] I. Rodríguez, M.P. Llompart, R. Cela, J. Chromatogr. A 885 (2000) 291.
[17] M. Llompart, M. Lourido, P. Landín, C. García-Jares, R. Cela, J. Chromatogr. A 963 (2002) 137.
[18] J.C. Miller, J.N. Miller, Estadística para Química Analítica, Addison-Weley Iberoamericana, Spain, 1988 (Chapter 5).