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Solid-phase microextraction for qualitative and quantitative determination of migrated degradation products of antioxidants in an organic aqueous solution

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Abstract

Low molecular weight aromatic substances may migrate out from plastic packaging to their contents, especially if they consist of organic aqueous solutions or oils. It is, therefore, extremely important to be able to identify and quantify any migrated substances in such solutions, even at very low concentrations. We have in this work investigated and evaluated the use of solid-phase microextraction for the specific task of extraction from an organic aqueous solution such as a simulated pharmaceutical solution consisting of 10 vol.% ethanol in water. The goal was furthermore to investigate the possibility of simultaneously identifying and quantifying the substances in spite of differences in their chemical structures. Methods were developed and evaluated for extraction both with direct sampling and with headspace sampling. Difficulties appeared due to the ethanol in the solution and the minute amounts of substances present. We have shown that a simultaneous quantification of migrated low molecular weight degradation products of antioxidants using only one fibre is possible if the extraction method and temperature are adjusted in relation to the concentration levels of the analytes. Comparions were made with solid-phase extraction.

Keywords: Solid-phase microextraction; SPME; Antioxidants; Transformation products; Migration

1. Introduction

Food and pharmaceutical packages are generally made of polypropylene since it can be sterilised by heat. Polypropylene, as well as other polyolefins, undergoes an irreversible deterioration process affected primarily by the processing and end-use environment [1]. Molecular oxygen, UV-radiation, thermal and mechanical breakdown, micro-organisms and interaction with other substances all have the potential to affect the polymer. In order to impede or minimize these effects, several methods have been developed. This paper focuses on antioxidants added to the polymer to prevent oxidation processes. Polypropylene is particularly susceptible to oxidation as a consequence of high concentrations of tertiary hydrogen atoms, which make radical formation more favourable. Unfortunately, antioxidants and their degradation products have a tendency to diffuse from the polymer matrix into the surrounding environment [2–5], e.g. a food or pharmaceutical solution. The antioxidants used today, especially in pharmaceutical packages, are well documented and shall not give any toxic effects. New antioxidants are, however, continuously introduced on the market, and since these antioxidants and their deterioration products may be toxic it is important to have them identified and quantified [6]. Difficulties arise, however, in the analyses which are especially attributable to the nature of the pharmaceutical or food solutions, the minute amounts of degradation products in the sample, and the large differences in their chemical structures.

The traditional methods used for this type of problem are, e.g. liquid–liquid extractions, purge and trap, headspace and solid-phase extraction (SPE). Despite the versatility and efficiency of SPE, the method has certain limitations. Only

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liquid samples are analysable and analytes may exhibit low breakthrough volumes, i.e. the analyte of interest is not completely retained by the sorbent. In addition, the possibility of concentrating the analytes is limited and this increase the detection limits. Merely evaporation is a way to increase the concentration of analytes in a solution without breakthrough related problems. The evaporation will, unfortunately, not only decrease the volume of the solution but also the amount of the volatile species of interest. Another technique, solid-phase microextraction (SPME), developed in 1989 [7], has been used by our group since 1997 [8] and its use has increased rapidly in recent years. The growing popularity is based on the easy sample preparation without the need for solvents and on the efficient concentration of volatile and semi-volatile analytes from both liquid and solid samples. SPME is not a solution for all kind of products, only volatile and semi-volatile, but the risk of evaporation of volatile species of interest during preconcentrations is avoided.

The SPME fibre is usually exposed to the aqueous or gaseous sample until an equilibrium stage is reached between the analyte in the sample and on the fibre. The time to reach equilibrium depends on the analyte. The fibre is subsequently placed directly in a gas chromatograph, or a liquid chromatograph using a specific interface. Complex samples with over 200 degradation products from polyethylene have been successfully identified by our group by inter alia extraction from headspace of solid samples using fibres with different polarities to reduce the complexity in the chromatograms [9]. SPME has also been successful for identification and quantification of degradation products from solid samples of polyamide 6-6, nitrile rubber and polyvinylchloride/polycaprolactone (PC) [10–13]. A new question is whether it is possible to use SPME for the identification and quantification of very low amounts of low molecular degradation products of antioxidatants in an organic aqueous solution such as a simulated pharmaceutical solution consisting of 10% ethanol in water. The ethanol makes extraction with SPME more difficult than in water because it increases the solubility of the analytes in the solution and competes with the analytes for the extraction efficiency of the fibre.

Organophosphate triesters, analogous to the common antioxidant and their degradation products, e.g. triphenyl phosphate with documented contact allergenic properties and haemolytic effects, have been detected in human plasma at very low levels, i.e. 0.13–0.15 μg/g plasma [14], and this emphasizes that there is a need to be able to detect small amount of antioxidants and their degradation products. Food and pharmaceutical solutions in general diverge in their physical properties from pure water, and this may lead to a higher degree of migration than to pure water and to the migration of other substances that are not soluble in pure water. Polypropylene containing Irganox 1010 and Irgafos 168 has, therefore, been aged in a simulated food and pharmaceutical solution consisting of 10 vol.% ethanol in water [15–17].

Table 1
Substances used for the method development and their abbreviations

Substance	Abbreviation
2,4-Ditertbutyl-phenol	Dtb-p
2,6-Ditertbutyl-*p*-benzoquinone	Dtb-bq
3,5-Ditertbutyl-4-hydroxyphenylpropionic acid	Dtb-hppa
2,6-Ditertbutyl-4-methoxyphenol	Dtb-mp
3,5-Ditertbutyl-4-hydroxybenzoic acid	Dtb-hba
Triphenyl phosphate	Tpp
Tri-*p*-tolyl phosphate	Ttp
Diphenyl phosphate	Dpp

The purpose of this work was to investigate the possibility of using SPME to identify and quantify low molecular degradation products from antioxidants that have migrated from polypropylene into an organic aqueous solution. In addition, the possibility of quantifying them simultaneously using the same fibre was studied. As mentioned in the introduction, difficulties arise due to the ethanol in the solution, the minute amounts of substances present and the large differences in their chemical structure. It is essential, at a very low concentration of analytes, to achieve as good an extraction as possible. Methods have been evaluated for extraction with the SPME fibre immersed in the sample solution (direct SPME) and with the fibre exposed to the headspace above the solution (HS-SPME). Two of the most common antioxidants, Irganox 1010 and Irgafos 168, were used as model substances. They are both approved for food and pharmaceutical applications and were chosen as model substances since their degradation products are well documented and have chemical structures similar to those of many other common antioxidants. To test the usefulness of SPME versus other extraction methods for analysation of very low concentrations of analytes, evaluation was also performed using solid-phase extraction.

2. Experimental

2.1. Materials

Unstabilised polypropylene reactor grade powder with a melt flow rate of 0.22 g/10 min (230 °C, 2.16 kg) and a density of 900–910 kg/m^3 was kindly provided from Borealis GmbH (Linz, Austria), from which films of 45 μm thickness where blown on a two-layer axon line with extruders having a screw diameter of 18 mm. Before the film blowing process, 0.1 wt.% of pentaerythritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate) (Irganox 1010) and tris(2,4-ditert-butylphenyl)phosphate (Irgafos 168), provided from Ciba Specialty Chemicals AB (Västra Frölunda, Sweden), were added. The substances used in the development of the method and their abbreviations are presented in Table 1. The 2,6-ditertbutyl-*p*-benzoquinone (98%) was purchased from Lancaster Synthesis (Morecambe, England), 3,5-ditertbutyl-4-hydroxyphenylpropionic acid (98%) from Alfa Aesar (Karlsruhe, Germany), diphenyl phosphate

(98%) from Sigma-Aldrich (Steinheim, Germany) and 2,4-ditertbutyl-phenol (97%), 2,6-ditertbutyl-4-methoxyphenol (97%), 3,5-ditertbutyl-4-hydroxybenzoic acid (99%), triphenyl phosphate (99%) and tri-*p*-tolyl phosphate (90%) were purchased from Acros Organics (Geel, Belgium).

2.2. Sample preparation

Samples were prepared by filling 20 ml glass vials with strips of polypropylene film containing antioxidants, approximately 5 cm long, with a total weight of about 0.3 g. Fourteen millilitres of 10% EtOH/H_2O solution (99.5% pure ethanol from Kemetyl AB, Haninge, Sweden and Mill-Q water), simulating a pharmaceutical solution, was subsequently transferred to each vial. Each vial was closed with a PTFE Butyl septum (Perkin-Elmer, Upplands-Väsby, Sweden). Samples extracted with SPME were stored in an oven at 80 °C (±1 °C) for 4 months. After this period, the samples were placed in a refrigerator at 4 °C where further degradation was considered negligible. Blank 20 ml vials containing 14 ml of 10% EtOH/H_2O were prepared later in order to detect any interfering peaks from sample matrix and septum. These vials were prepared after the ageing time of the samples and were, therefore, placed in an oven at 120 °C for 3 days to accelerate the influence of the thermal treatment. The samples were pH-adjusted to 2 with a 0.5 M HCl solution and saturated with NaCl (p.a. quality from Merck, Darmstadt, Germany) before extraction. Samples extracted with SPE were stored in an oven at 80 °C (±1 °C) for 4 months, 40 °C (±0.5 °C) for up to 12 months and at 60 °C (±1 °C) for 2.5 months. Some of the samples aged at 40 °C or 60 °C went through a sterilisation procedure using a Certo Clav A-4050 Tisch-Autoclav (purchased from VWR International, Stockholm, Sweden), at 120 °C for 20 min, before the ageing.

2.3. Gas chromatography–mass spectrometry (GC–MS)

All analyses of direct SPME were performed on a Varian 3400 Gas Chromatograph (Walnut creek, CA, USA) with a WCOT fused silica CP-Sil 8 CB low bleed column, 30 m × 0.25 mm × 0.25 μm (Varian purchased from Scantec Lab., Partille, Sweden). The GC was equipped with a FID detector. With the exception of the last step in the method development, the column temperature was kept at 40 °C for 3 min and subsequently programmed to 250 °C at 10 °C/min, after which the column was held at 250 °C for 10 min. The injector temperature was 250 °C and the detector temperature 275 °C. The splitter vent was closed and nitrogen (99.9999% purity from AGA, Stockholm, Sweden) was used as carrier gas.

The HS-SPME were analysed on a GCQ GC–MS from ThermoFinnigan (San José, CA, USA) equipped with a Gestel MPS2 (Mülheim and der Ruhr, Germany) SPME autosampler, except for the early comparisons with direct SPME on the instrument described above. The column was a CP-Sil 8 CB/MS from Varian with dimensions 30 m × 0.25 mm × 0.25 μm (purchased from Scantec Lab., Partille, Sweden). The temperature program and injector temperature were the same as above. The ion-trap mass spectrometer scanned in the mass range of 35–650 *m/z*. Helium (99.9999% purity from AGA, Stockholm, Sweden) was used as carrier gas. The analyses here were performed also with splitless injection. The filament was switched off during the first 4 min to avoid overloading the ion-trap.

2.4. High performance liquid chromatography (HPLC)

The HPLC analyses were performed on a Hewlett Packard 1090 HPLC series II equipped with a DAD detector. The column was a Discovery RP Amide C16 150 mm × 4.6 mm with 5 μm pore size, and a Vaco precolumn frit filter, 5 μm, and a Discovery RP Amide C16 Guard column 20 mm × 4.0 mm, 5 μm, were used to protect the column (all purchased from Sigma-Aldrich, Stockholm, Sweden). Acetonitrile was used as mobile phase A and water as mobile phase B, both of HPLC grade (Merck, Darmstadt, Germany). The mobile phases were degassed using helium (99.9999% purity from AGA, Stockholm, Sweden). Fifty microlitres was injected each time and detection was made at 220 nm. The gradient method was carried out in several steps, i.e. (1) 30% A with 0.25 ml/min for 5 min, (2) increase to 98% A during 40 min with 0.25 ml/min, (3) increase to 0.4 ml/min during 5 min with 98% A, (4) decrease to 0.25 ml/min during 20 min with 98% A, (5) 98% A with 0.25 ml/min for 5 min, (6) decrease to 30% A during 5 min, 0.25 ml/min, (7) 30% A with 0.25 ml/min for 5 min. The total time was 85 min.

2.5. Solid-phase microextraction (SPME)

SPME methods were investigated with the fibre either immersed in the sample solution (direct SPME) or with the fibre exposed to the headspace above the solution (HS-SPME). The extractions were performed in sealed 20 ml glass vials on 15 ml (direct) or 10 ml solutions (headspace) from aged samples or on solutions containing standard substances of known concentrations. The pH was adjusted to 2 and the samples were saturated with salt with the hypothesis that these conditions yield the most effective extraction [10–11,18–22].

The experiments with direct SPME sampling were executed using a manual fibre holder (Supelco, Bellefonte, PA, USA) and analysed using a GC with FID detector, in order to avoid salts entering the ion-trap mass detector otherwise used. During each extraction, the fibre was placed at the same height with respect to the surface of the aqueous phase in the sample, with 75% of the fibre immersed and the remaining 25% of the fibre in the headspace, in order to prevent the ethanol in the solution from destroying the means of attachment of the fibre. The vials were placed in a water bath with adjustable temperature. The solutions in the vials were agitated at a constant, maximum level, set to prevent the magnet from bouncing. Single or, when appropriate, several fibre

blanks were run daily to guarantee that no carry-over was present. To avoid systematic errors, all analyses were carried out in random order.

Five fibres were compared in order to determine theirusability: polydimethylsiloxane (PDMS), 100 μm; polydimethylsiloxane-divinylbenzene (PDMS/DVB), 65 μm; polyacrylate, 85 μm; Carboxen™/polydimethylsiloxane (CAR/PDMS), StableFlex 85 μm; and Carbowax©/divinylbenzene (CW/DVB), 65 μm (Supelco, Bellefonte, PA, USA). For this pilot step, solutions from 14 of the original sample vials were mixed in order to exclude deviations in results due to sample inconsistency. The water bath was kept at 40 °C, the extraction time was 60 min and the desorption time, 6 min. Prior to each sample analysis, an extraction and subsequent analysis of a blank vial was carried out for each fibre, in order to eliminate interfering peaks. Two additional analyses were performed using HS-SPME in order to compare the efficiencies of the two extraction techniques. These comparisons were made on standard solutions.

The evaluation of the extraction time and temperature and desorption time and temperature, and the analysis of the influence of pH and salt, were performed on extracts of standard solutions. Some of the substances in the standard solutions were not extractable using SPME, but these substances were included to give a matrix similar to the true sample solutions. Pre-extractions on standard solutions with different concentrations, using a PDMS/DVB fibre at 40 °C during 60 min, were obtained in order to determine the concentrations that would yield responses corresponding to the solutions of the aged samples.

Extractions were performed at 40 °C for 60 and 100 min, at 55 °C for 20, 40, 60, 80, 100 and 120 min, and at 70 °C for 60, 80 and 100 min with direct SPME to compare the extraction efficiencies under these conditions. The solutions were pH-adjusted and saturated with salt prior to extraction. The temperature of the water bath was limited to an upper temperature of 70 °C to remain below the boiling point of ethanol. The effects of pH and salt were examined, by extraction with and without pH adjustment to 2 and salt saturation, to investigate whether or not these sample modifications were essential.

Desorption temperatures at 235 and 275 °C, and desorption times of 2 and 10 min were examined and compared to 250 °C and 6 min, in order to determine the lowest temperature and shortest time for complete desorption, and thus minimise the wear of the fibre. Care was taken to keep the fibre at a sufficient depth in order to avoid any reduction in desorption of analytes from the fibre due to the temperature gradient that may occur in the injector [23]. Each run included three sub-steps: (a) an extraction under the previously determined conditions, (b) by desorption with the parameters above, and (c) a second desorption step at 250 °C for 6 min. The results were compared and the conceivable carryover was calculated. The extractions at 250 °C for 6 min were performed in triplet and the rest as single extractions. Coefficients of variation and detection limits were estimated for validation of the final method with direct SPME. The standards Dtb-hppa and Tpp were included in the standard solution for the validation analysis. The standard solution during the earlier extractions with direct SPME contained Dtb-p, Dtb-mp, Dtb-bq, Dtb-hba, Dpp and Ttp.

Headspace extractions were performed at 40 °C for 60 and 80 min, at 55 °C for 40, 60 and 80 min and at 70 °C for 20, 40 and 60 min, to determine good extraction conditions. Subsequent desorptions were carried out at 250 °C for 6 min. The extractions were made in duplicate on a standard solution containing all the standards in Table 1. The samples were pH-adjusted to 2 and saturated with NaCl and thereafter sealed with magnetic silicon/PTFE crimp caps (Varian, Lake Forest, CA, USA). The concentrations in the standard solutions were justified for the extraction from headspace, since some of the analytes had high responses compared to those of the real samples. Coefficiencts of variation and detection limits were estimated for validation of the final HS-SPME methods.

2.6. Solid-phase extraction (SPE)

Extractions were performed using SPE for comparison with SPME. The SPE sorbents used, C18 (octadedcyl), and 101 (polystyrene-divinylbenzene), were kindly provided from Sorbent AB, Västra Frölunda, Sweden. The cartridges with the sorbents were of different sizes depending on the amounts and kind of sorbent in them. Twenty-five milligrams of C18 and 25 mg of 101 were in 1 ml cartridges. One hundred milligrams of C18 and 100 mg of 101 were in 3 ml cartridges. The solvents used were methanol (Merck, Darmstadt, Germany) and isopropanol (Scharlau, Barcelona, Spain), both of HPLC quality, and ethanol (Kemetyl AB, Haninge, Sweden) of 99.5% purity.

The validation of the optimised method was performed using a 10% ethanol in water solution containing 0.0002 mg/ml of Dtb-p, Dtb-bq, Dtb-mp, Dpp, Irganox 1010, Irganox 168 and ethylated Dtb-hppa. The Dtb-hppa was ethylated using a 0.1 vol.% HCl in ethanol solution prior to mixing with the other analytes. The sorbents were conditioned in 1 ml of methanol. They were thereafter equilibrated using 2 ml of a 10% ethanol in water solution. Ten millilitres of the standard or sample solution was used for each extraction. Both the equilibrium solution and the standard or sample solutions were acidified to pH 2 using a 0.5 M HCl solution. The sorbents were dried using compressed air prior to the eluations. The solutions and solvents passed through the sorbent by gravitation force. The sample solutions were extracted after storage at 80 °C using 100 mg of C18 and 100 mg of 101 and after storage at 40 and 60 °C using 25 mg of 101 as sorbent. Five hundred microlitres isopropanol was used for the elution. The extractions were performed in duplicates if not stated otherwise.

3. Results and discussion

Polypropylene must contain antioxidants to prevent degradation during processing. These antioxidants and their degra-

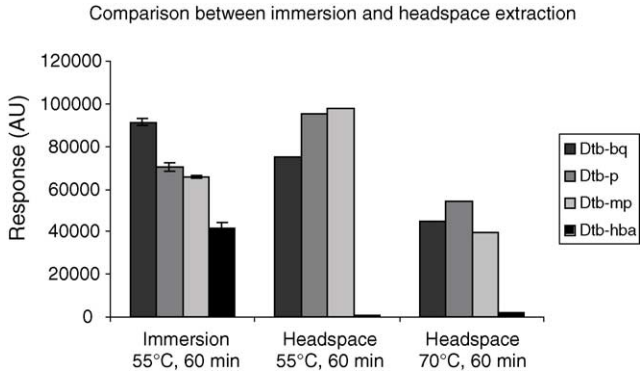

Fig. 1. Comparison of responses of standards after solid-phase microextraction using headspace and immersion techniques.

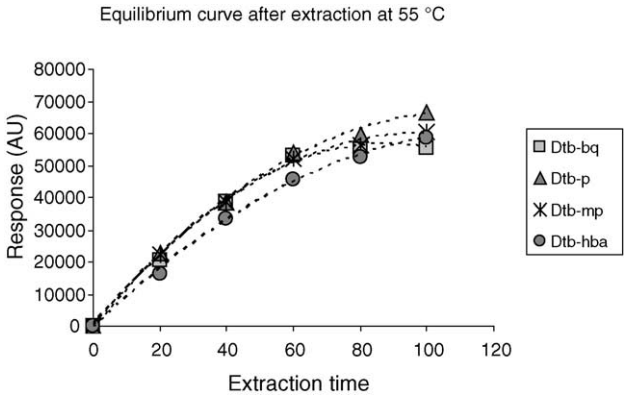

Fig. 2. Equilibrium curves for standards extracted at 55 °C with an immersed fibre.

dation products may migrate to the surrounding media. The use of solid-phase microextraction was investigated for the identification and quantification of low molecular degradation products of antioxidants which migrated from polypropylene into a simulated food and pharmaceutical solution consisting of 10% ethanol in water. The choice of polarity of the coating of the SPME fibre depends on the chemical properties of the analytes. The PDMS/DVB fibre was chosen for further evaluation after comparing the capability of five different fibres to extract analytes from real samples. Although the polyacrylate fibre exhibited slightly greater responses than the PDMS/DVB fibre for most of the identified peaks and greater responses for two unidentified peaks, the PDMS/DVB fibre demonstrated a more diverse spectrum and almost twice the response of Dtb-bq, showing potentially sufficient versatility for the application. The CW/DVB fibre underwent severe swelling during the extraction in the EtOH/H_2O solution and was not analysed in the GC in order to avoid contamination of the instrument.

In a comparison between HS-SPME and direct SPME using a PDMS/DVB fibre, rather varying results were obtained as indicated in Fig. 1. In this figure, the responses from the direct SPME are mean values of duplicate extractions while the responses from the HS-SPME are from single extractions. In the subsequent figures, the results are the mean values of duplicate tests. Although headspace was a more effective technique for Dtb-p and Dtb-mp, Dtb-hba was practically undetectable in the gaseous phase and the response of Dtb-hq was much lower. For this reson, parallel extraction methods with both direct SPME and HS-SPME were developed. Direct extraction facilitates extraction of semi-volatile substances, but the fibre undergoes more extensive wear than in extraction from the headspace.

3.1. Direct SPME

The efficiency of the direct SPME extraction can be increased by increasing the extraction temperature. Liquid samples can also be acidified and saturated with NaCl to change the equilibrium between the fibre, solution and gas phase. The extraction efficiency depends mainly on the extraction time and temperature. Extractions were performed at 40 °C for 60 and 100 min, at 70 °C for 60, 80 and 100 min and at 55 °C for 20, 40, 60, 80 and 100 min. The equilibrium curves at 55 °C are shown in Fig. 2. The responses from the duplicate extractions varied between 1% and 20%, which is considered adequate for manual sampling [19].

The curves for the different compounds varied slightly; equilibrium was not established for three of the standards, even after 100 min of extraction, whereas Dtb-hq had become unstable by the same time. Consequently, 80 min was the best extraction time for extraction at 55 °C since equilibrium is not vital for feasible quantification. Quantification is possible even when equilibrium has not been reached. Care must, however, then be taken to use the exact same extraction time and temperature for each extraction [19]. In addition, a shorter extraction time is favourable for multiple analyses. An even shorter extraction time, i.e. 60 min, is also possible, although the extraction yield and reproducibility are reduced. The results of the extractions at 55 °C for 80 min and the extractions at 40 °C and 70 °C for 60 and 100 min are compared in Fig. 3.

Extraction for 80 min at 55 °C was more effective than extraction for 60 min, regardless of temperature. The longer

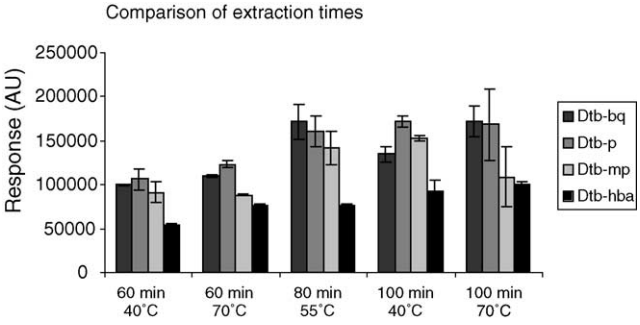

Fig. 3. Comparison of responses of standards after extraction with an immersed fibre for 60, 80 and 100 min at 40, 55 and 70 °C.

Table 2
Effect of pH and salt on extraction yield

Substance	pH	Salt
Dtb-bq	Not significant	Significant
Dtb-p	Not significant	Not significant
Dtb-mp	Not significant	Not significant
Dtb-hba	Significant	Significant

Results obtained from extraction with and without pH adjustment to 2 and salt saturation.

extraction time showed variations in responses. Dtb-p and Dtb-hba were not greatly affected, while Dtb-mp was better extracted at low temperatures and Dtb-hq at higher temperatures. The responses of 80 min extraction at 55 and 70 °C were very similar, although the response for Dtb-hba was slightly higher and that for Dtb-mp lower at 70 °C than at 55 °C. The coefficients of variation were not affected by the shorter extraction time. Care must though be taken to keep the extraction time as precise as possible. The results clearly indicate that the time affected the extraction yield more than the temperature. A decrease in response was seen for some of the compounds at high temperatures. Long extraction time at high temperature can cause the fibre to run hot and the affinity for some substances can change, due to desorption at elevated temperatures. In addition, the fibre undergoes more extensive wear at high temperatures and long extraction times. Extraction for 80 min at 55 °C was, therefore, chosen for subsequent tests to minimise the wear on the fibre.

The significance of acidification and saturation with salt was tested using a complete factor analysis with the extraction conditions determined in the previous step, 15 ml sample and 80 min extraction at 55 °C. Table 2 summarizes the results. Earlier results varied by at the most 15–20% between extractions performed under identical conditions, so that a factor was considered significant if the extraction yield was reduced by more than 20%. Dtb-hba was strongly dependent on an acidic environment, and was practically undetectable at pH 7. Dtb-hq and Dtb-hba showed considerably lower responses in the absence of salt, 60% and 40% of the original response, respectively. pH adjustment to 2 and salt saturation were, therefore, vital steps in the sample preparation.

Both desorption temperature and desorption time affected the amount released from the fibre in the injection port, as shown in Fig. 4. The shorter the desorption time and the lower the desorption temperature, the less wear will the fibre suffer. However, a sufficient desorption time at an adequate temperature is vital in order to avoid carry-over. The results

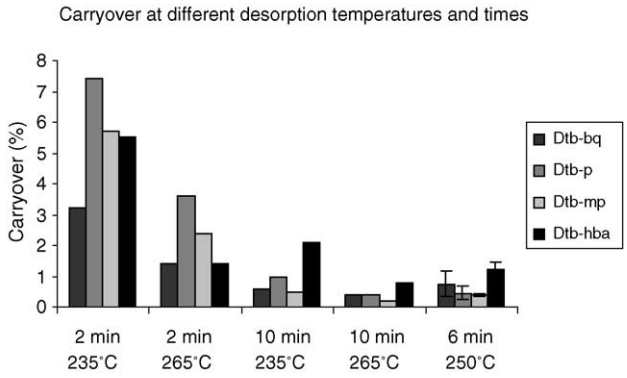

Fig. 4. Carry-over as a percentage of the response after desorption of standards from the fibre under different desorption conditions.

Table 3
Coefficient of variation from standard solution-SPME with an immersed fibre

Substance	Coefficient of variation of responses (%)	Coefficient of variation of responses/IS (%)	Detection limit (μg/l)
Dtb-bq	7	3	<1E−5
Dtb-p	8	5	<0.18
Dtb-hppa	6	1	<1E−10
Tpp	5	3	<1.8
Dtb-hba	7	–	–
Dtb-mp	8	–	–

indicated that desorption was in general influenced more by time than by temperature, although both factors were significant. Desorption at 265 °C for 10 min yielded the most effective desorption, but the difference compared to the original method was negligible, especially when the increased fibre wear under these conditions were taken into account. Consequently, the previously used desorption method, 250 °C for 6 min, was retained.

3.2. Validation of method with direct SPME

Dtb-mp and Dtb-hba were used as internal standards for validation of the chosen extraction method, direct sampling at 55 °C for 80 min. The relative standard deviation for four extractions on standard solutions can be seen in Table 3, together with the detection limits, and from three extractions on real samples in Table 4, together with the calculated concentrations. The coefficient of variation varied from 1% to 10% for the samples analysed after each other. Repeatability tests with an old fibre gave coefficient of variation

Table 4
Coefficient of variation and concentrations in real samples-SPME with an immersed fibre

Substance	Response AU	Coefficient of variation (%)	Response/IS	Coefficient of variation (%)	Concentration (μg/l)
Dtb-bq	22229	8	0.17	9	–
Dtb-p	6687	20	0.05	10	5
Dtb-hppa	60033	11	0.46	6	53

of 22% for Dtb-p and 16%, 13% and 5% for Dtb-bq, Tpp and Dtb-hppa, respectively. Dtb-p was less stable than the other substances which, together with the low concentration, explains the higher coefficient of variation. The detection limits were set at concentrations leading to responses corresponding to at least three times the noise level.

For quantification, the concentration of the analytes must be within the linear dynamic range of the PDMS/DVB fibre for the specific extraction conditions. The responses of the analytes were linear at least within the ranges of 0.18–18 μg/l for Dtb-p, 10–90 μg/l for Dtb-hppa and 9–900 μg/l for Tpp. The responses of Dtb-bq in the real samples were lower than the linear dynamic range (9–90 μg/l) and it was not, therefore, quantified. The detection limits were much lower for Dtb-hppa and Dtb-bq than for Dtb-p and Tpp. The concentrations of Dtb-p and Dtb-hppa were calculated to be 5 μg/l (0.08 μg/dm^2 film) and 53 μg/l (0.9 μg/dm^2 film), respectively.

3.3. HS-SPME

All headspace extractions were performed with a PDMS/DVB fibre above a 10 ml solution, acidified and saturated with salt. The extracted analytes were desorbed using the previously chosen condition, 6 min at 250 °C. Extractions were first made at 40 °C for 60 and 80 min, at 55 °C for 40, 60 and 80 min and at 70 °C for 20, 40 and 60 min to find a suitable extraction time and temperature.

Clear differences in extraction efficiency were seen for the substances, as shown in Fig. 5. The extraction efficiency of Dtb-bq was promoted by low temperatures, whereas a high temperature was better for Dtb-hppa and Dtb-hba. In general, larger amounts were extracted at longer extraction times. Due to the different requirements of the substances, two extraction conditions were chosen for validation, extraction for 60 min at 55 °C and for 60 min at 70 °C. The first-mentioned condition was chosen in spite of the greater efficiency of extraction at 55 °C for 80 min because of the shorter extraction time and lower standard deviation with extraction for 60 min and to avoid too high responses for Dtb-bq.

Table 5
Coefficient of variation from standard solution-SPME from headspace

Substance	Coefficient of variation of responses (%)	Coefficient of variation of responses/IS (%)	Detection limit (μg/l)
60 min at 55 °C			
Dtb-bq	8	19	<1E−14
Dtb-p	13	17	<6E−9
Dtb-hppa	4	16	<3E−5
Dtb-hba	15	–	–
60 min at 70 °C			
Dtb-bq	16	26	<1E−14
Dtb-p	9	18	<3E−6
Dtb-hppa	9	4	<6E−8
Dtb-hba	12	–	–

3.4. Validation of method with HS-SPME

The selected extraction conditions, headspace sampling 60 min at 55 and 70 °C, were validated using Dtb-hba as internal standard. Dtb-mp was unstable when kept for a long time in an acidified environment and could not be used as internal standard for the HS-SPME because the extractions were made using an auto-sample. To achieve lower detection limits, reconstructed ion chromatograms based on the two most abundant *m*/*z*-values in the mass spectrum of the substances were used instead of the total ion chromatogram.

Four extractions were made on a standard solution (Table 5) and two on real samples (Table 6) with both of the extraction conditions. Dtb-hba worked well as internal standard for Dtb-hppa with extractions at 70 °C leading to a lower standard deviation, but the standard deviation was higher in almost all other cases. The high response of Dtb-bq after extraction from the standard solution was most probably the reason for the higher standard deviations of the responses after extraction of the standard solution than after extraction of real samples, due to the limited absorption capacity of the SPME fibre. The detection limits were set at concentrations leading to responses corresponding to at least three times the noise level in the reconstructed ion chromatograms. The limits were controlled by comparison of mass spectra.

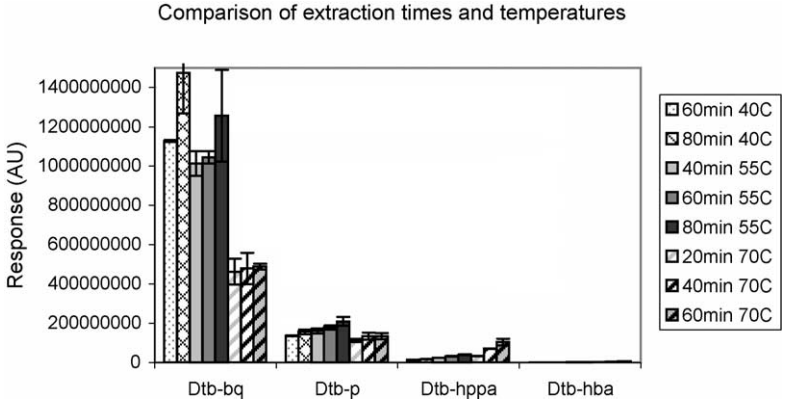

Fig. 5. Comparison of responses of standards after headspace extraction under different extraction conditions.

Table 6
Coefficient of variation and concentrations in real samples-SPME from headspace

Substance	Response AU	Coefficient of variation (%)	Response/IS	Coefficient of variation (%)	Concentration (μg/l)
60 min at 55 °C					
Dtb-bq	80335061	3	97.4	1	–
Dtb-p	34793025	4	42.2	8	6
Dtb-hppa	665463	4	0.8	8	12
Dtb-hba	824830	4	–	–	–
60 min at 70 °C					
Dtb-bq	32909432	4	3.4	7	–
Dtb-p	24951350	1	2.6	4	4
Dtb-hppa	3531074	4	0.4	1	12
Dtb-hba	9564468	3	–	–	–

The concentration of Dtb-hppa was calculated to be 12 μg/l (0.2 μg/dm^2 film) from both of the extraction methods, but the calculated concentrations of Dtb-p differed by 30% (0.07–0.1 μg/dm^2 film). The linear dynamic ranges were, for the method at 55 °C, at least 0.2–9 μg/l for Dtb-p and 3×10^{-5} to 45 μg/l for Dtb-hppa and, for the method at 70 °C, at least 1–9 μg/l for Dtb-p and 3×10^{-5} to 20 μg/l for Dtb-hppa. Extraction at 55 °C allowed quantification of Dtb-p at lower concentrations (0.2 compared to 1 μg/l) and of Dtb-hppa at higher concentrations (45 μg/l compared to 20 μg/l) than extractions at 70 °C, but the detection limit of Dtb-hppa was much lower at 70 °C than at 55 °C (6×10^{-8} μg/l compared to 3×10^{-5} μg/l). Quantification using an internal standard increases the repeatability of the measurements and the consumption time of the fibre. However, it unfortunately seemed to affect the linear dynamic ranges of the analytes by increasing the lower limit for Dtb-p (from 2×10^{-3} to 0.2 μg/l or 1 μg/l) and decreasing the upper limit for Dtb-hppa (from 90 to 20 μg/l or 45 μg/l) compared to quantification without using the internal standard. This effect would most likely be lowered by changing to a more suitable internal standard.

Extraction from the headspace made it possible to analyse the extract with an ion-trap mass spectrometry detector without the risk of salts coming into the detector. Reconstructed ion chromatograms could then be used to achieve lower detection limits. This was the reason for the low detection limit of Dtb-hppa using HS-SPME compared to using direct-SPME. The GC–MS instrument was also equipped with a SPME autosample which made the extractions much less time-consuming. On the other hand, a broader range of substances could be extracted using the direct SPME method. Direct sampling for 80 min at 55 °C gave a good extraction efficiency with relatively low wear of the fibre. The standard deviations were lower with direct SPME than with HS-SPME. It was the best method for extraction at high concentrations of Dtb-bq, and both ethylated and un-ethylated Dtb-hppa could be detected. The degree of ethylation in the standard solutions for the calibration curve has to be the same as in the samples to obtain a trustworthy quantification after HS-SPME. This was probably the reason for the large differences in the calculated concentrations of Dtb-hppa in the real samples after direct and headspace extractions. It was possible to extract Tpp with direct sampling but it had too low a volatility for extraction from the headspace. Headspace extraction at 55 °C for 60 min was the best method for Dtb-p, whereas headspace extraction at 70 °C for 60 min was found to be an alternative for extraction of Dtb-hppa with relatively low detection limit, if the degrees of methylation for the samples and for the Dtb-hppa standard in the calibration solutions were comparable. This is important for trustworthy quantification since it was impossible to extract un-ethylated Dtb-hppa from the headspace.

The results indicated that it is possible to quantify more than one degradation product simultaneously despite large differences in their chemical structures. The possibility can be enhanced by optimising extraction method and temperature to suit specific concentration levels of analytes. The minimal sample preparation facilitates the possibility to change method when the response of the analytes diverges from their linear dynamic ranges. The method development was based on volatile and semi-volatile products with chemical structure similar to those of many common antioxidants. One of the developed HS-SPME methods can be used for extraction, with following identification using GC–MS, of products in any sample of similar nature. Quantification can then be performed using standards of the identified analytes. New extractions with one of the other SPME methods may be necessary for quantification depending on if the responses of the analytes are within their linear dynamic ranges. The large differences in the detection limits depending on the extraction temperature can be seen as a useful tool rather than a problem, if the temperature is very well controlled, since it increase the possibility to optimise the method to suit specific concentration levels.

3.5. Comparison with SPE

Extraction using solid-phase extraction, also makes it possible to analyse non-volatile substances. However, SPME is a more effective technique in increasing the concentration of many volatile and semi-volatile compounds. Analysation

using GC–MS is possible with relatively low concentrations of the samples, but since non-volatile analytes will also be extracted using SPE high performance liquid chromatography (HPLC) would be a more suitable technique. Non-volatile products may remain within the gas chromatograph and come out as thermal degradation products, similar to possible analytes, in later analysis.

The use of SPE was evaluated in parallel to the SPME evaluation. HPLC analysis gave a detection limit of approximately 500 μg/l for the analytes discussed above and for two other less volatile analytes, i.e. Dpp and Irganox 1010. Extraction of a 10 ml sample with elution using 500 μl solvent gives a 20 times higher concentration. The sample solution would thus need to contain at least 25 μg/l for any utility of SPE in combination with HPLC. The efficiency of an SPE sorbent for extractions of analytes is very much dependent on the chemistry of the analytes of interest, the solution they are in and the choice of eluting solvent. Also the amount of the sorbent is of importance. SPE extractions were performed in triplicates on the real samples stored for 4 months at 60 °C using 100 mg of two different sorbents, C18 and 101, and with elution using 2 × 500 μl isopropanol. None of the analytes were detected in the extracts.

Later optimisation of a SPE method for the analytes showed that 25 mg sorbent of 101 with elution using 500 μl isopropanol gave better extraction yields. Tests performed at two different occasions with three extractions made at each occasion gave the yields in Table 7. Dtb-p showed a low coefficient of variation (6%) but a much higher yield than possible (220%). The high amount of Dtb-p was most likely an effect of degradation of 168 within the standard solution used for the method development. To evaluate this possibility, a solution with 0.1 mg/ml of Irganox 168 in a 10% ethanol in water solution was stored in room temperature for 1 month. Analysation of the solution showed a concentration of 0.0015 mg/ml of Dtb-p within the solution, which verifies our theory. The coefficient of variation was between 2.3% and 6.4% for all of the analytes within the standard solution. The variables investigated in the method development were the type of sorbent (C18, NH2, 101 or ENV+), the amount of sorbent (25–100 mg) the elution solvent (isopropanol or acetonitrile), the elution volume (300 to 1500 μl), the concentration of the analytes in the sample solution (0.002 mg/ml or 0.0002 mg/ml) and the effect of a decrease in the concentration of ethanol by dilution of the extracted solution.

Table 7
Yield and coefficient of variation from standard solution-SPE

Substance	Yield (%)	Coefficient of variation (%)
Dpp	40	5
Dtb-bq	34	3
Dtb-mp	100	3
Dtb-hppa	88	2
Irganox 1010	83	6

Extractions have been performed using the optimised method on solutions in which the same polypropylene film has been stored for up to 12 months at 40 °C and 2.5 months at 60 °C, both with and without foregoing sterilisation process using an autoclave. None of the analytes were detected in the samples even in the cases were the migration had been accelerated by the sterilisation process for 20 min at 120 °C. Other unidentified compounds, which were not seen after aging at 80 °C were, however, detected in quantitative amounts in some of the samples. The sterilisation process followed by storage for 2.5 months at 60 °C seemed to have led to at least as high degree of migration as the longer storage at 80 °C. This indicates that SPME was a more suitable technique than SPE for the searched analytes at low concentrations. SPE is, however, useful at higher concentrations and for less volatile compounds.

4. Conclusions

Using solid-phase microextraction, it was possible not only to identify but also to quantify low molecular aromatic substances, e.g. degradation products of antioxidants, in an organic aqueous solution such as a simulated food and pharmaceutical solution consisting of 10% ethanol in water. Difficulties appeared due to the ethanol in the solution and the minute amounts of substances present. SPME methods were developed and evaluated for extractions both with the fibre immersed in the sample solution (direct SPME) and as with the fibre exposed to the headspace above the solution (HS-SPME).

The findings supported our earlier studies which suggested that SPME is an excellent technique for detecting low molecular weight species at very low concentrations. SPME was useful for much lower concentrations than SPE in combination with HPLC. By selection of extraction method and temperature to suit the concentration levels of the analytes, it was possible to quantify more than one degradation product simultaneously.

Among the tested fibres, the one coated with polydimethylsiloxane–divinylbenzene was the most suitable. This fibre, in contrast to the others, demonstrated a more diverse spectrum. It was possible to identify and quantify a broader range of substances with direct SPME than with HS-SPME. Both ethylated and unethylated phenolic acids were detected with direct SPME, which made reliable quantification easier, whereas only the ethylated acids were detected in the headspace extraction. The detection levels of the quinone and 2,4-ditertbutyl-phenol were much lower after extraction from headspace than with direct extraction. The quinone was detectable with both methods, but with very large differences in sensitivity. The concentration in the real samples was too low for quantification with direct SPME and too high for headspace extraction. 2,4-Ditertbutyl-phenol in the real sample could be quantified with both methods but preferably at a relatively low temperature.

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References

[1] F. Khabbaz, A.-C. Albertsson, S. Karlsson, Polym. Degrad. Stab. 63 (1998) 127.
[2] W.L. Hawkins, M.A. Worthington, W. Matreyek, J. Appl. Pol. Sci. 3 (1960) 277.
[3] G. Scott, Biochem. Soc. Symp. 61 (1995) 235.
[4] G. Lawson, C.T. Barkby, C. Lawson, Fresenius J. Anal. Chem. 354 (1996) 483.
[5] K. Nagy, E. Epacher, P. Staniek, B. Pukanszky, Polym. Degrad. Stab. 82 (2003) 211.
[6] E. Földes, Angew. Makromol. Chem. 261–262 (1998) 65.
[7] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[8] M. Hakkarainen, A.-C. Albertsson, S. Karlsson, J. Environ. Polym. Degrad. 5 (1997) 67.
[9] F. Khabbaz, A.-C. Albertsson, Biomacromolecules 1 (2000) 665.
[10] M. Gröning, M. Hakkarainen, J. Chromatogr. A 932 (2001) 1.
[11] M. Hakkarainen, G. Gröning, A.-C. Albertsson, J. Appl. Pol. Sci. 89 (2003) 867.
[12] M. Hakkarainen, J. Chromatogr. A 1010 (2003) 9.
[13] M. Gröning, M. Hakkarainen, J. Chromatogr. A 1052 (2004) 61.
[14] O.B. Jonsson, E. Dyremark, U.L. Nilsson, J. Chromatogr. B 755 (2001) 157.
[15] D.R. Jenke, Int. J. Pharm. 224 (2001) 51.
[16] D.R. Jenke, J. Brennan, M. Doty, M. Poss, J. Appl. Polym. Sci. 89 (2003) 1049.
[17] M.R. Philo, A.P. Damant, L. Castle, Food Addit. Contam. 14 (1997) 75.
[18] C.L. Arthur, L.M. Killam, K.D. Buchholz, J. Pawliszyn, J.R. Berg, Anal. Chem. 64 (1992) 1960.
[19] R. Baciocchi, M. Attina, G. Lombardi, M.R. Boni, J. Chromatogr. A 911 (2001) 135.
[20] P. Castells, F.J. Santos, M.T. Galceran, J. Chromatogr. A 984 (2003) 1.
[21] K.D. Buchholz, J. Pawliszyn, Anal. Chem. 66 (1994) 160.
[22] J. Yang, M.-L. Cheng, Analyst 126 (2001) 881.
[23] C. Poole, A. Gunatilleka, R. Sethuraman, J. Chromatogr. A 885 (2000) 17.