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Development of a SPME-GC-MS/MS method for the determination of some contaminants from food contact material in beverages

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ABSTRACT

The development and validation of a simple, low-cost, and sensitive method for the determination of nine compounds expected in beverages and vinegar as a result of migration from food contact material (parabens, phenolic antioxidants, sulfonamide plasticizer, and flame retardant) is presented. The analytes were pre-concentrated using solid-phase microextraction and analyzed by gas chromatography – tandem mass spectrometry. The method required no derivatization procedure and an affordable chemical was used as internal standard. The LODs were in the range of 0.005–0.2 μg/L, the relative standard deviations 0.8–5.4%, and the mean recoveries 98–109%. Different alcoholic beverages and vinegars were analyzed. A crown cap migration study using several food simulants was conducted for 6 months. Moreover, migration from a home brewing plastic fermenter in a time span of 4 weeks was studied. Analyte concentrations up to 2220.99 μg/L were detected in real samples and up to 4.75 μg/L in migration experiments.

1. Introduction

Pure polymers without additives are unstable and show poor resistance against various external factors. Consequently the synthesis and processing of basic polymer compounds which result in safer, cleaner, more resistant and therefore useful plastic materials intended for food contact materials (FCM), requires the addition of various additives (Piringer and Baner, 2000). These are mostly lower molecular weight substances that have the tendency to migrate into the containing food and beverages (Sajiki et al., 2007; Kontominas et al., 2006; Elizalde et al., 2018). Because of their suspected or already proven toxic or endocrine disrupting potential, different additives have been subject to many controversies throughout the history of plastic materials and represent a global health issue (Geueke and Muncke, 2018; Ito et al., 2007; Lithner et al., 2009; Michałowicz, 2014). Article 3 of European Regulation No. 1935/2004 (repealing Directives 80/590/EEC and 89/109/EEC) requires materials, intended to be brought into contact with food, to be as safe and inert as possible ("Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC"). In order to verify the compliance of a certain plastic material, migration testing with appropriate food simulants mimicking chemical and physical properties exhibited by food should be performed ("Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food"). There are many types of potentially migrating compounds such as monomers, plasticizers, process aids, antioxidants, slipping agents, antimicrobial agents, flame-retardants etc., which should be monitored at trace levels.

Food packaging contaminants found in certain type of food depend on the type of food and the packaging material. In this study, we were interested in alcoholic beverages and vinegars. Based on preliminary leaching study, we focused on four types of compounds: parabens, synthetic phenolic antioxidants, sulfonamide plasticizers, and organophosphorous flame retardants.

Alkyl esters of *p*-hydroxybenzoic acid, more commonly known as parabens, are widely used as preservatives in the food industry, pharmaceuticals and personal care products (Khan and Mn, 2019; Soni et al., 2002). According to published studies, they are linked to breast tumors (Darbre et al., 2004), skin inflammation (Handa et al., 2006) and are suspected to have an impact on the endocrine system (Golden et al., 2005). Parabens are added to antimicrobial packaging and can therefore migrate and be released into the containing food (Chung et al., 2001; Lu et al., 2014). Studies by Chung et al. (2001) and Lu et al. (2014) are to our knowledge the only reports on the determination of parabens in FCM simulation studies. Liquid chromatography coupled with UV or MS detectors is the most commonly employed method for their analysis in different samples (González-Mariño et al., 2011; Zotou et al., 2010) with detection limits ranging from 0.004 to 0.2 μg/L. Due to their relatively non-volatile and polar nature, analysis of parabens by

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Abbreviations

BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
BP	butyl paraben
CAR/PDMS	carboxen/polydimethylsiloxane
CE	collision energy
dBHT	2,6-di(*tert*-butyl-d9)-4-methyl(phenol-3,5,*O*-d3)
DI	direct immersion
DVB/CAR/PDMS	divinylbenzene/carboxen/polydimethylsiloxane
EI	electron ionization
EP	ethyl paraben
FCM	food contact material
FS	food simulant
HRMS	high resolution mass spectrometry
HS	headspace
LOD	limit of detection
LOQ	limit of quantification
MMI	multimode inlet
MS/MS	tandem mass spectrometry
MP	methyl paraben
MS	mass spectrometry
NBBS	*N*-butylbenzenesulfonamide
PA	polyacrylate
PDCP	phenyl dichlorophosphate
PDMS	polydimethylsiloxane
PDMS/DVB	polydimethylsiloxane/divinylbenzene
PP	propyl paraben
PTFE	polytetrafluoroethylene
QQQ	triple-quadrupole mass spectrometry
RSD	relative standard deviation
SPA	synthetic phenolic antioxidants
SPE	solid phase extraction
SPME	solid-phase microextraction
SRM	selected reaction monitoring
TBEP	tris(2-butoxyethyl) phosphate
TBHQ	*tert*-butylhydroquinone
TIC	total ion chromatogram
UI	ultra inert
WC	working concentration

gas chromatographic (GC) methods usually requires derivatization. Traces of parabens have been detected by GC in samples of water (Canosa et al., 2006; López-Darias et al., 2010; Regueiro et al., 2009), urine (Azzouz et al., 2016), and in various solid samples (Farajzadeh et al., 2010). Different sample treatment and preconcentration methods are found in the literature (Azzouz et al., 2016; Canosa et al., 2006; Farajzadeh et al., 2010; Hui-Ting et al., 2017) including HS-SPME (Regueiro et al., 2009) and DI-SPME (López-Darias et al., 2010). In all reported methods except one (Farajzadeh et al., 2010), a derivatization step was necessary. Achieved detection limits ranged from 0.004 μg/L for derivatized parabens and up to 15 μg/L when analyzed directly.

Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and *tert*-butylhydroquinone (TBHQ) belong to synthetic phenolic antioxidants (SPA), which are added to food in order to prolong the shelf life and help retain its flavor (Shahidi and Ambigaipalan, 2015). Antioxidants can be added to food directly or via unintentional migration from the packaging (van Aardt et al., 2007). The wide use of SPA is driven by their chemically stability, low price and availability, but their safety, however, is questionable due to their potential teratogenic and carcinogenic effects (Okubo et al., 2003; Yang et al., 2018). Three selected reports for analyzing SPA as packaging migrants are detailed herein. For example, Jamshidian et al. studied the release of BHA, BHT and TBHQ from extruded polylactic acid film into food simulants based on ethanol. No additional sample treatment and preconcentration was performed before analysis with HPLC-UV (Jamshidian et al., 2012). Dopico-García et al. presented the study of SPA migration levels from low-density polyethylene films into water as a food simulant. Analytes were extracted by liquid-liquid extraction with *n*-hexane and analyzed by HPLC-UV (Dopico-Garcí;a et al., 2003). Cacho et al. reported a GC-MS method for the determination of SPA in soft drinks (Cacho et al., 2015).

N-butylbenzenesulfonamide (NBBS) is a member of sulfonamide plasticizers (Rider et al., 2012). Plasticizers are added to polymers in order to affect their flexibility and durability and can contribute up to several tens of percent by weight of the finished material. NBBS has been proven to show strong neurotoxic effects on laboratory mammals (Kumar et al., 2007; Rider et al., 2012) and poor biodegradability, which makes it an emerging contaminant in environmental samples such as wastewater (Huppert et al., 1998; Janzen et al., 2009; Trzcinski and Stuckey, 2010) and aerosol (Özel et al., 2011). The analysis of NBBS in these reports was mostly of qualitative or semiquantitative nature, required large sample amounts and the use of organic solvents in non-automated multistep sample preparation procedures. An exception to the latter and the only method implementing SPME was described by Huppert et al. who reported a rapid, inexpensive and solvent-free SPME-GC-MS method for the analysis of NBBS in wastewater samples with a detection limit of 0.1 μg/L (Huppert et al., 1998). Quantitative trace level determination of NBBS is poorly researched with the exception of the work by Duffield et al. (1994), who reported a method for the determination of NBBS in wines, ground water, and drinking water.

Tris(2-butoxyethyl) phosphate (TBEP) is a member of organophosphorus polymer flame retardants and plasticizers (Esch and International Programme on Chemical Safety, 2000). A study conducted by Ma et al. showed TBEP causes toxicity in the developing zebrafish (Ma et al., 2016). Giraudo et at. studied the chronic toxic effects of TBEP on aquatic organisms (Giraudo et al., 2015). Since TBEP is not chemically bonded to final products, it also poses a risk to migrate from the main polymer (Yang et al., 2019). Hakkarainen et al. reported detection of TBEP migration for quality control of polyamide 6.6 using SPME-GC-MS (Hakkarainen et al., 2003). TBEP was found in fish and human breast milk samples by semi-quantitative GC-HRMS analysis. Complex sample preparation with at least 10 steps resulted in a detection limit of 23 ng/g (Sundkvist et al., 2010). A TBEP determination by GC-MS in wastewater with a LOD of 1 ng/L was achieved by Fries et al. with the implementation of a multi-step SPE extraction (Fries and Puttmann, 2001).

The aim of this study was to develop a simple validated method for determination of food packaging contaminants in alcoholic beverages and vinegars. Analytes were chosen based on the findings from a controlled extraction experiment on beer bottle crown caps. This study proposes a simple, low-cost, precise, accurate and linear DI-SPME-GC-MS/MS method for simultaneous quantitative trace level determination of 9 compounds from 4 different groups of additives: 4 parabens, 3 synthetic phenolic antioxidants, NBBS, and TBEP in different food simulants. SPME sampling conditions such as NaCl addition, incubation temperature, fiber exposure time, sample volume, and fiber selection were studied. Under the final optimized conditions, the method was validated. Different alcoholic beverages and vinegars capped by crown caps with polymeric seals were analyzed. To the best of our knowledge, this is the first SPME-GC-MS/MS method for the direct analysis of parabens without derivatization and the first to simultaneously cover different packaging contaminants that can occur in real samples.

2. Materials and methods

2.1. Reagents and materials

Methyl paraben (MP, > 99%), ethyl paraben (EP, > 99%), propyl

paraben (PP, > 99%), butyl paraben (BP, > 99%), *tert*-butylhydroquinone (TBHQ, > 97%), *N*-butylbenzenesulfonamide (NBBS, > 99%), tris(2-butoxyethyl)phosphate (TBEP, > 94%), 2,6-di-*tert*-butyl-4-methyl-phenol (BHT, > 99%), 3-*tert*-butyl-4-hydroxyanisole (BHA, > 99%), phenyl dichlorophosphate (PDCP, > 95%), 2,6-di(*tert*-butyl-d_9)-4-methyl(phenol-3,5,*O*-d_3) (dBHT, > 98%), sodium chloride (NaCl, > 99%), and potassium chloride (KCl, > 99%) were purchased from Sigma-Aldrich (Steinheim, Germany). Concentrated hydrochloric acid (HCl, 37%) was from Carlo Erba reagents (Val de Reuil Cedex, France). Ethanol (96%), was from Honeywell (Seelze, Germany). For the migration testing of the plastic fermenter, denaturated ethanol purchased from a local supermarket was used. Purified water was obtained from the Merck Advantage A10 Milli-Q purification system (Darmstadt, Germany). The following SPME fibers were purchased from Supelco (Bellefonte, PA, USA): 85 μm PA, 100 μm PDMS, 65 μm PDMS/DVB, 50/30 μm DVB/CAR/PDMS and 75 μm CAR/PDMS. A 85 μm PA fiber was purchased from Restek (Bellefonte, PA, USA).

2.2. Preparation of solutions

Individual stock solutions (1 mg/mL) of each compound were prepared in ethanol and stored in amber glassware at 4 °C in the dark. Stock solution (1 mg/mL) of the internal standard PDCP was prepared in methanol.

Working standard solutions were freshly prepared every five days by appropriate dilution of stock standard solutions in water and following the method procedure. Since the responses of analytes at the same concentration differed significantly, the working concentrations of each analyte was adjusted in order to reach comparable peak areas between all the analytes in the reference solution. For exact working concentrations of each analyte see Table 2.

Solutions for optimization and validation of SPME method were prepared in food simulant C.

For the determination of ethanol residue in food simulant solutions, standard solutions of ethanol in water with concentrations ranging from 0.05% up to 5% (*v/v*) were prepared.

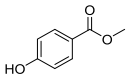
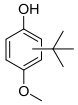
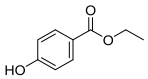
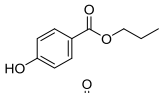
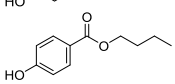
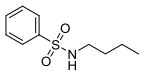
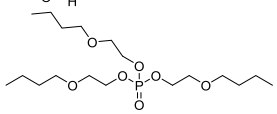
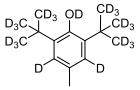
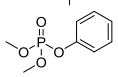
2.3. Samples

Crown caps, 0.33 L beer bottles and a 30 L plastic fermenter for home brewing were purchased at a local home brew shop (Ljubljana, Slovenia). Commercial samples of various alcoholic beverages and vinegars were purchased from local supermarkets. Homebrew beer was brewed by the authors of this article, stored in bottles, and sealed by the very crown caps used for the migration experiments. Samples of alcoholic beverages and vinegars, produced at home and stored for prolonged periods, were provided by friends and acquaintances.

2.4. GC-MS/MS method

GC-MS/MS system consisted of a MultiPurpose Autosampler (MPS, Gerstel, Mülheim an der Ruhr, Germany), 7890AGC and 7000B GC-MS/MS triple quadrupole system (both Agilent Technologies, Santa Clara, CA). Data acquisition and processing was performed using the corresponding Gerstel Maestro and Agilent Mass Hunter Qualitative and Mass Hunter Quantitative Analysis B.08.00 software. Chromatographic

Table 1
List of analytes, their structures, retention times, quantification and qualification transitions.

compound	structure	retention time (min)	quantifier transition (*m/z*)	qualifier transition (*m/z*)
MP		19.0	121 → 93	152 → 121
BHA		19.9	165 → 137	137 → 109
BHT		20.8	205 → 177	220 → 205
EP		20.9	121 → 93	166 → 138
TBHQ		21.8	151 → 123	166 → 151
PP		23.6	121 → 93	180 → 138
BP		25.4	121 → 93	194 → 138
NBBS		26.3	141 → 77	170 → 141
TBEP		31.8	125 → 99	85 → 57
dBHT		20.4	240 → 222	/
PDCP		18.2	202 → 90	/

separations were carried out using a J&W HP-5MS UI capillary column (30 m × 0.25 mm I.D., d_f: 0.25 μm) supplied by Agilent Technologies. Helium (purity 99.999%) was employed as a carrier gas at a constant flow of 1.4 mL/min. A multimode inlet (MMI) kept in splitless mode (held 1.25 min) at 250 °C was used. The oven temperature was programmed as follows: 50 °C (held 4 min) to 150 °C at 8 °C/min (held 5 min) to 280 °C at 12 °C/min (held 5 min) (total runtime = 37 min). The transfer line temperature was maintained at 280 °C. Electron ionization (EI) at 70 eV was used. The ion source temperature was set to 230 °C and temperatures of both quadrupole analysers were 150 °C. TIC chromatograms were recorded in the range 35–700 *m/z*. Optimized SRM transitions and collision energies of the corresponding quantifier and qualifier ions for each compound are presented in Table 1. For the identification of compounds, NIST mass spectral libraries were used (version 2.0, built May 19th, 2011).

2.5. Extraction experiments and migration testing

The polymer liner of each cap was mechanically separated from the metal cap. 5 g of polymer liners were heated under reflux in 50 mL of the following solvents: water, dichloromethane, isooctane, ethanol and ethanol 10% in water (*v/v*). The sample solutions were filtered and injected into the GC-MS/MS system. Additionally, a freshly prepared 10 mL aliquot of the water-based extract was extracted three times with 5 mL of dichloromethane. The combined extracts were dried with 5 g of anhydrous Na_2SO_4 and concentrated under reduced pressure to yield a total volume of about 1 mL and injected into the GC-MS/MS system.

Water-based food simulants were selected for migration testing, since we were primarily interested in alcoholic beverages and vinegars. The following food simulant solutions were prepared: food simulant A (ethanol 10% in water (*v/v*)), food simulant B (acetic acid 3% in water (*v/v*)), food simulant C (ethanol 20% in water (*v/v*)) and food simulant D1 (ethanol 50% in water (*v/v*)). Food simulant C was prepared using denaturated ethanol, previously confirmed for the absence of the analytes of interest. The plastic fermenter was filled with 20 L of food simulant C, tightly closed with a lid and water filled airlock and stored in a dry and dark place with temperatures ranging from 15 to 20 °C. 300 mL aliquots of the containing solution were sampled at different time intervals: immediately after filling the fermenter and then on days 1, 3 and 5 and later after 1, 2, 3, and 4 weeks.

Amber glass beer bottles were filled with 330 mL of water, ethanol, food simulant A, food simulant B, food simulant C, food simulant D1, tightly closed with a crown cap and kept in a KK-900CH stability testing climatic chamber supplied by Kambič (Semič, Slovenia) at 50 °C and 75% relative humidity for six months. Two replicates of each sample were prepared of which one was kept upright and the other was stored upside-down. Acquired samples were kept in darkness at 4 °C until analysis.

2.6. Sample preparation and SPME procedure

A 30-min ultrasonic degasification of samples was performed before further procedures. 100 mL of degassed sample was then transferred to a 250 mL round bottom flask and 500 μL of 50 μg/mL PDCP internal standard was added. Volatile compounds were removed under reduced pressure (5 min with the water bath temperature set to 60 °C) and the remaining content in the round bottom flask was quantitatively transferred to a 100 mL volumetric flask, to which 10 g of NaCl, 0.5 mL of 2.6 M KCl and 2.5 mL of 2 M HCl were previously added. The sample was diluted to 100 mL with purified water and shaken until the NaCl was completely dissolved. A sample aliquot of 8 mL was transferred to a 10 mL SPME vial and sealed with an aluminium cap furnished with a PTFE-faced septum. Before each analysis, the fibers were conditioned for 30 min at 250 °C and then, prior to starting a set of experiments, a blank analysis was performed to verify that no interfering compounds were desorbed from the fiber. The vials were incubated in the agitator operating at 50 °C. After pre-equilibrating for 5 min, the SPME fiber was inserted through the septum into the vial. Agitation speed was set at 250 rpm. The sorption of analytes was optimized at 25 min and after that, the SPME fiber was inserted into the injector fitted with a 0.75 mm I.D. liner. The SPME fiber was desorbed for 3 min. After each injection the fibers were additionally conditioned and cleaned in the bake-out station for 5 min at 250 °C.

2.7. GC-FID method for ethanol determination

GC-FID method was used for on-line evaluation of ethanol content in samples prior to their analysis by SPME-GC-MS/MS. A GC-FID system consisting of a G1888 Headspace sampler and 7890B GC chromatograph (both Agilent Technologies, Santa Clara, CA) was used. The installed column was an Rtx-1301 capillary column (60 m × 0.32 mm I.D., d_f: 1.8 μm) supplied by Restek. Helium (purity 99.999%) as a carrier gas at a constant column flow of 3.0 mL/min was employed. The inlet temperature was set to 140 °C with a split ratio of 100:1. An isothermal oven program at 60 °C (kept for 10 min) was employed. Ethanol residue in samples was evaluated online by pipetting 1.0 mL of sample into a 10 mL headspace vial, which was tightly sealed afterwards. Vials were equilibrated for 10 min at 80 °C and 1 mL of the corresponding headspace vapor was injected.

2.8. High resolution mass spectrometry method

High resolution mass spectrometric method (HRMS) was used to confirm the formation of the methylated analogue of PDCP (internal standard). Spectra were recorded using a LTQ Orbitrap XL Mass Spectrometer (supplied by Thermo Fisher Scientific Company, Villebon, France) equipped with a heated electrospray ionization source (HESI-II). Full-scan mode spectra were acquired with in the range 100–800 *m/z* in positive ionization mode.

3. Results and discussion

3.1. Crown cap extraction studies and selection of analytes

Crown cap plastic seals were extracted with different solvents as described in section “Extraction experiments and migration testing”. Multiple compounds were detected in each extract, out of which the majority were identified using mass spectral library. TIC chromatograms and some of the identified compounds are listed in Supplementary material SI 1, SI 2 and SI 3. Available toxicological and trace level research data background checks were performed for the identified compounds. Most of the latter belonged to various long chain hydrocarbons and alcohols. Some exceptions, however, were detected. These included the phenolic antioxidants BHA and BHT and the plasticizer NBBS. Based on the findings of the extraction study and the probability of their occurrence in real samples, nine compounds were selected as the analytes of interest.

3.2. MS^2 method optimization

GC-QQQ in SRM mode can provide high selectivity for target and even coeluting analytes in the most complex samples and in the presence of interferences of same *m/z*. First, TIC chromatograms of a mixture of diluted (0.1 mg/mL) stock standard solutions in ethanol were recorded to identify potential precursor ions of each compound. Three most abundant ions of each compound were selected and further fragmented in product ion scan mode at different collision energies (CE) to give potential product ion candidates. The CE studied were in the range from 2 to 60 eV in 5 eV steps. One most abundant product ion and one molecule-specific product ion were selected for each compound as the quantification and qualification transition. In order to refine the selected transitions, CE were again studied, this time as a fine

optimization around the optimal CE. The choice of 10 eV turned out to be a satisfactory option for all the analytes. The selected quantifier and qualifier transitions for each compound are presented in Table 1.

3.3. Choice and justification of the internal standard compound

The choice of a suitable internal standard compound is often difficult as it should show similar chemical and physical properties as (and at the same time not interfere with) the analytes of interest. Such criteria are most commonly met by the implementation of isotopically labeled analyte analogues, which are often not readily available due to their price or complicated synthetic pathways. In the present work, the performance of dBHT as a BHT analogue was compared to PDCP, which was found to show adequate properties to be used as an internal standard. During method development it was found that a methylated derivative was immediately formed at room temperature by preparing a stock solution of PDCP in methanol (1 mg/mL). The structure was recognized after the injection of the stock solution into the GC-MS/MS system and the detection of the molecular mass at 202 *m/z* in scan mode. The identity of the methylated analogue was additionally confirmed by directly injecting the stock solution into a HRMS mass spectrometer (see section 2.8.). $C_8H_{12}O_4P$ as the proposed $[M+H]^+$ molecular ion was obtained with a *m/z* of 203.0467. A quantitative conversion to the methylated analogue was confirmed since no *m/z* of the original chlorinated compound was detected. A comparison with dBHT in regards to method performance was evaluated. Basic validation parameters, such as method precision, accuracy and linearity were verified. Both internal standards have shown comparable performances, whereas for some parameters PDCP performed even better (lower RSD, better R^2).

The methylated analogue of PDCP belongs to the group of organophosphates and therefore represents a TBEP related compound. Since PDCP is used as a catalyst in organic synthesis, its presence in polymers and foods is highly unlikely.

Therefore, the choice of PDCP as a cheaper and more readily available internal standard was experimentally justified. Additional data to support the latter conclusion are shown in Supplementary material SI 4.

3.4. GC method development

Before starting the development of a GC method, the physical and chemical properties of the analytes were studied. Since no derivatization step was included in our sample preparation procedure, the properties of key importance were the polarity and the boiling points of the studied compounds. Satisfactory selectivity and fast retention times were achieved with the implementation of a (5%-phenyl)-methylpolysiloxane capillary column, specifically a HP-5MS UI column was chosen as the most appropriate. Fig. 1 shows the performance of a regular HP-5 column in comparison to a HP-MS UI column of the same dimensions. The latter was developed with additional focus on inertness, making it more suitable for analyzing more polar compounds. This allows the determination of even the most polar compounds without derivatization of polar functional groups (parabens, TBHQ).

3.5. Optimization of SPME parameters

Unless otherwise stated, the optimization of SPME parameters was performed using food simulant C (FS C).

3.5.1. Fiber selection

The efficiencies of five different fiber coatings (PA, PDMS, PDMS/DVB, DVB/CAR/PDMS, and CAR/PDMS) were evaluated as peak area responses of analytes from the standard aqueous solution. Results are shown in Fig. 2. PDMS and CAR/PDMS coatings did not sorb satisfactory quantities of any of the compounds to be further considered. Comparable peak areas for parabens were achieved with PA, DVB/CAR/PDMS, and PDMS/DVB coatings. DVB/CAR/PDMS and PDMS/DVB have shown the highest peak areas for BHA, BHT, TBHQ, and NBBS. PA and PDMS coatings sorbed the largest amounts of TBEP, followed by DVB/CAR/PDMS. Although for some compounds PDMS/

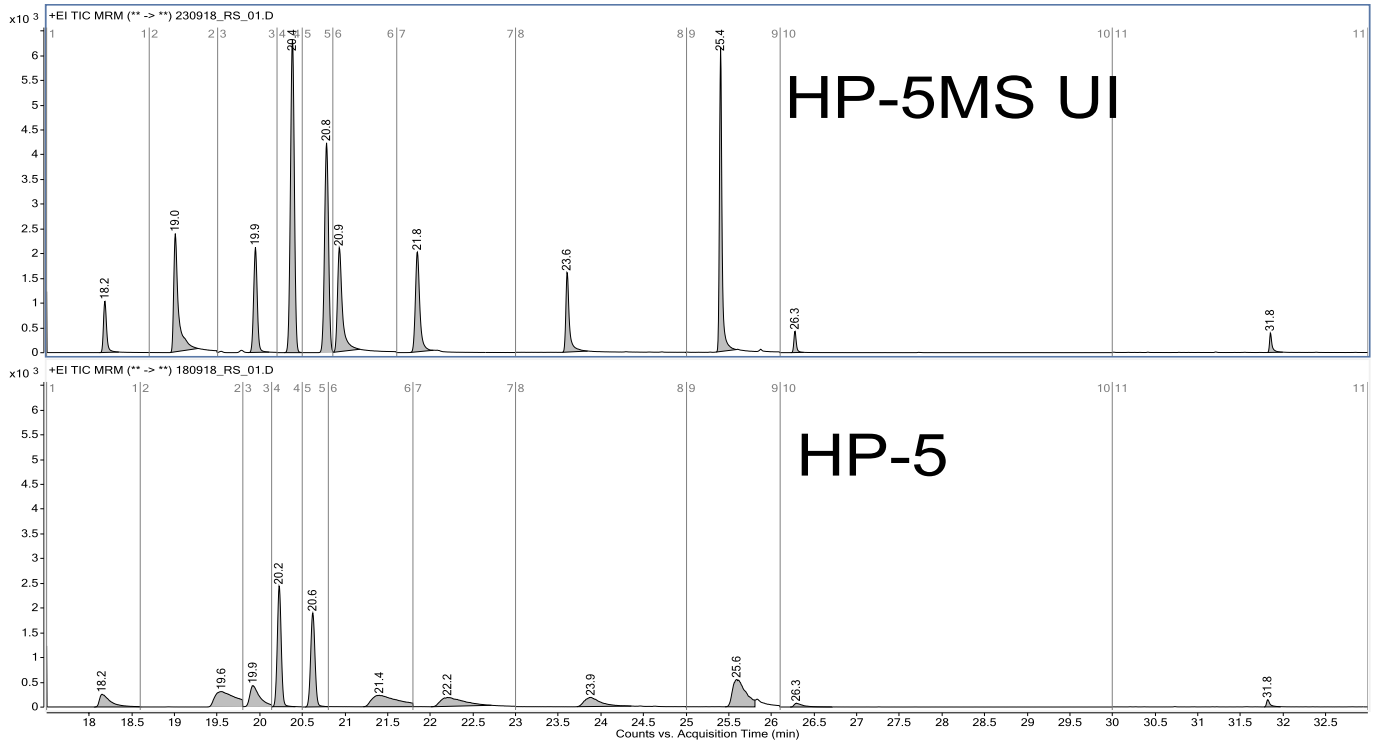


Fig. 1. Comparison of the performance between two differently modified columns of the same stationary phase and the same manufacturer. Chromatograms of the same reference solution at working concentration are shown.

DVB and DVB/CAR/PDMS coatings have shown better sorption, the PA coated fiber gave the best reproducibility in all cases and has proved itself as the most robust of all fiber coatings resulting in the longest fiber lifespan. To compromise between the best response and reproducibility for all compounds and at the same time retain a long fiber lifespan, the PA coated fiber was selected.

3.5.2. NaCl addition

Increasing the concentration of an electrolyte and raising the ionic strength of a solution can influence the sorption to the fiber in two ways: it can change the properties of the phase boundary and decrease the solubility of compounds with hydrophobic properties in the aqueous phase ("salting out" effect). The studied concentrations of NaCl were 0, 50, 100, and 200 g/L. Peak areas increased with increasing NaCl concentrations (see Fig. 3). The phenomenon was especially prominent for parabens, NBBS and the internal standard PDCP, which have all shown an increase in peak area by a factor more than 2 when comparing a 100 g/L concentration with a 200 g/L. BHA has shown a linear peak area response with respect to the increasing NaCl concentration. NaCl concentration did not affect BHT peak area response. In the case of TBHQ, a slight decrease of peak area was observed at 200 g/L NaCl. A trace-level analysis of TBEP without any presence of NaCl is not feasible since peak for TBEP at 0 g/L NaCl is barely detectable. Higher concentrations of NaCl, similar to BHT and TBHQ, slightly decrease the peak area response of TBEP. Although 200 g/L NaCl in general gave the highest peak area responses, the fiber lifespan was drastically reduced upon exposure to higher concentrations of NaCl. What is more, repeatability of peak areas in the case of 200 g/L NaCl was lower. Therefore, a 100 g/L addition of NaCl was selected as an optimal compromise between the highest peak area responses, peak area reproducibility and maintaining a longer fiber lifespan.

3.5.3. Incubation temperature

The effect of incubation temperature on peak areas was studied at 35, 50, and 75 °C. Findings indicate that higher incubation temperatures have a positive impact on peak areas of most of the studied compounds with BHT being an exception, its peak area decreases with increasing temperature. Similar to the NaCl effect, however, the repeatability of peak areas decreased along with higher temperatures with the internal standard PDCP being the most affected. A significantly more frequent fiber breakage was also observed at the highest incubation temperature. Thus, 50 °C was chosen as the optimal incubation temperature (Fig. 4).

3.5.4. Effect of sampling time

In order to reach good repeatability of peak areas, the partitioning of analytes between the solution and the fiber should reach or should at least be close to reaching an equilibrium state. Sampling times of 5, 15, 25, and 45 min were evaluated. The peak areas of all compounds increased with prolonged exposure time. At 5 min of exposure time, the repeatability of most compounds was unacceptable with RSD close to 50% (BHA, BHT, TBHQ). Since the relative differences between peak areas obtained after 45 min and 15 min of exposure time are negligible, analyzed compounds (with the exception of BHA and BHT) seem to reach satisfactory equilibrium even after 15 min. Therefore, a sampling time of 25 min was selected as a compromise between method sensitivity, repeatability and practicality. The latter affected method throughput, which was also subject to GC cycle time (Fig. 5).

3.5.5. Matrix effect

Food simulant A (without any modification), food simulant A (with the addition of NaCl, HCl, and KCl), food simulant C (without any modification) and food simulant C (with the addition of NaCl, HCl, and KCl) were studied. Additionally, variations of the method matrix in comparison with the optimized method parameters were studied. The results are presented in Fig. 6. The lowest peak area responses for all compounds were obtained in food simulant A and C based matrices without any sample preparation procedures applied (ultrasonic degasification, evaporation at reduced pressure, addition of NaCl and HCl – see section "Sample preparation and SPME procedure" for details), which confirms that organic solvents should be present only in low amounts when performing SPME. Due to lower ionic strength, the method performed in water only matrix has also shown poor sensitivity for parabens and especially for TBEP. A similar effect was also observed in the case of the matrices without NaCl with considerably lower peak areas of parabens, BHA, TBHQ, and NBBS. The addition of HCl and KCl (aside from pH stabilization) also contributes to a greater ionic strength. Absence of HCl, KCl or both does not considerably affect the peak areas of BHA, BHT, NBBS and TBEP. In the case of parabens and TBHQ, however, which are ionizable molecules, the absence of HCl resulted in slightly decreased peak areas. What is more, the absence of HCl has also caused larger standard deviations, which is most probably a consequence of unstable pH values. An exception to all the phenomena was BHT, which has shown similar peak area responses and repeatability in all matrices. In order to achieve the best sensitivity and repeatability of the method, the optimal sample matrix must have significant ionic strength, stabilized pH values in the acidic range and must contain as little interfering organic solvent residue as possible. Because of the latter it was necessary to remove as much of ethanol or other interfering volatile compounds as

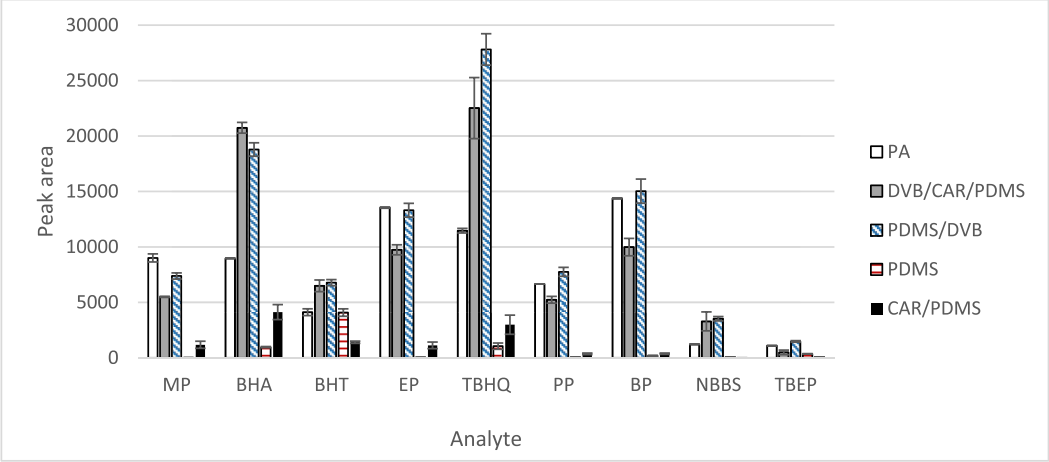


Fig. 2. Influence of fiber coating on obtained peak areas. Error bars represent the standard deviation (n = 3). Extraction was performed for 25 min at 50 °C at a NaCl concentration of 100 g/L.

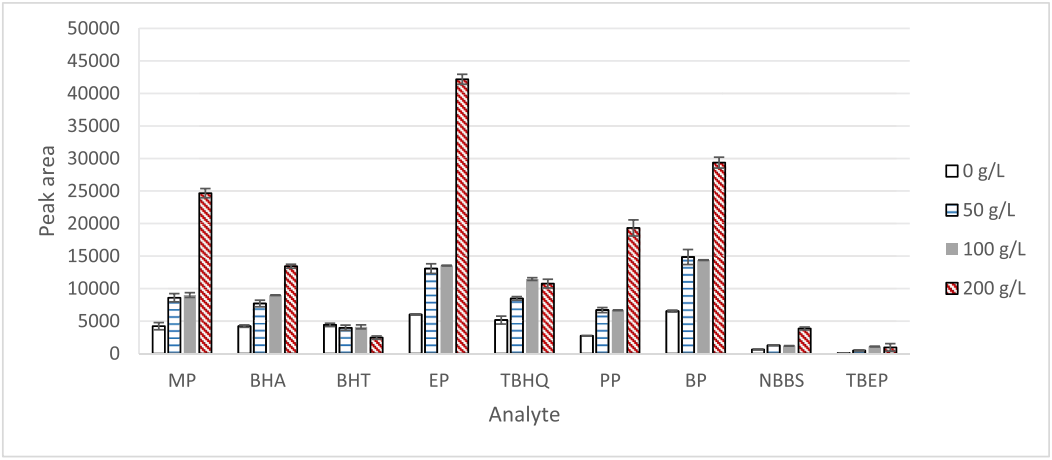


Fig. 3. Influence of NaCl addition on obtained peak areas. Error bars represent the standard deviation (n = 3). Extraction was performed for 25 min at 50 °C on a 85 µm PA fiber.

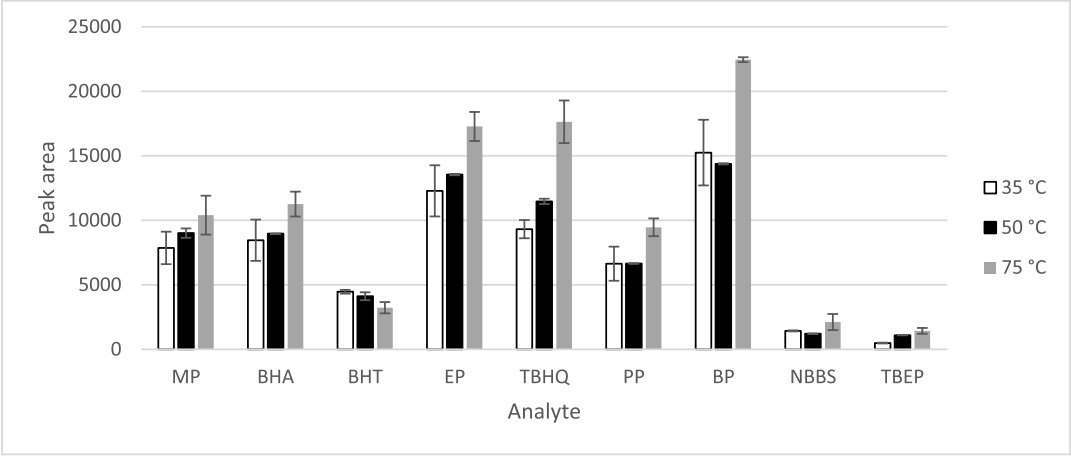


Fig. 4. Influence of incubation temperature on obtained peak areas. Error bars represent the standard deviation (n = 3). Extraction was performed for 25 min at a NaCl concentration of 100 g/L on a 85 µm PA fiber.

possible. We have striven to make the process of evaporation at reduced pressure as fast as possible. Therefore, the reduction of ethanol levels in food simulant C while performing evaporation at reduced pressure was monitored via GC-FID analysis (see section 2.7.). 5 min of evaporation with the water bath set at 60 °C has shown to sufficiently reduce ethanol levels down to approximately 1% (*v/v*).

3.6. Method validation

After optimization, the developed method was evaluated in terms of precision (repeatability and reproducibility), linearity, accuracy, detection and quantitation limits, and stability of solutions. Standard working concentrations (WC, see Table 2) of each compound were

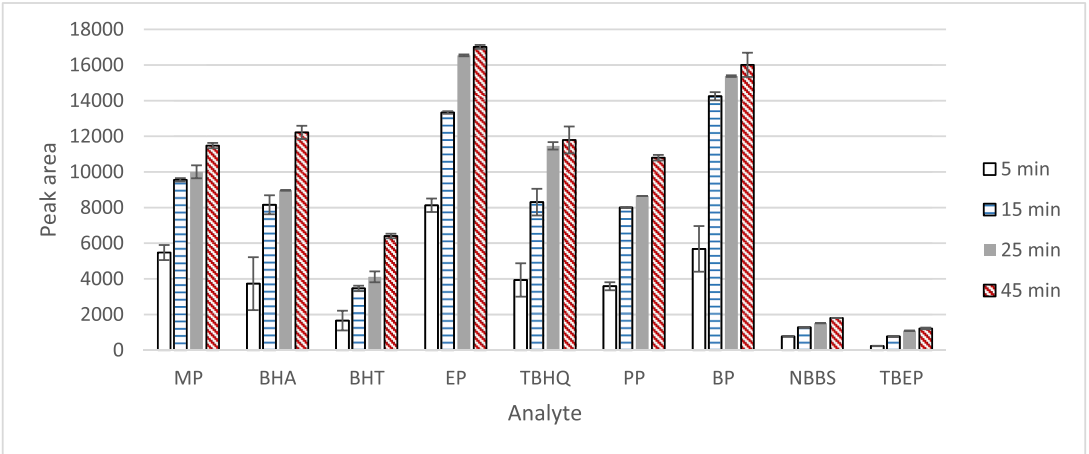


Fig. 5. Influence of fiber exposure time on obtained peak areas. Error bars represent the standard deviation (n = 3). Extraction was performed at 50 °C at a NaCl concentration of 100 g/L on a 85 µm PA fiber.

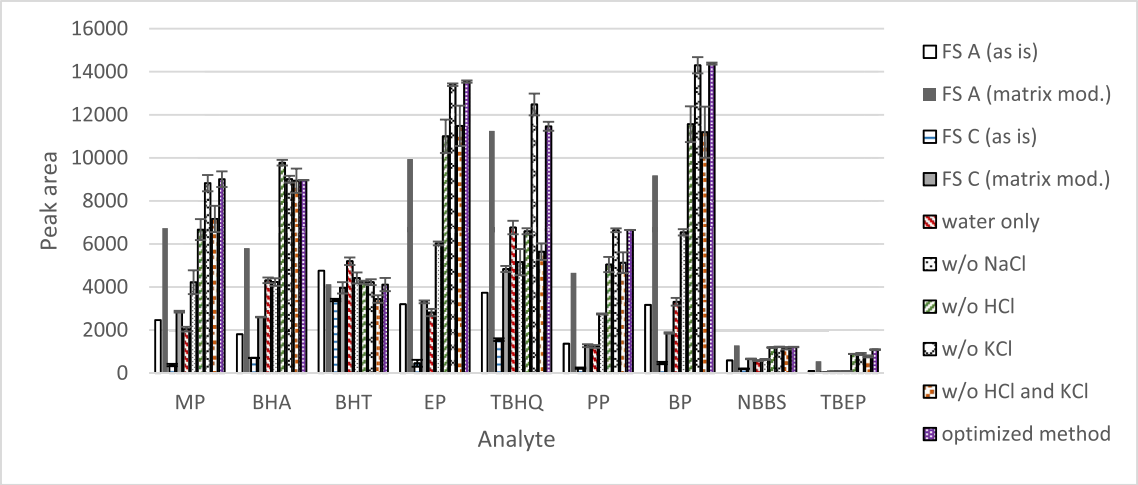


Fig. 6. Influence of matrix composition on obtained peak areas. Error bars represent the standard deviation (n = 3). »As is« - no additions; »matrix. mod.« - addition of NaCl, HCl, and KCl (see section 2.6.).

selected as to achieve comparable peak areas between compounds. Food simulant C as the main sample matrix was used for method validation.

3.6.1. Precision, repeatability and reproducibility

Intra-day and inter-day precision were validated by spiking food simulant C with analytes at their working concentrations and at their quantitation limits (LOQ). The replicate solutions were prepared according to the optimized method procedure. System intra-day precision was expressed as the relative standard deviation (RSD) of six replicates (n = 6) of peak area ratios between the analyte of interest and the internal standard. Method precision was expressed as the RSD (n = 6). The inter-day precision was evaluated by determining the RSD at the working concentration (n = 6) and at LOQ (n = 3) on three consecutive days. Results (Table 3), indicate good repeatability for all the analytes at the working concentration (values ranging from 1.5% for MP to 6.5% for BHT) and at LOQ (values from 4.3% for BHA and 16.6% for PP), respectively.

Reproducibility of the method was evaluated by the determination of RSD and recovery at LOQ (n = 3) and at the working concentration (n = 6) with a different batch of SPME fibers of the same coating (PA) and the same supplier (Supelco).

3.6.2. Accuracy

Accuracy of the method was determined in tandem with precision of the method. Food simulant C was spiked with analytes at their working concentrations (n = 6), at 10% of the working concentration (n = 3), at their quantitation limits (LOQ) (n = 3). Additionally, accuracy of the method at working concentration (n = 3) was also evaluated and verified in food simulant B, which represents a different (acidic) matrix with absence of a volatile organic solvent. Accuracy was presented as the ratio between the determined and the spiked amount. The determined values (see Table 3) indicate acceptable recoveries for all analytes with parabens being the most critical at lower concentrations and NBBS with TBEP reaching higher values at the working concentration in food simulant C.

3.6.3. Linearity

The linearity of the method was evaluated by analyzing three replicates of standard solutions of analytes prepared at ten concentration levels, ranging from the LOQ to about ten times the working concentration of each analyte. Linearity with determination coefficients equal or greater than 0.99 for all the studied analytes was obtained. Results are shown in Table 2.

3.6.4. Stability of analytical solutions

Stability of the standard analyte solutions and spiked food simulant C, representing a sample solution, was evaluated for five days. The solutions were stored in amber flasks at 4 °C in the dark. Acceptable stability of analytes both in standard as well as in the sample solutions with RSD less than 20% with respect to freshly prepared solutions was proven.

3.6.5. Limits of detection (LOD) and limits of quantitation (LOQ)

The limit of detection and limit of quantification are defined as the injected amount of analyte that gives a signal to noise ratio equivalent or larger than 3 and 10, respectively. The LODs of the method were in the range of 0.005–0.2 μg/L and the LOQs of the method were in the range of 0.015–0.5 μg/L. For LOD and LOQ values for each analyte see Table 3. Method detection and quantitation limits for parabens are approximately ten times greater than those reported by Regueiro et al. (2009) and

Table 2
Working concentrations (WC), linear ranges, determination coefficients (R^2), detection limits (LOD), quantitation limits (LOQ), precisions (RSD), and accuracy (recoveries) for all nine analytes of interest at three concentration levels (LOQ, 10% of WC, and WC) and in food simulant B (FS B) at WC.

Compound	WC (μg L^{-1})	Linear range (μg L^{-1})	R^2	LOD (μg L^{-1})	LOQ (μg L^{-1})	Recovery (%)			
						LOQ (n = 3)	10% WC (n = 3)	WC (n = 6)	WC FS B (n = 3)
MP	50	0.5–5000	0.9996	0.2	0.5	87	87	108	100
BHA	2.5	0.025–250	0.9993	0.01	0.025	83	91	98	99
BHT	1.5	0.015–150	0.9958	0.005	0.015	105	98	100	92
EP	25	0.25–2500	0.9989	0.05	0.25	83	81	100	105
TBHQ	20	0.2–2000	0.9985	0.05	0.2	107	103	108	113
PP	5	0.05–500	0.9973	0.025	0.05	86	91	103	104
BP	5	0.05–500	0.9971	0.025	0.05	87	88	99	101
NBBS	1	0.01–100	0.9984	0.001	0.01	119	112	107	115
TBEP	3	0.03–300	0.9975	0.005	0.03	120	102	109	116

Table 3
Intra-day precision, inter-day precision and method reproducibility.

Compound	Intra-day precision (%)			Inter-day precision (%)						Method reproducibility (%)			
	System precision		Method precision	Day 1		Day 2		Day 3		Precision (RSD, %)		Accuracy (%)	
	LOQ (n = 6)	WC* (n = 6)	WC* (n = 6)	LOQ (n = 3)	WC* (n = 6)	LOQ (n = 3)	WC* (n = 6)	LOQ (n = 3)	WC* (n = 6)	LOQ (n = 6)	WC* (n = 6)	LOQ (n = 6)	WC* (n = 6)
MP	12.4	1.5	1.8	0.7	1.9	4.8	3.5	3.2	3.2	12.5	4.3	89	98
BHA	4.3	4.2	3.6	3.0	2.5	8.6	3.3	2.1	1.2	8.9	6.0	85	98
BHT	6.4	6.5	4.2	2.9	4.0	9.7	3.4	2.1	2.0	20.5	8.8	100	95
EP	5.1	1.7	2.4	3.1	2.5	2.6	5.7	1.1	1.5	14.4	3.9	84	96
TBHQ	8.9	6.1	3.3	1.6	2.0	6.6	2.9	2.8	3.7	16.4	5.6	102	98
PP	16.6	5.6	1.4	4.0	2.8	7.8	3.3	2.9	1.7	16.3	5.3	85	101
BP	15.8	4.4	2.6	3.3	1.4	3.4	4.7	3.5	1.3	11.3	4.7	86	100
NBBS	8.7	6.3	0.8	4.3	2.2	15.8	5.2	5.7	1.7	16.0	4.6	114	105
TBEP	14.9	4.3	5.4	0.2	1.2	7.0	6.4	1.3	3.2	19.5	8.3	118	102

*WC = working concentration.

Azzouz et al. (2016). When taking into account the fact that no derivatization procedure was performed, however, and compare them with the detection limits reported by Farajzadeh et al. (2010), ours are approximately a hundred times lower. Comparable detection limits were achieved for SPA as reported by Cacho et al. (2015), however with our method no derivatization step was needed. The data on trace level determination of NBBS are limited with the exception of the work of Huppert et al. (1998) with a reported detection limit of 0.1 μg/L. A TBEP determination with a LOD of 0.001 μg/L by Fries and Puttmann (2001) represents an approximately five times lower detection limit.

3.6.6. Robustness of the method

Robustness of the method was evaluated by the determination of RSD (n = 6) and accuracy at the working concentration by using a SPME fiber of the same coating (PA) but by a different supplier (Restek). Robustness was also evaluated by modifying some of the SPME sampling parameters (incubation temperature, incubation time, NaCl addition) by ± 10%. The RSD and relative difference to the determined amounts are listed in Supplementary material SI 5. Results indicate that the selection of a fiber from a different manufacturer does not significantly affect the obtained accuracy. The repeatability (in the case of using the fiber of an alternative manufacturer) is higher for all the compounds except for BHA and BHT. With the exception of BHA, incubation time and NaCl addition did not turn out as crucial parameters that should be strictly controlled. Variations in incubation temperature, on the other hand, have shown to affect the results for all parabens, BHT and TBHQ with relative differences ranging up to 18.8% (BHT).

Food simulants A and D1 are similar in composition to food simulant C, the difference is in the percentage of ethanol. Since sample preparation included ethanol evaporation to below 1% (see section 3.5.5), we assumed the validity of our results also for FS A and D1. As for food simulant B, which contains acetic acid, it was shown that the method gives suitable accuracy for this matrix (92–116%, see Table 2).

Table 4
The average concentrations ± SD (n = 3) of detected analytes in the analyzed real samples. nd = not determined.

sample		concentration (μg/L)								
		MP	BHA	BHT	EP	TBHQ	PP	BP	NBBS	TBEP
liquor	1	nd	nd	0.11 ± 0.08	nd	nd	nd	nd	nd	nd
	2	nd	nd	0.02 ± 0.01	nd	nd	nd	nd	0.41 ± 0.04	nd
	3	nd	nd	0.06 ± 0.02	1.57 ± 0.28	nd	nd	nd	0.16 ± 0.03	nd
	4	nd	0.18 ± 0.4	0.38 ± 0.06	nd	nd	nd	nd	nd	nd
	5	nd	nd	0.73 ± 0.09	90.71 ± 2.15	nd	1.28 ± 0.12	nd	nd	nd
	6	nd	1.54 ± 1.10	nd	nd	nd	nd	nd	nd	nd
	7	nd	nd	nd	nd	nd	nd	nd	nd	nd
	8	0.34 ± 0.18	nd	0.05 ± 0.01	nd	nd	nd	nd	nd	nd
beer	1	nd	nd	0.01 ± 0.01	nd	nd	nd	nd	nd	nd
	2	nd	nd	nd	0.98 ± 0.18	nd	nd	nd	nd	nd
	3	1.49 ± 0.22	nd	0.07 ± 0.02	2.14 ± 0.23	nd	nd	nd	nd	nd
	4	3.33 ± 0.18	nd	nd	1.85 ± 1.10	nd	nd	nd	0.34 ± 0.08	nd
	5	1.45 ± 0.21	nd	0.07 ± 0.02	3.32 ± 1.21	nd	nd	nd	nd	nd
	6	1.12 ± 0.65	nd	nd	1.80 ± 0.73	nd	nd	nd	nd	nd
	7	3.38 ± 1.87	nd	0.92 ± 0.22	nd	nd	nd	nd	nd	nd
	8	nd	nd	0.08 ± 0.02	nd	nd	nd	nd	nd	nd
	9	nd	nd	nd	nd	nd	nd	nd	nd	nd
wine	1	9.34 ± 2.01	nd	0.02 ± 0.00	16.57 ± 1.81	nd	nd	0.09 ± 0.03	0.16 ± 0.02	nd
	2	nd	nd	0.02 ± 0.00	nd	nd	nd	nd	nd	nd
	3	6.81 ± 0.08	nd	nd	31.47 ± 1.22	nd	nd	nd	nd	nd
	4	nd	nd	nd	nd	nd	nd	nd	nd	nd
	5	9.63 ± 2.32	nd	0.03 ± 0.01	nd	nd	nd	nd	nd	nd
vinegar	1	nd	nd	0.09 ± 0.03	2.61 ± 0.91	nd	nd	nd	nd	nd
	2	nd	nd	0.07 ± 0.03	nd	nd	nd	nd	nd	nd
	3	nd	nd	0.08 ± 0.03	nd	nd	nd	nd	nd	nd
	4	nd	nd	1.11 ± 0.08	nd	nd	nd	nd	nd	nd
Tincture	1	3.12 ± 1.31	nd	0.06 ± 0.02	nd	nd	nd	nd	nd	nd
	2	8.55 ± 1.32	nd	0.02 ± 0.01	2220.99 ± 3.21	nd	0.40 ± 0.08	nd	nd	nd

Table 5
The average concentrations ± SD (n = 3) of detected analytes in the performed crown cap migration experiments. nd = not determined.

Sample			Concentration (μg/L)								
			MP	BHA	BHT	EP	TBHQ	PP	BP	NBBS	TBEP
Food simulant migration samples	water	↑	nd	nd	nd	nd	nd	nd	nd	nd	nd
		↓	nd	nd	nd	nd	nd	nd	nd	nd	nd
	ethanol 10% (*v/v*) – FS A	↑	nd	nd	nd	nd	nd	nd	nd	nd	nd
		↓	nd	nd	nd	nd	nd	nd	nd	nd	nd
	ethanol 20% (*v/v*) – FS C	↑	nd	nd	nd	nd	nd	nd	nd	nd	nd
		↓	nd	nd	nd	nd	nd	nd	nd	nd	nd
	ethanol	↑	nd	0.02 ± 0.00	1.24 ± 0.03	nd	nd	nd	nd	0.09 ± 0.01	nd
		↓	nd	0.06 ± 0.01	1.62 ± 0.02	nd	nd	nd	nd	0.33 ± 0.01	nd
	Acetic acid 3% (*m/v*) – FS B	↑	nd	0.03 ± 0.00	0.03 ± 0.01	nd	nd	nd	nd	3.76 ± 0.06	nd
		↓	nd	0.04 ± 0.01	0.04 ± 00	nd	nd	nd	nd	4.75 ± 0.03	nd

3.7. Analysis of samples

After optimization and validation, the method was used to analyze various commercial and homemade samples of alcoholic beverages and vinegars. As shown in Table 4, the most often occuring compound detected in the samples is BHT with values ranging from 0.03 μg/L in wines to 1.11 μg/L in vinegar. Frequently occuring compounds were also MP and EP with values of MP ranging from 0.34 μg/L in liquor up to EP values as high as 2220.99 μg/L in a tincture sample. NBBS concentrations ranging from 0.16 μg/L in wine and up to 0.41 μg/L in a liquor sample were detected. PP was detected in a liquor and tincture sample whereas traces of BP were detected in a wine sample. TBHQ and TBEP were not detected in any of the analyzed samples.

The proposed method was also applied to analyze the samples from the fermenter migration experiment and migration testing of bottles filled with food simulant solutions. From the results presented in Supplementary material SI 6, it is evident that the presence of MP and BHA is probably not a result of migration, since both are already present in the sample obtained immediately after filling the fermenter. A trend in increasing BHT concentrations with respect to the storage time is noted, which indicates the presence of BHT is most likely a consequence of migration from the contact surface of the fermenter. None of the remaining analytes were detected in the fermenter migration experiment.

In the upright and upside-down stored samples BHA, BHT, and NBBS were detected only in ethanol and food simulant B. In both solutions, we also observed some damage to the crown cap seals, which could result in additional extraction of analytes. The relative differences between the determined concentrations indicate that a direct contact only slightly affects BHA and BHT concentrations. In the case of NBBS, a threefold increase is observed in ethanol. NBBS concentrations in food simulant B were different between upright and upside-down stored samples. They are by far the largest in food simulant B with a value of 3.76 μg/L in the upright stored sample and 4.75 μg/L in the upside-down stored sample, respectively (see Table 5).

The fermenter migration study results indicate MP and BHA were most probably present on the surface of the fermenter since their values did not increase over time. In the case of BHT, however, an increase in concentrations from 0.02 to 0.49 μg/L is observed during prolonged exposure, which indicates BHT migrates from the fermenter contact surface.

4. Conclusion

In this work, a SPME-GC-MS/MS method for the detection of 9 compounds belonging to 4 different groups of additives was developed and validated. Since the proposed sample preparation procedure required little pre-treatment, we consider it as fast, affordable, and simple and therefore applicable for the analysis of larger amounts of samples. With the implementation of an internal standard, satisfactory recoveries and repeatabilities were achieved, demonstrating the reliability and practicability of this method. The proposed internal standard was proven to represent an affordable alternative to the commonly used isotopically labeled analyte analogues. Analysis of samples revealed the presence of almost all the studied analytes at barely detectable concentrations and up to the upper linearity limits. Based on the results of the performed migration experiments, BHT was confirmed as a fermenter contact surface migrant. Food simulant B and ethanol extracted the largest amounts of analytes in the performed crown cap migration experiments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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No conflicts of interest, financial or otherwise, are declared by the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.110829.

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