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Analysis of volatile contaminants in vegetable oils by headspace solid-phase microextraction with Carboxen-based fibres

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Abstract

The headspace solid-phase microextraction (HS-SPME) efficiencies from vegetable oil of the recently available Carboxen–poly(dimethylsiloxane) (PDMS) and divinylbenzene–Carboxen–PDMS fibres were found to be much greater than those of the PDMS fibre for a number of volatile contaminants. Using these Carboxen-based fibres, the commonly used HS-SPME equilibration times for aqueous matrices of 30–45 min at room temperature for a number of halogenated and aromatic analytes with volatilities ranging from 1,1-dichloroethylene to hexachlorobenzene were found to be insufficient for the effective extraction of the less volatile analytes from vegetable oil. HS-SPME at 100°C for 45 min, followed by rapid cooling to 0°C with a 10 min continuing extraction, however, significantly increased the SPME efficiencies for the less volatile analytes. Spiking solutions were prepared in vegetable oil instead of methanol as the latter was found to displace analytes from the Carboxen material. Using either of the Carboxen-based fibres and SPME at 100°C, all the target analytes could be determined at low or sub-μg kg^{-1} with repeatability ≤10%, even though an equilibrium SPME of the less volatile analytes was not achieved.

Keywords: Oils; Headspace analysis; Solid-phase microextraction; Extraction methods; Volatile organic components

1. Introduction

Solid-phase microextraction (SPME) is a rapid, versatile, sensitive, solventless and economical sample preparation technique and has been applied in a variety of analytical applications since first being described by Belardi and Pawliszyn [1]. Extensive studies on SPME, including its automation, optimisation and the dynamics of absorption, as well as a number of applications have been summarised in a recent book [2] and monograph [3]. Sampling by SPME employs a stationary phase coated on a fused-silica fibre which is either immersed in the aqueous sample or suspended over the sample in the headspace (HS). After equilibration between the sample and the coated fibre, the analytes are thermally desorbed in the injection port of a gas chromatography (GC) system, cryofocussed on column, and separated and detected by established GC procedures. The majority of the analytical applications using SPME have focussed on the determination of non-polar trace contaminants in environmental matrices at low or sub-μg kg^{-1} levels. Although SPME has also been applied to food products, most of these applications involve only a qualitative evaluation of the volatile profiles of naturally-occurring chemicals, particularly those related to aromas. Only few reported methods describe the use of SPME in the quantitative determination of volatile contaminants in foods. The use of quantitative methods is essential

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for data gathering purposes or, when the method is suitably validated, to enforce guidelines or regulations.

The SPME of volatiles from foods and environmental matrices differs in several ways. First, the volatile contaminants in foods may be inhomogeneously distributed in the bulk of the food and blending of the food itself or blending by dispersion in water is required to release volatiles from the interior of large food particles as well as to obtain a representative aliquot for analysis. Such homogenisation, and any other sample manipulations must be conducted with minimal volatile loss and contamination from the laboratory environment. Furthermore, the viscosity of the test portion for HS-SPME must permit efficient magnetic stirring, a requirement for time-efficient repeatable sampling. Experience and convenience in our laboratory has shown that a 15–20 g test portion, containing up to 5 ml of a liquid or 1–2 g of solid or semi-solid food dispersed in water with sufficient sodium chloride for saturation, gives good precision and low or sub-μg kg^{-1} sensitivity of non-polar volatiles by HS-SPME, providing lipid food components are not present [4]. A 30-ml HS vial is required.

The other important difference between food and environmental analysis using SPME affects the SPME efficiency, i.e., that fraction of the analyte initially present in the sealed HS vial which at equilibrium is extracted by the fibre coating and can be transferred to the GC injector for analysis. Foods usually heterogeneous and are normally extracted from the sample HS rather than with the coated fibre contacting the food or aqueous food dispersion as the fibre coating could become contaminated with non-polar lipid material or transfer particulates or dissolved non-volatiles (e.g., sugars or salts) to the GC injector. The SPME efficiency, as defined above, is strongly affected by the non-polar food components, mainly the triglycerides, and is a phenomenon only associated with foods [4]. The theoretical aspects of HS-SPME for the three-phase system has been described in detail by Pawliszyn and generalised for a multiphase situation [2]. With lipid-containing foods, the water immiscible triglyceride constitutes a fourth phase. The dielectric constants of the common triglycerides, such as tristearin (2.7), triolein (3.1), tripalmitin (2.9), and tributyrin (5.7) are similar to those of the two most commonly used fibre coatings, i.e., poly(dimethylsiloxane) (PDMS) (2.6–2.8) and poly(acrylate) (PA) (2.6–3.6), as well as the readily extracted organic compounds [2,5]. In a rough approximation, the food lipid could be considered as a separate or distinct part of the fibre coating in a three-phase system, located elsewhere in the sealed vial, with an equal or similar equilibrated analyte concentration, but with variable volume and resulting capacity. Assuming such equal equilibrated analyte concentrations, the respective capacities of the coating and food lipid (0.92 g ml^{-1}) will be proportional to their volumes: a fixed volume of 0.6 μl for the 100 μm thick PDMS fibre and a variable volume of about 11 μl for every 1% lipid in a 1 g food sample. Thus, a 1 g food sample with 1% lipid would contain about 18-times more analyte than the SPME coating because of its greater volume. When considering the effect of added lipid on the magnitude of the reduction in the amount of analyte extracted by the fibre coating, both the capacities of the lipid and the volatility of the particular analyte, i.e., the capacity of the headspace, are important [5].

With 100% lipid samples, a 1 g (1090 μl) vegetable oil sample with 10 ng of contaminant (10 μg kg^{-1}) would partition 0.6 μl /1090 μl (0.055%) or 5.5 pg to the SPME fibre coating for detection and analysis, an amount at or near the detection limits of most universal GC detectors. The equilibration theory for a three-phase system as outlined by Pawliszyn [2] describes the mass of analyte adsorbed by the SPME coating, n, as:

$$n = \frac{K_{fs}V_fC_0V_s}{K_{fs}V_f + K_{hs}V_h + V_s} \quad (1)$$

where K_{fs} and K_{hs} and are the fibre coating/sample and headspace/sample distribution constants, respectively; V_f, V_h and V_s are the volumes of the fibre coating, headspace and sample, respectively; and C_0 is the initial concentration of the analyte in the sample matrix. When the sample matrix is 100% vegetable oil, the equilibrated analyte concentrations in the sample matrix and the fibre coating would be similar as described above, and the K_{fs} distribution constant would approximate unity. Considering that

$V_f << V_s$, then the denominator term $K_{fs}V_f << V_s$ and $K_{fs}V_f$ can be ignored. In the denominator, K_{hs} is also small because the vapour pressure of the volatile analyte is significantly lower over lipid compared to aqueous media, and, as V_h and V_s are similar, then $K_{hs}V_h << V_s$. Thus Eq. (1) reduces to:

$$n = K_s V_f C_0 \quad (2)$$

This reiterates that the amount of analyte extracted by fibre coating is proportional to the initial concentration present in the vegetable oil, and that the fraction of total analyte present in the fibre is proportional to its volume compared to that of the lipid phase. This assumption is valid provided that $V_f << V_s$. Furthermore, the amount of analyte extracted is shown to be independent of the sample volume as the capacities of the headspace and the fibre are very small compared to that of the lipid sample and the concentration in the lipid will be virtually unchanged after equilibration occurs, again providing that $V_f << V_s$.

With lipid-containing foods, the dependence of extraction efficiency on both the sample lipid content as well as volatility of the particular analyte makes accurate quantitation of volatiles complex. Matrix matching of an otherwise identical pair of particular food samples, one found to contain contaminant(s), the other not containing the target residue(s) but spiked at approximately the same level can be used to perform the quantitation. The procedure of standard addition can be used when the sample matrix is expected to affect the HS-SPME extraction efficiency and matrix matched samples are not readily available [4]. Isotopically labelled internal standards can also be effectively employed when the mass spectrometer is used for detection. Internal standards of similar HS-SPME characteristics, i.e., similar volatility and polarity, and close GC elution to the target analytes could also be used to facilitate quantitation. In all cases the incurred and added standards or analytes must be properly equilibrated with the sample matrix and the SPME fibre coating. Linearity of response must be demonstrated when extrapolating beyond the spiking range.

Volatile contaminants in aqueous foods can typically be determined at low or sub-μg kg^{-1} levels by HS-SPME but method sensitivity can be dramatically reduced by food lipid. Several approaches to improve method sensitivity for foods with significant lipid content have been proposed including the use of steam distillation (SD) for the isolation of the volatile contaminants from the food lipid prior to the SPME step [6] and the development and use of SPME fibre coatings with improved extraction efficiency for small polar molecules [5].

In 1997 a new SPME fibre coating became available. The 75 μm Carboxen 1006–PDMS fibre coating (Car–PDMS) was designed for the efficient extraction of small polar molecules from aqueous solutions and is described as a porous carbon material with micro-, meso- and macropores of 6–50 Å blended with PDMS [7,8]. This fibre coating has been successfully applied in the intended application [9,10] as well as to determine pentanal, hexanal and other volatiles associated with off-flavours in milk [11,12]. However, larger analytes were found to be strongly adsorbed and the desorption of less volatile compounds in the GC injector was incomplete, even at 315°C for extended periods [13]. In 1998 another Carboxen-based fibre coating was developed [8,14]. The divinylbenzene–Carboxen 1006–PDMS (DVB–Car–PDMS) fibre coating is described as an inner 30 μm layer of Carboxen blended with PDMS on a strengthened fibre core with an outer 50 μm layer of DVB, also blended with PDMS. The outer DVB layer functions to trap the larger, less volatile analytes permitting the smaller, more polar analytes to pass through and be retained by the Carboxen. With this dual coated fibre a more efficient desorption at lower temperatures (280°C) becomes possible [12,14].

It is important to note that the primary sorption principle of these new Carboxen-based fibres is not the same as the PDMS or the PA material, which act as liquid coatings with the analytes absorbed into the bulk of the extracting phase [15]. The new porous fibres with their large surface area act primarily by adsorption, a surface phenomena. The capacity of the adsorbing porous phases is less than that of the liquid absorbent phases. Competitive displacement can occur with the former phases and the linearity of extraction may be limited to low concentrations and should be verified for each application [15]. The

Car–PDMS and the DVB–Car–PDMS fibre coatings, although developed for increased SPME efficiency for small polar analytes from aqueous media, could also provide a better approach to the analysis of non-polar volatile contaminants in lipids by SPME. This report describes such an application.

2. Experimental

2.1. Standards and reagents

The volatiles determined and studied are listed in Table 1. Certified standard solutions in methanol

Table 1
Volatile analytes studied in approximate order of elution (DB-5), reference numbers, ions monitored, relative response, and method limits of detection (LODs) from water for the PDMS, Carboxen–PDMS and the DVB–Carboxen–PDMS fibre coatings

No.	Compound	Ions[a]	Relative response[b]			LOD[c] (μg kg^{-1})		
			PDMS	Car–PDMS	DVB–Car–PDMS	PDMS	Car–PDMS	DVB–Car–PDMS
1	1,1-Dichloroethene	96, 61	1	190	70	0.2	0.001	0.005
2	Dichloromethane	84, 86	1	240	80	0.3	(0.20)	(0.15)
3	*trans*-1,2-Dichloroethene	96, 61	0.2	370	300	0.2	0.002	0.007
4	1,1-Dichloroethane	63, 65	5	550	200	0.3	0.002	0.001
5	*cis*-1,2-Dichloroethene	96, 61	0.4	420	280	0.2	0.002	0.005
6	Bromochloromethane	130, 128	0.1	120	50	3	0.009	0.002
7	Chloroform	83, 85	5	740	300	(0.10)	(0.14)	(0.20)
8	1,1,1-Trichloroethane	97, 99	9	220	230	0.05	0.003	0.001
9	1,2-Dichloroethane	62, 64	1	910	460	0.1	0.004	0.004
10	Benzene	78, 77	20	1800	1500	(0.006)	(0.003)	(0.004)
11	Carbon tetrachloride	117, 119	7	210	180	0.05	0.003	0.001
12	1,2-Dichloropropane	63, 112	14	630	470	0.03	0.002	0.001
13	Trichloroethene	95, 130	12	780	630	0.03	(0.0003)	(0.0003)
14	Dibromomethane	93, 95	3	260	160	0.9	0.009	0.02
15	Bromodichloromethane	83, 85	15	1400	910	0.05	(0.001)	0.007
16	Toluene	92, 91	30	1200	1100	(0.01)	(0.0065)	(0.01)
17	1,1,2-Trichloroethane	83, 97	4	390	340	0.2	0.003	0.001
18	Dibromochloromethane	129, 127	3	320	280	0.2	0.002	0.002
19	1,2-Dibromoethane	107, 109	4	390	360	0.2	0.003	0.02
20	Tetrachloroethene	166, 168	9	230	230	0.03	(0.002)	(0.0015)
21	Chlorobenzene	112, 114	50	1080	1100	0.006	0.003	0.003
22	Ethylbenzene	91, 106	140	2200	2300	(0.002)	(0.015)	(0.004)
23	*m*/*p*-Xylene	106, 91	90	1200	1300	(0.007)	(0.005)	(0.003)
24	Bromoform	173, 175	2	160	160	0.2	0.004	0.003
25	*o*-Xylene	106, 91	50	640	670	(0.004)	(0.006)	(0.003)
26	Styrene	104, 78	70	1100	1400	(0.004)	(0.015)	(0.04)
27	Bromobenzene	158, 156	20	290	310	0.007	0.003	0.001
28	1,3-Dichlorobenzene	146, 148	90	540	610	(0.015)	(0.009)	(0.008)
29	1,4-Dichlorobenzene	146, 148	110	590	680	(0.05)	(0.035)	(0.04)
30	1,2-Dichlorobenzene	146, 148	110	570	620	0.001	0.006	0.0003
31	1,2-Dibromo-3-chloropropane	157, 155	6	110	140	0.07	0.0003	0.0007
32	1,2,4-Trichlorobenzene	182, 180	120	260	290	(0.003)	(0.001)	(0.0003)
33	1,2,3-Trichlorobenzene	182, 180	110	270	290	(0.001)	(0.0002)	(0.0005)
34	1,2,4,5-Tetrachlorobenzene	216, 214	170	250	280	(0.001)	(0.002)	(0.0006)
35	1,2,3,4-Tetrachlorobenzene	216, 214	170	230	250	(0.007)	(0.002)	(0.0006)
36	Pentachlorobenzene	250, 248	250	290	330	(0.007)	(0.003)	(0.002)
37	Hexachlorobenzene	284, 286	60	70	70	0.003	(0.003)	(0.001)

[a] Primary and secondary ions; other secondary ions may also be used.
[b] Relative peak areas normalised to 4 ng each analyte/15 g water, 7.5 g sodium chloride, 45 min HS-SPME at room temperature.
[c] Method limit of detection with a $S/N = 5:1$. Bracketed values indicate level of interference in blank water, LOD not determined.

may be purchased from various suppliers and diluted as required. Alternatively, these primary stock solutions of standards may be prepared and stored as described by the US Environmental Protection Agency (EPA) procedures [16] using chemicals purchased separately. Stock solutions for microlitre spiking into aqueous solutions were prepared by accurate dilution of the primary solution in methanol suitable for trace volatile analysis (Burdick and Jackson, Muskegon, MI, USA). Stock solutions for spiking of vegetable oil, lard or shortening were prepared by accurate dilution of the primary solution in a suitable vegetable oil, selected by preanalysis to be low in interfering volatile contaminants. Dilutions for direct injections were prepared in 2-methylbutane.

2.2. Heating/cooling jacket

A special jacket as shown in Fig. 1 was used to heat and cool the HS sample vial.

The jacket consisted of a 270-ml (total capacity) wide-mouth glass Mason jar with an open-top screw cap fitted with a 0.8 mm thick brass lid with a 22 mm diameter centred hole. Inlet and outlet 6.25 mm copper tubes, protruding into the jar through the lid at 15 and 5 mm, respectively, were soldered in place side by side 7 mm inside the outer edge of lid. A Mason jar rubber ring was cut to fit and epoxied to the underside of the brass lid as a watertight seal. A 85 mm piece of 8 mm O.D. latex tubing was attached to the interior inlet to supply the steam or ice water to the bottom of the jar. A short piece of 6.25 mm O.D. stainless steel tubing bent at 90° was attached to the latex inlet to induce swirling of the steam or ice water. A rubber vacuum gasket (Büchi code KD-22 vacuum gasket) with the flange cut off, was fitted over the HS vial as a seal between the lid and the vial shoulder. Stopcock grease on the underside of the lid facilitated a water-tight seal. To hold the vial in place, 1.6 mm thick aluminium spacers with a 18 mm wide U-shaped slot were placed across the vial neck under the rim as the lid was pushed down to compress the rubber gasket. Steam heat was provided by a modified 1000 ml Garman SD apparatus [5,6] (not shown) with glass ball-joints and tubing connector (Quick Disconnect, Nalgene, VWR Scientific, Toronto, Canada) attached to the jacket inlet. A matching connector and tygon tubing provided pumped ice-water cooling. The jacket was clamped in position on a magnetic stirrer.

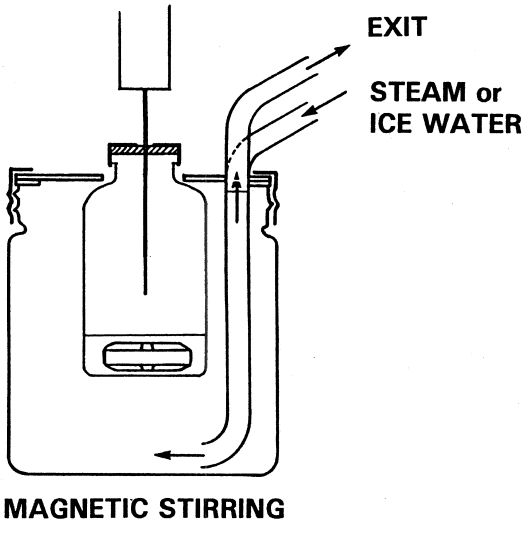

Fig. 1. Heating/cooling jacket for HS-SPME.

2.3. HS-SPME equipment and procedures

The SPME device consisting of a manual holder and three different fibre assemblies were obtained from Supelco (Sigma–Aldrich Canada, Oakville, Canada). The Carboxen–PDMS, the StableFlex DVB–Carboxen–PDMS and the 100 μm PDMS fibre coatings were studied. For HS-SPME extraction from lipid materials, 20 ml (nominal size) crimp-top HS vials (actual capacity about 27 ml), 20 mm×2.7 mm laminated silicone–PTFE (0.25 mm) septa, precored using a 23 ga hypodermic needle conically-ground to 90° for facile passage of the SPME needle, and aluminum seals (Supelco, Oakville, Canada) were used. PTFE-coated, 25×7.5 mm magnetic stirring bars were added to each vial. Clean vials with stirring bars were stored at 125°C in a forced air oven and cooled to room temperature immediately before use. Two gram test samples of vegetable oil, lard or shortening were accurately weighed into tared HS vials and immediately sealed. Similarly, parallel samples of vegetable oil, selected by preanalysis to be low in interfering volatile contaminants, were

spiked if required with appropriate standard solutions in vegetable oil and immediately sealed. Typically, 40 ng of each analyte listed in Table 1 in a 50-μl aliquot were added. For SPME at temperatures other than room temperature, the sealed HS vials were secured in the heating/cooling jacket. The outer needle of the SPME fibre assembly was then inserted through the precored septum, the fibre was extended so that its end was about 1 cm above the surface of the liquid, the fibre holder was clamped in place and the stirring started. For extractions from water, the same supplies, equipment, and techniques as for vegetable oil were used except 30 ml (nominal size) crimp-top HS vials (actual capacity about 37 ml) were required. Fifteen gram samples of purified water, shown to be low in interfering volatile contaminants, were saturated with 7.5 g of sodium chloride to enhance partition into the HS and sealed in HS vials. Using a chilled syringe, chilled standard solutions in methanol, typically 4 ng in a 2-μl aliquot, were added through the septum.

2.4. SPME desorption, chromatography and detection

After the HS-SPME step was completed, the fibre was then retracted, the fibre assembly needle withdrawn from the septum, the needle inserted into the GC injector, the fibre extended into the GC injector to about the midpoint of the heated zone and the injector and column oven programs initiated. The fibre was left in place for the required time and the integrated peak areas obtained. A Hewlett-Packard gas chromatograph (Model 5890 II) with a cool programmable on-column injector, cryogenic oven cooling, and a mass-selective detector (Hewlett-Packard, Model 5970B) operating in the selected-ion monitoring (SIM) mode was used for SPME desorption, analyte separation and detection. The ions monitored are given in Table 1. Additional ions were monitored to provide further confirmation as required. The analytes were desorbed from the fibre in the injector by a temperature program from 200°C (no hold) to 310°C at 100°C min^{-1} with a 19 min hold for the Car–PDMS fibre. For the DVB–Car–PDMS fibre the same program was used except a maximum temperature of 280°C was employed. The analytes were separated on 30 m×0.25 mm fused-silica capillary columns coated with either DB-624 (1.4 μm film) or DB-5 (0.25 μm film) (J&W Scientific, Folsom, CA, USA). The columns were connected to the injector using a 0.5 m×0.53 mm I.D. deactivated fused-silica retention gap and a press-fit capillary column union. Helium at 1 ml min^{-1} (35 cm min^{-1}) was used as a carrier gas. For the DB-624 column, the column oven was programmed from 10°C (1 min hold) to 80°C at 6°C min^{-1} (no hold) and then to 260°C at 20°C min^{-1} (3.25 min hold). The DB-5 column oven was programmed from −20°C (2 min hold) to 60°C at 5°C min^{-1} (no hold) and then to 270°C at 17°C min^{-1} (no hold).

2.5. Studies in aqueous systems

To compare the HS-SPME efficiencies of the two Carboxen-based fibres and 100 μm PDMS fibre, the test analytes listed in Table 1 were spiked into 15 g of water in 30-ml sealed HS vials each containing 7.5 g of sodium chloride to give 0.27 μg kg^{-1} of each analyte. After extraction for 45 min at room temperature with magnetic stirring, the fibres were desorbed.

2.6. Displacement effects of spiking solvents – methanol

To investigate the potential effects of methanol in the competitive displacement of the target analytes from the two Carboxen-based fibres, 2, 4, 6, 8 or 20 μl of methanol were added to 2 g oil samples in addition to a 2 μl methanol spike and mixed to ensure proper incorporation of the analytes. The HS-SPME was then conducted for 45 min at room temperature. In a second study, also for 45 min at room temperature, 2, 5, 10 or 25 μl of methanol were added to 125-ml HS flasks (actual volume, 153 ml) each containing 2 μl of a methanol spiking solution of the target analytes, a stirring bar but no oil. Other solvents were also evaluated to determine their suitability as a non-competitive spiking solvent.

2.7. Studies in vegetable oil

The HS-SPME of the test analytes listed in Table 1 at different extraction temperatures and times from

2 g of vegetable oil using mainly the two Carboxen-based fibres but also the 100 μm PDMS fibre was studied. The effect of extraction time on the analyte response was studied at room temperature for periods from 45 min to 21 h. A HS-SPME temperature of 100°C was evaluated for time-efficient extraction, with times of 45 min and 2 and 5 h investigated. After a 45 min extraction at 100°C, the effect of a temperature reduction to 0°C with continuing extraction of 0, 10, 20, 30 and 45 min was also investigated. The actual percent of each analyte extracted by each fibre coating was determined by comparing the extracted areas of each analyte from the HS-SPME to those from a direct injection (injector at 50°C with a 0.5 min hold) of the same amount of analyte in 2-methylbutane.

2.8. Method performance

The repeatability ($n=5$) for the HS-SPME procedure using the Car–PDMS and DVB–Car–PDMS fibres from 15 ml of water spiked at 0.27 μg kg^{-1} for each analyte as noted in Section 2.5 above was determined. The method limits of detection (LODs, $S/N \geq 5$) were determined in the same aqueous system using all three fibres by spiking water at levels from 0.0007 to 2.7 μg kg^{-1}. When contamination of the blank water to be spiked was observed, the determined levels of the particular contaminants were estimated by a single level standard addition. The linearity of response of the target analytes for the HS-SPME procedure for the three fibres was also determined.

The repeatabilities ($n=5$) of the HS-SPME extraction procedure for the target analytes from 2 g of vegetable oil at 100°C for 45 min with a continuing extraction at 0°C for 10 min were determined for the Car–PDMS and the DVB–Car–PDMS fibres. The vegetable oil was spiked with standard solutions prepared in vegetable oil to give levels of each analyte at 20 μg kg^{-1}. The linearity of response of the target analytes for the HS-SPME procedure for the two fibres was determined from 2 g of oil spiked at levels from 0.2 to 12 μg kg^{-1} using the same extraction conditions. The LODs ($S/N \geq 5$) by HS-SPME using the two fibres were also determined for the target analytes in vegetable oil. The oil chosen for spiking, selected by preanalysis to be low in interfering volatile contaminants, did contain low levels of a number of common volatile contaminants. When such contamination was observed, the determined levels of the particular contaminants were estimated by a single level standard addition.

2.9. Quantitation

The HS-SPME of 2 g samples of vegetable oil was conducted as noted above in Section 2.3. with stirring at 100°C for 45 min with a continuing extraction at 0°C for 10 min. Quantitation was by comparison to a spiked vegetable oil, selected by preanalysis to be low in interfering volatile contaminants. The results were corrected for any interfering volatiles in the spiked standard. Ten samples, including sunflowerseed, cottonseed, canola, olive, olive–canola mixed and canola–soya mixed oils as well as a shortening and lard sample were analysed to evaluate the applicability of the procedure to a variety of lipid samples.

3. Results and discussion

3.1. HS-SPME equipment and procedures

The jacket shown in Fig. 1 provides effective heating/cooling of all the HS vial and its contents except for the protruding aluminum seal and septum. When a water sample is heated, condensing water vapour will heat these areas. The effect on the overall partition of any condensate on the underside of the septum will be small as it will be replaced by the continuing condensation, furthermore, its volume, compared to that of the 15 g sample is small. Vegetable oil volatiles may condense on the underside of the septum and affect the HS-SPME partition between the oil, the HS and the fibre coating. However, it is believed that this effect on the described partition is negligible, because of the small volume of the condensate. The heating/cooling apparatus permits the effective magnetic coupling between the stirrer and stir bar. The action of the stir bar is readily observable. As the temperature is reduced from 100°C to 0°C the viscosity of the oil increases and the rate of stirring was observed to decrease.

3.2. Aqueous systems

The relative responses, corrected for incurred contamination, for the test analytes using the three fibres are given in Table 1. Compared to the PDMS fibre, the more volatile, earlier eluting, relatively polar analytes are extracted with much greater efficiencies by both Carboxen-based fibres. Only the tetra-, penta- and hexachlorobenzenes were extracted with similar efficiencies with all three fibres tested. With the di- and trichlorobenzenes the efficiencies of the two Carboxen-based fibres were about 5- and 2.5-times, respectively, that of the PDMS fibre. As the analyte volatility further increases, the two Carboxen fibres extracted the analytes more efficiently than the PDMS fibre by factors ranging from about 10 to well over 100. The Car–PDMS and the DVB–Car–PDMS fibres were found to be very similar in their extracting capabilities for analytes eluting after toluene. Analytes eluting before toluene, however, were more efficiently extracted by the Car–PDMS coating by a factor of 2–3, possibly reflecting the greater amount of the Carboxen material in this coating. Illustrative chromatograms of the three fibres studied are presented in Fig. 2A–C. It is important to note the differences in the ion abundance scale of the three chromatograms. The scales of those for the Carboxen-based fibres (Fig. 2B and C) were about three-times greater than that of the PDMS fibre (Fig. 2A). The method LODs are also given in Table 1 and generally correlate with the respective relative responses. The water used in the determination of these LODs contained small amounts of contaminants and did not permit the actual measurement of the LOD for these analytes. In these situations the estimated level of the contaminant is presented as a bracketed value, the actual LOD being less, and could be estimated from analytes with similar volatility, polarity and relative response. These LODs using the HS-SPME procedure and either of the Carboxen-based fibres were all <0.01 μg kg^{-1} and are lower than those reported [16] for the EPA Method 524.2 (purge-and-trap–GC–MS) for all analytes studied. The most common contaminants found in the water used in this study were chloroform, styrene, 1,4-dichlorobenzene, dichloromethane and the BTEX aromatics, i.e., benzene, toluene, ethylbenzene and the xylenes. These contaminants were either present in the water or were contributed by the laboratory environment. With the Carboxen-based coatings, carryover was evaluated by a second desorption and not found to be a problem.

3.3. Vegetable oil

The initial studies of the HS-SPME of the target analytes from vegetable oil were conducted at room temperature (23°C) for 45 min, i.e., essentially the same conditions used for aqueous systems. When 2 g of oil was spiked to give 20 μg kg^{-1} of each analyte only small peaks were detected when the oil was extracted using the 100 μm PDMS fibre as shown in Fig. 3A. The responses using the two Carboxen-containing fibres were adequate in detecting the more volatile analytes as shown in Fig. 3B for the Car–PDMS fibre. Note that the ion abundance scale for Fig. 3B is about 50-times that of the PDMS fibre (Fig. 3A). Extending the extraction time for the Carboxen-based fibres increased the response of the later-eluting analytes, indicating a non-equilibrated extraction. In addition, the analyte responses were lower than expected when the analyte concentration was doubled, indicating possible competitive displacement effects in the analyte adsorption. This non-linearity was not observed, however, with aqueous samples, nor was it observed with the PDMS coating. It was apparent that studies on factors affecting method linearity, i.e., competitive displacement, and an optimisation of the extraction time and temperature were required. These aspects are described below.

3.3.1. Displacement effects of spiking solvent – methanol

As the increase of the analyte concentration also increased the methanol spiking solvent, the possible competitive displacement effect of methanol in the analyte adsorption by the two Carboxen-based fibres was studied. The small amounts of added methanol were expected to dissolve completely in the oil and not significantly alter the properties of that phase. Accordingly, studies as described above in Section 2.6 were conducted and showed the progressive decrease in HS-SPME as the amount of methanol was increased. For example, the room temperature

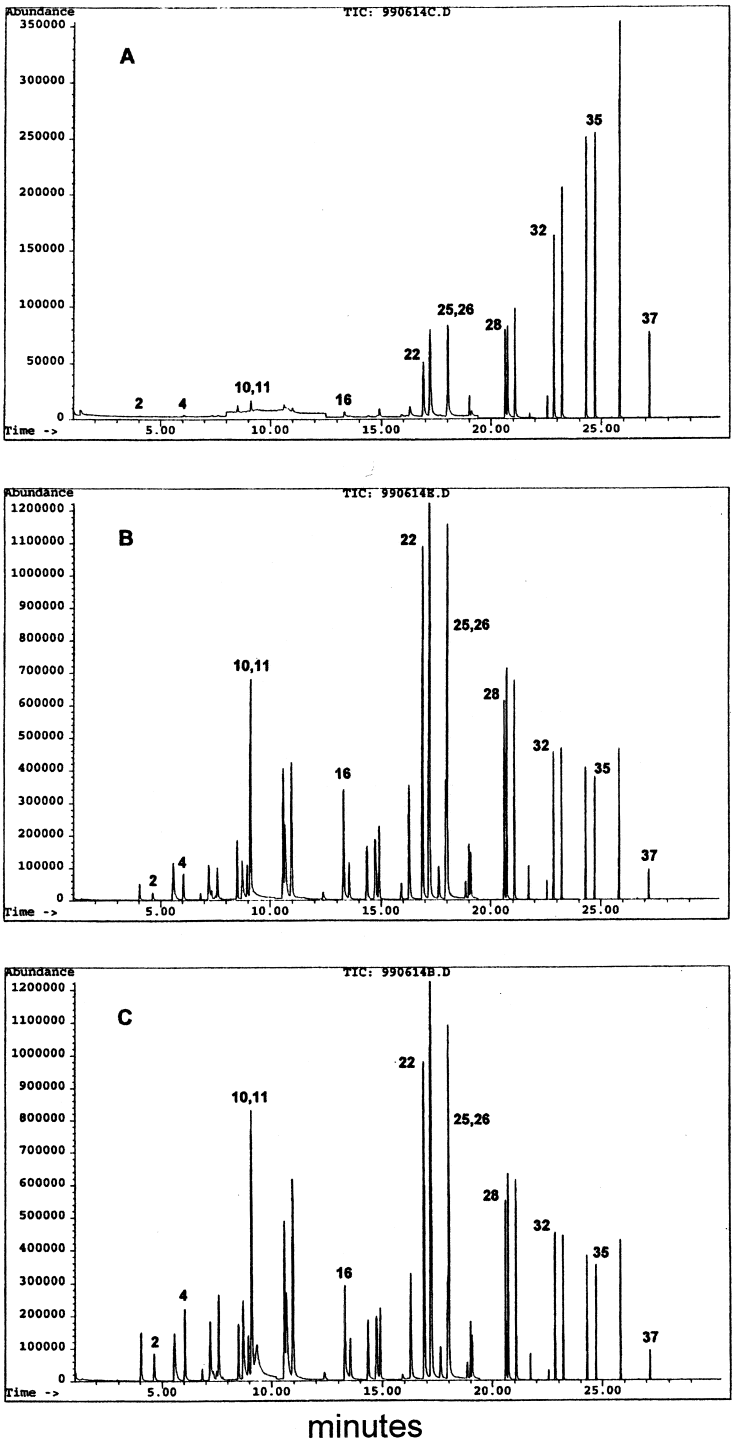

Fig. 2. GC–MS chromatograms of the target analytes at 0.27 μg kg^{-1} listed and identified in Table 1 extracted by HS-SPME at 23°C for 45 min from 15 ml of salt-saturated water using the following film coatings: (A) 100 μm PDMS; (B) DVB–Car–PDMS; and (C) Car–PDMS. Time scales in min.

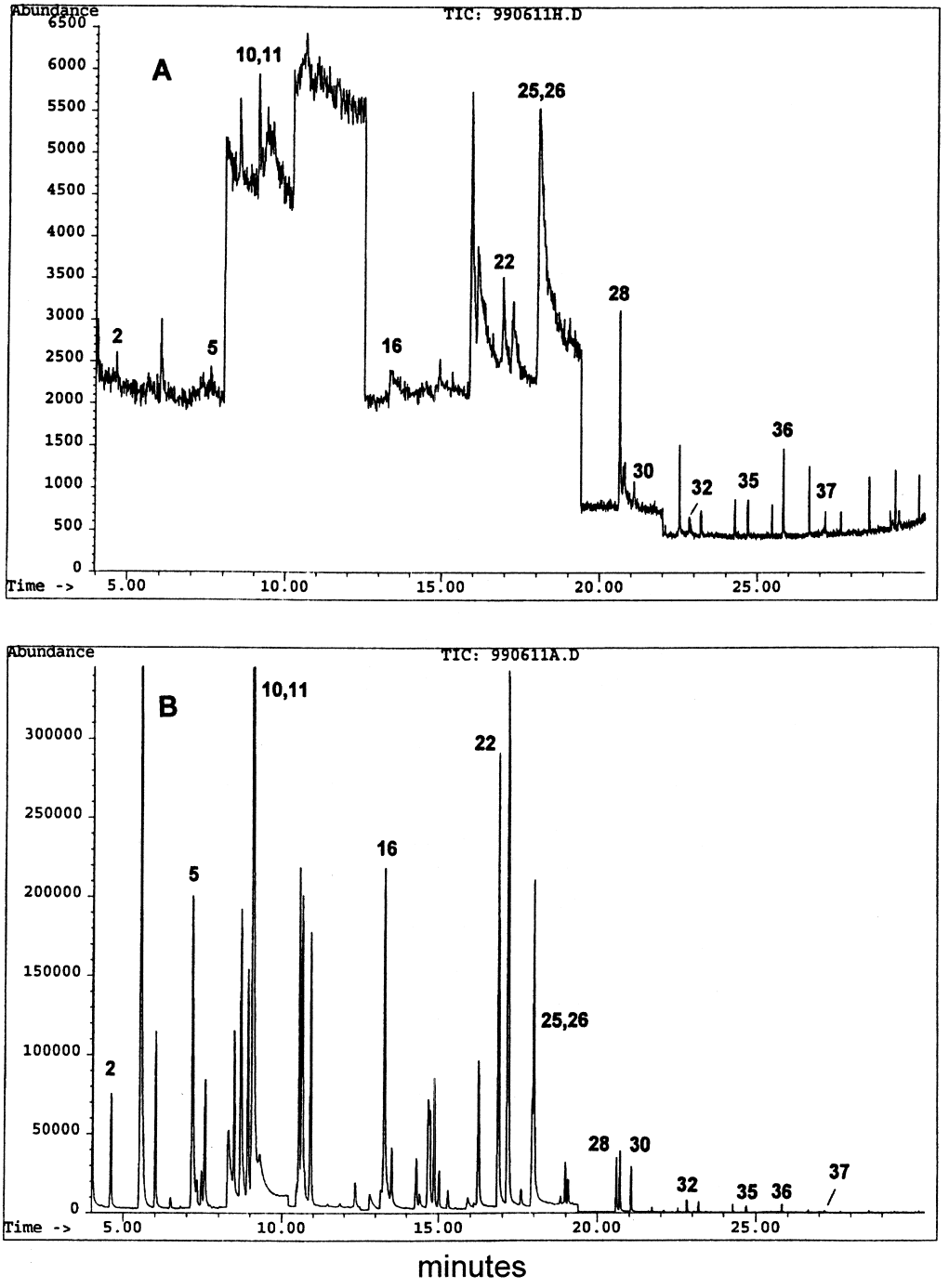

Fig. 3. GC–MS chromatograms of the target analytes at 20 μg kg^{-1} listed and identified in Table 1 extracted by HS-SPME at 23°C for 45 min from 2 g of vegetable oil using the following film coatings: (A) 100 μm PDMS and (B) Car–PDMS. Time scales in min.

HS-SPME of 2 g oil samples spiked with 2 μl of methanol analyte solution using the Car–PDMS fibre showed that, compared to an oil sample with no additional added methanol, the extracted analytes 1,1-dichloroethene to tetrachloroethene were reduced to 38–81% for an added 2 μl of methanol; to 20–67% for 4 μl; to 14–53% for 6 μl; to 10–44% for 8 μl; and to 2–21% for 20 μl, respectively. Bromoform also fell within these ranges. The effects of added methanol on the aromatic hydrocarbons and the monohalogenated benzenes was less pronounced; no effects were observed with additions of 2, 4, 6 or 8 μl methanol but the 20 μl addition reduced the extracted amount to 56–84%. The dichlorobenzenes and later-eluting analytes were found to be poorly extracted under the study conditions and could not be studied. Comparable decreased extractions for the DVB–Car–PDMS fibre for 1,1-dichloroethene to tetrachloroethene were 34–70%, 29–57%, 13–39%, 14–41% and 5–29% for the 2, 4, 6, 8 and 20 μl added methanol, respectively. For the aromatic hydrocarbons and the monohalogenated benzenes the 20 μl addition of methanol decreased the HS-SPME to 64–99%. Thus, the decrease in extraction attributed to methanol was less for the DVB–Car–PDMS fibre coating than that of the Car–PDMS fibre.

A simplified two-phase model was used to study the competitive displacement phenomena by methanol in the absence of vegetable oil. As described in Section 2.6 above, a 2-μl methanol spike at 1/5 the concentration of the previous study and 2, 5, 10, 15, 20 or 25 μl of methanol were added to 125-ml HS vials. The larger vial ensured that the added methanol was all in the gas phase and the absence of oil allowed all the analytes to be readily extracted and observed. Again increasing methanol showed a decrease in the HS-SPME similar to that noted above when oil was present. As with the oil study, the methanol affected the DVB–Car–PDMS fibre coating less than that of the Car–PDMS fibre. The least volatile analytes, as characterised by their GC retention after that of the dichlorobenzenes, were only slightly affected by the added methanol or not at all. These effects were not observed with the PDMS fibre. Other spiking solvents were also studied including acetonitrile, ethanol, 1-propanol and 2-propanol. Effects similar to those noted above for methanol were observed. To overcome the non-linearity attributed to competitive displacement of the analyte by the spiking solvent, all dilutions of the primary standards were into vegetable oil, selected by preanalysis to be low in interfering volatiles. Thus, for a commercially available multiple analyte standard at 200 $\mu g\ ml^{-1}$ in methanol when diluted in oil to give 0.8 $\mu g\ ml^{-1}$ of each analyte, a 50-μl spike would only contribute 0.2 μl of methanol to the 2 g sample. For more concentrated commercial standards, the amount of methanol in the vegetable oil spiking solvent would be proportionately less. As noted below in Section 3.3.3 linear responses were obtained with vegetable oil spiking.

3.3.2. Extraction times and temperatures

The extraction time of 30 min used for the equilibrated HS-SPME of water [4] was extended to 45 min at room temperature for the comparison of the three fibres in the HS-SPME of vegetable oil. The smaller sample size (2 g) and reduced volume of the HS vial suggested that the equilibration should readily occur within the 45 min time frame. As shown in Fig. 3A and B, the HS-SPME of the less volatile analytes from vegetable oil was inefficient for the later-eluting target analytes. However, when the extraction time was increased to 21 h, the HS-SPME response for these later-eluting analytes was observed to increase as much as 20-fold for the Carboxen-based fibres. Increases were not noted for the PDMS coating. This suggested that an equilibrated extraction was not reached with the Carboxen-based fibres and the later-eluting analytes would be extracted more efficiently with a longer extraction period. Rather than using an increased extraction time it was decided to carry out the extraction at an elevated temperature.

We had previously employed an increased temperature for the HS-SPME of semi-volatile organochlorine contaminants from water using a PDMS fibre coating [17]. For convenience, heating with steam was selected for our particular application. Initial HS-SPME studies were conducted with the Carboxen-based fibres at 100°C for periods of 45 min and 2 and 5 h. The results showed that for those analytes eluting after the BTEX aromatics, 45 min gave only about 50–60% of the response of that for the 2 h extraction using the DVB–Car–PDMS fibre and about 30–50% for the Car–PDMS fibre. For the

BTEX aromatics and those eluting before, responses after 45 min were greater by up to 65% than those of the 2 h extraction for both fibres. This indicates a loss with an extended extraction, possibly by leakage from the thermally pressurised HS vial. Extractions at 5 h showed increased efficiency for only the penta- and hexachlorobenzenes for both fibres. The absence of a precipitous loss of any analyte indicated that decomposition at 100°C was not a problem. Although a 2 h extraction may represent an optimum extraction, shorter extraction times were considered to be more time-efficient. Therefore, further HS-SPME were conducted at 100°C for 45 min, recognising that an equilibrated HS-SPME need not be attained for accurate quantitation. With good temperature control and consistent stirring non-equilibrated situations have been shown to be theoretically [18] and practically valid [1] for quantitation.

With the more volatile compounds, the extraction at 100°C significantly increases the headspace capacity while decreasing those of the fibre coating and the sample matrix. For the less volatile compounds, however, such a temperature increase makes their HS-SPME possible within a reasonable timeframe. In order to benefit from the increased HS-SPME rate and maintain the HS-SPME efficiency noted at room temperature it was decided to follow the 100°C extraction with a continuing extraction at 0°C. Both the upper and lower extraction temperatures are convenient to achieve and the heating/cooling is easily managed with the jacket shown in Fig. 1. A thermometer inserted through a hole in the septum and positioned in 15 ml of water indicated that using steam the vial contents reached a temperature of >98°C in less than 3 min. When the steam was disconnected and ice water pumped through the jacket a temperature of <3°C was attained in less than 5 min. The temperature profile of 2 g of oil in the 20-ml HS vial was not monitored but more rapid temperature equilibrations would be expected. The HS-SPME at 100°C for 45 min with continuing extractions at 0°C for 0, 10, 20, 30 or 45 min of 2 g samples of vegetable oil spiked to give 20 μg kg^{-1} of each analyte were carried out using the Car–PDMS and the DVB–Car–PDMS fibres. Compared to the extraction without cooling, increases from about 1.2- to 6-times and from 2- to 10-times, respectively for the two fibres, were observed for the analytes with volatilities up to and including the dichlorobenzenes, for the 10, 20, 30 and 45 min cooling periods. No changes were noted with the later-eluting analytes and little differences were noted in the extraction for the different cooling periods with either fibre coating.

To further characterise the partition phenomena at 100°C for 45 min with continuing extractions at 0°C for 10 min, the actual percentages of each analyte added to a 2 g samples of oil at 20 μg kg^{-1} extracted using each of the two Carboxen-based fibres were determined by comparisons to direct injections. These results are given in Table 2 and show the Car–PDMS fibre to be more efficient than the DVB–Car–PDMS fibre for the HS-SPME of most analytes studied by factors up to about 10. Higher HS-SPME efficiencies of the later-eluting analytes would be expected if the extraction times were extended up to 2 h, thus approaching an equilibrated extraction as described above.

The chromatograms shown in Fig. 4 illustrate the differences between the absorbent PDMS fibre coating (Fig. 4A) and the adsorbent DVB–Car–PDMS and Car–PDMS fibre coatings shown in Fig. 4B and C, respectively. Note the 50-fold difference in their respective ion abundance scales. With the PDMS fibre coating, little differences are noted between the chromatograms of Fig. 3A, a 23°C (45 min) extraction, and that of Fig. 4A, a 100°C (45 min) then 0°C (10 min) extraction. This suggests equilibrium situations with similar capacities for both extraction conditions. On the other hand, with the Car–PDMS fibre coating, chromatograms in Figs. 3B and 4C differ significantly. In particular, the later-eluting peaks in Fig. 4C are much greater than those in Fig. 3B demonstrating that the extraction at 23°C is much farther from equilibration than the extraction demonstrated in 4C at 100°C (45 min) then 0°C (10 min).

3.3.3. Method performance

Using the chosen HS-SPME conditions for 2 g samples of oil spiked at 20 μg kg^{-1}, the repeatability of the method was determined for each analyte using both Carboxen-based fibre coatings. Using a range of spiking concentrations, the linear dynamic range as well as the LODs for each analyte were also determined. The results for repeatability are given in Table 2. The average precision of 7.1% for the

Table 2
SPME of target analytes from 2 g vegetable oil, % analyte extracted at 100°C/0°C[a], repeatability, and method limits of detection (LODs) for the Carboxen–PDMS and the DVB–Carboxen–PDMS fibre coatings

Analyte No.	% in fibre		Repeatability[b] (%)		LOD[c] (μg kg^{-1})	
	Car–PDMS	DVB–Car–PDMS	Car–PDMS	DVB–Car–PDMS	Car–PDMS	DVB–Car–PDMS
1	6.9	1.2	13	5.5	0.35	0.15
2	7.8	1.6	17	6	(0.95)	(1)
3	12	2.6	14	9.6	0.15	0.3
4	–[d]	–	14	9.1	0.2	0.2
5	7.9	1.7	16	4.1	0.15	0.3
6	3.7	0.94	11	6.8	0.4	1.5
7	1.6	0.58	10	7.6	0.3	0.3
8	0.69	0.86	11	7.3	0.25	0.2
9	9.9	1.8	11	7.3	(0.5)	0.1
10	7	1.8	10	7.1	(4)	(4.5)
11	0.9	0.82	9.4	6.6	0.25	0.2
12	1.5	1	9.1	8.8	0.5	0.5
13	6.3	1.3	13	7.9	0.1	0.1
14	4.4	1	12	9.8	0.2	0.4
15	1.7	0.57	13	12	0.5	0.35
16	17	3	24	6.4	(6.5)	(1.5)
17	3.9	1	9.1	12	2	1
18	1	0.44	8.5	10	0.5	0.5
19	8.8	1.3	4.1	6.1	0.1	0.2
20	5.4	1.1	4.4	7.8	0.5	0.4
21	22	2.8	2.4	4.7	0.05	0.1
22	16	1.4	3.1	6	(4)	(7.5)
23	20	2.2	3.5	6.1	(0.6)	(1)
24	14	0.32	4.2	6.2	0.7	2.5
25	11	0.92	7.7	6	(2.5)	(1.5)
26	15	1.3	7	7.8	(2.5)	(10)
27	18	2.7	7.1	3.1	0.04	0.1
28	15	2.9	10	2.4	(0.15)	(0.4)
29	14	3.4	12	2	(0.8)	(1.5)
30	10	1.8	9.9	4.7	0.03	0.06
31	1.9	0.37	13	9.9	0.4	0.8
32	6.7	2.1	19	4.9	0.05	0.2
33	5.6	1.8	6.8	4.3	0.06	0.2
34	3.2	1.5	3.4	8.2	0.1	0.2
35	2.3	1	9.2	10	0.1	0.2
36	0.92	0.52	11	15	0.3	1
37	0.29	0.2	7.8	3.9	2	4

[a] 40 ng each analyte/2 g vegetable oil, 45 min HS-SPME at 100°C, then 10 min continuing extraction at 0°C vs. direct injection.
[b] $n=6$.
[c] Method limit of detection $S/N=5:1$. Bracketed values indicate level of interference in blank oil, LOD not determined.
[d] Not determined, direct injection solvent interference.

DVB–Car–PDMS is slightly better than the 10% average for the Car–PDMS fibre coating. Both average repeatabilities may be considered acceptable for a non-equilibrated extraction involving a thermally pressurised HS vial. Furthermore, these results compare well to the average reproducibilities we determined for water spiked at 0.27 μg kg^{-1} where an equilibrated extraction at room temperature gave values of 8.0% and 9.5% for the respective fibres. The LODs are also presented in Table 2 for both the Carboxen-based fibres and suggest similar detection capabilities for both fibres. In general, most analytes are readily detected at less than 1 μg kg^{-1}. The higher LODs for the penta- and hexachlorobenzenes

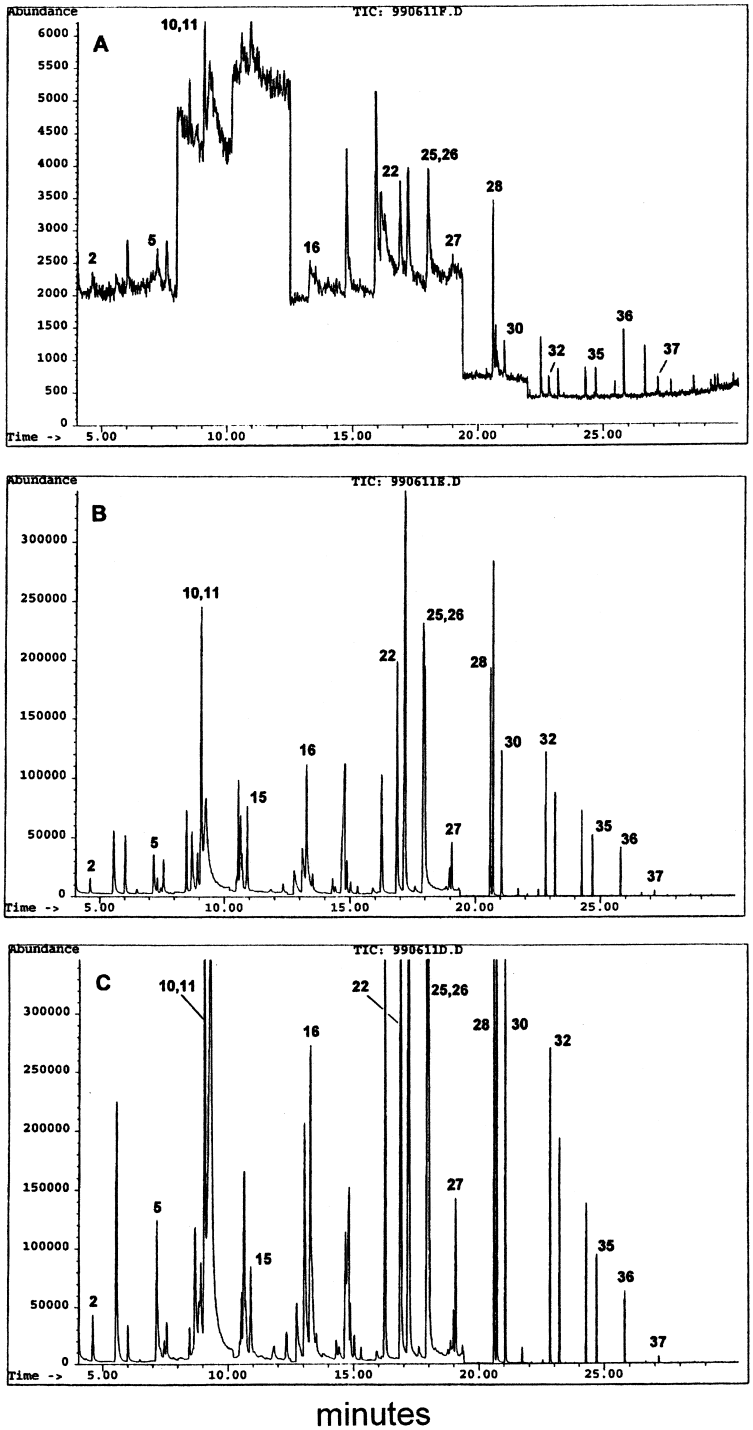

Fig. 4. GC–MS chromatograms of the target analytes at 20 μg kg^{-1} listed and identified in Table 1 extracted by HS-SPME at 100°C for 45 min with continuing extraction at 0°C for 10 min from 2 g of vegetable oil using the following film coatings: (A) 100 μm PDMS; (B) DVB–Car–PDMS; and (C) Car–PDMS. Time scales in min.

reflect the incomplete equilibration. As pointed out above, longer extractions times would improve these values for the least volatile analytes. The oil blank used in the determination of these LODs contained small amounts of contaminants and did not permit the actual measurement of the LOD for these analytes. As with water, the LODs could be estimated from analytes with similar volatility, polarity and relative response.

The linearity of the HS-SPME procedure applied to 2 g vegetable oil samples using the two Carboxen-based fibres was evaluated for the target analytes at concentrations from 0.2 to 12 μg kg^{-1}. Correlation coefficients were >0.99 for all analytes except the penta- and hexachlorobenzene which were ≥ 0.98.

3.3.4. Volatiles in oil products – analytical results

The analytical findings from the analysis of the oils, lard and shortening samples using both Carboxen-based fibre coatings showed the presence of analytes also found in the water samples as noted above in Section 3.2. In most cases, the low levels of these analytes could be attributed to contamination of the sample itself and not contamination arising from the laboratory environment. The main exception is the finding of dichloromethane which is ubiquitous in our laboratory. Low levels of the following common contaminants were found: chloroform was detected in 7 of 10 samples at levels up to 2.1 μg kg^{-1}; 1,1,1-trichloroethane, in 3 of 10 samples at levels up to 1.5 μg kg^{-1}; benzene, in all samples at levels ranging from 1.1 to 122 μg kg^{-1}; carbon tetrachloride, in 3 of 10 at levels up to 0.5 μg kg^{-1}; trichloroethylene, in 2 of 10 samples at levels up to 0.4 μg kg^{-1}; toluene, in all samples at levels from 1.5 to 50 μg kg^{-1}; 1,1,2-trichloroethane, in one sample at 5.3 μg kg^{-1}; tetrachloroethylene, in 6 of 10 samples at levels up to 13.5 μg kg^{-1}; chlorobenzene, in 8 of 10 samples at levels up to 0.24 μg kg^{-1}; ethylbenzene, in all samples at levels from 5.8 to 7.2 μg kg^{-1}; *m*/*p*-xylene, in all samples at levels from 0.9 to 13 μg kg^{-1}; *o*-xylene, in all samples at levels from 1.8 to 7.4 μg kg^{-1}; styrene, in all samples at levels from 1.7 to 17 μg kg^{-1}; 1,3-dichlorobenzene, in all samples at levels from 0.2 to 1.3 μg kg^{-1}; and 1,4-dichlorobenzene in all samples at levels from 0.3 to 18 μg kg^{-1}.

Some differences between the oil samples and the two lard and shortening samples were apparent. The levels of 1,4-dichlorobenzene were considerably higher in the lard and shortening samples, 18 and 11 μg kg^{-1}, respectively, compared to the range of 0.3 to 5.1 μg kg^{-1} for the vegetable oils. The highest level of tetrachloroethylene, 13.5 μg kg^{-1}, was found in the lard sample. The two lowest levels of benzene, 1.1 and 0.94 μg kg^{-1}, were found in the lard and shortening. In general, the results agreed fairly well between the two fibre coatings employed. The results for styrene given above were obtained only using the Car–PDMS fibre. The results using the DVB–Car–PDMS fibre were variable and higher and not included.

4. Conclusions

Studies were conducted to compare the two newer Carboxen-based SPME fibre coatings to the older 100 μm SPME fibre coating for the extraction of a number of halogenated and aromatic analytes with volatilities ranging from 1,1-dichloroethylene to hexachlorobenzene from both aqueous and lipid matrices. The results confirmed the previously reported greater extraction efficiency of the Car–PDMS fibre compared to the PDMS fibre for small polar analytes in aqueous samples. Similar results were obtained with the newer DVB–Car–PDMS fibre. With both fibres and a 15 g water sample, method LODs at <0.01 μg kg^{-1} for all analytes studied were attained. The first reported quantitative application HS-SPME to the determination of the above analytes in lipid samples was made possible by using either of the two Carboxen-based fibres. In this application, the HS-SPME was conducted at 100°C for 45 min followed by a continuing extraction at 0°C for 10 min. A non-linear analyte response was observed for both Carboxen-based fibres when the methanol spiking volume (and amount of analyte) was increased. This effect resulted from the competitive displacement by the analyte solvent methanol of analytes adsorbed to the fibre coating. Linear responses were obtained when all dilutions of primary standards were made in vegetable oil rather than methanol. Quantitation of vegetable oils, lards, and shortening was performed

by comparison to standards prepared in vegetable oil. Other quantitation procedures, such as the use of isotopically labelled internal standards when available, and standard additions, could also be used.

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