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SPME–GC determination of potential volatile organic leachables in aqueous-based pharmaceutical formulations packaged in overwrapped LDPE vials

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Abstract

A direct liquid immersion solid-phase microextraction–gas chromatographic (SPME–GC) method was developed and validated for the determination of 11 potential volatile organic compounds that may leach from preprinted foil laminate overwrap into aqueous pharmaceutical formulations filled in low-density polyethylene (LDPE) vials. The target compounds namely, ethanol, acetone, isopropyl alcohol, ethyl acetate, 2-butanone, *n*-heptane, isopropyl acetate, *n*-propyl acetate, toluene, diacetone alcohol and 1-propoxy-2-propanol, were suitably extracted from aqueous sample solutions by SPME using a 100-μm PDMS fiber, desorbed inside the GC inlet port, and analyzed using a J&W Scientific DB-1701 (86% polydimethylsiloxane/14% cyanopropylphenyl, 30 m × 0.53 mm i.d., 1.5-μm film thickness) capillary column with FID detection. The variables affecting the SPME absorption and desorption conditions were optimized and discussed. The average recoveries for all analytes varied from about 97.9 to 116.7% with the exception of *n*-heptane and toluene where the mean recoveries ranged from about 73.6 to 100.0% presumably due to their poor solubility in the aqueous sample matrix. The standard curves for all compounds were linear over the concentration range investigated with coefficient of correlations, $r^2 \geq 0.98$. The detection and quantitation limits ranged from approximately 0.6 ng/ml to 1.7 μg/ml and 5 ng/ml to 4.2 μg/ml, respectively, and the intra- and inter-day precision was considered adequate (R.S.D. ≤ 16%) for low-level determination of the target analytes in the sample matrix. The method was successfully applied for determination of the target compounds from preprinted foil laminate overwrap in selected aqueous-based pharmaceutical formulations.
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Keywords: SPME–GC; Potential leachables; Container closures; Method validation; Aqueous pharmaceutical formulations

1. Introduction

The container-closure systems of a finished drug formulation usually contain primary packaging components such as low-density polyethylene (LDPE) vials from resins, screw caps, glass bottles and secondary packaging materials including labels, inks, foil laminate overwraps and cardboard boxes. These packaging materials contain a number of chemicals used during their manufacture such as organic solvents, plasticizers, adhesive materials, and photo stabilizers that could be directly or indirectly in contact with the pharmaceutical formulation [1,2]. Regulatory authorities require that extractables or leachables, generally defined as minute contaminants in a pharmaceutical product originating from the product's packaging components or manufacturing equipment, must be controlled to ensure the quality of the drug product throughout the shelf life [3–6]. The identification and/or quantitation of these compounds present perhaps one of the greatest challenges in routine analysis of the pharmaceutical product due to their usual low levels (sub-ppm), when present.

Solid-phase microextraction (SPME), introduced in 1990 by Arthur Pawliszyn for analysis of organic contaminants in water [7], is a simple, sample preparation method that combines sampling, extraction, concentration, and sample introduction into a single solvent-free step. The technique consists of two distinct steps: (i) exposure of a polymer-coated silica fiber to the liquid sample or its headspace to allow for absorption of the analytes according to their affinity toward the fiber coating, and (ii) desorption of the extracted analytes from the coated fiber in an analytical instrument for analysis. For gas chromatography

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(GC), analyte desorption is achieved by inserting the fiber into a hot GC injector port. As a result of the significant reduction in sample preparation times and waste disposal costs in addition to better detection limits over the conventional methods of purge-and-trap, liquid–liquid extraction and other sample preparation techniques, SPME has provoked considerable interests in numerous monitoring applications including environmental, food and beverages, forensic, biological and clinical [6–17].

Although the technique has not been published in the pharmacopoeias, SPME is becoming an attractive analytical tool for control of residual solvents and volatile process impurities in drug substances, excipients or drug products in the pharmaceutical industry [18–21]. Several authors have investigated the use of SPME coupled with gas chromatography-flame ionization detector (FID) or mass spectrometer (MS) for the determination of residual solvents in pharmaceutical products with excellent sensitivity and precision [22–26]. Other reported applications include the analysis of pharmaceutical packaging off-odor [27], determination of methyl and ethyl esters of various sulfonic acids in drug substances [28], and identification of an unknown volatile degradant in the excipient of a capsule formulation [29]. In addition to presenting acceptable validation data, majority of these studies revealed the advantages of SPME over the traditional liquid–liquid and solid-phase extraction methods as well as the direct-injection GC and headspace-GC for the analysis of the target compounds in pharmaceuticals. However, to our knowledge, none of the published articles described a SPME method designed for determination of leachable compounds in the pharmaceutical formulations, which hitherto are often determined by more than one analytical techniques including high-performance liquid chromatography (HPLC) utilized at our facility depending on the results of the initial screening analysis [30].

In this paper, the application of a simple and fast SPME–GC method for determination of 11 potential leachables from a preprinted foil laminate overwrap into aqueous pharmaceutical formulations filled in LDPE vials in a single run was investigated. The solvents of interest include acetone, ethyl acetate, 2-butanone, *n*-propyl acetate and toluene, which are associated with the foil laminate overwrap; and ethanol, isopropyl acetate, ethyl acetate, *n*-propyl acetate, diacetone alcohol and 1-propoxy-2-propanol associated with the color inks used to print the product information on the foil. The proposed method was validated and successfully used for evaluating the presence of these solvents in selected aqueous-based pharmaceutical formulations.

2. Experimental

2.1. Chemicals

Methanol, isopropyl alcohol (IPA), ethyl acetate, acetone, 2-butanone (MEK), isobutyl alcohol and *n*-heptane were obtained from VWR (West Chester, PA, USA). *n*-Propyl acetate, toluene, 1-propoxy-2-propanol, diacetone alcohol and isopropyl acetate were purchased from Sigma–Aldrich (St. Louis, MO, USA). All solvents were of ACS grade or better, and used as received. SPME fiber assembly was obtained from Supelco Inc. (Bellefonte, PA, USA). USP grade sodium chloride was purchased from VWR (West Chester, PA, USA). In-house purified water was used throughout the study.

2.2. Gas chromatograph

Chromatographic separations were performed using on Agilent 6890 series gas chromatograph (Wilmington, DE, USA) equipped with a flame ionization detector, a Gerstel MultiPurposeSampler MPS (Baltimore, MD, USA) and fitted with PerkinElmer Turbochrom Client/Server Data System—Version 6.1.2 (Shelton, CT, USA). Unless stated otherwise, the desorbed analytes were separated on a J&W Scientific DB-1701 (86% polydimethylsiloxane/14% cyanopropylphenyl, 30 m × 0.53 mm i.d., 1.5-μm film thickness) capillary column (Folsom, CA) operated with temperature programming from 35 °C (held for 20 min) to 200 °C at 10 °C/min (held for 3 min), and helium and nitrogen as carrier gas (1.7 ml/min) and make-up gas (30 ml/min), respectively. The GC injector port was set at 250 °C in a splitless mode, and detector temperature was kept at 300 °C.

2.3. Preparation of standard solutions

A 30% sodium chloride solution was prepared by dissolving 300 g of sodium chloride in 900 ml of purified water in a 1 l volumetric flask, and once dissolved, the solution was diluted to volume with purified water. The stock and intermediate standard solutions of the 11 organic solvents and the internal standard (isobutyl alcohol) were prepared in methanol at the concentrations listed in Table 1. The working standards were prepared by adding 0.4 ml of intermediate standard solution followed by 0.4 ml of the internal standard solution into a 100 ml volumetric flask and diluted to volume with 30% sodium chloride solution to obtain the standard concentrations presented in Table 1. To avoid any loss of the highly volatile organic solvents, each solution was mixed gently by inverting the flask for at most three times upon dilution.

2.4. SPME procedure

SPME was performed using a 100-μm PDMS fiber conditioned at 250 °C for 30 min prior to use. The compounds were extracted by immersing the absorption fiber into a 9 ml sample solution placed in a septum-capped glass vial for 30 min at room temperature under constant agitation at 100 rpm. After extraction, the fiber was inserted into the GC injection port kept at 250 °C where the analytes were desorbed for 5 min. Unless stated otherwise, all the extraction solutions contain about 30% sodium chloride.

2.5. Method validation

Throughout the course of this study, the system suitability parameters listed in Table 2 were monitored. Standard calibration curves were prepared from analysis of appropriate dilutions

Table 1
Solvent density and concentrations of the standard solutions

Analyte	Density [31] (g/ml)	Stock standard (mg/ml)	Intermediate standard (mg/ml)	Working standard (μg/ml)
Ethanol	0.789	18.9	1.89	7.6
Acetone	0.788	10.1	1.01	4.0
Isopropyl alcohol	0.785	18.8	1.88	7.5
Ethyl acetate	0.898	3.6	0.36	1.4
2-Butanone	0.805	3.2	0.32	1.3
n-Heptane	0.684	2.7	0.27	1.1
Isopropyl acetate	0.870	0.9	0.09	0.3
n-Propyl acetate	0.887	0.5	0.05	0.2
Toluene	0.866	0.07	0.007	0.03
Diacetone alcohol	0.931	12.1	1.21	4.8
1-Propoxy-2-propanol	0.885	3.5	0.35	1.4
Isobutyl alcohol	0.806	8.1	0.81	3.2

of the intermediate stock standard in 30% sodium chloride solution from limit of quantitation (LOQ) to 150% of the working standard (nominal) concentration as described in Section 2.3. The plots of peak area responses against solvent concentrations were evaluated by linear least square regression analysis. The accuracy of the method in terms of percent recovery was determined at the lower, middle and higher concentrations corresponding to 50, 100 and 150% of the nominal analytical concentration for each solvent. The method precision, expressed as %R.S.D. (relative standard deviation) of the determined concentration for each analyte, was evaluated from five replicate preparations of a standard solution at the target concentration for the intra-day precision. A second analyst performed a similar determination on a different day for inter-day precision. The limits of quantitation and detection were estimated from the measured signal-to-noise ratios of the analytes' responses in the standard solution. The robustness of the method was examined by investigating the effect of small, but deliberate changes in the SPME extraction and desorption times as well as the GC carrier gas flow rate, inlet port temperatures, and capillary column purchased from different manufacturers on the system suitability parameters. Finally, the stock standard and standard solutions stability were evaluated from the recovery data obtained for separate aliquots of each solution stored at refrigerated and room temperature conditions against a freshly prepared standard. All analytes' concentrations were calculated from their peak area ratios relative to the internal standard at the optimum SPME–GC conditions.

Table 2
System suitability report

Parameter	Criteria	Result
Retention time (t_R) for 1-propoxyl-2-propanol peak from a standard injection	NMT 32 min	30.3 min
Precision (%R.S.D.) for 1-propoxyl-2-propanol/internal standard response ratio from three standard injections	NMT 15%	0.9%
Resolution (R_s) between acetone and IPA peak from a standard injection	NLT 1.5	2.9
Tailing factor (T) for ethanol from a standard injection	NMT 2.5	1.3

3. Results and discussion

3.1. Method development

3.1.1. Optimization of SPME procedure

3.1.1.1. SPME fiber. The selection of the fiber was based on its ability to render maximum sensitivity, least amount of interfering peaks, and good precision of the extraction process. Four fibers ranging from non-polar to polar, 100-μm PDMS, 65-μm PDMS/DVB, 75-μm PDMS/carboxen and 85-μm polyacrylate were exposed to the standard solution by direct immersion and desorbed in the GC inlet port. The selectivity, as illustrated in Fig. 1, and the precision (%R.S.D. of peak area ratio of analyte to internal standard) from triplicate extractions (see Fig. 2) for the fibers varied with the polarity of the solvents. Good sensitivity was achieved with both the PDMS and PDMS/DVB fibers while the solvents were poorly resolved using the PDMS/carboxen fiber for extraction. The polyacrylate fiber poorly extracts majority of the solvents from the samples matrix as demonstrated by the low peak intensities. As presented in Fig. 2, better precision was obtained for all analytes on the selected fibers with the exception of *n*-heptane and toluene where precision as high as 37.5 and 75.5%, respectively, were obtained with carboxen fiber. The extremely poor precision for *n*-heptane and toluene with this fiber may be attributed to low desorption presumably caused by condensation of the analyte molecules trapped in the carboxen pores [32]. However, the 100-μm PDMS fiber was chosen since it exhibited good precision (R.S.D. $\leq$ 13.5%) for all the solvents with little or no interferences from the sample matrix when compared with the 65-μm PDMS/DVB fiber. Also, since most of the target analytes are volatile with relatively low molecular weights, a thicker fiber coating (100 μm) was selected to increase the retention of the compounds with acceptable precision. Subsequently, other SPME parameters including extraction time, desorption temperature and time, and matrix effect were optimized with the PDMS fiber.

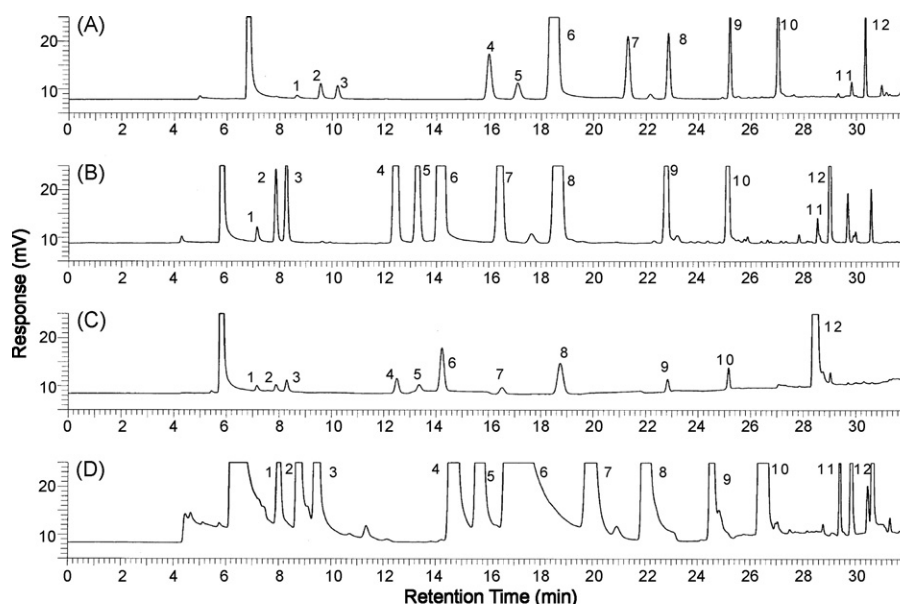

Fig. 1. Chromatogram of a standard solution of the leachable compounds extracted with (A) PDMS, (B) PDMS/DVB, (C) polyacrylate, and (D) PDMS/carboxen fibers. Peaks: 1 = ethanol, 2 = acetone, 3 = isopropyl alcohol, 4 = ethyl acetate, 5 = 2-butanone, 6 = *n*-heptane, 7 = isopropyl acetate, 8 = isobutyl alcohol (internal standard), 9 = *n*-propyl acetate, 10 = toluene, 11 = diacetone alcohol, and 12 = 1-propoxy-2-propanol.

3.1.1.2. PDMS fiber extraction time. The extraction time was determined by exposing the 100-μm PDMS fiber to equal portions of the standard solution for 5, 10, 15, 20, 25, 30, 35 and 40 min with constant agitation at room temperature, and the analytes were desorbed at 250 °C for 5 min. As shown in Fig. 3, the optimum extraction time for most analytes is between 20 and 30 min with the exception of toluene, which tends to increase further with time. Since the goal of the study is to analyze all the solvents in a single run with acceptable sensitivity and precision, and the fact that full equilibration is not necessary as long as the sampling time and other SPME parameters are monitored and kept constant [32–34], an extraction time of 30 min was considered adequate for the analysis. A similar extraction time was established in other SPME studies for determination of a wide variety of volatile organic solvents in pharmaceutical products [22,23,25,28]. Moreover, this extraction time almost equaled the GC run time, thus allowing for maximum sample throughput.

3.1.1.3. Desorption temperature and time. Upon completion of the extraction of analytes, the fiber is desorbed in a hot GC inlet port. Although the extraction step controls several experimental parameters such as accuracy, speed, precision and sensitivity, the quality of the data obtained (i.e. peak shapes, interference) is also affected by the desorption step due to carryover. Desorption temperatures ranging from 200 to 270 °C were investigated, and as illustrated in Fig. 4, the plot of desorption temperature against peak response flattens at about 250 °C for all analytes. Therefore, desorption temperature of 250 °C was chosen for the selected PDMS fiber. The desorption time for the analytes was examined by exposing the analyte-coated fiber in the GC inlet

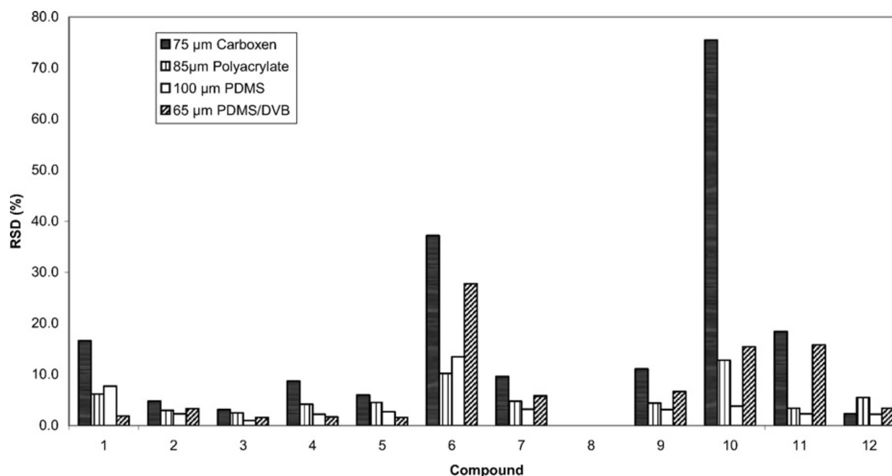

Fig. 2. Comparison of precision for extracted solvents with four different SPME fibers. Compound identifications are as described for chromatographic peaks in Fig. 1.

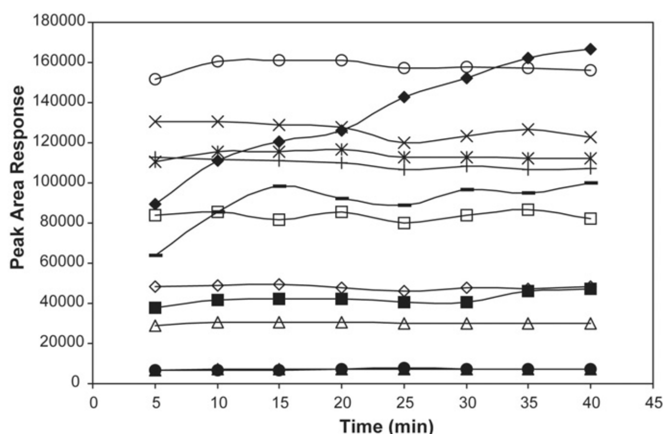

Fig. 3. Extraction time profiles for leachable compounds using a 100-μm PDMS fiber. Symbols: (●) ethanol, (■) acetone, (△) isopropyl alcohol, (×) ethyl acetate, (◊) 2-butanone, (▬) *n*-heptane, (◯) isopropyl acetate, (+) isobutyl alcohol (internal standard), (✳) *n*-propyl acetate, (♦) toluene, (▲) diacetone alcohol, and (□) 1-propoxy-2-propanol.

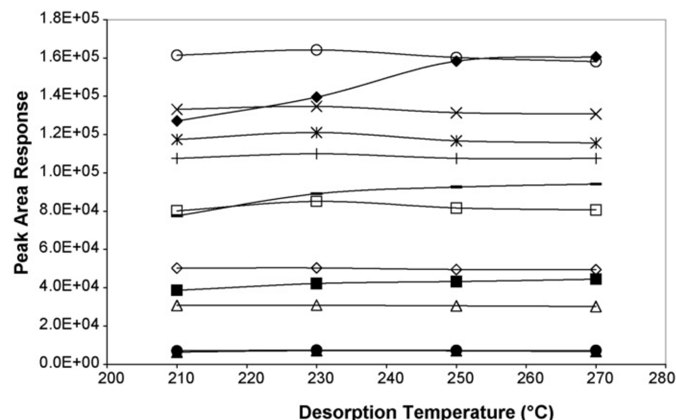

Fig. 4. Desorption temperature profiles for leachable compounds using a 100-μm PDMS fiber. See Fig. 3 for analyte identification.

port at 250 °C for 0.5, 1, 3, 5, 7 and 10 min. Fig. 5 shows that the optimum desorption time for most analytes is about 5 min, and no carryover or memory effect was observed when the fiber was reinserted after the desorption, demonstrating the adequacy of

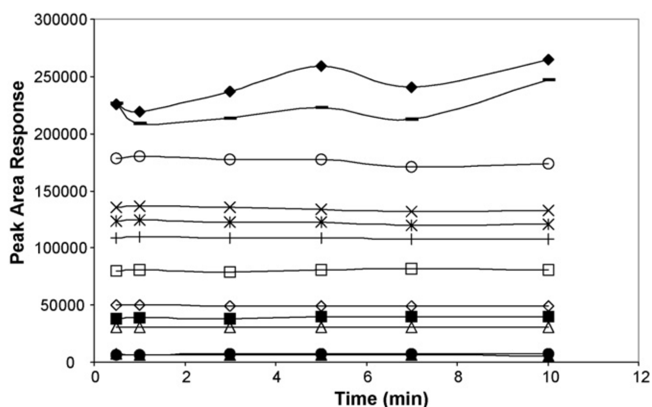

Fig. 5. Desorption time profiles for leachable compounds using a 100-μm PDMS fiber. See Fig. 3 for analyte identification.

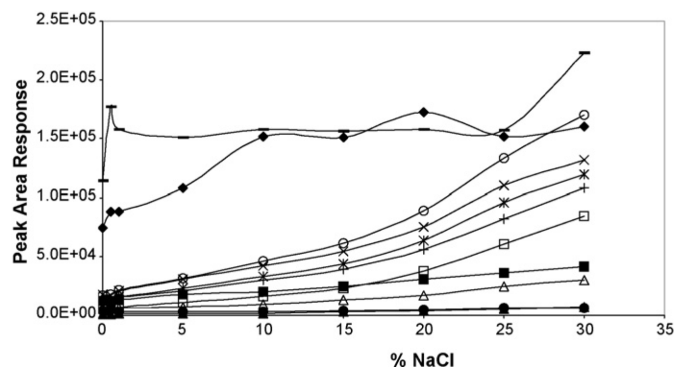

Fig. 6. Effect of ionic strength on the extraction of leachable compounds in aqueous solutions using a 100-μm PDMS fiber. See Fig. 3 for analyte identification.

the 5 min desorption time. Also, blank runs were periodically performed throughout the analyses to eliminate or minimize cross-contamination.

3.1.1.4. Matrix effects. The addition of sodium chloride is expected to enhance analytes extraction since their solubilities in aqueous matrix generally decrease with increasing ionic strength [35]. The effect of adding sodium chloride to the solutions was examined by preparing samples in 0–30% (w/v) sodium chloride solutions. The solutions were extracted using the PDMS fiber and analyzed by GC. The peak area response for each analyte was plotted against percent sodium chloride as shown in Fig. 6. As expected, the peak area responses for all analytes increased with increasing sodium chloride concentration with the exception of *n*-heptane and toluene. The lack of trend exhibited by *n*-heptane and toluene with increasing salt concentration may be due to low water solubilities, and consequently, the extraction efficiencies for these compounds were not significantly affected at high ionic strength [32,36,37]. Based on the data obtained, a sodium chloride concentration of 30% (w/v) was chosen for the preparation of the standard and sample solutions to enhance the method sensitivity.

3.2. Gas chromatographic analysis

In addition to the SPME parameters, the GC operating conditions such as the column dimensions, injection port and column oven temperature, injection mode, and the carrier gas flow rate were optimized, but not discussed in detail in the present paper for the sake of brevity, to achieve maximum resolving power with minimal run time. Fig. 7 compares the chromatograms of liquid immersion SPME against the direct liquid injection, headspace and headspace-SPME sampling techniques for a standard solution of the volatile compounds analyzed under the optimized GC conditions provided in Section 2.2. As shown in Fig. 7A, the direct liquid injection gave poor peak shapes for the early eluting compounds even when several split ratios and sample volumes were used. All attempts with this technique resulted in peak distortion and poor precision for the early eluting peaks presumably due to flashback arising from large volume of water introduced into the injection port. Analysis of these

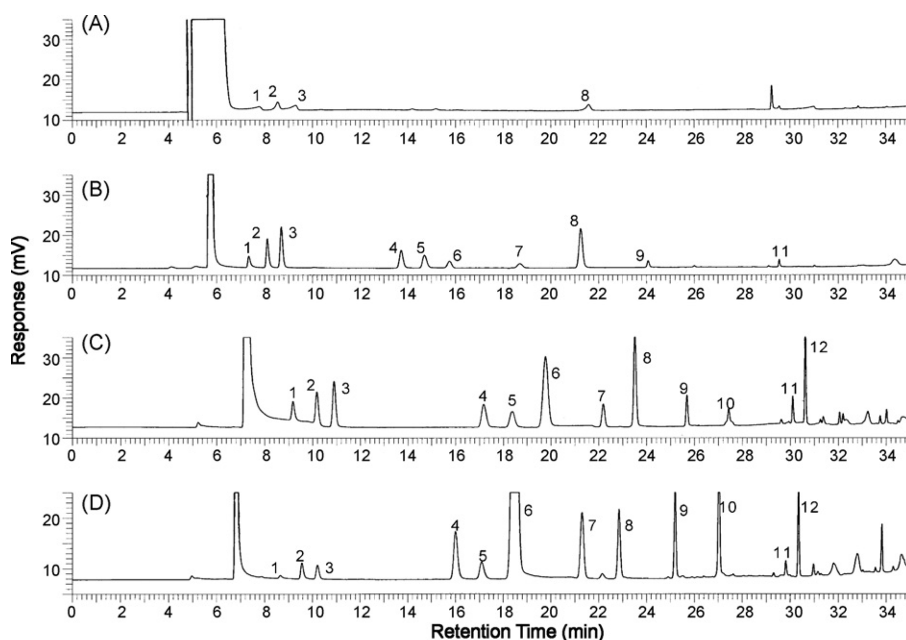

Fig. 7. Comparison of chromatograms for leachable compounds obtained from four different sampling techniques: (A) direct liquid injection, (B) headspace, (C) headspace-SPME, and (D) liquid immersion SPME. Peak identifications are as described in Fig. 1.

compounds using headspace injection after incubating a known volume of the analysis solution at 80 °C for 35 min showed lack of sensitivity for the late eluting peaks but greater responses for the early eluting peaks (see Fig. 7B). Several attempts at optimizing the incubation temperatures and times did not produce any improvement in separation of the late peaks. Even though Fig. 7C showed the headspace-SPME sampling technique to be equally good for the separation of the analytes, the immersion SPME generally gave the best repeatability with acceptable precision, which as illustrated in Fig. 8, varied from about 1 to 13% compared to about 3 to 30% using the headspace-SPME. It can be seen in Fig. 8 that the repeatability was worse for the late eluting peaks using the headspace-SPME presumably due to their low volatility. Therefore, the liquid immersion SPME was chosen for the analysis of the target compounds consisting of volatile and semivolatile organic solvents in a single run and with high sample throughput being more reliable and precise than headspace-SPME in the present study.

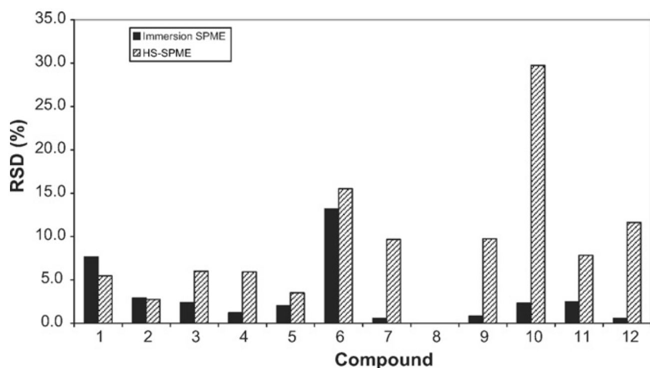

Fig. 8. Repeatability for extracted solvents using the immersion and headspace-SPME sample preparation techniques with 100-μm PDMS fiber. Compound identifications are as described for chromatographic peaks in Fig. 1.

3.3. Method validation

3.3.1. Quantitation and detection limits

The limit of detection (LOD) and limit of quantitation for the method were determined from the analysis of standard solutions containing known concentrations of the analytes. The LOD and LOQ for each analytes were estimated from the concentration that produces a signal-to-noise of about 3 and 10 for detection and quantitation limits, respectively. As shown in Table 3, the detection and quantitation limits varied widely amongst the analytes ranging from 0.6 ng/ml to 1.7 μg/ml for detection limits, and 5 ng/ml to 4.2 μg/ml for quantitation limits. These values, which compared favorably well with those reported in the literature for similar solvents [23,38], are low enough to permit the determination of these compounds in real samples.

3.3.2. Linearity

A plot of detector response expressed as peak area was found to be linear from about the limit of quantitation to 150% of the nominal concentration for each solvent. As presented in Table 3, the coefficient of correlation for the analytes varied from 0.9755 for *n*-heptane to 1.000 for isopropyl alcohol. In addition to achieving acceptable linearity ($r^2 \geq 0.98$) for low-level determination of these compounds in the aqueous matrix, the data demonstrates the suitability of the PDMS fiber for extraction of the target analytes over the concentration range examined.

3.3.3. Accuracy

The accuracy of the method was evaluated from solutions containing about 50, 100 and 150% of the nominal concentration for each solvent. The average recoveries for polar analytes listed in Table 4 ranged from 97.9 to 116.7% with R.S.D. of

Table 3
Linearity and limits of detection and quantitation of the SPME–GC method

Analyte	Linearity range (μg/ml)	Regression equation ($y = mx + c$)	Coefficient of correlation, r^2	LOD (μg/ml)	LOQ ± R.S.D.[a] (μg/ml)
Ethanol	2.0–11.8	$y = 445x + 382$	0.9911	1.7	4.2 ± 5.0
Acetone	0.4–6.1	$y = 7179x + 2444$	0.9898	0.1	0.4 ± 3.7
Isopropyl alcohol	1.5–11.3	$y = 2323x + 798$	1.0000	0.4	1.5 ± 0.4
Ethyl acetate	0.05–2.2	$y = 71{,}800x + 3199$	0.9981	0.01	0.05 ± 2.0
2-Butanone	0.2–1.9	$y = 26{,}920x + 6235$	0.9925	0.06	0.2 ± 1.0
n-Heptane	0.01–1.6	$y = 1{,}337{,}548x - 9489$	0.9755	0.002	0.01 ± 10.9
Isopropyl acetate	0.02–0.5	$y = 350{,}244x + 1560$	0.9829	0.004	0.02 ± 4.7
n-Propyl acetate	0.01–0.3	$y = 436{,}602x + 8601$	0.9916	0.003	0.01 ± 2.8
Toluene	0.005–0.039	$y = 3{,}246{,}637x + 19{,}228$	0.9897	0.0006	0.005 ± 5.9
Diacetone alcohol	1.2–7.4	$y = 1485x + 866$	0.9989	0.3	1.2 ± 9.9
1-Propoxy-2-propanol	0.05–2.1	$y = 37{,}275x + 2086$	0.9975	0.03	0.05 ± 3.3

[a] Mean ± R.S.D. from triplicate determinations.

0.0–5.9%, while those for the non-polar solvents, that is, *n*-heptane and toluene ranged from 73.6% for *n*-heptane to 100.0% for toluene with R.S.D. of 0.0–24.1%. The recovered amounts are well within those reported in the literature for volatile organic compounds in water as well as for residual solvents and organic volatile impurities (OVIs) in drug substances with SPME [32,38,39]. The relatively low recovery for *n*-heptane is in line with the observation that the solvent prefers to remain in the vapor phase (high headspace-liquid partition coefficient), and only a small quantity of the solvent was probably extracted from solution [32]. For such compound, the preferred method of analysis is headspace-sampling technique. Nevertheless, the recovery level is considered acceptable for low-level determination of the target analytes in the sample matrix.

3.3.4. Precision

The data for intra- and inter-day precision is summarized in Table 4. The method showed good intra-day precision with R.S.D. varying from 0.0% for the acetates and 2-butanone to 16.0% for toluene, and the mean values agreed reasonably well with the nominal concentrations. The inter-day precision, evaluated by a second analyst on a different day, ranged from 0.0% for *n*-propyl acetate to 11.6% for *n*-heptane with mean values that agreed between 92.9 and 100.0% with the those obtained by the first analyst. The results of both analysts indicate that the method is suitable precise and reproducible for its intended purpose.

3.3.5. Robustness

The method was found to be robust when small deliberate changes made in the GC and SPME parameters did not significantly affect the separation profile of the compounds (see Table 5). As expected, the retention time of the analytes decreased with increasing column temperature and carrier gas flow rate, and vice versa. With the exception of alternate column, none of the altered GC parameters produced a significant change in resolution and tailing factor, and the system precision (%R.S.D.) from all alterations was far below the acceptance criterion of not more than (NMT) 15%. Furthermore, the separation profile of the analytes remained unaffected with slight but deliberate changes in the SPME extraction and desorption times.

3.3.6. Standard solution stability

The stability of the stock and standard solutions was determined by analyzing separate aliquots of each solution stored in

Table 4
The recovery and reproducibility data for leachable compounds

Analyte[a]	Recovery (%)[a]			Reproducibility (μg/ml)[b]		
	Low level	Middle level	High level	Analyst 1, mean ± R.S.D.	Analyst 2, mean ± R.S.D.	Agreement (%)[c]
Ethanol	105.6 ± 2.3	102.4 ± 2.9	105.1 ± 2.7	7.4 ± 2.0	6.9 ± 8.2	93.2
Acetone	107.7 ± 3.6	105.2 ± 2.8	99.6 ± 0.8	3.8 ± 3.5	3.7 ± 6.6	97.4
Isopropyl alcohol	97.9 ± 3.7	103.5 ± 2.1	106.3 ± 1.1	7.2 ± 3.5	6.9 ± 5.6	95.8
Ethyl acetate	100.0 ± 0.0	104.2 ± 3.5	111.1 ± 2.2	1.4 ± 0.0	1.4 ± 6.0	100.0
2-Butanone	100.0 ± 0.0	106.3 ± 5.9	106.9 ± 2.2	0.8 ± 0.0	0.8 ± 5.7	100.0
n-Heptane	79.2 ± 24.1	87.5 ± 7.7	73.6 ± 11.8	1.1 ± 13.7	1.1 ± 11.6	100.0
Isopropyl acetate	100.0 ± 0.0	100.0 ± 0.0	116.7 ± 0.0	0.4 ± 0.0	0.4 ± 6.0	100.0
n-Propyl acetate	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	100.0
Toluene	91.1 ± 4.2	100.0 ± 0.0	88.9 ± 8.7	0.03 ± 16.0	0.03 ± 9.1	100.0
Diacetone alcohol	103.8 ± 3.7	103.2 ± 2.2	102.1 ± 3.6	4.7 ± 3.2	4.4 ± 8.7	93.6
1-Propoxy-2-propanol	100.0 ± 0.0	102.1 ± 3.5	104.2 ± 0.0	1.4 ± 0.0	1.3 ± 9.9	92.9

[a] Mean ± R.S.D. of triplicate preparations at 50, 100 and 150% of the nominal concentrations.
[b] Mean ± R.S.D. of five independent preparations at the nominal concentrations, $n = 5$.
[c] Agreement of the mean values between analysts 1 and 2.

Table 5
Robustness

Condition	Variation	Result[a]			
		t_R (min)	%R.S.D.	R_s	T
Column temperature (°C)	33/198[b]	30.6	0.6	3.2	1.4
	35/200	30.3	0.9	2.9	1.3
	37/202	30.1	1.0	2.6	1.3
Flow rate (ml/min)	1.5	31.1	0.6	2.8	1.1
	1.7	30.3	3.2	2.9	1.3
	1.9	29.7	0.6	3.0	1.3
GC inlet temperature (°C)	248	30.3	0.8	2.9	1.3
	250	30.3	0.8	2.9	1.4
	252	30.3	1.6	2.9	1.3
Extraction time (min)	28	30.3	1.3	2.9	1.3
	30	30.3	0.4	2.9	1.3
	32	30.3	1.5	2.9	1.3
Desorption time (min)	3	29.7	1.0	3.0	1.3
	5	29.7	1.0	2.9	1.3
	7	29.7	0.6	2.9	1.3
Column type	DB-1701	30.3	0.9	2.9	1.3
	Rtx-1701	31.0	0.2	2.5	1.1

[a] System suitability parameters as described in Table 2.
[b] Initial/final GC oven temperature.

a refrigerator (5 ± 3 °C) and at room temperature over a period of 7 days. The concentrations of the solvents in the aged solutions were evaluated against a freshly prepared standard. As presented in Table 6, analyte recoveries after 4 days of storage of the working standard solution in a refrigerator and at room temperature were significantly lower than those obtained after 24 h, demonstrating that the solution was only stable for a day at both storage conditions. While the stock standard solution was only stable at room temperature for 24 h with 96.2–113.0% recovery, the recovered values of 96.2–119.3% under the refrigerated storage condition indicate that the solution was stable for up to 7 days. Therefore, a working standard solution of the analytes is considered stable for 24 h if refrigerated or stored at room temperature, and the stock standard solution is stable only for 24 h at room temperature, and up to 7 days if refrigerated.

Table 6
Standard solution stability

Analyte	Recovery (%)				
	Working standard		Stock standard		
	Day 1	Day 4	Day 1	Day 4	Day 7
Refrigerated					
Ethanol	105.3	109.6	102.1	97.9	111.7
Acetone	100.0	96.2	100.0	98.1	96.2
Isopropyl alcohol	103.2	101.1	101.1	101.1	105.3
Ethyl acetate	106.7	100.0	106.7	100.0	106.7
2-Butanone	100.0	93.8	100.0	100.0	100.0
n-Heptane	100.0	64.3	100.0	100.0	100.0
Isopropyl acetate	100.0	100.0	100.0	100.0	100.0
n-Propyl acetate	100.0	100.0	100.0	100.0	100.0
Toluene	103.3	72.5	102.9	103.3	119.3
Diacetone alcohol	110.6	106.4	108.5	117.0	112.8
1-Propoxy-2-propanol	106.7	106.7	100.0	106.7	106.7
Room temperature					
Ethanol	109.6	101.1	102.1	108.5	105.3
Acetone	100.0	100.0	96.2	103.8	100.0
Isopropyl alcohol	105.3	101.1	100.0	105.3	102.1
Ethyl acetate	106.7	100.0	106.7	106.7	106.7
2-Butanone	100.0	93.8	100.0	106.3	100.0
n-Heptane	85.7	42.9	108.6	78.6	57.1
Isopropyl acetate	100.0	100.0	100.0	100.0	100.0
n-Propyl acetate	100.0	100.0	100.0	100.0	100.0
Toluene	89.3	82.3	113.0	111.5	79.4
Diacetone alcohol	106.4	110.6	106.4	115.5	119.1
1-Propoxy-2-propanol	106.7	106.7	106.7	113.3	106.7

3.4. Application to aqueous formulations

The validated method was used to determine the amount of organic leachable compounds in selected drug formulations filled into LDPE vial with and without preprinted foil laminate overwraps. Depending on the fill volume, appropriate number of drug-vials was composited, and 9 ml of the solution was transferred into the SPME glass vial. Next, about 2.7 g of sodium chloride was added to the vial and closed with a Teflon-lined crimp cap ready for extraction. In addition, aqueous extract of preprinted foil pouches with different color inks was ana-

Table 7
Leachable compound contents range in aqueous pharmaceutical formulations and foil pouch extracts

Compound	Content (μg/ml)			
	Unpouched LDPE vial	Pouched LDPE vial (n = 12)[a]	Plain foil pouch extract	Preprinted foil pouch extract (n = 5)[a]
Ethanol	ND	ND	ND	ND
Acetone	0.1	0.04–0.15	ND	ND
Isopropyl alcohol	2.3	ND–3.2	ND	ND–0.26
Ethyl acetate	ND	ND–0.29	ND	ND
2-Butanone	ND	ND–0.26	ND	ND
n-Heptane	1.4×10^{-3}	1.0×10^{-3} to 2.0×10^{-3}	2.1×10^{-3}	1.0×10^{-3} to 1.6×10^{-3}
Isopropyl acetate	ND	ND	ND	ND
n-Propyl acetate	2.2×10^{-3}	1.1×10^{-3} to 3.8×10^{-3}	1.7×10^{-3}	ND–1.1×10^{-3}
Toluene	0.1×10^{-3}	0.1×10^{-3} to 0.8×10^{-3}	0.6×10^{-3}	2.1×10^{-3} to 3.2×10^{-3}
Diacetone alcohol	4.6	1.1–3.8	1.2	0.8–1.1
1-Propoxy-2-propanol	0.04	ND–0.06	0.01	8.5×10^{-3} to 0.018

ND = not detected.
[a] n = number of samples tested.

lyzed and compared with a plain foil pouch. In this case, the foil pouches (2 in. × 4 in.) containing about 10 ml of purified water was sealed and incubated at 70 °C for 24 h after which 9 ml of the extract was transferred into a SPME glass vial. Next, about 2.7 g of sodium chloride was added and the vial was closed with a Teflon-lined crimp cap ready for SPME–GC analysis. As summarized in Table 7, the levels of leachable compounds are either absent or present below the target limits (analytes' concentrations in the standard solution), which are by far lower than the control limits for residual solvents in pharmaceuticals [40]. With the exception of isopropyl alcohol, ethyl acetate and 2-butanone in pouched LDPE vials and diacetone and isopropyl alcohols in unpouched LDPE vial, all other solvents were present below or at near the quantitation limits, where detected. It can be seen that both the pouched and unpouched drug-vials did not show any significant difference in the amount of leachable compounds detected in the formulations. Also, there were no significant differences in the levels of leachable compounds found in the print versus non-print foil pouch extracts. The results obtained demonstrate that immersion SPME is a valuable sample introduction technique for quality control of the target compounds in aqueous formulations with maximum sample throughput.

4. Conclusion

An immersion SPME–GC method has been developed and validated for determination of potential organic compounds that may leach from preprinted foil laminate into aqueous drug formulations. The PDMS fiber was found to be more suitable than PDMS/DVB, PDMS/carboxen and polyacrylate fibers for the extraction of the analytes in the presence of a matrix modifier (30% sodium chloride solution). The method was found to be accurate and linear from the limit of quantitation to about 150% of the nominal concentration for the target analytes with $r^2 \geq 0.98$. The relative standard deviations obtained from replicate preparations by two different analysts were less than 16%, demonstrating acceptable intra- and inter-day precision. In addition to exhibiting good specificity and sensitivity, analyte extraction with PDMS fiber proved efficient for simultaneous determination of semivolatile and volatile leachable compounds in aqueous-based pharmaceutical formulations.

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