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Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 507–518

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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# High-speed gas chromatographic analysis of solvents in pharmaceuticals using solid phase microextraction

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Received 4 February 2002; received in revised form 7 February 2002; accepted 1 March 2002

**Abstract**

A simple, inexpensive and rapid analytical approach for the determination of organic volatile impurities in pharmaceutical drug substances is developed, where sample preparation step was conducted using solid phase microextraction (SPME), followed by a fast GC separation. With an extraction time between 3 and 5 min and separation of 13 solvents in less than 3 min employing fast temperature programming using resistively heated column, organic volatile impurities can be analyzed within a total analysis time of 6–9 min. Various SPME phases were evaluated towards sensitivity and selectivity for the extraction of 13 commonly found solvents in drug substances dissolved in dimethyl sulfoxide and water. A2-cm Carboxen/polydimethyl siloxane/divinylbenzene (Carboxen/PDMS/DVB) phase and a 65-μm DVB/PDMS phase showed better sensitivity towards these solvents when extracted from organic and aqueous matrix in comparison with the sensitivity obtained with direct injection approach. Extraction parameters such as extraction time, extraction stir rate, etc. are discussed. %RSD of peak area of replicate extraction was between 2 and 10% when 100 μm PDMS was used for extracting solvents from aqueous matrix. When DVB/PDMS fiber was evaluated for precision, %RSD of peak area from replicate extractions of solvents from organic matrix was between 2 and 8%. One-hundred micrometer PDMS showed excellent linearity from 10 to 500 μg/ml for analytes extracted from water solutions. On the other hand, DVB/PDMS phase showed better linearity than Carboxen/PDMS/DVB fiber when it was used to extract analytes in the concentration range of 10–5000 μg/ml from organic matrix. 

*Keywords:* High-speed gas chromatography; Solid phase microextraction; Residual solvents; Pharmaceutical analysis

## 1. Introduction

Analysis of residual solvents in samples is one of the most demanding tasks for analytical chemists in pharmaceutical industry. The analytical procedure used for the quantitation of residual solvents in these samples must be sensitive, accurate and precise. Gas chromatography (GC) with flame ionization detection is a commonly employed method for quantitation of organic volatile impurities in pharmaceutical products. Requests for analysis of solvents in pharmaceutical prod-

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0731-7085/02/$ - see front matter 
PII: S0731-7085(02)00085-7

ucts, intermediates and process samples are extremely high. At the same time, a quick sample turn around time is expected for samples submitted for such analyses. One approach to satisfy quick sample turn around is application of a rapid analysis cycle time where both the sample preparation time as well as chromatographic run time must be curtailed. Various approaches are employed to reduce chromatographic run time [1]. With the development of narrow-bore (0.1 mm i.d.) capillary GC columns, it is now possible to achieve a much shorter (in a few minutes) GC run time. However, limited sample loading capacity is an issue when such capillary GC columns are used, consequently resulting in rather poor sensitivity for the quantitation of residual solvents [2]. Alternatively, with the advent of high-speed GC achieved by rapid temperature programming using resistively heated capillary column, it is possible to achieve faster run times. High-speed GC based on fast temperature programming using resistively heating of the column has been successfully employed for the analysis of environmental [3], petroleum, [4] food and flavor [5] analysis. However, one of the limitations of high-speed chromatography using fast temperature programming is the unavailability of commercial guard column to protect analytical column from contamination when samples with difficult matrixes are directly injected.

Various sample preparation and injection approaches are used for the analysis of solvents in pharmaceuticals. In direct injection technique using conventional GC, sample is dissolved in an organic solvent at a high concentration (10–l00 mg/ml) to achieve desired sensitivity. A guard column is connected to analytical column to minimize contamination of analytical column from non-volatile matrix. Other approaches for the quantitation of solvents in pharmaceutical matrixes include static headspace, purge and trap, etc. However, these methods are rather time consuming and are not within the time frame of fast separation. If the sample preparation time significantly exceeds chromatographic run time, the sample turn around time is not substantially reduced and therefore, it negates the benefit of high-speed GC.

Most recently, solid phase microextraction (SPME) has gained popularity for determination of organic volatile impurities in pharmaceutical compounds [6,7]. SPME is a solventless technique for the extraction of analytes from difficult matrix. Analytes are adsorbed or absorbed by a fiber coated on a fused silica capillary. This fiber is exposed to the sample by its immersion or to its headspace. Once equilibrium is established between the sample and the fiber, the extracted analytes by the fibers are thermally desorbed by exposing the fiber in the injection port of a gas chromatograph. Extraction from headspace SPME allows for the analysis of samples from various matrixes such as biological [8], environmental [9], food [10], forensic [11], etc. SPME extraction times usually vary from a few minutes to an hour or longer, depending on the matrix, analytes, fiber phase used for extraction and a target to attain desired sensitivity. Longer extraction times may be acceptable as long as they are within the time frame of gas chromatographic run time. However, longer extraction times and longer GC run times limit the throughput of laboratories analyzing numerous samples on a daily basis. One approach to gain acceptable throughput is to apply high-speed GC in combination with faster sample preparation technique such as SPME using non-equilibrium conditions.

One of the major applications of the method discussed in this research is analysis of solvents in pharmaceutical process samples where solvents are typically present from 0.1 to 5% (w/w). The goal of the approach outlined in this work is not to achieve the best sensitivity but to develop a fast, simple and inexpensive analytical method for the analysis of solvents in pharmaceutical process samples, intermediates, drug substances and their raw materials. The rapid analysis time was accomplished by applying non-equilibrium extraction conditions for the sample preparation such that the extraction time does not significantly exceed the chromatographic run time of 2.25 min. This method was not intended

to be used as a release method for a drug substance.

## 2. Experimental

An Agilent HP6890 equipped with split/splitless inlet and flame ionization detector was converted to fast GC by coupling EZFlash™ unit purchased from Environmental Sample Technology, Inc, OH. A 50/30 μm divinylbenzene/Carboxen™ on polydimethylsiloxane (DVB/Carboxen/PDMS) on a 2 cm fused silica fiber, 70 μm Carbowax/DVB, 85 μm polyacrylate, 100 μm PDMS, 65 μm DVB/PDMS SPME fibers used for the method development were purchased from Supelco, Inc, MO. These fibers were conditioned prior to their use as recommended by the manufacturer. A stock solution containing analyte solvents was prepared in dimethylsulfoxide (DMSO). This stock solution was further diluted with either DMSO or water to obtain standards in the concentration range of 0.1–5000 μg/ml. Analytes from standards and samples were extracted using a SPME fiber exposed to the headspace of a 3 ml aliquot taken in a sealed 10 ml vial and stirred on a magnetic stir plate. Crimp caps of headspace vials were conditioned overnight at 150 °C to remove any residue. After analytes are extracted, SPME fiber was subsequently injected manually into a splitless injector of the gas chromatograph. A Thermedics TDX RTX-1301, 10 m, 0.25 mm i.d., and 1.0 μm film thickness was employed for separation. Helium was used as a carrier gas at a linear velocity 50 cm/s. Injector with a 0.75 mm i.d. inlet liner was maintained at 250 °C. Flame ionization detector was kept at 240 °C and nitrogen was used as a make-up gas at a flow rate 25 ml/min. The column temperature was programmed from initial 35 °C and ramped from 35–40 °C in 60 s, 40–100 °C at 2.00 °C/s, 100–140 °C at 2.66 °C/s, 140–210 °C at 2.33 °C/s. The inlet was purged with the carrier gas for 12 s immediately after each injection. The total chromatographic run time was 2.25 min. For direct injection, inlet temperature was set at 210 °C and a splitless injection of 1.0 μl was made using an autosampler.

## 3. Results and discussion

It has been our experience that most process samples are soluble either in DMSO or in aqueous medium. Therefore, the purpose of the preliminary work was to develop an approach where process solvents can be quantitated from the solutions prepared in either of these matrices. In order to minimize introduction of difficult matrix into fast GC analytical column, all extractions were done by sampling headspace. In addition, sampling the headspace would allow analyzing concentrated sample as high as 100–150 mg/ml or to the maximum allowable by solubility limits of sample in a diluent to attain desired sensitivity. Several SPME fibers were evaluated for the extraction of mixture of 13 solvents from the pharmaceutical samples. Fig. 1 shows a typical high-speed GC separation of solvents after their extraction from a DMSO solution. Except benzene, these solvents are the most commonly used solvents in pharmaceutical synthesis.

### *3.1. Comparison of sensitivity with SPME fibers*

Preliminary work involved the selection of fiber that is able to extract at least 6 out of 13 analytes from a standard solution prepared at 10–100 μg/ml in water or DMSO. Any fiber not meeting this criterion was not further evaluated for method development. The mixture of 13 analytes was extracted for 5 min at a stir rate of 700 rpm using various commercially available fibers. Only 65 μm DVB/PDMS, 2 cm long DVB/Carboxen/PDMS fiber, 100 μm PDMS fiber showed acceptable sensitivity when a mixture of analytes were extracted from aqueous media. DVB/Carboxen/PDMS fiber demonstrated the best sensitivity towards the extraction of these solvents, in that it extracted 11 analytes out of 13 analytes from aqueous matrix. One-hundred micrometer PDMS extracted only 6 analyte solvents and showed lowest sensitivity among the three fibers. Sixty-five micrometer DVB/PDMS fiber showed moderate sensitivity where 8 out of 13 solvents were extracted from aqueous matrix. Similar sensitivity was demonstrated by DVB/PDMS and DVB/Carboxen/PDMS fibers when the solvents were ex-

tracted from organic (DMSO) matrix. One-hundred micrometer PDMS was not used for the extraction of solvents from DMSO because it caused its swelling in organic vapor and consequently stripping of the coated phase from silica fiber.

Direct injection of this mixture of solvents resulted in the detection of only 3 components out of 13 solvents.

### 3.2. Optimization of extraction time and agitation rate

There are numerous approaches to maximize sensitivity using SPME. These include increasing ionic strength of sample [12], heating sample [13], optimizing extraction time [14] and agitating sample solution [15], etc. Only two parameters were optimized in the present work, namely, extraction time and agitation rate since one of the objectives of the research was to develop a simple sample preparation approach.

In order to maximize the extraction uptake to achieve the optimum sensitivity, extraction time profiles were constructed from the three fibers that showed an acceptable sensitivity, as discussed in the preceding section. Again, a mixture of 13 analyte solvents at a concentration range of 10–100 μg/ml in aqueous as well as organic matrix was extracted using these fibers from extraction time of 0.5–10 min. The mixture was constantly stirred at 700 rpm. Extraction times longer than 10 min were not studied since the goal was to keep the SPME time as close to GC run time (which is 2.25 min) as possible. Fig. 2 shows extraction time profiles of these fibers for toluene in water solutions. For the purpose of presentation, extraction profile of only toluene is shown in this figure.

It is evident from these profiles that all three fibers show a consistent increase in the response of toluene for an extraction time up to 5 min, after which there was no significant gain in the response. This information proved valuable in that the extraction time up to 5 min is compatible with the chromatographic run time of 2.25 min (plus 1 min of cooling time to reach initial temperature of 35 °C). Similar extraction profiles were observed with other analytes studied in this work when extracted from aqueous and organic matrix.

To determine the effect of agitation rate on the uptake of analytes to the headspace, aqueous and organic solutions of a mixture of analytes pre-

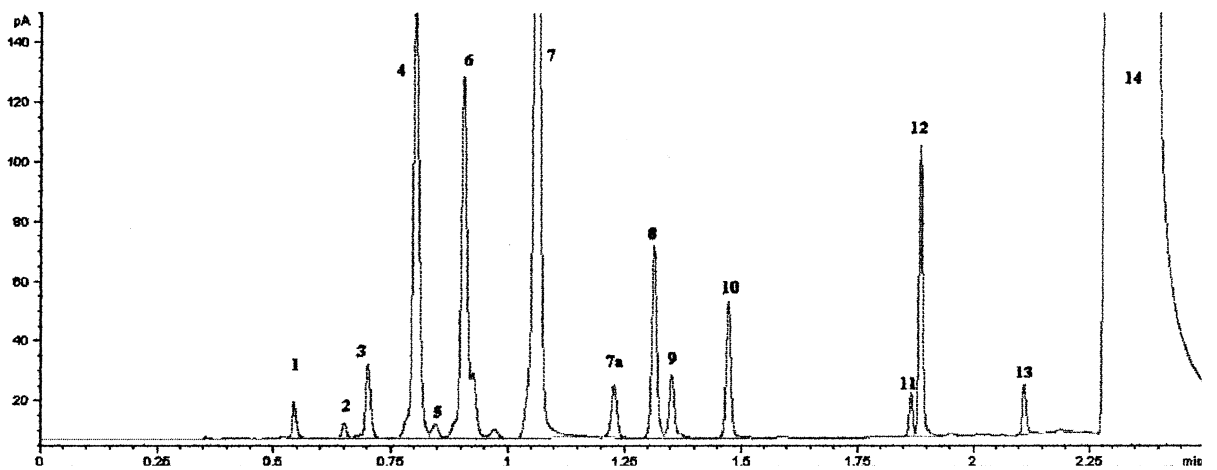


Fig. 1. Fast separation of commonly found solvents in pharmaceutical samples. Analyte concentration: approximately 5–50 μg/ml in DMSO; extraction time: 5 min; stir rate: 900 rpm, extracted from headspace with a DVB/Carboxen/PDMS fiber. 1—Methanol, 2—Methylene chloride, 3—Ethanol, 4—Acetone, 5—Isopropyl alcohol, 6—Acetonitrile, 7—Hexane, 7a—Hexane impurity, 8—Ethyl acetate, 9—Tetrahydrofuran, 10—Benzene, 11—Methyl isobutyl ketone, 12—Toluene, 13—Dimethyl formamide, 14—Dimethyl sulfoxide.

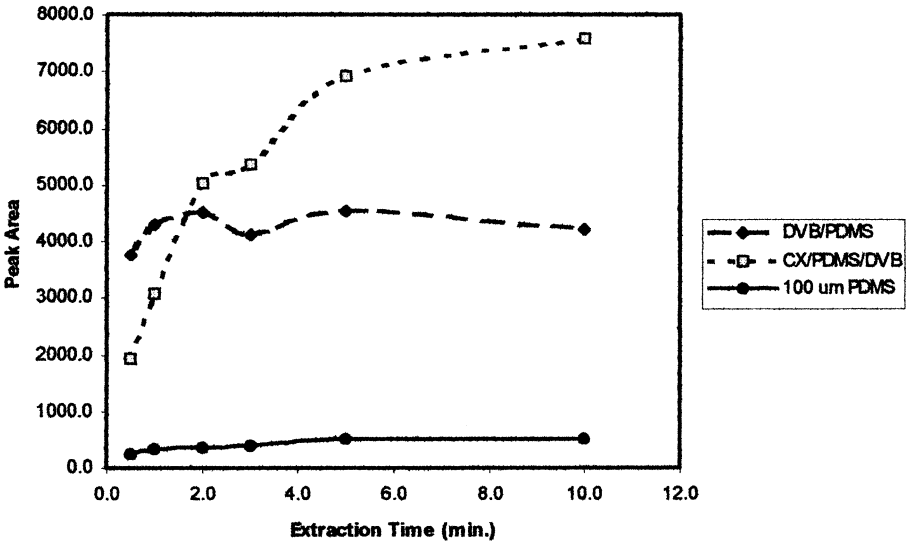


Fig. 2. Extraction time profile of toluene using DVB/PDMS, DVB/Carboxen/PDMS and 100 μm DVB fibers from aqueous matrix.

pared in water as well as in DMSO at a concentration level of 10–100 μg/ml were stirred at various rates from 300 to 1100 rpm. Fig. 3 shows the effect of stir rate on the response of toluene extracted from water when extracted for 5 min. Again, toluene was chosen as a representative analyte for the sake of simplicity for presentation of data. It is apparent from these plots that the rate of agitation has a little effect on the response of toluene when extracted with 100 μm PDMS and 65 μm DVB/PDMS fibers. On the other hand, there is a consistent increase in the amount of toluene extracted from stir rate of 300–700 rpm when DVB/Carboxen/PDMS fiber is used. After 700 rpm, there was no significant gain in response of toluene. Similar trend was followed by all the analytes prepared in both aqueous as well as in organic matrix. For further studies, an agitation rate of 700 rpm was employed.

The optimum conditions of 5 min extraction time and agitation rate of 700 rpm were employed for evaluating performance characteristic of the current approach, with the emphasis of selecting one fiber among the three fibers, which would show acceptable precision, linearity and accuracy for both aqueous and organic matrix. Acceptance criteria for precision was set at %RSD of peak area of six replicate injections less than 10% and that for linearity was set at correlation coefficient values exhibiting higher than 0.99. An accuracy value between 80 and 120% was considered acceptable for the intended purpose of this approach.

### 3.3. Precision

To demonstrate the precision of extraction and subsequent thermal desorption, six replicates of aqueous solution of a mixture of analyte solvents prepared at 10–100 μg/ml were extracted for 5 min at 700 rpm using 65 μm DVB/PDMS, DVB/Carboxen/PDMS, 100 μm PDMS fibers. Percent relative standard deviations of peak area of detected analytes were obtained from these six replicate extraction and injection cycles and these values were used as a measure of precision. Table 1 gives %RSD values of peak area of analytes extracted from water and analyzed under optimum conditions. Most analytes showed rather unsatisfactory precision as high as 30% RSD when these analytes were extracted from aqueous matrix using DVB/PDMS phase. On the other hand, DVB/Carboxen/PDMS fiber demonstrated lower %RSD ($< 5\%$) for non-polar analytes and higher %RSD (5–25%) for polar analytes. One-hundred micrometer PDMS fiber showed satisfac-

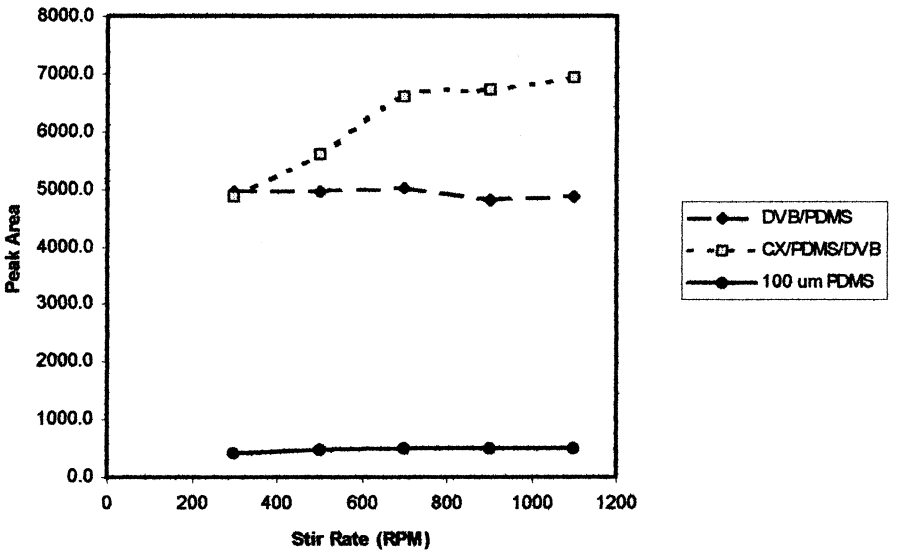


Fig. 3. Effect of rate of agitation on response of toluene extracted from aqueous matrix using 65 μm DVB/PDMS, DVB/Carboxen/PDMS and 100 μm PDMS fiber.

tory precision values ( < 10%) for both polar as well as non-polar analytes, with the exception of hexane, which resulted in %RSD value of 10.5%. Hexane is not completely soluble in DMSO and water, therefore, would result in variable amount of hexane in all six aliquots used for precision determination. Evidently, variation in response of hexane extracted from six aliquots is expected, thus resulting in rather unsatisfactory %RSD values.

Precision of extraction, followed by gas chromatographic separation was studied for analyte mixture extracted from DMSO matrix using DVB/Carboxen/PDMS and DVB/PDMS fibers. One-hundred micrometer PDMS fiber was not used for extraction of analytes from organic matrix. These values are summarized in Table 2. Most analytes demonstrated less than 10% RSD of peak area when they were extracted with DVB/Carboxen/PDMS and DVB/PDMS fibers. Overall, these precision values may be acceptable considering the fact that extraction and injection steps were carried out manually. With the use of an SPME autosampler, it may be possible to improve precision of the method, however, such an autosampler was not available for the present work.

### 3.4. Linearity

Linearity of the method was evaluated by extracting and injecting standard solutions of mixtures of analytes prepared in water as well as in

Table 1
Precision of extraction and chromatographic response for various analytes extracted from aqueous matrix using DVB/PDMS, DVB/Carboxen/PDMS and 100 μm DVB fibers

| Solvent | %RSD (peak area) | | |
|---|---|---|---|
| | DVB/PDMS | DVB/Carboxen/PDMS | 100 μm PDMS |
| Methanol | 34.4 | 13.7 | n.d. |
| Ethanol | 32.3 | 22.8 | 7.1 |
| Acetone | 30.1 | 4.0 | 2.6 |
| Isopropyl alcohol | 13.9 | 16.4 | 6.0 |
| Acetonitrile | 26.6 | 8.7 | 4.6 |
| Hexane | 21.5 | 13.3 | 10.5 |
| Ethyl acetate | 11.9 | 2.1 | 3.0 |
| THF | 19.3 | 5.6 | 2.4 |
| Benzene | 15.5 | 2.7 | 4.5 |
| MIBK | 12.6 | 9.2 | 4.7 |
| Toluene | 16.8 | 3.4 | 4.7 |
| DMF | 24.2 | 17.2 | n.d. |

n.d.: not detected.

Table 2
Precision of extraction and chromatographic response for various analytes extracted from organic matrix using DVB/PDMS and DVB/Carboxen/PDMS

| Solvent | %RSD (peak area) | |
|---|---|---|
| | DVB/PDMS | DVB/Carboxen/PDMS |
| Methanol | 5.0 | 3.6 |
| Ethanol | 5.1 | 3.0 |
| Acetone | 4.7 | 4.6 |
| Isopropyl alcohol | 7.2 | 1.1 |
| Acetonitrile | 4.0 | 4.4 |
| Hexane | 8.1 | 20.2 |
| Ethyl acetate | 3.9 | 3.3 |
| THF | 3.7 | 4.8 |
| Benzene | 1.8 | 3.7 |
| MIBK | 3.5 | 4.7 |
| Toluene | 3.2 | 3.8 |
| DMF | 4.4 | 6.5 |

Table 3
Correlation coefficients of analyte solvents extracted from organic matrix using DVB/PDMS and DVB/Carboxen/PDMS

| Solvent | Correlation coefficient | |
|---|---|---|
| | DVB/Carboxen/PDMS | DVB/PDMS |
| Toluene | 0.9981 | 0.9984 |
| Acetonitrile | 0.9740 | 0.9996 |
| Ethanol | 0.9937 | 0.9979 |
| Ethyl acetate | 0.9910 | 0.9994 |
| THF | 0.9999 | 0.9997 |
| DMF | 0.9991 | 0.9979 |
| MIBK | 0.9998 | 0.9992 |
| Acetone | 0.9804 | 0.9997 |
| Hexane | 0.9998 | 0.9973 |
| Methanol | 0.9987 | 0.9974 |
| Isopropyl alcohol | 1.0000 | 0.9982 |
| Benzene | 0.9975 | 0.9992 |

DMSO in a concentration range of 10–5000 μg/ml. For the purpose of comparison, linearity curves were constructed with two of the most sensitive fibers i.e. DVB/Carboxen/PDMS and DVB/PDMS. Once again, the linearity curves with 100 μm PDMS fiber for the analytes extracted from DMSO were not constructed due to its swelling in organic vapor. Fig. 4 shows standard curves for acetone extracted with DVB/Carboxen/PDMS and DVB/PDMS from DMSO matrix. DVB/Carboxen/PDMS fiber showed a non-linear response with increasing concentration

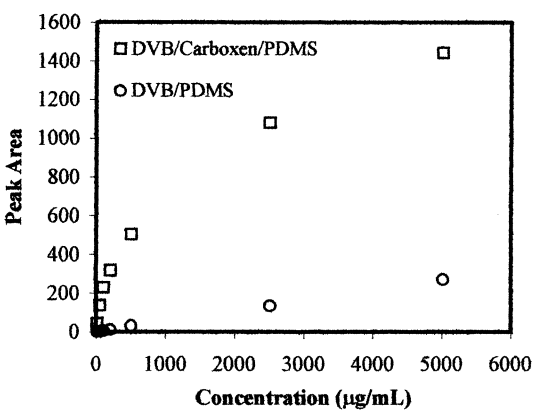


Fig. 4. Standard curves for acetone extracted with DVB/Carboxen/PDMS and DVB/PDMS obtained from DMSO matrix.

of acetone whereas DVB/PDMS fiber showed an acceptable linearity with $R^2$-value of 0.9996. A similar non-linear trend was observed for all the analytes in the mixture when the solvents were extracted from DVB/Carboxen/PDMS from organic matrix. At the same time, excellent linearity was demonstrated by DVB/PDMS for all the analytes extracted from DMSO. Correlation coefficient obtained for analyte solvents extracted from organic matrix using DVB/PDMS and DVB/Caboxen/PDMS are given in Table 3. $R^2$-values for linearity obtained with DVB/PDMS are calculated using linear regression whereas that obtained with DVB/Carboxen/PDMS fiber are calculated using second order polynomial fit.

Because DVB/PDMS showed excellent linearity towards the analytes extracted from organic matrix compared to that demonstrated by DVB/Carboxen/PDMS fiber, only DVB/PDMS fiber was further evaluated for the extraction of analyte solvents from aqueous solutions for determining its linearity. Standard solutions of mixture of analytes prepared in water in a concentration range of 10–500 μg/ml were used to construct calibration curves. The results were compared with the linearity data obtained with a 100 μm PDMS fiber since this fiber showed acceptable precision for extraction of analytes from water. It

was not possible to use 100 μm PDMS phase for extraction of analytes at a higher concentration than 500 μg/ml due to its swelling in organic vapor. Fig. 5 shows standard curves for acetone extracted from 65 μm DVB/PDMS and 100 μm PDMS phases used for extraction of analytes from water. Again, non-linearity was observed, however, this time with DVB/PDMS. On the other hand, correlation coefficient of acetone extracted from aqueous matrix with 100 μm PDMS fiber was at an acceptable value of 0.9998. A similar trend of non-linearity with DVB/PDMS and excellent linearity ($R^2$-values $>0.999$) with 100 μm PDMS was observed for all other analytes except hexane when extracted from aqueous solutions. Non-linearity of hexane is attributed to its incomplete solubility in DMSO used as a diluent to prepare stock solution. Correlation coefficients obtained for analyte solvents extracted from aqueous matrix using DVB/PDMS and 100 μm PDMS are given in Table 4. Many polar organic analytes were not detected at lower concentration levels when extracted from aqueous medium; therefore, this table includes only correlation coefficients of 9 out of 13 solvent analytes. $R^2$-values for linearity obtained with l00 μm PDMS are calculated using linear regression whereas those obtained with 65 μm DVB/PDMS fiber are calculated using second order polynomial fit.

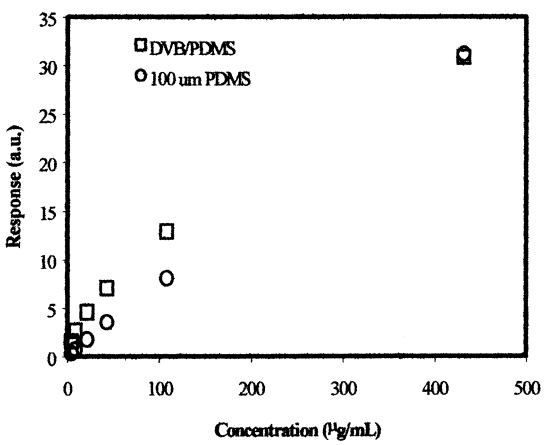


Fig. 5. Standard curves for acetone extracted from 65 μm DVB/PDMS and 100 μm PDMS phases used for extraction of analytes from water.

Table 4
Correlation coefficients of analyte solvents extracted from aqueous matrix using DVB/PDMS and 100 μm PDMS

| Solvent | Correlation coefficient | |
|---|---|---|
| | l00 μm PDMS | DVB/PDMS |
| Toluene | 0.9993 | 0.9953 |
| Acetonitrile | 0.9993 | 0.9992 |
| Chloroform | 0.9993 | 0.9970 |
| Ethyl acetate | 0.9998 | 0.9978 |
| THF | 0.9992 | 0.9984 |
| MIBK | 0.9998 | 0.9910 |
| Acetone | 0.9999 | 0.9986 |
| Hexane | 0.9789 | 0.9785 |
| Benzene | 0.9996 | 0.9983 |

Non-linearities observed with DVB/Carboxen/PDMS and DVB/PDMS fibers are consistent with the fact that these fibers extract analytes based on adsorption mechanism. Analytes compete with each other for the limited number of adsorption sites available with these fibers and displace other analytes with low distribution ratio by compounds with high distribution ratio, therefore, causing non-linear responses with increasing concentration of analytes [16].

### 3.5. Accuracy

Accuracy of the method was evaluated by determining recovery of analyte solvents spiked in a drug substance. In our experience, among the samples we receive for solvent analysis, the estimated concentration of solvents in the process samples has been mostly in the range of 0.1–5% (w/w). Therefore, accuracy of analysis was evaluated in this concentration range. A mixture containing 100–5000 μg/ml solvents prepared in DMSO was spiked in a drug substance dissolved in DMSO. This concentration range corresponds to 0.1–5% (w/w) based on a sample prepared at a concentration of 100 mg/ml. For organic matrix, only 65 μm DVB/PDMS fiber was employed for the extraction of the analyte solvents from spiked DMSO-soluble drug substance since this fiber showed an acceptable linearity and precision when used for extracting analytes from organic matrix. Table 5 outlines accuracy values for ana-

Table 5
Accuracy values for analytes spiked in a drug substance dissolved in DMSO

| Solvent | % Spike recovery |
|---|---|
| Methanol | 84.4 |
| Methylene chloride | 87.0 |
| Acetone | 91.3 |
| Isopropyl alcohol | 87.7 |
| Acetonitrile | 92.8 |
| Hexane | 96.2 |
| Ethyl acetate | 104.8 |
| Chloroform | 93.6 |
| Benzene | 92.4 |
| MIBK | 96.9 |
| Toluene | 98.7 |
| DMF | 92.6 |

lytes spiked in a drug substance dissolved in DMSO. As evident from this table, most analytes exceeded 80%, which is acceptable for the intended purpose of the method for the analytical support of pharmaceutical synthesis process development.

Accuracy of the method was also evaluated for analytes extracted from aqueous solutions using 100 μm PDMS fiber. For the extraction of analytes from aqueous solutions, 100 μm PDMS fiber was used since this fiber showed most acceptable linearity among all the three fibers. Table 6 outlines spike recovery data for the analytes spiked in a water-soluble drug substance and extracted with 100 μm PDMS fiber. Ten out of 13 spiked analytes were detected at the concentration levels mentioned above. The spike recoveries for the polar analytes such as ethanol, isopropyl alcohol, etc. were found at satisfactory level of 80–100%. However, spike recovery of non-polar components such as benzene and toluene were not an acceptable value (i.e. not greater than 80%).

Table 6
Spike recovery data for the analytes spiked in a water-soluble drug substance and extracted with 100 μm PDMS fiber

| Solvent | % Spike recovery |
|---|---|
| Ethanol | 100.0 |
| Acetone | 101.3 |
| Isopropyl alcohol | 106.7 |
| Acetonitrile | 81.1 |
| Ethyl acetate | 88.6 |
| THF | 98.9 |
| Chloroform | 72.7 |
| Benzene | 66.7 |
| MIBK | 71.7 |
| Toluene | 72.7 |

Because most analytical samples soluble in water are also soluble in DMSO, a 65 μm DVB/PDMS fiber could be used for extraction of solvents from DMSO. Therefore, it is recommended to use 65 μm DVB/PDMS fiber as general-purpose extraction fiber with having additional advantage of better sensitivity than that shown by 100 μm PDMS fiber for most analytes, as discussed in preceding section.

### 3.6. Pharmaceutical sample analysis

#### 3.6.1. Determination of methyl isobutyl ketone in HPLC samples

Often times, analysts supporting pharmaceutical synthesis process development get requests for the analysis of solid drug substance for purity determination by HPLC analysis. Simultaneously, for the same set of samples, quantitation of residual solvents in that drug substance sample by GC analysis is requested. This often requires preparation of two sets of standards and samples for these analyses by HPLC and GC. For purity determination by HPLC, samples and standards are typically prepared in a concentration range of 0.5–5.0 mg/ml using HPLC mobile phase as a diluent. On the other hand, for residual solvents quantitation by direct injection GC, samples are dissolved in an organic solvent to obtain their solutions at a concentration of 20–50 mg/ml. High sample concentration is used to achieve desired sensitivity when direct injection technique is used. Because SPME also serves as a sample enrichment technique, this application of SPME was used with fast GC analysis of residual solvents. The same samples and standards prepared in 50% acetonitrile in water for HPLC analysis were used for extraction of methylisobutyl ketone (MIBK) as a residual solvent in these samples using 65 μm DVB/PDMS fiber. When MIBK was extracted from sample matrix containing 50% acetonitrile in water, there was no significant gain in

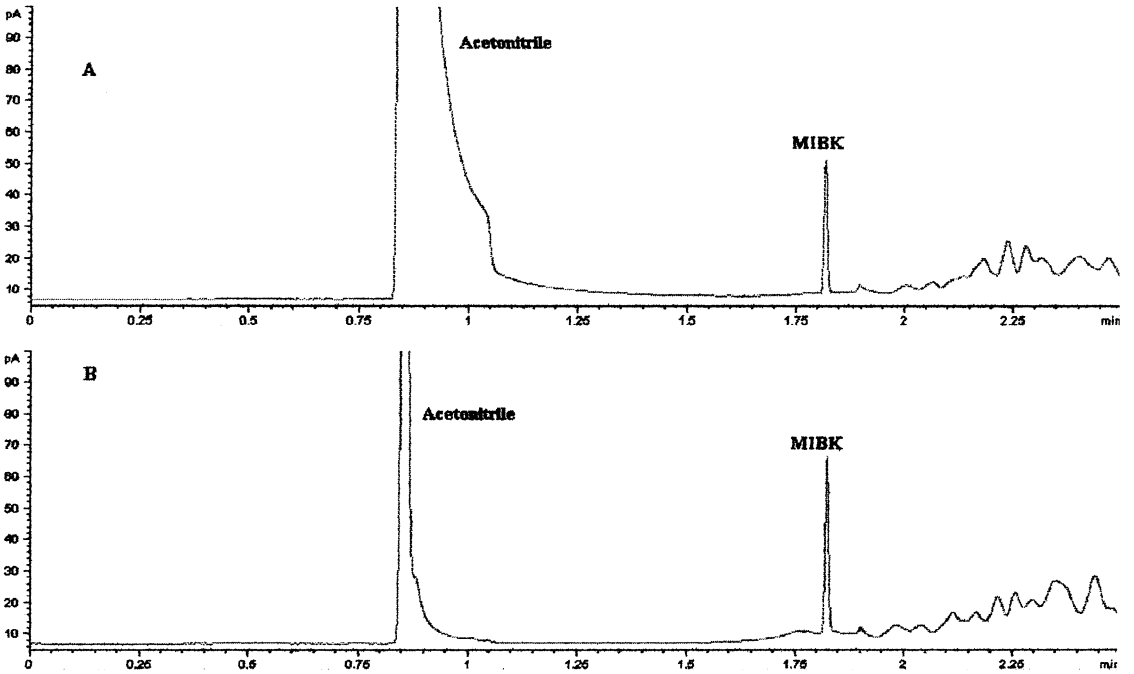


Fig. 6. Effect of acetonitrile concentration on extraction of MIBK (A) from 50% acetonitrile in water solution (B) from 0.25% acetonitrile in water.

response by SPME over that observed with direct injection. Lower than expected response of extracted MIBK is attributed to the competition between acetonitrile and MIBK for the available adsorption sites on DVB/PDMS fiber. To demonstrate the effect of acetonitrile competition with MIBK for the available adsorption sites and to facilitate removal of MIBK from acetonitrile/water phase to headspace, all the standards and samples were diluted 200-fold with water and subsequently MIBK was extracted using a 65 μm DVB/PDMS fiber. Fig. 6 shows chromatograms comparing MIBK extracted from 50% acetonitrile in water and that extracted from 0.25% acetonitrile in water. Surprisingly, there was no loss in sensitivity of MIBK even after 200-fold dilution of this sample with water. This implies that higher concentration of acetonitrile in the extraction matrix had a detrimental effect on the sensitivity of MIBK. A calibration curve was constructed from diluted MIBK standards originally prepared in 50% acetonitrile in water. Similarly, HPLC samples prepared in 50% acetonitrile in water were also diluted with water for the purpose of quantitation. Fig. 7 shows the calibration curve of MIBK extracted from diluted standard solution. As evident from the standard curve, a slight non-linear response was observed with increasing concentration. However, when a linear fit was used, an acceptable correlation coefficient of $R^2$ of 0.9919 was obtained for the narrow range of concentration (0.015–0.750 mg/ml) used for

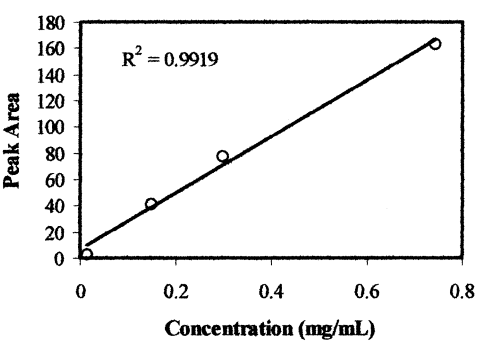


Fig. 7. Calibration curve obtained for diluted MIBK standards originally prepared in 50% acetonitrile in water.

MIBK standards. The results obtained by another analyst using direct injection technique on a conventional GC were compared. A good correlation on the amount of MIBK found by conventional GC and by the method under this presentation was observed (1.40% by conventional GC versus 1.54% by SPME-Fast GC method for one sample, and 1.47% by conventional GC versus 1.71% by SPME-Fast GC method for the other sample).

In this application, it took approximately 30 min, for extraction and chromatographic run for all four standards to obtain calibration curve. In addition, it took approximately 7.5 min for extraction and chromatographic run time per sample. Most importantly, the sample preparation for GC analysis time was substantially reduced since same set of samples and standards that was used for HPLC was used for GC analysis after they were diluted with water. Although some time was spent on dilution of standards and samples originally prepared for HPLC analysis, this length of time was much shorter than the time an analyst would have to spend for the preparation of standards and samples exclusively for analysis of solvents by direct injection GC.

### *3.6.2. Determination of ethyl acetate in a raw material*

Ethylacetimidate hydrochloride is a raw material for the synthesis of a drug substance. A sample of ethylacetimidate hydrochloride contained 4.6% (w/w) ethyl acetate when analyzed by conventional GC technique. This sample was also analyzed by SPME extraction using a 65 μm DVB/PDMS fiber followed by high-speed GC for ethyl acetate. A standard addition method was used for quantitation where ethyl acetate solution prepared in DMSO was spiked in this raw material sample and amount of ethyl acetate present in the original sample was quantitated by back extrapolation. Fig. 8 depicts a standard addition curve for ethyl acetate spiked in ethylacetimidate hydrochloride sample. Amount of ethyl acetate found was 4.8%, which correlates well when quantitated by conventional GC. For this analysis, quantitation was conducted by standard addition method. However, it may be possible to quantitate solvents by external standard method, which would significantly reduce time for such analysis.

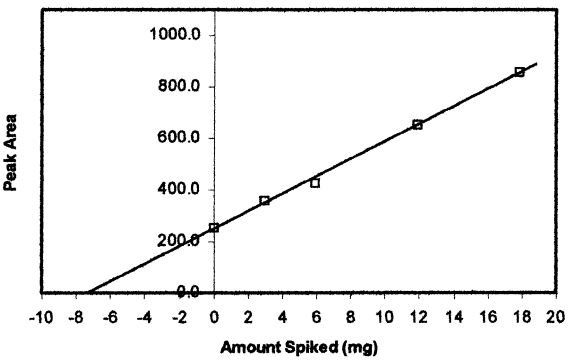


Fig. 8. Standard addition curve for ethyl acetate spiked in ethylacetimidate hydrochloride sample.

## 4. Conclusions

A rapid method for the quantitation of solvents in pharmaceutical process samples using SPME and high speed GC was developed. This method correlates well for the quantitation results obtained with conventional direct injection gas chromatographic method. Although DVB/Carboxen/PDMS fiber showed highest sensitivity, it resulted in non-linear responses to solutions prepared in both organic and aqueous matrix when extracted from solutions with a wide analyte concentration levels. One-hundred micrometer PDMS showed lower sensitivity for analytes extracted from aqueous medium. Although excellent linearity was demonstrated by 100 μm PDMS fiber, it failed to demonstrate an acceptable accuracy when used for extracting analytes from aqueous matrix. Sixty-five micrometer DVB/PDMS SPME coupled with fast GC was found to be the best choice in terms of linearity, accuracy and precision. This fiber showed an acceptable sensitivity for the intended purpose of its use.

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