Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 818 (2005) 147–157
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Sol–gel-based solid-phase microextraction and gas chromatography-mass spectrometry determination of dextromethorphan and dextrorphan in human plasma

Habib Bagheri[a,*], Ali Es-haghi[a], Mohammad-Reza Rouini[b]

[a] *Department of Chemistry, Sharif University of Technology, Azadi Av., P.O. Box 11365-9516, Tehran, Iran*
[b] *Biopharmaceutics and Pharmacokinetic Division, Department of Pharmaceutics, Faculty of pharmacy, Tehran University of Medical Sciences, 14155-6451 Tehran, Iran*

Received 13 August 2004; accepted 21 December 2004
Available online 21 January 2005

Abstract

A novel solid-phase microextraction (SPME) method was developed for isolation of dextromethorphan (DM) and its main metabolite dextrorphan (DP) from human plasma followed by GC-MS determination. Three different polymers, poly(dimethylsiloxane) (PDMS), poly(ethylenepropyleneglycol) monobutyl ether (Ucon) and polyethylene glycol (PEG) were synthesized as coated fibers using sol–gel methodologies. DP was converted to its acetyl-derivative prior to extraction and subsequent determination. The porosity of coated fibers was examined by SEM technique. Effects of different parameters such as fiber coating type, extraction mode, agitation method, sample volume, extraction time, and desorption condition, were investigated and optimized. The method is rapid, simple, easy and inexpensive and offers high sensitivity and reproducibility. The limits of detection are 0.010 and 0.015 ng/ml for DM and DP, respectively. The precisions for both analytes are below 5% ($n = 5$). The correlation coefficient was satisfactory ($r^2 > 0.99$) for both DM and DP. Linear ranges were obtained from 0.03 ng/ml to 2 μg/ml for DM and from 0.05 ng/ml to 2 μg/ml for DP.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Sol–gel technology; Solid-phase microextraction; Gas chromatography-mass spectrometry; Dextromethorphan; Dextrophan; Plasma samples

1. Introduction

Dextromethorphan (DM) is a safe perorally administered antitussive that is widely available without prescription for which the metabolite fate in humans has been well-characterized (Fig. 1). The metabolism of DM is primarily by O-demethylation to dextrorphan (DP), a reaction that is mediated by cytochrome P450 enzyme CYP2D6. DM is also metabolized to 3-methoxymorphinan and 3-hydroxymorphinan, but these appear to be minor pathways mediated by CYP2D6 and CYP3A3/4, respectively. Because the CYP2D6 enzyme displays polymorphism, DM metabolism to DP has been used to phenotype subjects [1,2].

Several methods have been reported for the determination of DM and DP in biological fluids including high-performance liquid chromatography (HPLC) with UV [3,4], fluorescence detection [5–8,9], and tandem mass spectrometry (MS/MS) [2,10–12], gas chromatography (GC) [1,13,14], capillary electrophoresis [15,16] and thin-layer chromatography (TLC) [17]. However, all of the reported methods, except LC/MS/MS methods suffer from lack of sensitivity and poor limit of quantification. In most of methods described, liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are the most commonly used techniques for isolation and/or enrichment of DM and DP prior to chromatographic analysis. These approaches have disadvantages, as they are tedious, labor-intensive and time-consuming procedures. Although, the use of robotics has greatly decreased the level of tediousness, labor and time required for SPE and

* Corresponding author. Tel.: +98 21 6005718; fax: +98 21 6012983.
E-mail address: bagheri@sharif.edu (H. Bagheri).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.12.031

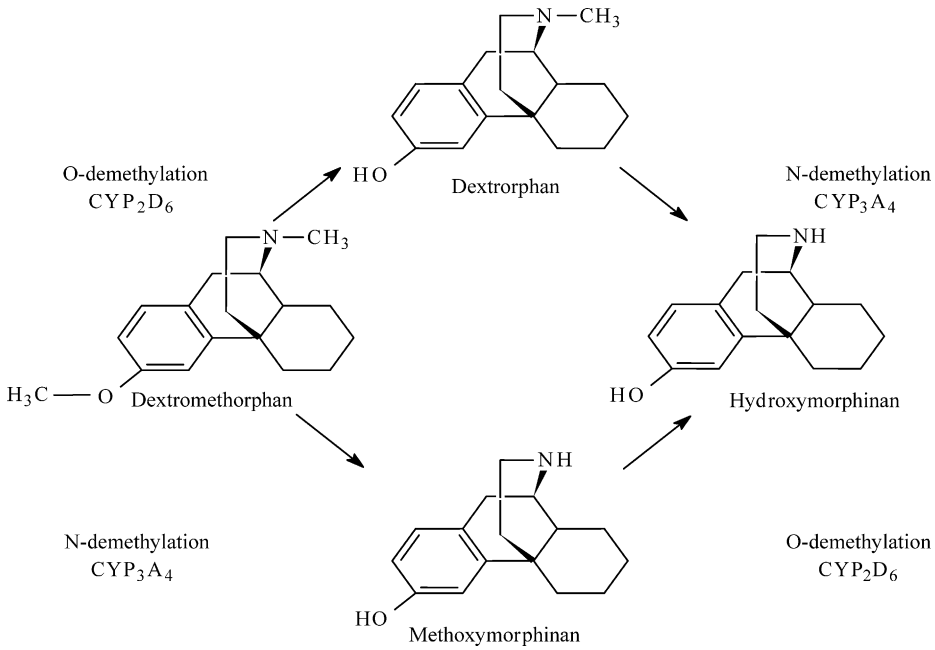

Fig. 1. Chemical structures and metabolic pathway of DM.

LLE but usually such equipment is rather expensive and may not be practical for all laboratories. LLE produces an emulsion and requires the use of large amount of highly-purity solvents, which are often hazardous and result in the production of toxic laboratory waste. Prior to the chromatographic analysis, when LLE and SPE are employed, there is a need for solvent evaporation, in order to preconcentrate the samples. Although SPE is less time-consuming than LLE, it still requires an appreciable amount of toxic solvent for analyte desorption.

Solid-phase microextraction (SPME) technique, initially introduced for analysis of volatile compounds, has gained an increasingly important role for isolation of vast varieties of compounds from aqueous media [18]. SPME overcomes the above difficulties by reducing or eliminating the use of organic solvents and by allowing sample extraction and preconcentration to be performed in a single run. The technology is more rapid and simple than the conventional methods. It is also inexpensive, portable and sensitive. In SPME, sorbent coated silica fibers are used to extract analytes from solid, aqueous or gaseous samples. After extraction, the fibers are directly transferred into the injection port of a gas chromatograph or special interface coupled to HPLC, via a modified syringe, after which the analytes are desorbed, thermally or by solvents, and subsequently analyzed [19–24,25].

SPME is predominantly performed on commercially available SPME fibers coated with different sorbents having various polarities [20]. The recommended operating temperatures for these fibers are generally within the range of 200–270 °C [26,27], which is not suitable for analysis of some polar compounds. Moreover, all these fibers are often prepared by physical deposition of the polymer coating on the surface of the fused silica fiber and that is the most likely reason for the low thermal and chemical stability. Sol–gel chemistry offers a simple and convenient pathway for the synthesis of advanced material systems to overcome this problem, by providing efficient incorporation of organic components into the inorganic polymeric structure in solution under quite mild thermal conditions. Clearly, creating chemical binding between the solid-phase coating and the substrate surface leads to more stable performance of the coating and subsequently extends the SPME application field toward less volatile and more polar compounds [28–31].

In the continuation of our research interests on trace determination of organic compounds in aqueous media [32–34], a simple, rapid, sensitive and reproducible SPME-GC-MS method for the determination of DM and DP in plasma is described. Three different coated fibers including poly(dimethylsiloxane) (PDMS), poly(ethylenepropyleneglycol) monobutyl ether (Ucon) and PEG were prepared, based on sol–gel technology, and evaluated for the SPME of these analytes from human plasma followed by GC-MS analysis.

2. Experimental

2.1. Chemicals and reagents

Tetramethoxysilane, poly(methylhydrosiloxane) (PMHS), trifluoroacetic acid (TFA), hexamethyldisilazane (HMDS), polyethylene glycol 4000 (PEG), methylene chloride, methanol, sodium hydroxide, hydrochloric acid, an-

hydride acetic acid, benzoyl chloride, 2,4-dinitro benzoyl chloride and pentaflourobenzoyl chloride (PFBC), were purchased from Merck (Darmstadt, Germany). Hydroxy-terminated PDMS, trimethylmethoxysilane, poly(ethylene-propylenglycol) monobutyl ether (Ucon HTF 14) were purchased from Fluka (Buchs, Switzerland). Dextromethorphan hydrobromide and dextrophan tarterate (>99%) were kindly provided by Toulid daru (Tehran, Iran).

2.2. Preparation of standards

Stock solutions (100 μg/ml as free base) of DM and DP were prepared in methanol, and stored at −20 °C. Other standard solutions were prepared daily by diluting these solutions. Blank plasma samples were spiked with different concentrations of DM and DP solutions, in a way that, final concentration of DM and DP were achieved as desired. After SPME of spiked samples the calibration curves for DM and DP were plotted using the GC-MS response for each analyte against its corresponding concentration.

2.3. Apparatus

An SPME syringe was purchased from Azar Electrode Co. (Uromieh, Iran). All samples were extracted from 4 ml clear glass vials with silicon septa and open-top phenolic caps. Samples were heated in a homemade glass water bath connected to a refrigerated circulating water bath (Neslab) and stirred using a Snijders hot plate stirrer (Tilburg, Holland). A Heraeus Sepatech Model Labofuge 1500 centrifuge (Osterode/Harz, West Germany) was used to separate the sol solution from any possible precipitate. The scanning electron microscopy (SEM) experiments on the sol–gel fiber coatings were carried out on a JEOL JXA-840 (Tokyo, Japan) scanning electron microscope.

2.4. SPME fiber preparation

Three different fibers with different polarities were prepared. The sol solution for each polymer was prepared separately.

2.4.1. Preparation of the PDMS sol solution

A volume of 300 μL of tetramethoxysilane (precursor), 180 mg of hydroxyl-terminated PDMS (coating polymer), 30 mg of PMHS (deactivation reagent), and 200 μL of 95% TFA (acid catalyst containing 5% water) were thoroughly shaken in a borosilicate culture tube. The mixture was then transferred into an Eppendorf micro centrifuge tube and centrifuged at 15,000 rpm for 5 min. The precipitate at the bottom of the tube was removed and the top clear sol solution was used for fiber coating.

2.4.2. Preparation of the Ucon sol solution

An amount of 0.187 g of Ucon HTF 14 (coating polymer) was dissolved in 500 μL of methylene chloride in a borosilicate culture tube. A 100 μL volume of tetramethoxysilane (precursor) and 200 μL of 95% TFA (acid catalyst containing 5% water) were then sequentially added with thoroughly mixing. The mixture was then transferred into an Eppendorf micro centrifuge tube and centrifuged at 15,000 rpm for 5 min. The clear liquid (sol) from the top was transferred to a clean vial and used for fiber coating.

2.4.3. Preparation of the polyethylene glycol sol solution

A volume of 400 μL of tetramethoxysilane (precursor), 200 mg of polyethylene glycol (coating polymer), 200 μL acetone and 150 μL of 95% TFA (acid catalyst containing 5% water) were thoroughly mixed in an Eppendorf micro centrifuge tube and centrifuged at 15,000 rpm for 5 min. The top clear liquid (sol) solution was transferred to a clean vial and used for fiber coating.

2.4.4. Sol–gel coating of SPME fiber

Details of sol–gel coating were previously described [29,30]. The total length of an SPME fiber was 2 cm, from which a 1.2-cm end segment was chosen to be coated by the sol–gel methodology. Prior to sol–gel coating, it was important to remove the polyimide layer from a 1.2 cm segment of the fiber at one of its ends. This was performed by burning off the polyimide layer using a naked flame. The burnt section of the fiber was cleaned with methanol, dried, and then dipped into the 1 M sodium hydroxide solution for an hour, washed with double distilled water and put into hydrochloric acid 0.1 M for 20 min then washed with double distilled water and dried. The fiber was then dipped vertically into the sol solution. It was held inside the sol solution for 20 min, during which a sol–gel coating was formed on the bare outer surface of the fiber end. For each fiber, this coating process was repeated three times, using a freshly prepared sol solution each time. This was followed by the end-capping process. For this, the coated fiber end was dipped into a trimethylmethoxysilane/methanol solution (4:1, v/v). After one min the fiber was removed from the end-capping solution and placed in a desiccators at room temperature for 24 h. The end-capping procedure was carried out just on PDMS coated fiber.

2.4.5. Conditioning of the fiber

The sol–gel coated fiber was initially conditioned at 250 °C under a stream of helium for approximately 2–3 h in the GC injection port. After removal from the injector, the fiber was cooled to room temperature. The fiber was then conditioned again at desired temperature using the same GC condition, for 30 min. The 30-min conditioning cycle was repeated a few more times until a stable GC baseline was obtained. The fiber was then ready for SPME and SPME-GC-MS experiments.

2.5. Derivatization of DP and SPME sampling

All vials were silanized prior to use according to the following procedures: the vials were rinsed with 5% solution of

HMDS in methylene chloride, followed by placing them in an oven at a temperature of 250 °C for 8 h. The vials were then sequentially rinsed with methylene chloride, methanol, and double distilled water. Sample vials prepared by first introducing 0.5 ml of plasma, containing DM and DP, into empty vials. Then, 1.5 ml of sample modification solution containing 0.4 g sodium chloride, 0.3 g potassium carbonate and 5 μl of methanol were added, using an adjustable-volume pipetter. The derivatizing agent, anhydride acetic acid, was added directly to the sample using a Hamilton syringe. The acetyl form of DP was formed after continuous stirring for 2 min. The vials were sealed with a phenolic hole cap and silicon septum. Sample vials were heated in a glass water bath, connected to a circulating water bath, and mixed by magnetic stirrer. The SPME fiber was exposed to the headspace by piercing the septum with the needle of the fiber assembly and then depressing the plunger. Care was taken to prevent any direct contact between the fiber and the liquid sample. After extraction, the fiber was withdrawn into the needle and removed from the sample vial.

2.6. SPME-GC-MS analysis

All gas chromatographic separations were performed on an Hewlett-Packard (HP) 6890 series gas chromatograph, equipped with an HP 5973 mass selective detector (MSD) (Hewlett-Packard, Palo Alto, CA, USA), and split–splitless injector. Helium was used at a flow-rate of 1 ml/min as the carrier gas. The injection port temperature was 280 °C. The electron impact (EI) ion source, quadrupole mass analyzer, and the interface temperature were maintained at 230, 150, and 280 °C, respectively. The MS was operated in the total ion current (TIC) mode, scanning from *m*/*z* 25 to 350. For quantitative determination, the MS was operated in the time-scheduled selected ion monitoring (SIM) mode. At a dwell time of 100 ms, two sets of characteristic ions at *m*/*z* 271, 150, 59, and 214 within 15–16.2 min and 150, 299, 231, and 157 after 16.2 min were selected for GC-MS analysis of DM and derivatized DP, respectively. The fiber, which analytes were extracted on, was inserted into the GC injection port. A duration of 5-min was allowed for the analytes to be desorbed from the fiber and transferred into the GC column for analysis. The thermal desorption step was carried out in the splitless mode, maintaining the column temperature at 50 °C. Low column temperature ensured effective solute focusing at the column inlet. On completion of the thermal desorption step, the split vent was opened and kept in that position for the remainder of the chromatographic run. All separations were performed using an HP-5 column (Hewlett-Packard, Avondale, PA) with a 0.25 μm film, 30 m × 0.25 mm. After completion of the sample introduction step, the column temperature was raised to a final temperature of 280 °C at a rate of 20 °C/min and remained at this temperature for 10 min. The total run time was 26 min.

3. Results and discussion

3.1. Sol–gel coating in SPME

Nowadays, SPME is regarded as a well-established methodology predominantly performed on commercially available SPME fibers coated with different sorbents. To extend this technique to the analysis of polar compounds and less volatile substances, there is a need to make stable stationary-phase coatings at the fibers end. Polysiloxanes are the most popular SPME coating materials due to their high thermal stability and enhanced solute diffusion capabilities. Mostly, the thermal stability of such coatings is less than 270 °C, which limits the molecular weight range of analytes that can be analyzed by SPME-GC [26,27]. Nevertheless, SPME has been coupled with liquid chromatographic methods in order to analyze larger molecular weight analytes, however, there are some problematic issues [19,20,35–37]. In those techniques, liquid solvents are being used to desorb the analytes from the fiber, suffering from the lack of adequate speed for analyte diffusion in liquid and long sample desorption/introduction process. Instability of coating in these cases is mostly due to the use of solvents rather than exposing the fiber to high temperatures.

Our primary investigations revealed that using routine PDMS fibers, prepared by physical deposition process, were not the suitable choice. Sol–gel technology, on the other hand, seemed to be a feasible option to enhance the operational stability of SPME fibers. In sol–gel approach the stationary phase coating is chemically bonded to the surface of the fiber under extremely mild thermal conditions [27–31]. The thickness of chemically bonded polymeric layer can be controlled by varying the dipping time and the concentrations of the solution ingredients. Higher film thickness can be achieved through repeated dipping operations. Sol–gel coating technology leads to a higher degree of flexibility in coating composition and selectivity. The surface coating obtained from sol–gel chemistry has a composite nature. The composition of this organic–inorganic coating can be controlled by varying the proportions of the sol solution ingredients, leading to much higher selectivity in SPME.

The PDMS coated fibers, prior to any conditioning, were treated with a solution of trimethylmethoxysilane in order to reduce the silanol contents of the coating. As the trimethylmethoxysilane molecules have a smaller molecular size than PMHS or hydroxyl-terminated PDMS, they should have greater access to the porous structure of the coating, providing added deactivation to sol–gel coating [27]. Detailed structures of surface-bonded sol–gel PDMS (deactivated by PMHS), Ucon, and PEG coatings are illustrated in Fig. 2.

The surface characteristics of the sol–gel coated fibers were investigated by scanning electron microscopy (SEM) technique. The SEM micrograph provided an estimated film thickness of 10 μm for the sol–gel PDMS, Ucon and PEG coatings. This thickness is almost ten times smaller than the

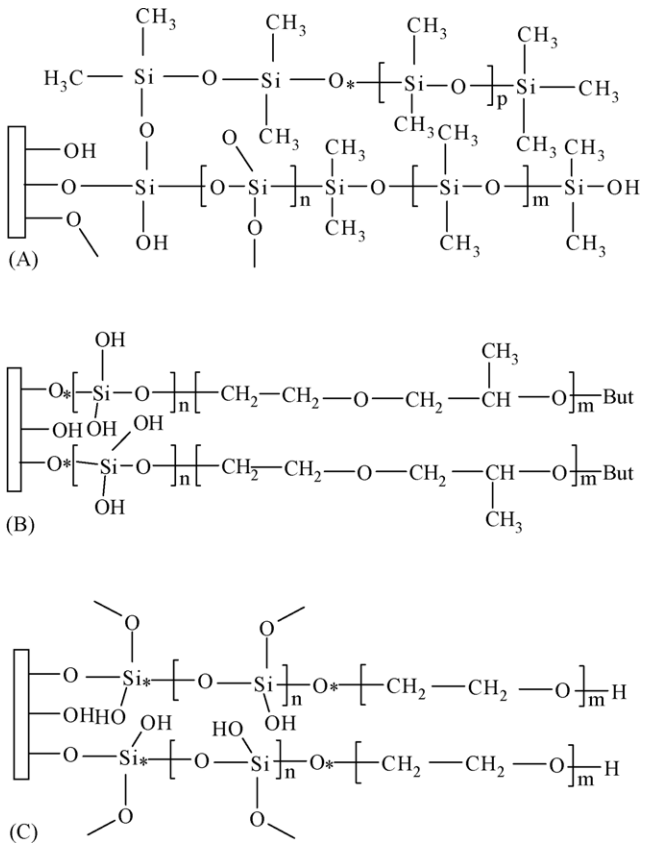

Fig. 2. Structures of surface-bonded sol–gel: (A) PDMS (deactivated by PMHS), (B) Ucon, and (C) PEG coatings.

conventional 100-μm coating thickness used on commercial fused-silica fibers. From this perspective, sol–gel coatings might have some disadvantageous for SPME. On the other hand, as it is seen from the SEM of the coated surface presented in Fig. 3, the sol–gel coatings possess porous structures, which should significantly increase the surface area availability on the fiber. Consequently, even an apparently thinner coating will be able to provide enhanced stationary-phase loading and, therefore, high fiber sample capacity. It should be also mentioned that even using the untreated fiber substrate with a diameter of 200 μm, providing higher stationary-phase loading, was not satisfactory. Higher surface area of the sol–gel coating should also provide enhanced extraction efficiency in SPME. The smaller coating thickness should help much faster mass transfer during extraction as well as analyte desorption process during sample introduction as will be seen in our results. Sol–gel coated PEG coating was not as porous as PDMS and Ucon coatings. This might be due to the facts that PEG 4000 is solid under ambient condition.

3.2. SPME optimization

In SPME, the rules based on the equilibrium between various phases can be employed. In a three-phase system, the equilibrium constants of analyte between each two phases

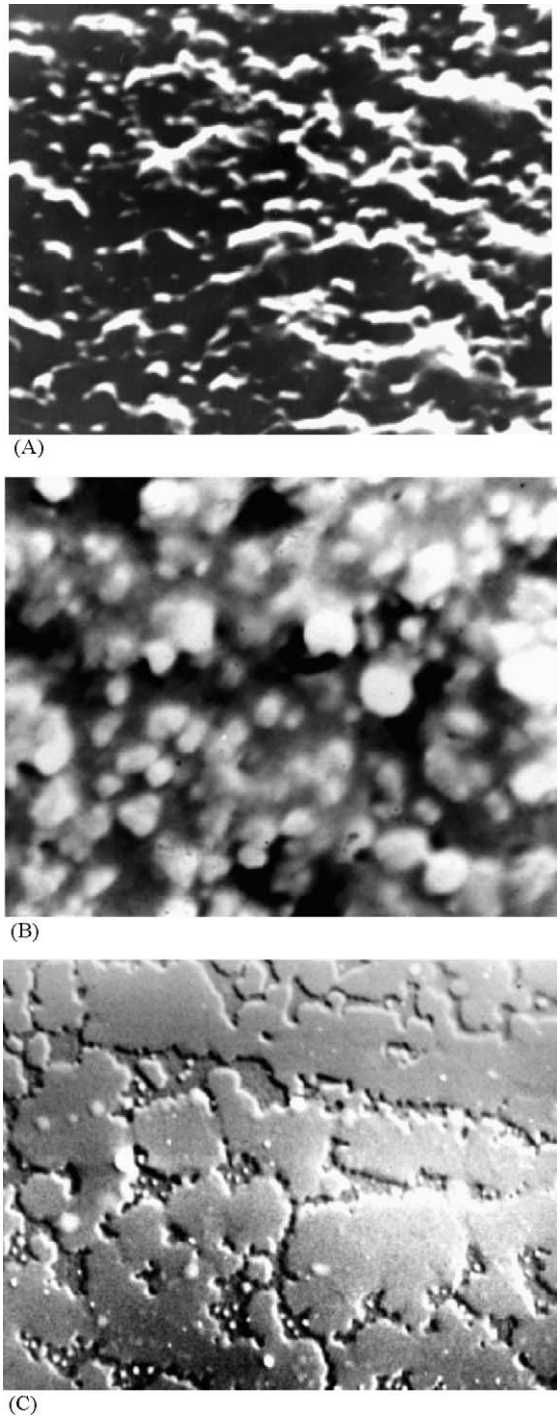

Fig. 3. Scanning electron micrograph of sol–gel coated fibers at 11,000-fold magnification (surface view), (A) PDMS, (B) Ucon, and (C) PEG.

can be written:

$$K_{fw} = \frac{C_f}{C_w}; \quad K_{hw} = \frac{C_h}{C_w}; \quad K_{fh} = \frac{C_f}{C_h} \tag{1}$$

where C_f is the equilibrium concentration of the analyte in the fiber coating; C_w, equilibrium concentration of the analyte in the aqueous medium; and C_h, equilibrium concentration of the analyte in the headspace. It is possible to replace the

molar concentrations by the number of analyte in the corresponding phase volume. Also, considering that n_f is the number of molecules in the fiber; n_w, number of molecules in the aqueous phase; and n_0, initial number of molecules in the aqueous phase and replacing their corresponding equilibrium constants and phase volumes, n_f can be written:

$$n_f = \frac{K_{fh}K_{hw}V_f n_0}{K_{fh}K_{hw}V_f + K_{hw}V_h + V_w} \quad (2)$$

where V_w is the volume of aqueous phase; V_f, volume of coating; and V_h, volume of headspace. Considering the Henry's Law constants, it would be possible to reach to the following equation.

$$n_f = \frac{K_{fw}V_f n_0}{K_{fw}V_f + K_{hw}V_h + V_w} \quad (3)$$

A linear relationship between n_f and n_0 can be derived from Eq. (3). However, due to the non-exhaustive nature of SPME, the maximum recovery of SPME in equilibrium, R_{max}, can be defined:

$$R_{max} = \frac{n_f}{n_0} = \frac{K_{fw}\ V_f}{K_{fw}V_f + K_{hw}V_h + V_w} \quad (4)$$

The equilibrium constants values in Eq. (4) are affected by temperature, addition of salt, pH and organic solvent and modifiers. SPME method often involves a number of stages. A univariate approach was employed to optimize influential factors, including fiber coating selection, extraction mode and conditions, agitation method, sample volume, extraction time, and desorption condition, in the developed method. In some aspects, prior knowledge with the procedure or analytical requirement will influence decisions to be made. In other cases, experimental work will be required to determine the most suitable condition [38–43].

3.2.1. Extraction mode

It was possible to use direct or headspace sampling for DM and DP analysis, however, headspace extraction was preferred. Firstly, it was important to analyze the free forms of these drugs if they would have adequate vapor pressure to penetrate into the headspace. Secondly, isolating the fiber coating from direct contact with the sample matrix would prevent and/or minimize adverse effect due to harsh sample conditions. As Fig. 1 shows, DM and DP have tertiary amine group on one side while on the other side, DM has a methoxy group whereas, DP has a hydroxyl group. Different conditions for headspace sampling of DM and DP are, therefore, required. Also, for headspace sampling, acidity of matrix should be different for analytes in order they are partitioned into headspace. Thus, for performing simultaneous extraction of both analytes in a single step, the derivatization of DP into a more volatile form was necessary.

Several methods for derivatization of phenolic groups have been reported [44–48]. In these methods anhydride acetic acid, benzoyl chloride and some of its derivatives such as, 2,4-dinitro benzoyl chloride, pentaflouro benzoyl chloride (PFBC) were used for derivatization of phenolic compounds and their determination were carried out using GC or HPLC. In some instances, DP was derivatized by PFBC and subsequently determined by GC-ECD by Salsali et al. [1]. Derivatization of DP using benzoyl chloride and 2,4-dinitro benzoyl chloride was not successful but the derivatization process was fully accomplished using PFBC and anhydride acetic acid. Both of these derivatzing agents were used in SPME sampling of DM and DP employing 1 ml of sample containing 100 μg/L of DP in alkaline condition. It should be noted that PFBC showed adverse effects on fiber coating, leading to an increased background. Due to the mild and rapid derivatization condition, anhydride acetic acid was preferred for this purpose. Mass spectrum of acetyl-derivative of DP (Fig. 4) confirms the derivatization process as it contains a base and molecular ion peak at *m*/*z* 299, which is due to the replacement of hydrogen in the hydroxyl group of DP ($C_{17}H_{22}NO$, *m*/*z* 257) by the acetyl group (–OC–CH_3, *m*/*z* 43).

3.2.2. Sample volume

Due to the nature of biological samples and considering Eqs. (3) and (4), a volume of 2 ml in a 4 ml vial was selected. This provided enough space for the fiber to be positioned in the headspace above the sample, without contacting the sample. Considerations were made to use the sample volume closely controlled at 2 ml/vial, and identical vials were used for all samples. The method optimization was performed by developing an extraction time profile based on following conditions: An amount of 0.5 ml plasma containing 2 μg DP and 1 μg DM, 1.5 ml solution containing 0.3 g potassium carbonate, 0.3 g sodium chloride, and 10 μl methanol were transferred to a silanized glass vial, then 15 μl anhydride acetic acid was added and stirred for 2 min in order to complete the derivatizatization of DP into its acetyl form. Since carbon dioxide was liberated during derivatization of DP, the vial was not sealed, completely. Samples were stirred at 100% efficiency of magnetic stirrer at 70 °C for different times ranging from 10 to 60 min. Sol–gel coated PDMS was used for extraction. Desorption was 5 min at 280 °C. The results obtained were used as an indication to assess improvements.

3.2.3. Temperature effect and equilibrium time

According to SPME theory the fiber equilibration process is an exothermic process and any increase in sampling temperature will decrease both analyte recovery and equilibrium extraction time [19–22]. The headspace/sample partition coefficient of the analyte increases with enhanced temperature, while at the same time, the fiber coating/headspace partition coefficient decreases. The first phenomenon leads to a higher concentration of the analyte in the headspace and the latter results in a lower equilibrium amount of analyte that the fiber coating is able to extract. Because the mass of analyte required to transfer from the headspace to the coating is smaller, a shorter equilibration time results. When sampling temperature are elevated, and the samples are equilibrated prior to the introduction of the fiber, the fiber coating extracts

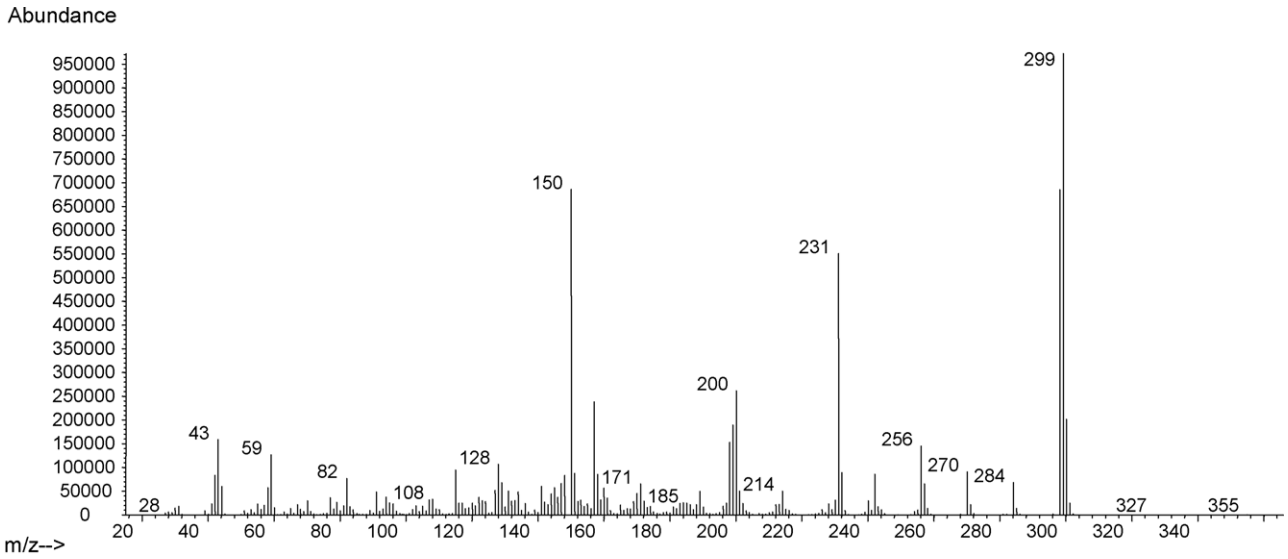

Fig. 4. Electron impact mass spectrum of acetyl-derivative of DP.

a smaller amount of analyte from a headspace containing a higher concentration of analyte and the amount of analyte required to move from the liquid sample to the headspace in order to satisfy the three-phase equilibrium balance is reduced. As the mass transfer of analyte from the liquid sample to the headspace is often the limiting factor in equilibration, the equilibrium extraction time will be further reduced with higher sampling temperatures.

However, comparing the extraction time profiles obtained at different temperatures (Fig. 5) reveals that shorter extraction times and higher sampling temperatures will increase the speed of equilibrium in comparison with those of lower sampling temperatures, having a non-equilibrium status. At 40–50 °C, neither compound was extracted at equilibrium levels. At temperatures between 60 and 90 °C, equilibrium extraction is achieved in 30 min. A temperature of 60 °C was, therefore, selected as the extraction temperature for future work, as it gave a higher recovery level. Sampling under equilibrium condition is preferred as extractions performed under non-equilibrium conditions suffer from poor precision. Under the extraction conditions selected, the equilibrium extraction time has been reduced to a practical level.

3.2.4. Selection of fiber coating

Among the sol–gel coated PDMS, Ucon and PEG SPME fibers, the PEG coated fiber showed insufficient extraction recovery, which could be due to the nonporous structure of polymeric coating (Fig. 3). The sol–gel coated Ucon exhibited slightly higher recoveries than PDMS, but it did not reach the equilibrium in the 30-min time experiment.

As is known, conventionally coated PDMS fibers do not show sufficient selectivity for polar compounds. The difference between PDMS coatings (conventionally and sol–gel coated) can be explained by comparing the compositional differences between these two types of coatings. The conventional PDMS coating contains a thick film of PDMS material, which is inherently non-polar. Normally, this non-polar coating has less affinity toward polar compounds. The situation is significantly different for sol–gel coated PDMS coatings. Firstly, individual molecules of hydroxy-terminated PDMS used for sol–gel coating contain terminal silanol groups that

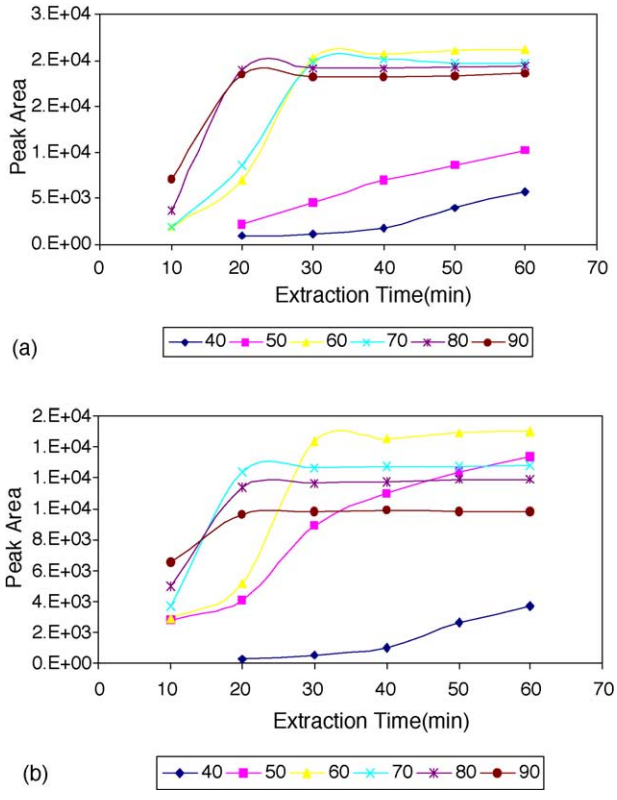

Fig. 5. Temperature effect on absorption time profile, analyte recovery and equilibration time. Condition was as described in Section 3.2.3. (a) DM and (b) acetyl-derivative of DP.

are absent in PDMS molecules used for conventional coatings. These hydroxyl groups are meant for chemical bonding of the polymer to the sol–gel network through condensation reaction. Such a chemical bonding requires only one hydroxyl group per molecule. So the second terminal hydroxyl group might be free, at least for some of the bonded PDMS molecules. The presence of such a hydroxyl group will make the coating more polar compared with analogous coatings used on conventionally coated PDMS fibers. Secondly, the sol–gel PDMS coating is an organic–inorganic composite material in which the hydroxyl-terminated PDMS molecules are attached to the polar silica network through chemical bonding. The polarity of inorganic and organic components of the composite sol–gel PDMS coating makes it suitable for extraction of polar compounds. The sol–gel PDMS fiber was, therefore, used for the further experiments and the other two coatings were excluded from further investigations.

3.2.5. *Effect of agitation*

Usually, sample agitation enhances extraction efficiency and reduces extraction time. For the purpose of the present study various stirring efficiency of magnetic stirrer, from 10 to 100% of maximum efficiency was performed. This was accomplished using the method described in Section 3.2.3 and a 60 °C sampling temperature and 30 min sampling time. Our results showed that the maximum responses for analytes were obtained at above 70% of maximum stirring efficiency. The relative standard deviation (%RSD) values for this section was ranged from 10 to 15%.

3.2.6. *Effect of pH and selection of base*

Due to the higher polarity of DP, its conversion to more volatile derivative by anhydride acetic acid was necessary. Derivatization of phenolic groups with anhydride acetic acid usually needs to be carried out under basic condition and that is why potassium carbonate is usually used. Also, as Fig. 1 shows, DM should be extracted under alkaline condition. Addition of potassium carbonate could adjust the pH value in the range of 11.5–12, which led to satisfactory extraction efficiency. This might be due to the pH and ionic strength effects caused by this divalent species. In order to check the whole alkaline pH range, sodium hydroxide, in the absence of potassium carbonate, was used. A decrease in extraction efficiency of both analytes was observed for the alkaline pH ranges. Effect of potassium bicarbonate was also examined to obtain a rather lower pH, ranging from 8 to 9, but the results were poorer in comparison with the data obtained when potassium carbonate was used. Potassium carbonate was, therefore, selected for the pH adjustment because a buffered solution can be formed while, due to its doubly charged ionic structures, it can have greater ionic strength as well. It must be noted that for SPME of biological fluids, the extraction solution should be buffered by appropriate buffer because from sample to sample pH is different, and potassium carbonate is one of the best choices. Effect of potassium carbonate quantity on extraction efficiency was also tested using a range of 0.1–0.8 g

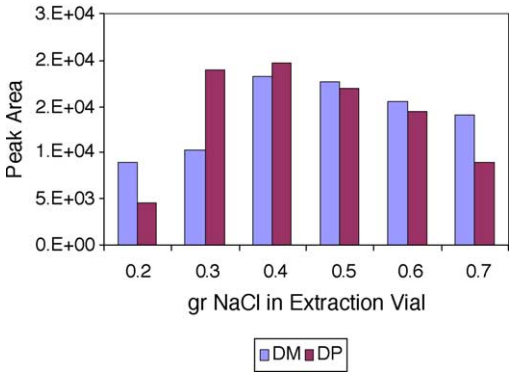

Fig. 6. Influence of sodium chloride quantities on extraction efficiencies.

of potassium carbonate per vial. The results (data not shown) showed that using 0.3 g of potassium carbonate per vial, initially was chosen, results the maximum efficiency. Due to the medium polarity of DM and acetyl-derivative of DP, excess amount of potassium carbonate will increase the ionic strength of solution causing a reduction in analytes volatility and extraction efficiency.

3.2.7. *Effect of sodium chloride*

The influence of salt addition on the efficiency of SPME was also investigated. Usually, the presence of salt increases the ionic strength of aqueous solution and would affect the solubility of organic solutes. This can be explained by the engagement of water molecules in the hydration spheres around the ionic salt. These hydration spheres reduce the concentration of water available to dissolve solute molecules. This should, then, drive additional solutes into a non-polar sorbent or extractant. In this experiment, sodium chloride concentrations of 0.2–0.7 g/vial were tested. Other conditions were as used for Fig. 5 except that extractions were performed at 60 °C for 30 min. An increase in extraction efficiency was observed (Fig. 6) by adding sodium chloride and the efficiency started to decrease when amounts higher than 0.4 g of NaCl/vial were employed. An amount of 0.4 g of NaCl/vial was, therefore, used the optimum quantity. DM and acetyl-derivative of DP are not completely polar in character and ex-

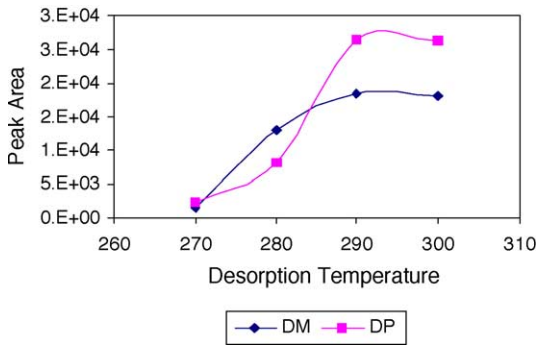

Fig. 7. Effect of desorption temperature on extraction efficiency; conditions were as described in the text.

cess amount of sodium chloride will increase ionic strength resulting a decrease in analytes volatility and extraction efficiency. The %RSD values for this section ranged from 8 to 12%.

3.2.8. Effect of organic modifier on extraction efficiency

The use of organic additives has been recommended for matrices with polymer components such as plasma. It is suggested that the binding of target analyte to the proteins can be decreased and, therefore, the sensitivity of the method can be substantially increased. Four modifiers including methanol, ethanol, acetone and acetonitrile (10 μl) were tested in our work. Results showed an increase of extraction efficiency when methanol was used as organic modifier. An amount of 5 μl of methanol led to higher extraction efficiency. The %RSD values for this section were ranged from 8 to 10%.

3.2.9. Effect of desorpton temperature and time on extraction efficiency

It is quite important to keep the time interval required for the desorption as short as possible and carryover effects must be avoided. Thus, using the highest possible temperature without damaging the fiber coating and the smallest diameter of the injector insert should be applied. This is due to the fact that partition coefficient between fiber coating and headspace is decreased with increased temperature, and the linear flow-rate is increased with smaller diameter of the insert. In all cases an insert with diameter of 0.75 mm was, therefore, used for SPME-GC-MS. Fig. 7 shows the effect of desorption temperature on extraction efficiency, condition was as described in Section 3.2.3 with the exceptions that sodium chloride was 0.4 g, extra amount of methanol was 5 μl and stirring rate was 70% of maximum stirring efficiency. Clearly, the maximum efficiency is obtained at 290 °C. Also, desorption time was optimized and after each desorption process, carry over effect was evaluated. A desorption temperature of 290 °C for 5 min was, finally, selected.

3.3. Method validation

Choosing a suitable separation and detection technique depends on many parameters [49]. In this work, GC-MS was chosen as separation and detection technique because it is robust, rapid, highly sensitive and selective. In addition, cou-

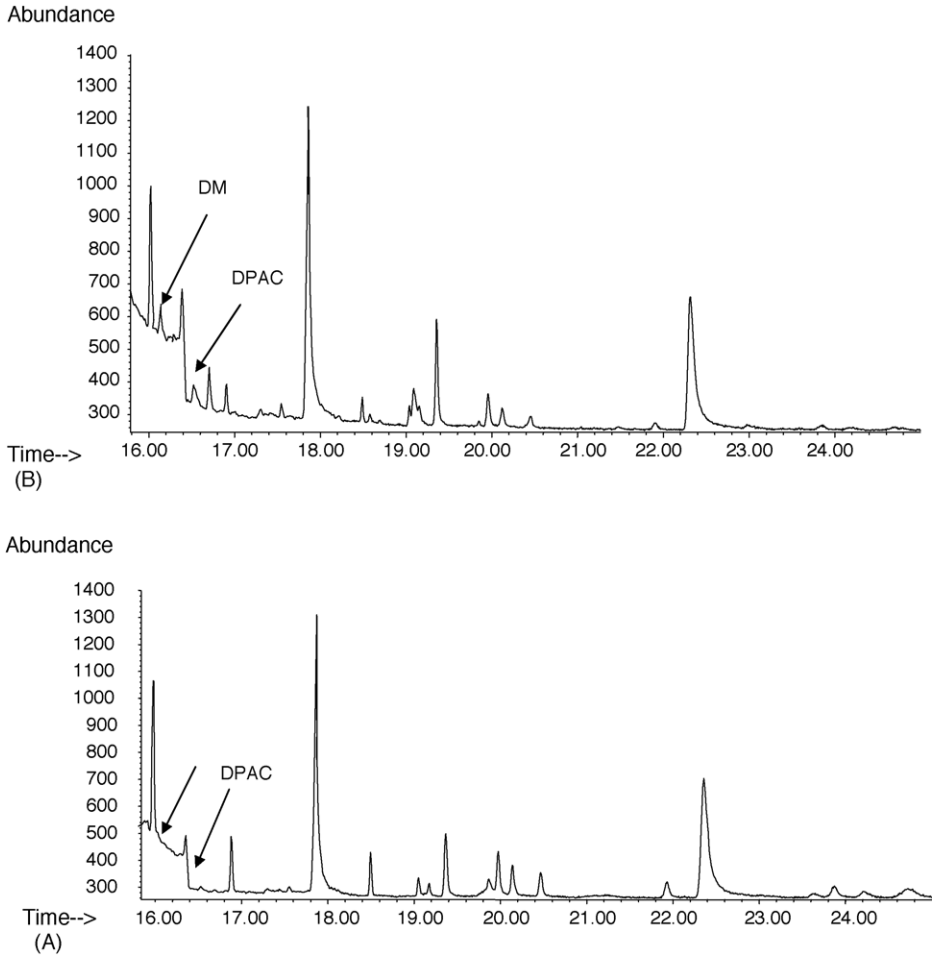

Fig. 8. Mass chromatograms obtained after SPME of (A) blank human plasma, and (B) plasma spiked with DM and DP (0.05 ng/ml), conditions were as described in Section 3.3. DP was derivatized into its aceryl form.

Table 1
Regression equations, correlation coefficients, accuracy, intra- and inter-day precision obtained after SPME-GC-MS determination of DM and DP in plasma samples

Analyte	Concentration (ng/ml)	Regression equation	r^2	Accuracy (%)	Intra-day R.S.D. (%) ($n = 5$)	Inter-day R.S.D. (%) ($n = 15$)
DM	10	$y = 28.759x + 3887.8$	0.999	104.5	4.49	4.57
	50			99.86	4.23	4.51
	500			100.2	3.54	3.63
DP	10	$y = 21.689x + 318.51$	0.994	103.8	4.81	4.98
	50			99.94	4.58	5.04
	500			100.7	3.29	3.38

pling of SPME to GC-MS is rather convenient and there is no need to any particular interface in comparison with HPLC.

Preliminary works were carried out using the GC-MS in the full scan mode to check the retention times of analytes and any other possible co-extracted compounds. However, no interferences from co-extracted compounds were observed and eventually the GC-MS was operated in the SIM mode to enhance the sensitivity.

The optimized method was examined for the extraction and determination of DM and DP in plasma samples. The SPME-GC-MS analysis was performed using a 30 min extraction time at 60 °C, samples were stirred at 70% of maximum stirring efficiency, sodium chloride was 0.4 g/vial, 5 μl extra amount of methanol was also added to the vial and desorption temperature was 290 °C for 5 min. A typical chromatogram obtained under these conditions is shown in Fig. 8. Plasma samples were spiked with different concentrations of DM and DP solutions, in a way that, final concentration of DM and DP in vials were in the range of 1 pg/ml up to 4 μg/ml. After extraction and GC-MS analysis, calibration curves for DM and DP were plotted. A linearity range of 0.03 ng/ml to 2 μg/ml for DM and 0.05 ng/ml to 2 μg/ml for DP was obtained. The correlation coefficient was satisfactory ($r^2 > 0.99$) for both analytes. Detection limits, based on a signal-to-noise ratio of $S/N = 3$, were at 0.01 ng/ml and 0.015 ng/ml for DM and DP, respectively. Intra- and inter-day precision were evaluated by spiking plasma samples with three different amounts of each analyte and extracting them five times in a day and three different days. As Table 1 shows the R.S.D. values for both analytes are less than 5%. Clearly, the proposed method can be easily used for the analysis of real clinical samples because; method optimization was carried out using real plasma samples.

4. Conclusion

In this work, three different sol–gel based polymers were synthesized and, for the first time, applied as the SPME fiber coatings for extraction of semi volatile drugs such as DM and DP from plasma samples followed by their determinations by GC-MS. Since the invention of SPME, variety of commercial fibers have been employed for analysis of different drugs, however, these fibers exhibit carryover effects due to the limitation of relatively lower desorption temperature, and lower lifetimes, while pretreatment of samples are almost essential prior to the extraction process [38–43]. Sol–gel chemistry offers a simple and convenient methodology for the production of advanced material systems with desired structure, composition, and properties. Both polar and non-polar surface-bonded coatings can be made using this technology. Because of chemical bonding between sol–gel coatings and fused-silica fibers, sol–gel coated fibers exhibit higher thermal stability. Enhanced thermal stability of sol–gel coated fibers allowed us to analyze DM and its metabolite, under higher temperature and strong basic conditions. Sol–gel coatings possess a porous structure and reduced coating thickness that provide enhanced extraction and mass transfer rates in SPME. High-temperature conditioning of sol–gel-coated PDMS fibers leads to consistent improvement in peak area reproducibility. Influential parameters such as fiber coating type, extraction mode, agitation method, sample volume, extraction time, and desorption condition, were investigated and optimized. Among different agents tested for derivatization of DP, for the first time, anhydride acetic acid was successfully applied and the converting reaction was proceed under a gentle condition within 2 min. The proposed method provides a rather easy, simple, rapid and inexpensive SPME method for the determination of DM and its metabolite DP with sufficient sensitivity and reproducibility. The use of automated SPME would definitely increase the speed of the method to be comparable with the dilute and shoot method previously developed for LCMS/MS [2,10,11]. Due to the use of head space sampling the matrix effect from biological samples are minimized and the method can be used easily for real clinical plasma samples as well as pharmacokinetic studies.

Acknowledgment

Financial support for this research was provided by the Research Council of Sharif University of Technology (SUT).

References

[1] M. Salsali, R.T. Coutts, G.B. Baker, J. Pharmacol. Toxicol. 41 (1999) 143.
[2] S.S. Vengurlekar, J. Heitkamp, F. McCush, P.R. Velagaleti, J.H. Brisson, S.L. Bramer, J. Pharm. Biomed. Anal. 30 (2002) 113.

[3] Y.H. Park, M.P. Kullberg, O.N. Hinsvark, J. Pharm. Sci. 73 (1984) 24.
[4] J.M. Hoskins, G.M. Shenfield, A.S. Gross, J. Chromatogr. B 696 (1997) 81.
[5] H.P. Hendrickson, J. Bill, W.D. Gurley, Wessinger, J. Chromatogr. B 788 (2003) 261.
[6] E. Bendriss, N. Markoglu, I.W. Wainer, J. Chromatogr. B 754 (2001) 209.
[7] D.R. Jones, J.C. Gorski, M.A. Hamman, S.D. Hall, J. Chromatogr. B 678 (1996) 105.
[8] M. Afshar, M.R. Rouini, M. Amini, J. Chromatogr. B 802 (2004) 317.
[9] B. Mistry, J. Leslie, N.E. Edington, J. Pharm. Biomed. Anal. 16 (1998) 1041.
[10] T.H. Eichhold, M. Quijano, W.L. Seibel, K.R. Wehmeyer, J. Chromatogr. B 698 (1997) 147.
[11] D.L. Mccauley-Myers, T.H. Eichhold, S.H. Hoke II, J. Pharm. Biomed. Anal. 23 (2000) 825.
[12] R.D. Bolden, S.H. Hoke II, T.H. Eichhold, K.R. Wehmeyer, J. Chromatogr. B 772 (2002) 1.
[13] M. Furlanut, L. Cima, P. Benetello, P. Giusti, J. Chromatogr. 140 (1977) 270.
[14] Y.J. Wu, Y.Y. Cheng, S. Zeng, M.M. Ma, J. Chromatogr. B 784 (2003) 219.
[15] H.T. Kristensen, J. Pharm. Biomed. Anal. 18 (1998) 827.
[16] M.R. Gomez, R.A. Olsina, L.D. Martinez, M.F. Silva, J. Pharm. Biomed. Anal. 30 (2002) 791.
[17] S.P. Singh, D.E. Moody, J. Pharm. Biomed. Anal. 13 (1995) 1027.
[18] R.P. Berladi, J. Pawliszyn, Water Pollut. Res. J. Can. 24 (1989) 179.
[19] J. Pawliszyn, Solid Phase Microextraction, Theory and Practice, J. Wiley and Sons, New York, 1997.
[20] J. Pawliszyn, Application of Solid Phase Microextraction, Royal Society of Chemistry, Cambridge, 1999.
[21] Z. Zhang, J. Pawliszyn, Anal. Chem. 65 (1993) 1843.
[22] Z. Zhao, A. Malik, M.L. Lee, Anal. Chem. 65 (1993) 2747.
[23] H.B. Wan, H. Chi, M.K. Wong, C. Yew Mok, Anal. Chim. Acta 298 (1994) 219.
[24] W. Chang, Y. Sung, S. Hung, Anal. Chim. Acta 495 (2003) 109.
[25] H. Bagheri, A. Salemi, Chromatographia 59 (2004) 501.
[26] Manufacturer Data Sheet, Supelco Corp., Bellefonte, PA, 1996.
[27] S.L. Chong, D. Wang, J.D. Hayes, B.W. Wilhite, A. Malik, Anal. Chem. 69 (1997) 3889.
[28] L.L. Hench, J.K. West, Chem. Rev. 90 (1990) 33.
[29] D. Wang, S.L. Chong, A. Malik, Anal. Chem. 69 (1997) 4566.
[30] Z. Wang, C. Xiao, C. Wu, H. Han, J. Chromatogr. A 893 (2000) 157.
[31] J.J.D. Hayes, A. Malik, J. Chromatogr. B 695 (1997) 3.
[32] H. Bagheri, A. Mohammadi, A. Salemi, Anal. Chim. Acta 513 (2004) 445.
[33] H. Bagheri, M. Saraji, D. Barceló, Chromatographia 59 (2004) 283.
[34] H. Bagheri, A. Saber, S.R. Mousavi, J. Chromatogr. A 1046 (2004) 27.
[35] Y. Liu, M.L. Lee, K.J. Hageman, Y. Yang, S.B. Hawthorne, Anal. Chem. 69 (1997) 5001.
[36] K. Jinno, T. Muramatsu, J. Pawliszyn, J. Chromatogr. A 754 (1996) 137.
[37] H. Kataoka, H.L. Lord, J. Pawliszyn, J. Chromatogr. B 731 (1999) 353.
[38] L. Heather, J. Lord, Pawliszyn, Anal. Chem. 69 (1997) 3899.
[39] S. Ulrich, J. Chromatogr. A 902 (2000) 167.
[40] U. Staerk, W.R. Kulpmann, J. Chromatogr. B 745 (2000) 399.
[41] N.H. Snow, J. Chromatogr. A 885 (2000) 445.
[42] G.A. Mills, V. Walker, J. Chromatogr. A 902 (2000) 267.
[43] T. Kumazawa, X. Lee, K. Sato, O. Suzuki, Anal. Chim. Acta 492 (2003) 49.
[44] M. Llompart, M. Lourido, P. Landýn, C. Garcýa-Jares, R. Cela, J. Chromatogr. A 963 (2002) 137.
[45] P.A. Vollmer, D.C. Harty, R.A. Dean, J. Chromatogr. B 685 (1996) 370.
[46] H. Bagheri, M. Saraji, J. Chromatogr. A 910 (2001) 87.
[47] H. Bagheri, M. Saraji, J. Chromatogr. A 986 (2003) 111.
[48] H. Bagheri, A. Mohammadi, J. Chromatogr. A 1015 (2003) 23.
[49] K.K. Stewart, R.E. Ebel, Chemical Measurements in Biological Systems, J. Wiley and Sons, New York, 2000.