Ultrasonic assisted SPME Coupled with GC and GC–MS using Pencil Lead as a Fiber for Monitoring the Organic Volatile Impurities of Ceftazidime

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Abstract

A simple, rapid, non-destructive, and *in-situ* method for the isolation and sampling of organic volatile impurities in Ceftazidime is developed using solid-phase microextraction (SPME). For the monitoring of the extracted compounds, gas chromatography (GC) and GC–mass spectrometry analyses are used. The effective factors such as nature of the fiber, SPME mode, extraction temperature, and ultrasonic assistance have been investigated and detailed here. Qualification studies reveal the existence of pyridine (as a degradation product of ceftazidime) and the residual solvents; acetone, methylene-chloride, and diethylamine are the main impurities in the studied pharmaceutical. External standard method is used for quantitative analysis. The % relative standard deviation values are below 10%, and the limits of detection for the detected solvents are 1.06, 0.98, 0.83, and 0.51 ppm, respectively. The proposed method is both accurate and linear and could be used in quality control of ceftazidime and also its stability investigations.

Introduction

Using organic solvents is essential in many steps of pharmaceutical processes. A typical drug synthesis route consists of three to eight reaction steps and four or more different solvents may be employed in these steps (1). The solvents are not completely removed by practical manufacturing techniques such as freeze-drying and drying at high temperature under vacuum, and their traces may remain in the final products (2). The presence of these unwanted and toxic chemicals even in small amounts may influence the efficacy, safety and stability of the pharmaceutical products (1). Typically, the final purification step in many pharmaceutical drug-substance processes involve a crystallization step and the crystals thus formed can entrap a finite amount of solvent from the mother liquor that may cause degradation of drug (3). The total amount of these compounds is called organic volatile impurities (OVI).

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Drug manufactures are strongly required to monitor and limit the presence of OVI in their products (4). If the residual solvents in pharmaceutical products occur at levels higher than that can be supported by safety data, there may be harm to the human body or to the environment. Thus, determination of residual solvents receives a great deal of attention (5). The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) formulated guidelines for residual solvents control on 17 July 1997 (4,5). This guideline classifies residual solvents in four classes in terms of their level of hazard to humans and the environment, and to regulate the level of each solvent. Currently, residual solvent determination has been adopted by pharmacopoeias. The guideline has been accepted by the European Pharmacopoeia, Japanese Pharmacopoeia, the United States Pharmacopoeia, and the Chinese Pharmacopoeia (5).

The most commonly used method for the analysis of residual solvents is conventional gas chromatography (GC). Drug powder commonly dissolves in HCl or NaOH and then a certain volume of solution is injected into the GC. To achieve acceptable detectability, large amounts of the product need to be injected, which generally leads to clogging of the injector and requires frequent changes of the insert glass (6). Also, less volatile compounds or dissolution media would remain on the column, which could reduce the lifetime of the column and interfere with the subsequent analyses (7). Other approaches for the quantitation of residual solvents in pharmaceutical matrices include static headspace (HS), purge and trap followed by GC analysis. However, these methods are rather time consuming and are not within the time frame of fast separation and analysis (8). Most recently, solid-phase microextraction (SPME) has gained popularity for determination of OVI in pharmaceutical compounds (6,8,9). SPME is a solvent-free technique for the extraction of analytes from different matrices (10–15). In SPME, a 1–2 cm long and ~0.2 mm diameter fiber is exposed to the sample by its immersion into sample solution or headspace of the sample. Once equilibrium is established between the sample and the fiber, the extracted analytes are thermally desorbed by exposing the fiber in the injection port of the GC.

In this work, an effective analysis of organic volatile impurities in Ceftazidime using SPME coupled with GC and GC–MS was performed. Four residual solvents: acetone, pyridine, methylene-chloride, and diethylamine were identified by GC–MS. We have evaluated the efficiency of different SPME fibers even modified pencil-lead fiber in extraction of these solvents. The effects of some other experimental factors, such as extraction time and desorption temperature and time and ultrasonic-assistance were investigated.

Experimental

Chemicals and reagents

Ceftazidime was sampled from a batch of industrial production. Pyridine, acetone, diethylamine, methylene chloride, and all other chemicals were purchased from E. Merck (Darmstadt, Germany) and were all of analytical grade. SPME syringe, polydimethylsiloxane (PDMS) 100 µm, and polyacrylate (PA) 85 µm fibers were from Supelco (Dorset, UK). HB pencil-lead was purchased from Rotring Company (Hamburg, Germany).

Apparatus

Monitoring of the analytes was performed using a GC (PerkinElmer Auto system XL, Waltham), equipped with an FID. Recognition of volatile impurities was performed by Varian GC (model 3200, Palo Alto) coupled to a mass spectrometer (model 2000, Varian). Extraction of the analyte was performed from a vial sealed with a silicone-rubber septum cap (Supelco). Samples were agitated vigorously for 1 min and then subjected to SPME.

Preparation of pharmaceutical samples

Blank powder

In order to prepare a blank sample, it was necessary to remove all residual solvents from the drug matrix. Due to considerable instability of ceftazidime, cefalexin powder was chosen as matrix, which is from cephalosporins family and has enough stability. For this purpose, 1 g of cefalexin was homogenously scattered in the watch glass and placed into a dark oven at room temperature for 24 h. A portion of this drug was transferred into 10-mL sampling vial sealed with a silicone-rubber septum cap.

Standard powders

For the preparation of standard pharmaceuticals containing 2.5, 5, 10, 20, 40, 60, 80, and 100 µg/g of each solvent, appropriate volume of spiking solvents were introduced into blank pharmaceutical powder. The samples were shaken carefully to homogenize and stored at 4°C.

Standard solutions

1 g of standards or real sample were dissolved in 5 mL of water and transferred into a 10-mL sampling vial separately.

Real sample

Ceftazidime produced by a pharmaceutical industry in Iran was selected as a real sample. The extraction and GC or GC–MS analyses were performed directly from commercial vials of ceftazidime.

Extraction of residual solvents

SPME fiber was introduced into a sample vial, via silicone-rubber septum cap and exposed to headspace of the vial containing the desired samples. The vial and fiber were placed in ultrasonic bath for 15 min at room temperature, so that only the sample was exposed to the ultrasonic field. After extraction, the fiber was introduced into the GC injector.

Modification of pencil-lead fibers

Commercial pencil-leads were modified as described in a previous paper (17). The main step in this process is carbonization to give high adsorption capacity, which is accomplished by heating for 60 min in the presence of water vapor in the furnace. Before using, they were conditioned inside the GC injection port for 15 min at 260°C.

GC operating conditions

Analytes were separated using a PTE-5 column (Supelco) 30 m × 0.25 mm i.d., with a film thickness of 0.25 µm. The column temperature was held at 35°C for 4 min, then programmed at 40°C/min to 240°C and held for 2 min. Analyte desorption from fiber (injection) was performed in a splitless mode at a temperature of 260°C for 1 min. Detector temperature was optimized at 250°C. The helium velocity as carrier gas was 2 mL/min, and the make-up gas flow was 30 mL/min.

GC–MS operating conditions

The GC–ion trap mass spectrometer (GC–MS) used for the

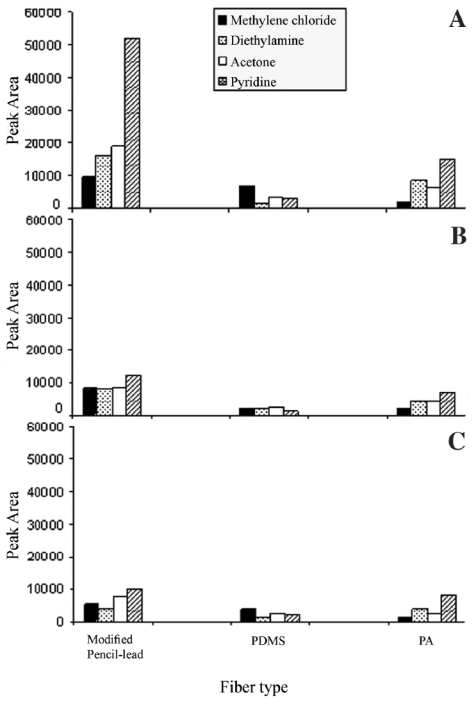

Figure 1. Comparison of the efficiency of different SPME fibers for extraction of the studied compounds from: headspace of powder standard (A), headspace of standard solution (B), and standard solution (C). The sample weight was 1 g, the concentration of each solvent was 5 ppm, SPME was performed for 15 min at room temperature. Compounds were desorbed by split/splitless injection (splitless desorption for 1 min) at an injector temperature of 260°C.

identification of residual solvents was a Varian-Saturn 2200 system. The chromatographic column used for GC–MS was CP-Sil5-CB, 30 m × 0.25 mm i.d. (Chrompack, Palo Alto, CA). The ion trap temperature was set at 220°C, the manifold and transfer line temperatures were 120°C and 160°C, respectively. All other chromatographic conditions were the same as the GC conditions explained in the "GC operating conditions" section.

Results and Discussion

Selection of extraction and sampling method

To prevent any external contamination of the pharmaceutical samples and also entrance of undesired compounds in GC injector port and capillary column, headspace sampling either by Hamilton syringe or SPME was employed in this work. To investigate the efficiency of each of them, two identical standard samples containing 5 µg/g of each compound were prepared. Sampling and injection were performed in the optimum conditions through SPME and Hamilton syringe (10 µL from headspace). Extraction efficiencies and method repeatability were presented in Table I by means of chromatographic peak area of each compound. These results reveal that, the SPME method leads to a better extraction efficiency and repeatability.

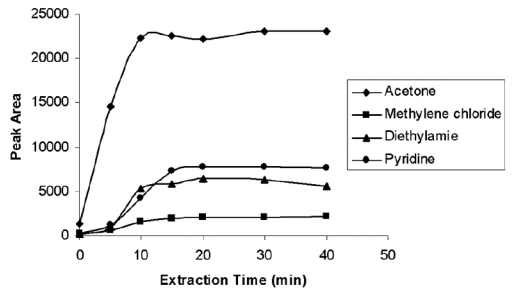

Figure 2. Extraction-time profiles of studied compounds. The experimental conditions were as given for Figure 1A.

Selection of optimum SPME mode and fiber

SPME procedures were performed from standard samples containing 5 µg/g of each compound, using three types of fibers: polydimethylsiloxane (PDMS) 100 µm, polyacrylate (PA) 85 µm, and modified pencil-lead at room temperature for 15 min from: (*i*) headspace of standard cefalexin powder; (*ii*) headspace of aqueous solution of cefalexin, and (*iii*) direct immersion of the fibers into aqueous solution. The SPME fiber was withdrawn from extraction vial and introduced immediately into the GC or GC–MS injector port for temperature desorption and analyses. The results obtained are illustrated in Figure 1 by means of chromatographic peak area. From these results, SPME using modified pencil-lead promises higher extraction efficiency regardless of extraction mode. On the other hand, the extraction efficiency of all the studied solvents from headspace of standard powder is far better than the solutions. Consequently, we have chosen modified pencil-lead as SPME fiber and extraction from headspace of pharmaceutical powder for further investigations.

Optimization of extraction time

Extraction-time profiles of the studied compounds were obtained using pencil-lead fiber from headspace of standard powders containing 5 µg/g of each compound. All other conditions were the same as those described in the Experimental section. The results are given in Figure 2. From these results, after exposure of fiber on the headspace of standard powder for 10 min, extraction-time profiles reach a plateau for acetone, methylene chloride, and diethylamine, whereas for pyridine with less volatility, equilibrium state was reached after 15 min. Consequently, 15 min was chosen for unifying the procedure because the equilibrium was occurred for all the studied solvents after this period.

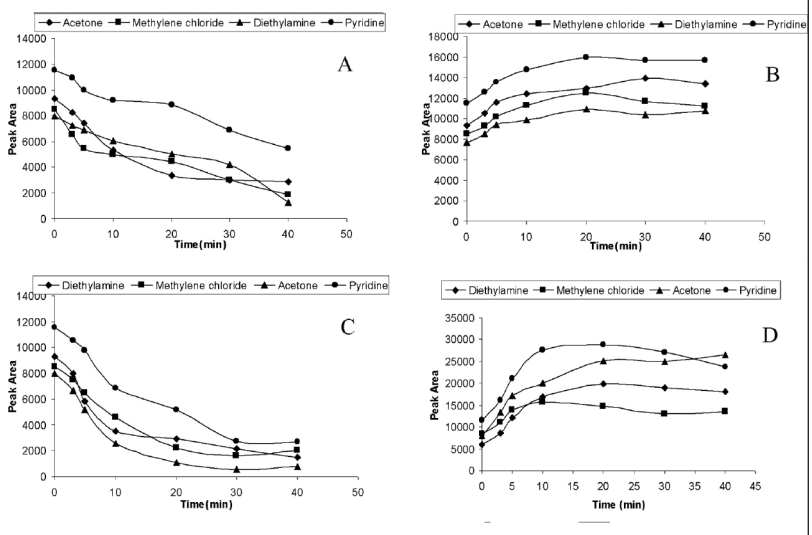

Figure 3. Effect of ultrasound field on the extraction efficiency of the studied compounds were studied at different modes; Direct SPME from standard solution (A), Headspace SPME from standard solution (B), Headspace SPME from standard powder which sample and fiber were exposed on ultrasonic field (C), and Headspace SPME from standard powder which only the sample was exposed on ultrasonic field (D). The experimental conditions were as given for Figure 1A.

Effect of ultrasonic field

We have investigated the effect of ultrasonic waves on the extraction efficiency of the analytes. SPME procedures were performed at room temperature for 0–40 min from standard sample of cefalexin containing 5 µg/g of each compound (solvent) in different modes: (*i*) immersion SPME from standard solution of cefalexin. Exposing of sample and fiber to ultrasonic field; (*ii*) HS-SPME from standard solution of cefalexin. Exposing of sample to ultrasonic field, while the fiber is away; (*iii*) HS-SPME from standard powder of cefalexin. Exposing of sample and fiber to ultrasonic field. (*iv*) HS-SPME from standard powder of cefalexin. Exposing of sample to ultrasonic field, while the fiber is away.

Extracted compounds were monitored by GC and the extraction efficiencies were measured by means of chromatographic peak area and are represented in Figure 3. From these results, when

both sample and SPME fiber are exposed in ultrasonic field, the extraction efficiency decreases by increasing the extraction time regardless of the sample character (Figure 3A and 3C), whereas when the fiber is out of ultrasonic field, extraction efficiency increases by extraction time for the samples either in aqueous solution or powder forms (Figure 3B and 3D). Comparison of these figures reveals that the ultrasonic field releases the studied compounds from either aqueous sample solution or powder samples. However, ultrasound can prevent most effectively the adsorption of analytes on the surface of SPME fiber. Consequently, for the further experiments, ultrasonic assisted SPME from powder standard or real sample was performed when the fiber was keep out of ultrasonic field (mode D).

Table I. Comparison of HS-SPME and Direct Headspace Sampling Ability for the Extraction of OVI from Powder Standard of Ceftazidime*

Compounds (Solvents)	Chromatographic peak area (n = 3)	
	HS-SPME	HS-Direct Injection
Acetone	8225 ± 309	5251 ± 470
Pyridine	17401 ± 180	7784 ± 420
Diethylamine	7824 ± 340	6578 ± 390
Methylene chloride	4352 ± 210	4478 ± 440

* Experimental conditions were as given for Fiure 1A.

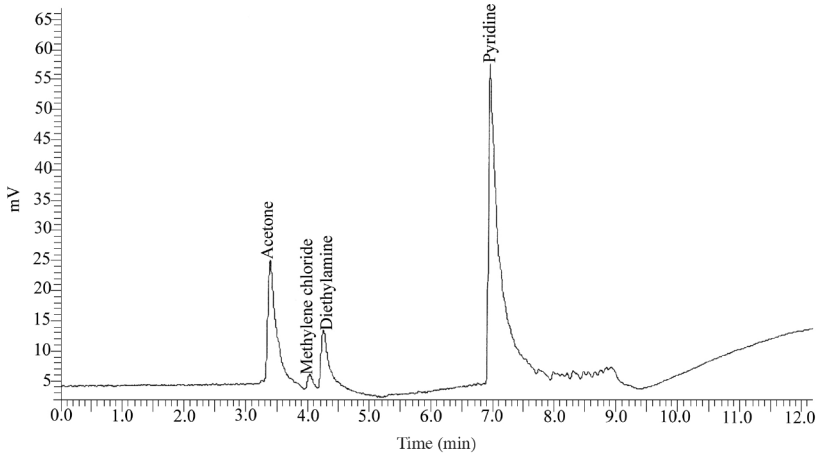

Figure 4. Typical chromatogram obtained after extraction using pencil-lead as SPME fiber. The experimental conditions were as given for Figure 1A.

Analytical approach

Figure 4 shows a typical gas chromatogram obtained under optimized extraction and chromatographic conditions from HS-SPME of 1 g cefalexin powder. Characteristic quantitation data for triplicate analyses at eight levels over a range of 2.5–100 ppm for all of the studied compounds, including reproducibility, limits of detection, dynamic range, limit of quantitataion (calculated as three times the LOD value), correlation coefficient of calibration curves, and relative standard deviations obtained by using the proposed fiber are presented in Table II.

Limit of detection

The limit of detection (LOD) defined as the lowest concentration of analyte that can be detected is estimated as three times the signal to noise ratio. The LODs obtained for the compounds studied are in the range of 0.51–1.06, which shows the ability of the developed SPME-GC method to perform simultaneous extraction and analysis of the four solvents.

Linearity

Linear calibration plots were obtained over the concentration ranges tested. For all analytes, good linearity was observed and the correlation coefficients (r) for acetone, pyridine, methylene chloride, and diethylamine were 0.996, 0.998, 0.997, and 0.997, respectively.

Precision

The precision of the method for triplicate analyses is also presented in Table II. As shown in this table, the RSD% values for all solvents are below 10%. Taking into account the very low concentration levels of the analytes, the precision is acceptable.

Recovery (%)

To investigate the method efficiency for the extraction and recovery of four analytes, the same concentration of each analyte was added to the sample vial once in the presence of the cefalexin blank powder and next time in the

Table II. Characteristic Calibration Graphs and Analytical Data for the Determination of OVI in Ceftazidime using Modified Pencil-Lead as SPME Fiber and Capillary GC*

Analyte	Calibration curves Equation	LOD (ppm)	LOQ (ppm)	LDR (ppm)	RSD (%) (n = 3)	Correlation coefficient (R)	Resolution (Rs)
Acetone	$y = -614 + 1178x$	1.06	3.18	2.5-100	9.1	0.996	2.12
Methylene chloride	$y = 7064.8 + 2926.3x$	0.83	2.49	2.5-100	9.8	0.997	1.13
Diethylamine	$y = -1569.9 + 2175.6x$	0.51	1.53	2.5-100	9.4	0.997	4.55
Pyridine	$y = 12735.2 + 5351x$	0.98	2.94	2.5-100	9.7	0.998	

* The experimental conditions were as given for Figure 1A.

absence of cefalexin powder. Recovery (%) values were calculated considering peak areas and illustrated in Table III.

Pharmaceutical applications of the proposed method

Monitoring of organic volatile impurities in ceftazidime

In order to measure the OVI in Ceftazidime real samples, three Ceftazidime vials containing 1 g Ceftazidime produced from a batch of pharmaceutical industry were taken. Ultrasonic assisted HS-SPME procedures followed by GC analyses were performed in optimum conditions. The obtained results and the permitted levels of the OVI were presented in Table IV where the OVI levels in the selected pharmaceutical sample were within the acceptable range.

Stability indicating assay

Ceftazidime is degraded in solid state and/or in its solutions; pyridine is one of the main degradation products, and its levels have been considered as a stability indicator of the drug in the pharmaceutical solutions (18–20). Therefore, the proposed method is capable of monitoring the stability of ceftazidime and could be used as a stability-indicating assay. To show this capability, the photodegradation of ceftazidime was investigated as an example for this application. The ceftazidime samples were exposed to sunlight for up to 25 days, the peak area of the OVI were determined at three day time intervals, and the results are shown in Figure 5. The concentration of acetone, methylene chloride, and diethylamine increased, then reached to the maximum, and decreased after 8–12 days. An increasing pattern was observed for pyridine because it originates from the degradation process of ceftazidime (21). These preliminary results revealed that the proposed method could be used in the stability investigations of ceftazidime.

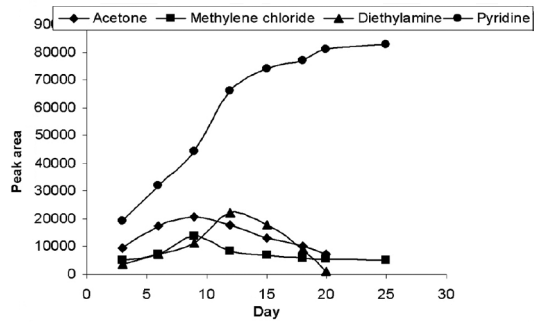

Figure 5. Effect of sunlight on extraction of solvents and degradation of ceftazidime. The experimental conditions were as given for Figure 1A.

Table III. Analyte Recovery (%) Values from Cephalexin*

Solvents	Recovery (%) (*n* = 3)
Acetone	87.03 ± 1.38
Methylene chloride	64.23 ± 1.78
Diethylamine	84.60 ± 2.07
Pyridine	85.63 ± 0.98

* Experimental conditions were as given for Figure 1A.

Table IV. Measured Amounts of OVI in the Vial Containing 1 g of Ceftazidime*

OVI	Measured concentration ± SD (*n* = 3)	Permitted limit (ppm)†
Acetone	65.8 ±1.6	5000
Diethylamine	11.9 ± 1.9	1600
Methylene chloride	3.7 ± 1.4	600
Pyridine	4.3 ± 0.6	200

* Experimental conditions were as given for Figure 1A.
† From the literature (4).

Conclusions

This study suggests that ultrasonic assisted headspace-SPME using modified pencil-lead fiber can be used successfully for the extraction and sampling of OVI directly from pharmaceutical vials followed by GC–FID analysis. This method represents a clean approach compared with the direct injection of large amounts of sample into the GC system. Low cost and high temperature resistance are the advantages of this fiber and it can be used in routine analyses for a wide range of different compounds.

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References

1. S. Nojavan, A. Ghasempour, Y. Bashour, M. Khalilian, and H. Ahmadi. Determination of residual solvents and investigation of their effect on ampicillin trihydrate crystal structure. *J. Pharm. Biomed. Anal.* **36:** 983 (2005).
2. M. Rocheleau, M. Titley, and J. Bolduc. Measuring residual solvents in pharmaceutical samples using fast gas chromatography techniques. *J. Chromatogr. B* **805:** 77 (2004).
3. C. Camarasu, C. Madichie, and R. Williams. Recent progress in the determination of volatile impurities in pharmaceuticals. *Trends Anal. Chem.* **25:** 768 (2006).
4. Harmonised Tripartite Guideline on Impurities: Residual Solvents (Q3C), International Conference on Harmonisation of Technical Requirements for Registrations of Pharmaceuticals for Human Use (ICH), Geneva. 1998.
5. Y. Liu and C. Hu. Establishment of a knowledge base for identification of residual solvents in pharmaceuticals. *Anal. Chim. Acta.* **575:** 246 (2006).
6. S. Legrand, J. Dugay, and J. Vial. Use of solid-phase microextraction coupled with gas chromatography for the determination of residual solvents in pharmaceutical products. *J. Chromatogr. A* **999:** 195 (2003).

7. K. Urakami, A. Higashi, K. Umemoto, and M. Godo. Matrix media selection for the determination of residual solvents in pharmaceuticals by static headspace gas chromatography. *J. Chromatogr. A* **1057:** 203 (2004).
8. A.R. Raghani. High-speed gas chromatographic analysis of solvents in pharmaceuticals using solid phase microextraction. *J. Pharm. Biomed. Anal.* **29:** 507(2002).
9. C.C. Camarasu, M. Mezie, and G.B. Varga. Residual solvents determination in pharmaceutical products by GC-HS and GC-MS–SPME. *J. Pharm. Biomed. Anal.* **18:** 623 (1998).
10. G. Theodoridis, E.H.M. Koster, G.J. Jong, and M.H. Abraham. Solid-phase microextraction for the analysis of biological samples. *J. Chromatogr. B* **745:** 49 (2000).
11. M.F. Alpendurada. Solid-phase microextraction: a promising technique for sample preparation in environmental analysis. *J. Chromatogr. A* **889:** 3 (2000).
12. A. Zander, A.G. Bishop, and P. Prenzler. A solid phase microextraction method to fingerprint dissolved organic carbon released from Eucalyptus camaldulensis (Dehnh.) (River Red Gum) leaves. *Anal. Chim. Acta* **530:** 325 (2005).
13. C.C. Camarasu. Headspace SPME method development for the analysis of volatile polar residual solvents by GC-MS. *J. Pharm. Biomed. Anal.* **23:** 197 (2000).
14. H. Kataoka, H.L. Lord, and J. Pawliszyn. Applications of solid-phase microextraction in food analysis. *J. Chromatogr. A* **880:** 35 (2000).
15. M. Chiarotti, R. Marsili, and A.M. Pineiro. Applications of solid-phase microextraction in food analysis Gas chromatographic–mass spectrometric analysis of residual solvent trapped into illicit cocaine exhibits using head-space solid-phase microextraction. *J. Chromatogr. B* **772:** 249 (2002).
16. R. Porra, A. Farina, V. Cotichini, and R. Leche. Analysis of ceftazidime and related compounds by micellar electrokinetic chromatography. *J. Pharm. Biomed. Anal.* **18:** 241 (1998).
17. Dj. Djozan and Y. Assadi. Modified pencil lead as a new fiber for solid-phase microextraction. *Chromatographia* **60:** 313 (2004).
18. A.R. Barnest and S. Nash. Stability of ceftazidime in a viscous eye drop formulation. *J. Clin. Pharm. Ther.* **24:** 299 (1999).
19. H. Servais and P.M. Tulkens. Stability and compatibility of ceftazidime administered by continuous infusion to intensive care patients. *Antimicrob. Agents Chemother.* **45:** 2643 (2001).
20. M. Arsene, P. Favetta, B. Favier, and J. Bureau. Comparison of ceftazidime degradation in glass bottles and plastic bags under various conditions. *J. Clin. Pharm. Ther.* **27:** 205 (2002).
21. A. Farina, R. Porra, V. Cotichini, and A. Doldo. Stability of reconstituted solutions of ceftazidime for injections: an HPLC and CE approach. *J. Pharm. Biomed. Anal.* **20:** 521(1999).

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