Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 954–957

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Measurement of residual solvents in a drug substance by a purge-and-trap method

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ARTICLE INFO

Article history:
Received 13 August 2007
Received in revised form 7 March 2008
Accepted 10 March 2008
Available online 16 March 2008

Keywords:
Class1 solvents
Drug
P&T-GC-FID
Purge-and-trap
Residual solvents

ABSTRACT

The purge-and-trap (P&T) gas extraction method combined with gas chromatography was studied for its suitability for quantitative residual solvents determination in a water-soluble active pharmaceutical ingredient (API). Some analytical method performance characteristics were investigated, namely, the repeatability, the accuracy and the detection limit of determination. The results show that the P&T technique is – as expected – more sensitive than the static headspace, thus it can be used for the determination of residual solvents pertaining to the ICH Class 1 group. It was found that it could be an alternative sample preparation method besides the static headspace (HS) method.

1. Introduction

The quality of products is very strictly regulated in the pharmaceutical industry so the qualitative and quantitative knowledge of residual solvent impurities in the bulk materials and in the final products is essential. The residual solvents are volatile organic compounds; therefore they can be separated and determined qualitatively and quantitatively by gas chromatography. As the prescribed limits are at ppm levels, in most cases sample preparation includes the enrichment of the sample. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [1] and the Pharmacopoeias [2] (based on the ICH Q3C guideline) provide limits and offer analytical methods, which in most cases are static headspace sampling coupled to gas chromatography (HS-GC). Besides, headspace solid phase micro extraction (HS-SPME) sample preparation is often applied, and nowadays various full evaporation techniques are also popular [3–5].

In purge-and-trap (P&T) sample preparation technique the purge gas stream, divided into bubbles, passes through the dissolved sample, saturates it, thereby reducing the solubility of the volatile components in the liquid, thus the removal of residual solvents is more efficient and faster. The volatile compounds are extracted from the gas flow and trapped. The advantage of the method is that theoretically all volatile compounds from the liquid can be removed and trapped. The method was first described in the 1960s [6] and not much later a variation of it became known [7], where the purge gas is circulated in closed loop. The trapping can be performed by freezing or adsorption. A few reviews discussed the theoretical bases of purging [8,9] and trapping on adsorbent [10,11]. The method is more advantageous than the HS if the equilibrium constant of gas–liquid phase is lower than 1000 [12]. This is true in case of apolar molecules and which do not dissolve well in water. The partition coefficient can be decreased by increasing the temperature or by salting out [11]. The method was mainly used in the pharmaceutical industry to analyse solid polymer matrices [3] because of its low detection limit but was applied rarely for the analysis of liquid samples because of its complexity and its disadvantages [12]. Due to the reduced numbers of practical application of P&T the United States Pharmacopoeia (USP) dropped the method in 1994 [13].

We examined whether the P&T sample preparation using a modern automated apparatus, successfully used in environmental analysis and in food analysis [14], can be used in residual solvents analysis of an active pharmaceutical ingredient (API).

2. Experimental

2.1. Apparatus

2.1.1. Purge-and-trap apparatus

A SOLATek 72 Autosampler and a Velocity XPT Sample Concentrator (both from Tekmar-Dohmann Instruments, USA) were

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doi:10.1016/j.jpba.2008.03.009

Table 1
The parameters of SOLATek 72 Autosampler and Velocity XPT Sample Concentrator

Variable	Value	Variable	Value
Rinse water temp.	90 °C	Sample preheat time	1.00 min
Sample cup temp.	30 °C	Preheat temp.	40 °C
Sample needle temp.	30 °C	Purge time	Variable
Transfer line temp.	125 °C	Purge temp.	0 °C
Soil valve temp.	125 °C	Purge flow	40 ml/min
Sample sweep time	0.50 min	Dry purge time	1.00 min
Needle rinse volume	7 ml	Dry purge temp.	40 °C
Needle sweep time	0.50 min	Dry purge flow	200 ml/min
Bake rinse volume	7 ml	GC start	Start of desorb
Bake sweep time	0.20 min	Desorb preheat temp.	200 °C
Bake drain time	0.50 min	Desorb drain	On
Number of bake rinses	3	Desorb time	2.00 min
Valve oven temp.	200 °C	Desorb temp.	200 °C
Transfer line temp.	200 °C	Desorb flow	100 ml/min
Sample mount temp.	90 °C	Bake time	10.00 min
Purge ready temp.	35 °C	Bake temp.	225 °C
Dryflow standby temp.	200 °C	Dryflow bake temp.	300 °C
Standby flow	0 ml/min	Bake flow	250 ml/min
Pre-purge time	0.50 min	Focus temp.	120 °C
Pre-purge flow	40 ml/min	Inject time	1.00 min
Sample heater	Off	Inject temp.	130 °C

used and controlled by VOC TekLink 2.4 software (Teledyne Tekmar Company, USA). The purge gas was high purity nitrogen; the trap was Tenax/silica gel/charcoal (Tekmar-Dohmann Instruments, USA) as adsorbent. The volume of purge vessel was 5 ml, the volume of the sample vial was 40 ml. The sample vial had a screw cap with 2.5 mm wide butyl red/PTFE grey septa (La-Pha-Pack GmbH, Germany) covered with aluminium foil. The instrument allows after thermal desorption a secondary trapping before injection to the GC by cryofocusing. Injection could be quicker from this trap than from adsorbent. The fast injection decreases the width of chromatogram peaks, allowing improvement of the separation and detection limits. Still, the advantages of cryogenic focusing were not seen in the preliminary investigations so this unit was not used, but it was turned on because of technical considerations. The employed experimental parameters are presented in Table 1.

2.1.2. GC

A Shimadzu GC-14A capillary gas chromatograph (Shimadzu Corporation, Japan) with split/splitless injector and flame ionisation detector (FID) was used. The column was Rtx-1MS, length: 30 m, i.d.: 0.32 mm, thickness: 1.0 μm 100% dimethylpolysiloxane stationary phase (Restek Corporation, USA). Hydrogen was used as carrier gas with constant 0.75 bar inlet pressure. The temperature of injector was 160 °C, the split ratio was 1:10. The operation must be in split mode because of the speed of injection [15]. The detector temperature was 300 °C, the detector gases were adjusted at the optimum values given in the user's manual: the flow rate of air at 400 ml/min, hydrogen gas flow was set at 40 ml /min and nitrogen was used as make-up gas at 40 ml/min. The oven temperature was 35 °C at the beginning for 3 min, then programmed at 10 °C/min rate to 75 °C, then 30 °C/min rate to 200 °C. Finally it was kept at 200 °C for 5 min. Using these experimental parameters the retention times of 1,1-dichloroethene, 1,2-dichloroethane, benzene and carbon tetrachloride are 3.08, 4.09, 5.02 and 5.12 min, respectively. For data collection and processing a Clarity-Preliniary 2.4.1.43 software (DataApex Company, Czech Republic) was used.

2.2. Sample preparation

The target drug compound was a water-soluble purine derivative: pentoxyfilline (CAS Registry Number: 6493-05-6). Ultra pure water (Millipore Corporation) and minimum 99.5% purity standard chemicals were used. The stock solution was made from the standards by weighing. The stock solution contains 7.73 mg/ml benzene (Merck), 13.60 mg/ml carbon tetrachloride (Sigma), 21.30 mg/ml 1,2-dichloroethane (Sigma) and 24.62 mg/ml 1,1-dichloroethene (Sigma), dissolved in *N*,*N*-dimethylformamide (Fluka). The working solutions were diluted from the stock solution with water. The concentration of the API in all working solutions was 10 mg/ml. Accuracy, repeatability, detection limits and range of linearity were studied. The solvents concentrations of working solutions to study accuracy and repeatability are included in Table 2. The measurements were repeated five times.

Table 2
Concentrations of working solutions (ppm is calculated relative to the API)

	1,1-Dichloroethene (ppm)	1,2-Dichloroethane (ppm)	Benzene (ppm)	Carbon tetrachloride (ppm)
1	0	0	0	0
2	1.53	1.33	0.48	0.85
3	3.07	2.66	0.97	1.70
4	4.60	3.99	1.45	2.55

The linearity was studied in two series, in low and high concentration ranges, repeated three times at every point. The ranges are included in Table 3.

3. Results and discussion

The goal of the experiments was to study the applicability of a modern automated P&T apparatus, which is successfully used in environmental analysis and food analysis, in the residual solvents analysis of an API.

The given API decreases the surface tension of the water and the sample strongly foamed during purging. Based on the preliminary investigation, the concentration of API could be maximum 10 mg/ml and the purge flow maximum 40 ml/min; otherwise the tubes of the apparatus would be contaminated by over foaming of the sample. It was found that the Teflon coated septa adsorbs or can be permeable for the investigated volatile organic compounds. The decrease of peak areas in the case of the most volatile analytes (carbon tetrachloride and 1,1-dichloroethene) was especially notable. The septa were packed into approx. 0.015 mm thick aluminium foil and this proved to eliminate the phenomenon. Afterwards only packed septa were used for the measurements. At first the purge time was optimised. Fig. 1 shows the influence of purge times on analytes peak areas (the composition of samples was kept constant).

The experimental profiles from Fig. 1 follow well the theoretical curves [12].

As 1,2-dichloroethane has the highest water solubility among investigated residual solvents and because of its polarity, it requires

Table 3
Ranges of linearity

Compounds	Range (ppm)	Number of measuring levels
1,1-Dichloroethene	0.08–10	6
	30–160	4
1,2-Dichloroethane	0.05–7	6
	26–110	4
Benzene	0.04–3	6
	9.7–35	4
Carbon tetrachloride	0.1–6	6
	17–340	5

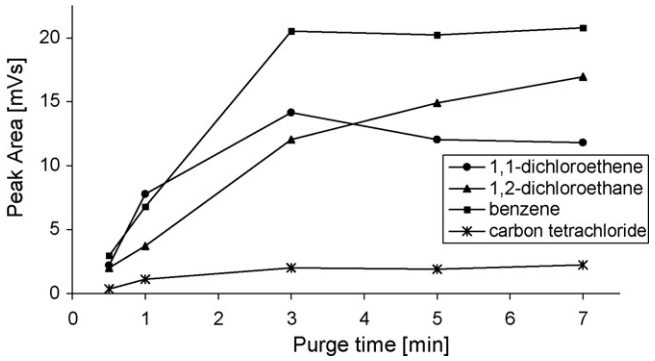

Fig. 1. Optimization curves of purge time. 1,1-Dichloroethene 1.5 ppm, 1,2-dichloroethane 1.6 ppm, benzene 1.1 ppm, carbon tetrachloride 2.0 ppm.

the longest purge time. For all other solvents the optimum purge time is 3 min.

It was found that a purge time of 7 min is optimal for maximizing the extraction efficiency of all investigated analytes.

The bubbling of purge gas begins about 20 s later than the start of the purge gas flow. This time lag can be also seen in Fig. 1.

In residual solvents analysis using HS and HS-SPME sometimes the standard addition method is used for the quantitative analysis, in order to eliminate matrix effects [2,4,15]. Traditionally, the purge-and-trap method uses internal standard quantitation [14] to compensate for the inaccuracy of its complex injection system. During our experiments the relative standard deviation (R.S.D.%) of peak area was found to be lower than 5%. As it is much lower then the requirements in the Pharmacopoeia [2], the standard addition method was chosen instead of the internal standard method, thus eliminating the impact of the strongly foaming matrix.

The method was found to be linear over the ranges presented in Table 3 (in both low and high concentration ranges), with correlation coefficients (R^2) greater than 0.99 for all investigated analytes. The upper limits of linear range in the case of benzene, 1,2-dichloroethane and 1,1-dichloroethene were determined from the upper limits of the linear range of the detector. The measurement of samples with higher concentrations is also possible by applying a suitable dilution to the sample solutions. The high concentration ranges could eventually be used for residual solvents determination for in-process samples of different intermediates from the manufacturing process. The linearity curves are presented in Fig. 2.

From the calibration curves it can be seen that benzene is the most sensitive and carbon tetrachloride is the least sensitive (as is having a low FID response), 1,1-dichlotoethene and 1,2-dichloroethane being between these two extremes. The relative sensitivities are similar to the calculated relative sensitivities from the effective carbon number data [17]. The detection limits

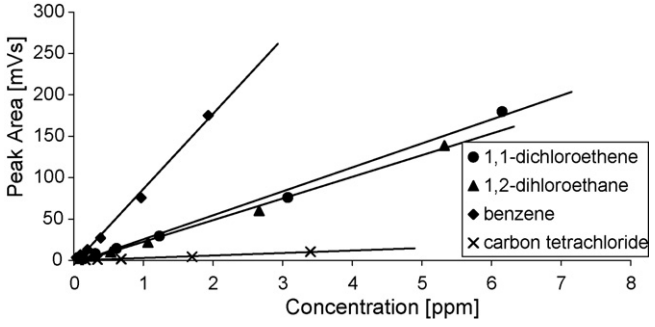

Fig. 2. Linearity curves at ICH limit and at lower range.

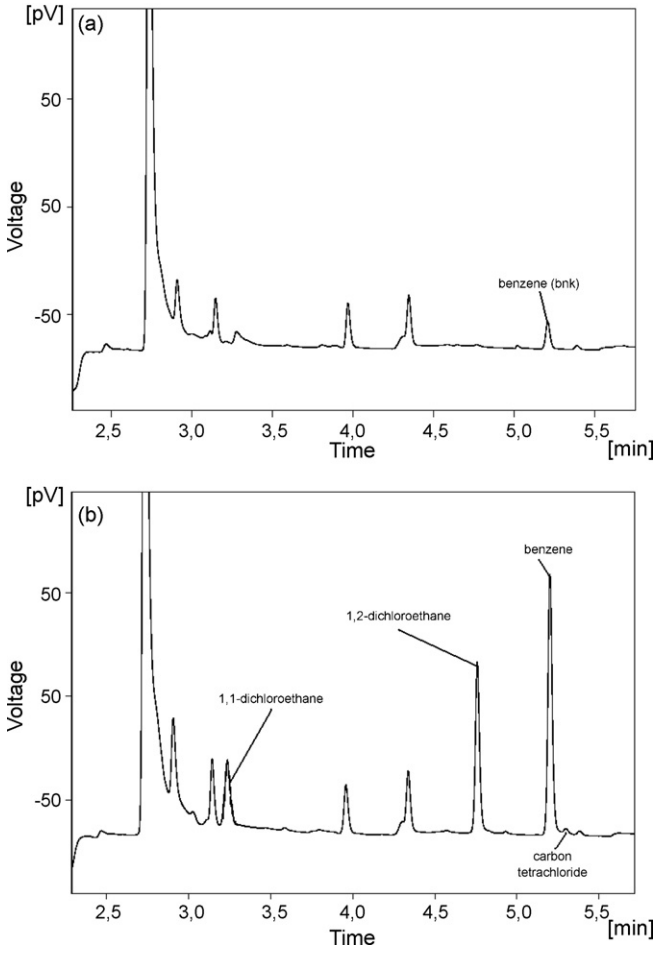

Fig. 3. Chromatograms of drug without added solvent (a), with added solvent (b).

(DL) were calculated from the slope (S) and the standard deviation(s) of blank sample [18] and showed values between 0.001 and 0.037 ppm.

The repeatability of five injections was less than 4.6% for all investigated analytes. The percent recoveries were between 99 and 108%, with a relative standard deviation ranging between 4.6% for benzene and 10.8% for 1,1-dichloroethene.

After each sample the tubes of autosampler and the glass purge vessel were rinsed with hot water and the gas tubes were flushed with nitrogen flow at 200 °C for 10 min (see Table 1, appliance parameters). Carry-over was noticed for all analytes besides benzene at approx. 500 ppm solvent concentration, probably due to the adsorption on the plastic tubes of the autosampler. In the case of benzene, a peak at the same retention time as benzene was observed in the blank chromatograms (using clean water samples), in the samples of the API without any spiked solvents and when using empty purge vessels with approximately the same peak area in all cases. This phenomenon was caused by benzene, which came from the adsorbent [16], therefore, the peak areas of benzene had to be corrected with the peak area of the blank sample. This increased the detection limit of benzene. A chromatogram of blank sample and a sample are shown in Fig. 3. The negative peak in the first part of the chromatogram is caused by the instant variation in nitrogen flow after the injection by the purge-and-trap apparatus. The other peaks are those of volatile compounds already present in the pharmaceutical base material.

The sensitivity changed slightly day-to-day, and this was produced mainly by the changes in the state and in the cleanness

Table 4
Summary of results

Compound	ICH limit (ppm)	Linearity		Precision (RSD%)	Detection limit (ppm)	Recovery (%)	RSD% of recovery	RSD% of sensitivity from day-to-day
		Range (ppm)	R^2					
1,1-Dichloroethene	8	0.08–10 30–160	0.9955 0.9995	4.5	0.002	99	10.8	9
1,2-Dichloroethane	5	0.05–7 26–110	0.9933 0.9997	4.0	0.001	108	8.0	10
Benzene	2	0.04–3 9.7–35	0.9952 0.9994	4.2	0.002	104	4.6	14
Carbon tetrachloride	4	0.1–6 17–340	0.9927 0.9952	4.6	0.037	99	10.2	14

of the detector, but this fluctuation is acceptable. The inter-day sensitivity relative standard deviation ranged between 9% for 1,1-dichloroethene and 14% for benzene and carbon tetrachloride (See Table 4), which can be explained in the case of carbon tetrachloride by its low sensitivity and in the case of benzene by the presence of a blank benzene peak having variable peak areas during different days.

The comparison of the results of reported P&T-GC-FID method with other methods shows that our method has lower detection limits than HS-GC-FID system [19], (where the detections limits are 0.1 ppm to benzene, 0.4 ppm to carbon tetrachloride and 0.2 ppm to 1,2-dichloroethane) and is similar to the HS-SPME-GC-FID system [20] (where detection limit to benzene is 0.002 ppm). The detector is FID in the most publications of residual solvent analysis [3], but in the recent experiments mass spectrometers (MS) are used. Theoretically the MS (in full scan mode) and FID detectors have similar detection limits [21] but in practice the detection limits are often better with MS than with FID. According to the comparison by Pavon et al. [5], the detection limit to benzene is ten times lower with HS-GC-MS [22] than with HS-GC-FID [19]. According to Fliszar et al. [19], the MS with selective ion monitoring is hundred times more sensitive to carbon tetrachloride than the FID. In some cases lower detection limit can be obtained with HS-GC-MS [23] and HS-SPME-GC-MS [24] and with full evaporation technique than with P&T-GC-FID. Our method nevertheless can be advantageous against HS or HS-SPME because the significantly shorter sample preparation time. When compared to the SPME technique the P&T shows much better robustness, as the SPME fibre extraction efficiency might change with usage and number of injections, and at the same time SPME fibres sometimes show poor inter-batch reproducibility of sensitivity.

4. Concluding remarks

The utility of purge-and-trap sample preparation and GC-FID separation to analyse residual solvent in an API was studied. Class 1 solvents including benzene, carbon tetrachloride, 1,2-dichloroethane and 1,1-dichloroethene were analyzed in a water-soluble drug. Detection limit, repeatability, accuracy and the linearity were examined. These characteristics of the method were proved to be suitable, and detection limits were much lower than the required values described in various international pharmacopoeias. The purge-and-trap sample preparation with modern, automated apparatus can also be applicable for residual solvent analysis in pharmaceutical active ingredients.

Acknowledgement

Many thanks to Bálint Analitika Kft. for the possibility to use their apparatus in our research.

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