Development and validation of a headspace gas chromatographic method for determination of residual solvents in five drug substances

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ABSTRACT : A simple and sensitive method for the simultaneous determination of ethanol, ethyl acetate, tetrahydrofuran, 2-propanol, hexane, dichloromethane and methanol in five drug substances by headspace techniques with FID detection is described. The method was validated for repeatability, linearity, limit of detection, limit of quantification and recovery according to the International Conference on Harmonization guidelines. Excellent results were obtained, within the globally accepted validation reference values, particularly taking into account the low concentration levels investigated

Keywords: Validation; Residual solvents, GC-HS, Drugs

I. INTRODUCTION

Residual solvents, or organic volatile impurities, are a potential toxic risk of pharmaceutical products and have been a concern of manufacturers for many years [1]. Moreover, residual solvents can also affect the quality and stability of not only drug substances but also drug products [2,3]. Thus, acceptable levels of many are included in regulatory guidance documents; in particular in guideline Q3C issued by the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) [4]. Residual solvents are classified into four classes on the basis of the toxicity level and the degree to which they can be considered an environmental hazard [5]. Class 1 solvents are known carcinogens and are strongly suspected of being harmful to humans and the environment, so they should be avoided. Class 2 solvents are nongenotoxic animal carcinogens. Solvents of this class should be limited in pharmaceutical products because of their inherent toxicity. Class 3 solvents have low toxic potential to humans and should be used only where it would be impractical to remove them. Finally, Class 4 solvents are those for which no adequate toxicological data have been found. These last three classes of solvents are the ones most commonly analyzed. Residual solvents are typically determined using chromatographic techniques such as static headspace gas chromatography (HS-GC) [6]. Here I report a full validation of a HS-GC analytical method for determination of seven residual solvents (Class 3: ethanol, ethyl acetate, 2-propanol; Class 2: hexane, dichloromethane, tetrahydrofuran, and methanol) commonly used during the manufactories of drug substances and purification steps. Additionally, the method was tested on five drug substances: Midazolam, Flumazenil, Ciprofibrate, Bromazepam and Alprazolam, considering that the solvents mentioned in the validation are normally used in the synthetic routes to produce the drugs in question.

Chromatographic Condition

II. EXPERIMENTAL

All gas chromatography experiments were conducted with a Shimadzu 17A Ver. 3 gas chromatograph interfaced with a Shimadzu HSS-4a headspace auto-sampler -. The chromatographic oven temperature program was as follows: the initial temperature of 35 °C was held for 10 min after injection; it was then ramped up at a rate of 15 °C/min to 40 °C and the temperature was maintained for 10 min; it was then ramped up again at a rate of 18 °C/min to 235 °C and, after holding for 8 min at 235 °C, the temperature was returned to its initial value. Total run time was 40 minutes. The headspace conditions corresponded to those described in the Eur. Ph. for water as sample solvent. The oven temperature was set at 80 °C for 60 min, with gentle shaking. The transfer line and loop temperatures were 85 °C. The pressurization time was 0.5 min, the loop fill and loop equilibration times were 0.1 min and 0.05 min, respectively, and the injection time was 1.5 min. Vial pressure was set at 18 p.s.i. and the headspace carrier was regulated at 25 mL min⁻¹.

Reagents and preparation of solutions and samples

All the drug substances (Midazolam, Flumazenil, Ciprofibrate, Bromazepam and Alprazolam) were synthesized by Sintefina Almirall (Diadema-SP, Brazil). The solvents ethanol, ethyl acetate, tetrahydrofuran, 2-propanol, hexane, dichloromethane and methanol were provided by Merck (Darmstadt, Germany).

The solvents and internal standard were prepared together. The amounts of solvents were diluted in 100 mL of DMSO. 1 mL of the previous solution was diluted to10 mL of DMSO, and then 2 mL of this solution was diluted with 100 mL of DMSO, after which 10 mL of this solution was transferred to a 20-mL vial. The test solutions were prepared using 1 g of each sample (Midazolam, Flumazenil, Ciprofibrate, Bromazepam and Alprazolam) dissolved in 10 mL of DMSO in a 20-mL vial (100 mg mL⁻¹).

III. RESULTS AND DISCUSSION

In this study, a HS-GC analytical method was developed and validated for the quantitative determination of the solvents methanol, ethanol, 2-propanol, dichloromethane, hexane, ethyl acetate and tetrahydrofurane (THF) in drug substances. The proposed method uses the standard addition technique with internal standard quantitation for determination of seven solvents. The method was validated within ICH guidelines Q2A and Q2B. Selectivity, limits of detection and quantitation, linearity, range, precision (system repeatability), recovery and robustness (changes in HS and GC conditions and solution stability) were determined. Excellent results were obtained, within global validation reference values, particularly taking into account the low concentration levels investigated. The test method was validated and had good reproducibility and linearity for the solvents used in the manufacturing process. The recovery was good and justified the preparation of the standard in DMSO without the product as matrix.

Selectivity

The ZB-624 column, in the 30 m x 0.32 mm I.D. configuration, was chosen because this column has a standard stationary phase, which is recommended by the European and American Pharmacopeias, and has provided baseline separations of all solvents used in the validation, including the internal standard (Dioxane) and diluent (DMSO). The method showed good peak shape, and the narrow peak width resulted in excellent column efficiency. The blank chromatogram did not show any interference with the solvent peaks (Figs 1-2), for which the retention times are reported in the Table 1.







Figure 2: Gas chromatogram (GC) of the blank (DMSO).

Table 1: Names of s	solvents and their r	espective relative re	etention times to dioxane

Solvents	Retention Times (min)						Relative	Relative	Relative
Name	Absolute	Relative to	Absolute	Relative	Absolute	Relative to	time	time	time
		Dioxane		to		Dioxane	average	SD	RSD
				Dioxane					
Methanol	2.148	0.140	2.136	0.140	2.135	0.140	0.140	0.0002	0.16%
Ethanol	3.017	0.197	2.999	0.196	2.994	0.196	0.196	0.0004	0.20%
2-Propanol	3.825	0.250	3.803	0.249	3.795	0.249	0.249	0.0005	0.20%
Dichloromethane	4.230	0.276	4.207	0.275	4.199	0.275	0.275	0.0005	0.18%
Hexane	5.267	0.344	5.242	0.343	5.225	0.342	0.343	0.0007	0.19%
Ethyl acetate	7.527	0.491	7.493	0.490	7.477	0.490	0.490	0.0006	0.13%
Tetrahydrofuran	7.997	0.522	7.970	0.521	7.952	0.521	0.521	0.0004	0.07%
Dioxane*	15.326	-	15.289	-	15.261	-	-	-	-



Linearity and range

To carry out this study, six concentrations were prepared of each solvent. All concentrations were prepared in triplicate, by individually weighing amounts of solvents. The experimental results were represented graphically to obtain a calibration curve and carry out the corresponding statistical study (Anova). The method is linear within a wide range for the solvents included in the validation. The correlation coefficients were all above 0.99 and linear regression showed a positive response throughout the range (Fig 3).

The specified range is normally derived from linearity studies and depends on the intended application of the procedure [7]. In this paper it was characterized as the interval between the lowest (52 ppm) and highest (1000 ppm) concentration, which can be determined using a given method, with assumed precision, trueness and linearity. The wide measurement range allows determination with adequate precision of different analyte contents in various matrices. The measurement ranges are shown in the Table 2 with the respective RSD values.



Figure 3. Linear regressions of HS/GC determinations for the residual solvents methanol, ethanol, 2propanol, dichloromethane, hexane, ethyl acetate and tetrahydrofurane.

Table 2. Results of the range study													
Metha	nol	Ethano	ol	2-Prop	anol	Dichlor	omethane	Hexan	e	Ethyl a	acetate	Tetrahy	drofuran
Level	RSD	Level	RSD	Level	RSD	Level	RSD	Level	RSD	Level	RSD	Level	RSD
Ppm		ppm		ppm		ppm		ppm		ppm		ppm	
52	3.70%	50	3.27%	64	0.77%	65	1.01%	50	2.10%	66	2.01%	53	2.95%
208	3.00%	209	2.05%	207	2.54%	210	0.05%	104	3.50%	207	3.37%	210	0.49%
402	1.90%	410	1.19%	402	2.65%	410	0.75%	202	1.59%	404	0.55%	407	0.25%
610	2.50%	609	3.35%	601	2.96%	609	1.20%	275	2.58%	602	0.63%	602	0.88%
803	3.00%	816	1.44%	807	1.95%	801	0.79%	402	2.85%	802	0.99%	807	0.20%
1000	1.00%	1005	1.58%	1010	1.91%	1030	1.45%	613	2.01%	1011	0.58%	998	0.56%

Table 2 Results of the range study

Repeatability

Repeatability was determined in accordance with ICH guidelines, i.e.: nine independent determinations were carried out during single day and on their basis the values of the standard deviations were established. The repeatability, representing the spread of the results, was expressed as RSD (Table 3).

Table 3. Repeatability (n = 9) of the determination of residual solvent at a level
Corresponding to approximately 100 ppm.

Solvents	RSD
Methanol	2.13%
Ethanol	1.20%
2-Propanol	0.61%
Dichloromethane	1.88%
Hexane	2.32%
Ethyl Acetate	0.59%
Tetrahydrofuran	0.44%

Detection (LODs) and LOQs) quantification limits

LODs were calculated as those concentrations that gave an S/N ratio of approximately 3. LOQs were calculated as those concentrations that gave an S/N ratio \geq 10 and low-residual linearity values. The sensitivity of the method was demonstrated by the low-LOD values obtained for all the solvents analyzed (Table 4).

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Detection limit			Quantification limit				
Solvents	Level (ppm)	RSD %	Solvents	Level (ppm)	RSD %		
Methanol	11.75	1.49	Methanol	28.45	3.33		
Ethanol	14.40	3.37	Ethanol	31.00	2.96		
2-Propanol	20.85	1.04	2-Propanol	47.50	2.66		
Dichloromethane	9.20	4.13	Dichloromethane	18.85	2.50		
Hexane	3.85	3.84	Hexane	8.00	1.81		
Ethyl acetate	15.65	3.93	Ethyl acetate	22.00	1.37		
Tetrahydrofuran	8.60	3.27	THF	21.80	2.99		

Table 4. Detection and quantification limits in ppm of the solvents methanol, ethanol, 2-propanol,
dichloromethane, hexane ethyl acetate and THF.

Recovery

The mean recoveries for all the solvents were between 97.0-105.3 and were lower than tabulated t for p = 0.05 (Table 5), so the recoveries and 100% values were not significantly different.

IV. CONCLUSIONS

The analytical method proposed for the quality control of five active ingredients (Midazolam, Flumazenil, Ciprofibrate, Bromazepam and Alprazolam) in relation to the residual methanol, ethanol, 2-propanol, dichloromethane, hexane, ethyl acetate and acetone contents, met the validation requirements. Excellent results were obtained, within globally accepted validation reference values, particularly taking into account the low concentration levels investigated. The method was sensitive, linear, accurate and precise.

Three randomly selected batches of each drug substance were analyzed under validated method conditions and the concentrations of residual methanol, ethanol, 2-propanol, dichloromethane, hexane, ethyl acetate and acetone were much lower than their maximum ICH limits. Moreover, the validated method can be applied to others drug substances.

Solvents	Level (ppm)	% Recovery
	463	100 1
Methanol	658	99.1
Witthanoi	792	101 4
	192	101.4
	578	98.2
Ethanol	612	97.8
	800	102.7
	458	98.5
Propapol-2	612	99.7
r topunor 2	831	103.2
	001	103,2
	499	97.8
Dichloromethane	599	101.1
	777	97.0
	458	98.5
Hexane	612	99.7
	831	103.2
	473	99.9
Ethyl acetate	611	105.3
	831	104.5
	446	103.3
Tetrahydrofuran	633	99.3
	760	98.0

Table 5. Recovery average values of HS/GC determinations of residual solvents for methanol, ethanol, 2-propanol, dichloromethane, hexane, ethyl acetate and acetone in Midazolam, Flumazenil, Ciprofibrate, Bromazepam and Alprazolam.

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