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Static headspace gas chromatographic method for quantitative determination of residual solvents in pharmaceutical drug substances according to European Pharmacopoeia requirements

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Abstract

A static headspace (HS) gas chromatographic method for quantitative determination of residual solvents in a drug substance has been developed according to European Pharmacopoeia general procedure. A water–dimethylformamide mixture is proposed as sample solvent to obtain good sensitivity and recovery. The standard addition technique with internal standard quantitation was used for ethanol, tetrahydrofuran and toluene determination. Validation was performed within the requirements of ICH validation guidelines Q2A and Q2B. Selectivity was tested for 36 solvents, and system suitability requirements described in the European Pharmacopoeia were checked. Limits of detection and quantitation, precision, linearity, accuracy, intermediate precision and robustness were determined, and excellent results were obtained.

Keywords: Validation; Headspace analysis; Standard addition; Pharmaceutical drug substance; Residual solvents; European Pharmacopoeia

1. Introduction

Since the late 1970s, a large number of investigations have been performed to establish specifications and methods for the control of residual solvents in pharmaceuticals [1,2]. In 1997, limit contents for residual solvents in relation to their permitted daily exposure (PDE) were issued by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) in guideline Q3C [3,4], which became effective in March 1998. In this guideline, the ICH classified solvents in three categories and set limits depending on toxicity data for each solvent. The US Food and Drug Administration (FDA) published their guidance in December 1997 [5] and the European Pharmacopoeia (Eur. Ph.) included the guideline in the chapter "Residual Solvents" [6].

The current review (2003) of alternative techniques for residual solvents testing in pharmaceuticals published by B'Hymer [7] still refers to static headspace (HS) as the most widely used sampling technique for gas chromatography (GC). It is preferred against Purge and Trap, solid phase microextraction or direct injection. In 1997, Witschi and Doelker [2] published an up-to-date compendium of the different GC techniques available. Although standard addition is the quantitation technique most recommended by different authors [1,7,8] to overcome matrix effects in HS analyses, few methods have been published in comparison with external standard quantitation [7,9].

The Eur. Ph. general method for Identification and Control of Residual Solvents in drug substances [10] defines a general procedure and describes two complementary GC conditions for the identification of unknown solvents. "System A" is recommended for general use and is equivalent to "Methods IV and V" of the US Pharmacopoeia for analysis of volatile organic impurities [11]. "System B" is used to confirm identification and to solve coelutions. Implementation

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of this general method is a subject of major concern in the pharmaceutical industry. However, few references to applications and validations have been published. Some studies of technical problems have been reported, for instance artefact formation during analyses of hydrochlorides of basic compounds in *N,N*-dimethylformamide (DMF) [12]; modifications (direct-injection [13]); or system performance tests and matrix effects studies [14]. But, currently there is a lack of references concerning the development and validation of quantitative standard addition methods following procedures and conditions described in the Eur. Ph.

In this study, the Eur. Ph. general method is applied to the qualitative analysis of residual solvents in a new drug substance and the quantitative determination of those used in the synthesis. Some problems have been overcome, for instance sample insolubility in water and DMF at working concentrations or low-flame ionization detection (FID) sensitivity to some solvents such as carbon tetrachloride (Class 1) and pyridine (Class 2) at ICH levels (4 ppm and 200 ppm, respectively).

The method has been adapted to achieve two main goals: (1) to detect all Classes 1 and 2 solvents at ICH limits and the most common Class 3 solvents using a flame ionization detector and (2) to quantify the known solvents used in the last steps of the synthetic route, ethanol, toluene and tetrahydrofuran (THF) by the standard addition technique.

Eur. Ph. describes a limit test for the quantitation of Classes 1 and 2 solvents and requires the development and validation of a standard addition method for the quantitation of Class 3 solvents. A limit test is suitable as a routine test, however during the development of a drug substance (changes in process, scale-up, etc.) accurate quantitation is necessary. In this study, the standard addition method has been validated for toluene, THF (Class 2 solvents, ICH limits of 890 ppm and 720 ppm[1], respectively) and ethanol (Class 3, ICH limit 0.5%) according to ICH requirements Q2A and Q2B [15,16].

2. Experimental

2.1. Reagents and chemicals

The drug substance was synthesised by Almirall (Barcelona, Spain). Standard substances for trace analysis of ethanol, toluene and *n*-propanol (internal standard), Class 1 solvents (benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethylene and 1,1,1-trichloroethane) and Class 3 solvents (heptane, *tert*.-butyl methyl ether, acetone, ethyl acetate, methyl acetate, methyl ethyl ketone, 2-propanol and methyl isobutyl ketone) were provided by Merck (Darmstadt, Germany). THF was purchased from Aldrich (Steinheim, Germany). Standard mixtures of Class 2 residual solvents used were Eur.Ph./ICH Class 2 Mix A and Eur.Ph./ICH Class 2 Mix B, provided by Supelco (Bellefonte, PA, USA).

HPLC-grade water was supplied by Merck (Darmstadt, Germany), DMF and dimethyl sulfoxide (DMSO) for trace analysis by Fluka (Buchs, Switzerland).

2.2. Analytical method

2.2.1. Qualitative standard solutions for system suitability

Four standard solutions were prepared to check Eur. Ph. system suitability requirements. A total of 36 solvents were included in these standard solutions: (a) *Reference Solution A*, containing Class 1 solvents at ICH limit values (2 ppm of benzene, 4 ppm of carbon tetrachloride, 5 ppm of 1,2-dichloroethane, 8 ppm of 1,1-dichloroethylene) except 1,1,1-trichloroethane (10 ppm instead of the ICH limit of 1500 ppm). (b) *Reference Solution A1*, sample spiked with Class 1 solvents at ICH limit values (except 1,1,1-trichloroethane). (c) *Reference Solution B*, containing 21 Class 2 solvents at ICH limit values. (d) *Reference Solution C*, containing tetrahydrofuran and some common Class 3 solvents (ethanol, heptane, *tert*.-butyl methyl ether, acetone, ethyl acetate, methyl acetate, methyl ethyl ketone, 2-propanol and methyl isobutyl ketone) used in the manufacture of pharmaceutical substances.

Reference Solution A was prepared by transferring to a 20 ml of HS vial 5.0 ml of water–DMF (3:2) and 1.0 ml of *Class 1 Standard Solution* containing 0.4 μg/ml of benzene, 0.8 μg/ml of carbon tetrachloride, 1.0 μg/ml of 1,2-dichloroethane, 1.6 μg/ml of 1,1-dichloroethylene and 2.0 μg/ml of 1,1,1-trichloroethane in water–DMSO (4:1). *Reference Solution A1* was prepared in the same way as *Reference Solution A*, with the addition of 0.20 g of sample to the vial.

Reference Solution B was prepared by transferring to a 20 ml of HS vial 5.0 ml of water–DMF (3:2) and 1.0 ml of a *Class 2 Standard Solution* containing Class 2 solvents (from Eu. Ph./ICH Class 2 Mix A and Eu. Ph./ICH Class 2 Mix B solutions) at concentrations, in μg/ml, five times lower than ICH limit values in water–DMSO (4:1). THF (Class 2) was not included, neither in Eu. Ph./ICH Class 2 Mix A, nor in Eu. Ph./ICH Class 2 Mix B, due to the recent change from Class 3 to Class 2 [4]. It was therefore included in *Reference Solution C*.

Reference Solution C was prepared by transferring to a 20 ml of HS vial 5.0 ml of water–DMF (3:2) and 1.0 ml of a *Class 3 Standard Solution* containing 100 ppm (v/v) of each solvent, ethanol, heptane, *tert*.-butyl methyl ether, acetone, ethyl acetate, methyl acetate, methyl ethyl ketone, 2-propanol, methyl isobutyl ketone and tetrahydrofuran (Class 2 solvent according to Q3C(M) [4]) in water–DMSO (4:1).

2.2.2. Quantitative standard addition solutions of ethanol, toluene and tetrahydrofuran

Standard Solutions P0, P1, P2 and *P3* were prepared in water–DMSO (4:1). All of them contained 803.5 μg/ml of *n*-propanol as internal standard (I.S.). *Standard Solution P0*

[1] The new ICH level for THF came into operation in March 2003 [4].

Table 1
Standard additions (μg) in the analytical method in *Reference Solutions P0, P1, P2* and *P3*

Solvent	*Reference solution P0*	*Reference solution P1*	*Reference solution P2*	*Reference solution P3*
Ethanol	0	158	316	526
THF	0	178	356	593
Toluene	0	34.7	69.4	116

contained only the I.S. and *Standard Solutions P1, P2* and *P3* contained the values in μg/ml of ethanol, THF and toluene stated in Table 1, as well as the I.S.

Reference Solutions P0, P1, P2 and *P3* were prepared in 20 ml of HS vials. 0.20 g of sample were weighed accurately into four 20 ml of HS vials. Then, 5.0 ml of water–DMF (3:2) and 1.0 ml of *Standard Solutions P0, P1, P2* and *P3* were transferred to each vial, respectively. The blank solution was prepared by transferring 5.0 ml of water–DMF (3:2) and 1.0 ml of *Standard Solution P0* to a 20 ml of HS vial. The four-capped vials were sonicated for 5 min. Table 1 shows standard addition amounts of ethanol, toluene and tetrahydrofuran in each *Reference Solution.*

2.2.3. Headspace gas chromatography

A 6890 series Hewlett-Packard GC system with a FID system (Waldbronn, Germany) and a 7496 Hewlett-Packard headspace autosampler equipped with a 1.0 ml sample loop were used. Chromatographic data were collected and processed by Software Millenium 32 of Waters (Milford, MA, USA). An OVI-G43 capillary column (30 m × 0.53 mm i.d. and 3 μm film thickness) (Supelco) was used.

In this study, "System A" conditions from the Eur. Ph. were selected for method development and validation. "System B" is not described in this study.

The carrier gas was helium at a flow rate of 5.0 ml/min. Injection was carried out in split mode, with a total split flow of 25 ml/min. The injector temperature was 140 °C and the detector temperature was 250 °C. The oven temperature was initially set at 40 °C for 20 min, then it was raised by 10 °C/min to 240 °C and left constant for 20 min.

Headspace conditions correspond 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. Pressurization time was 0.5 min, 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 (p.s.i. = 68 g, 4.76 Pa).

2.2.4. Quantitation

The quantitation of ethanol, toluene and THF was performed by the standard addition technique. The relative areas of ethanol, toluene and THF obtained in *Reference Solutions P0, P1, P2* and *P3* were plotted versus standard addition amounts in μg (presented in Table 1). The calibration curve was calculated by the least-squares method. The absolute *x* value when the *y*-axis equals zero is the residual solvent amount (μg) in the sample added to the HS vial.

2.3. Validation procedure

The validation parameters required in ICH guidelines Q2A and Q2B were determined for ethanol, toluene and THF: the limits of detection (LODs) and quantitation (LOQs), linearity, accuracy, system repeatability, method precision, intermediate precision and robustness. LODs and LOQs, linearity and accuracy were determined by adding standard amounts to a sample of a solvent-free batch of the drug substance (free of ethanol, toluene and THF). The absence of the three residual solvents in this batch was checked previously using the method described.

2.3.1. Selectivity and system sensitivity

Selectivity and system sensitivity requirements defined in the Eur. Ph. for "System A" conditions were checked for Classes 1 and 2 solvents: (a) the S/N ratio for 1,1,1-trichloroethane in the chromatogram of *Reference Solution A* must be at least 5; (b) all Class 1 solvents in the *Reference Solution A1* (spiked sample) should still be detected and (c) the resolution between acetonitrile and methylene chloride in the chromatogram of *Reference Solution B* must be at least 1.0.

2.3.2. Limits of detection and quantitation

The LODs and LOQs were determined by adding small amounts of ethanol, THF and toluene to the sample (solvent-free batch). Low-concentration standard solutions containing the three solvents were prepared in water–DMSO (4:1). One microliter of each standard solution was transferred to a vial containing 0.20 g of sample and 5.0 ml of water–DMF (3:2). LODs were calculated as those concentrations that gave a S/N ratio of approximately 3. LOQs were calculated as those concentrations that gave a S/N ratio ≥10 and low-residual linearity values. Average peak-to-peak noise was calculated in time intervals of 30 s (between 5.0 min and 5.5 min for ethanol and THF and 19–19.5 min for toluene).

2.3.3. Linearity

Linearity was determined in duplicate by adding amounts of ethanol, tetrahydrofuran and toluene to the sample (solvent-free batch). ICH Q2B specifies minimum linearity ranges to be considered, from reporting level (normally LOQ) to 120% of the limit value. In this study (which used standard addition quantitation), linearity was checked from

LOQs to the sum of 120% of the limit value (1200 μg of ethanol, 173 μg of THF and 214 μg of toluene) plus the highest amount of standard addition defined in the method (*Reference Solution P3*, 526 μg of ethanol, 593 μg of THF and 116 μg of toluene as shown in Table 1). Therefore, ranges studied were from the LOQ to the total calculated nominal amounts of 1726 μg of ethanol, 766 μg of THF and 330 μg of toluene.

Reference solutions for the determination of linearity were prepared by transferring 1.0 ml of standard solution [of the corresponding concentration level in water–DMSO (4:1)] and 5.0 ml of water–DMF (3:2) to a vial containing 0.20 g of sample (of solvent-free batch). Seven quantity levels were added to the sample ranging from 3.5 μg to 347 μg for toluene; nine from 3.9 μg to 1754 μg for ethanol; and ten from 7.1 μg to 889 μg for THF.

2.3.4. Accuracy

Accuracy was determined in triplicate at three concentration levels (0.1%, 0.5% and 1.1% of ethanol; 356 ppm, 711 ppm and 889 ppm of THF; and 433 ppm, 867 ppm, 1084 ppm of toluene) in a solvent-free batch. The residual solvents were added to 0.20 g of sample with the sample solvent water–DMF (3:2) in 20 ml-headspace vials. *Reference Solutions P0* (in triplicate), *P1*, *P2* and *P3* were prepared for each spiking level according to method description (Section 2.2.2).

Quantitation was performed by the standard addition technique as described in paragraph 2.2.4. Nine calibration curves (three per spiking level) were recorded. The amounts of the solvents recovered were obtained by *x*-axis intersection of the standard addition curve.

2.3.5. Precision

Three parameters were determined to evaluate precision: system repeatability at working values and at LOQs, method precision and intermediate precision.

2.3.5.1. System repeatability. Seven vials containing 1.0 ml of standard solution (263 μg/ml of ethanol, 116 μg/ml of toluene, 593 μg/ml of THF and the I.S.) and 5.0 ml of water–DMF (3:2) were analysed to determine system repeatability at working amounts. Similarly, seven replicated solutions with I.S. and LOQ amounts of ethanol, THF and toluene were analysed to determine system repeatability at the limits of quantitation.

2.3.5.2. Method precision. The method precision was calculated as the relative standard deviation of the recoveries obtained in the nine accuracy determinations (three levels in triplicate).

2.3.5.3. Intermediate precision. To evaluate the intermediate precision, a representative drug substance batch was analysed by two analysts using different batches of capillary columns and different GC instruments. Each analyst carried out six replicated determinations. Intermediate precision was determined by comparing standard deviations of the results obtained by both analysts. The *F*-test was performed to check significant differences between standard deviations at 95% of confidence interval. The experimental *F* was calculated using the ratio of the variances of the two populations.

2.3.6. Robustness

2.3.6.1. Changes in HS and GC conditions. The Eur. Ph. selectivity requirements were checked for variations of ±10% on the carrier gas flow, ±5 °C on the initial oven temperature, ±1 °C/min on the temperature rate and for different batches of columns and instruments. Eur. Ph. system sensitivity requirements were also checked for variations of ±4 °C on HS oven temperature, ±10 min on equilibrium time and for different columns and instruments.

2.3.6.2. Solutions stability. The stability of the solutions was checked at 0 h, 12 h, 18 h and 24 h after sample preparation in duplicate. Eight replicated *Reference Solutions P3* were prepared simultaneously and analysed at each time from preparation.

3. Results and discussion

The drug substance taking part in this study is quite insoluble at the working concentration and at room temperature in water and DMF. At HS oven temperature, the sample is soluble in DMF but not in water. However, water is preferred in the headspace analysis to increase method sensitivity. Water–DMF mixtures, able to solubilize the sample, were considered the most suitable solution to obtain good recoveries and to increase method sensitivity in this case. Higher sensitivity for most solvents, especially Class 1 solvents, was obtained with water–DMF (3:2), and carbon tetrachloride and pyridine were detected.

3.1. Selectivity and system sensitivity

Selectivity and system sensitivity requirements defined for "System A" in the Eur. Ph. method for Classes 1 and 2 solvents were within limits: The S/N ratio of 1,1,1-trichloroethane obtained in the chromatogram of *Reference Solution A* was greater than 5 (46), and all Class 1 solvents were also detected in *Reference Solution A1* (Fig. 1). Fig. 2 shows a blank (*Reference solution P0*) to identify drug related peaks. Chromatographic profiles obtained for *Reference Solution B* (Class 2 solvents) and *Reference Solution C* (Class 3 solvents) are shown in Figs. 3 and 4. Resolution between acetonitrile and dichloromethane in *Reference Solution B* was 3.7, a value ≥1.0 as required by the Eur. Ph. The most critical resolution in *Reference Solution C* was obtained between acetone and 2-propanol (1.7), and was greater than 1.0. Good separation is obtained between the solvents used in the synthetic route of the drug substance and the internal standard,

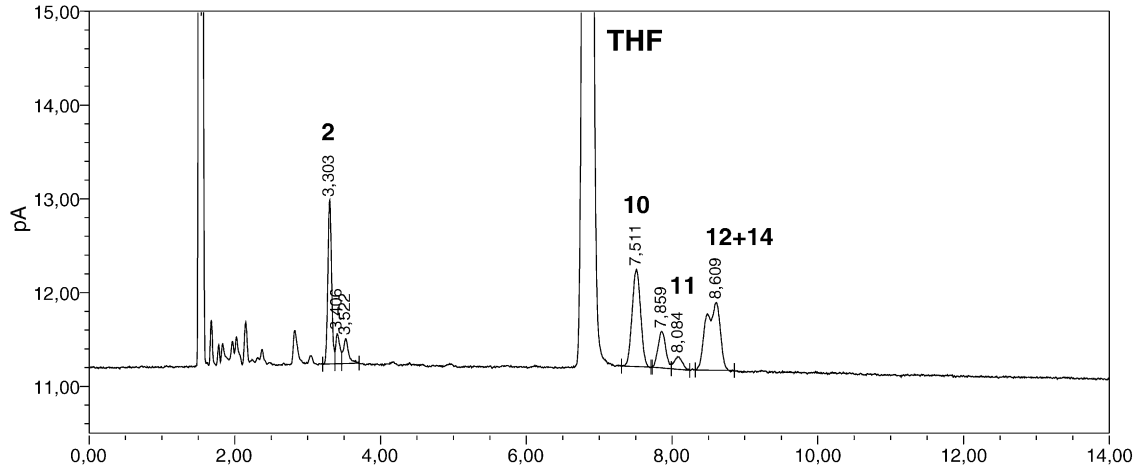

Fig. 1. Chromatogram of *Reference Solution A1* (Class 1 solvents in sample). 1,1-Dichloroethylene (2), 1,1,1-trichloroethane (10), carbon tetrachloride (11), benzene (12) 1,2-dichloroethane (14).

as shown in Fig. 5 (relative retention times of 0.55, 1.35 and 3.90 for ethanol, THF and toluene, respectively).

3.2. Limits of detection and quantitation

The sensitivity of the method was demonstrated by the low-LOD values obtained for ethanol, toluene and THF, 7.9 ppm, 0.3 ppm and 0.9 ppm, respectively. Sample concentrations of 20 ppm of ethanol, 4.5 ppm of THF and 0.5 ppm of toluene gave a S/N ratio slighly higher than 10 (12, 14 and 11, respectively), but high-residual linearity values were obtained at these concentrations for THF and toluene. Whereas, ethanol presented an acceptable residual linearity value at LOQ (36% at 20 ppm of ethanol, 3.9 μg of added amount), THF and toluene obtained residuals of 360% and 1200% at 4.5 ppm (0.9 μg) and 0.5 ppm (0.09 μg), respectively. Both deviations were too high to be accepted. For this reason, higher limits of quantitation were established: 36 ppm (7.1 μg) of THF and 18 ppm (3.5 μg) of toluene with lower residual values of linearity (51% and 61%, respectively), acceptable values for LOQ.

3.3. Linearity

The experimental linearity ranges and equations obtained for the standard addition curves are presented in Table 2. The regression coefficients of the three curves were ≥0.999. The experimental ranges include nominal ranges defined in Section 2.3.3.

3.4. Accuracy

The results obtained in triplicate at the three spiking levels studied are given in Table 3. The mean recoveries obtained for ethanol, THF and toluene were 98%, 102% and 117%, respectively. The mean values of the nine determinations for the three solvents studied were from 80% and 120%, criteria accepted world-wide.

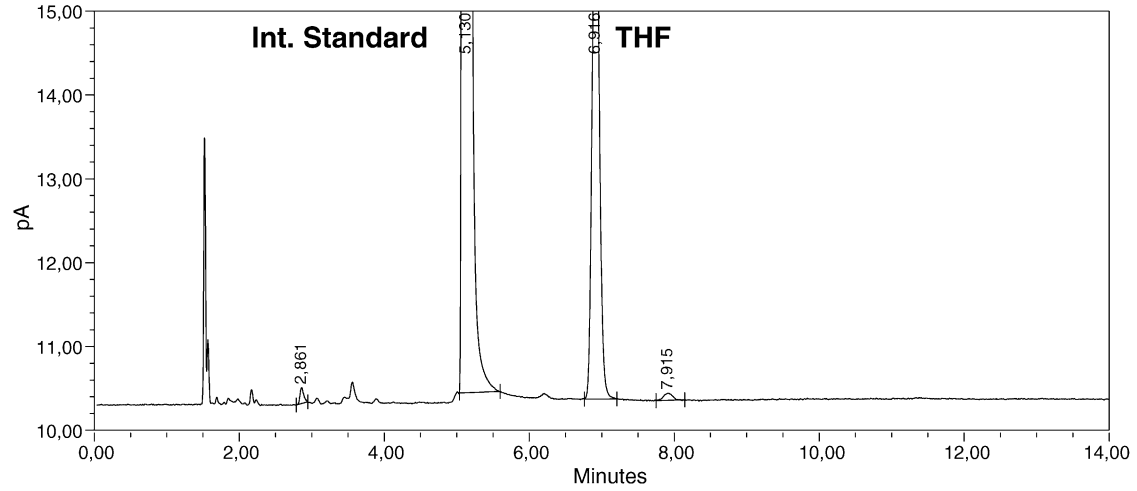

Fig. 2. Chromatogram of non-spiked sample (*Reference solution P0*).

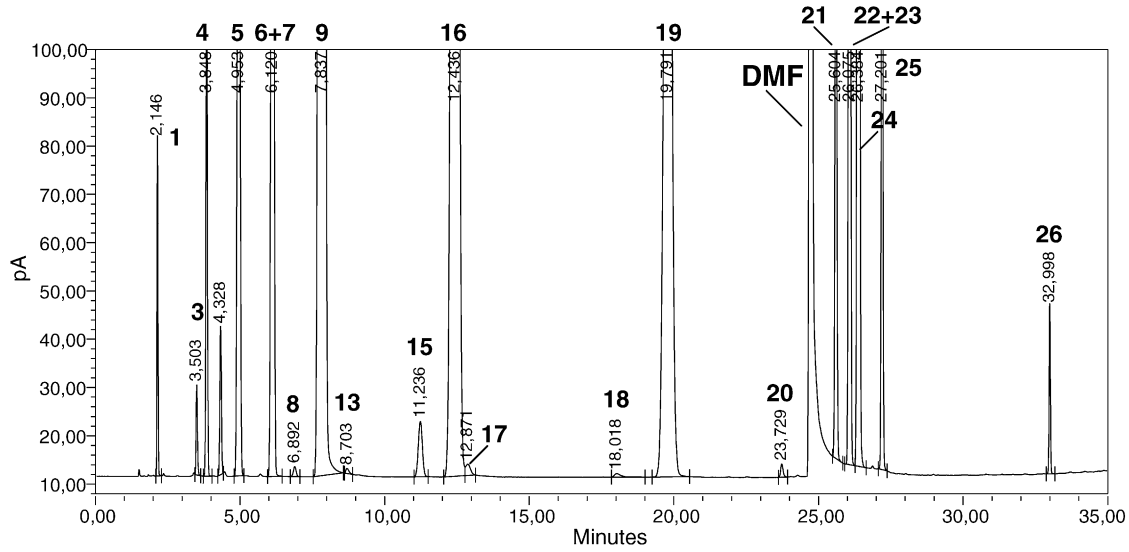

Fig. 3. Chromatogram of *Reference Solution B* (Class 2 solvents). Methanol (1), acetonitrile (3), dichloromethane (4), hexane (5), *cis*1,2-dichloroethylene (6), nitromethane (7), chloroform (8), cyclohexane (9), 1,2-dimethoxyethane (13), 1,1,2-trichloroethylene (15), methylcyclohexane (16), 1,4-dioxane (17), pyridine (18), toluene (19), 2-hexanone (20), chlorobenzene (21), ethylbenzene (22), *m*-xylene (23), *p*-xylene (24), *o*-xylene (25), tetraline (26).

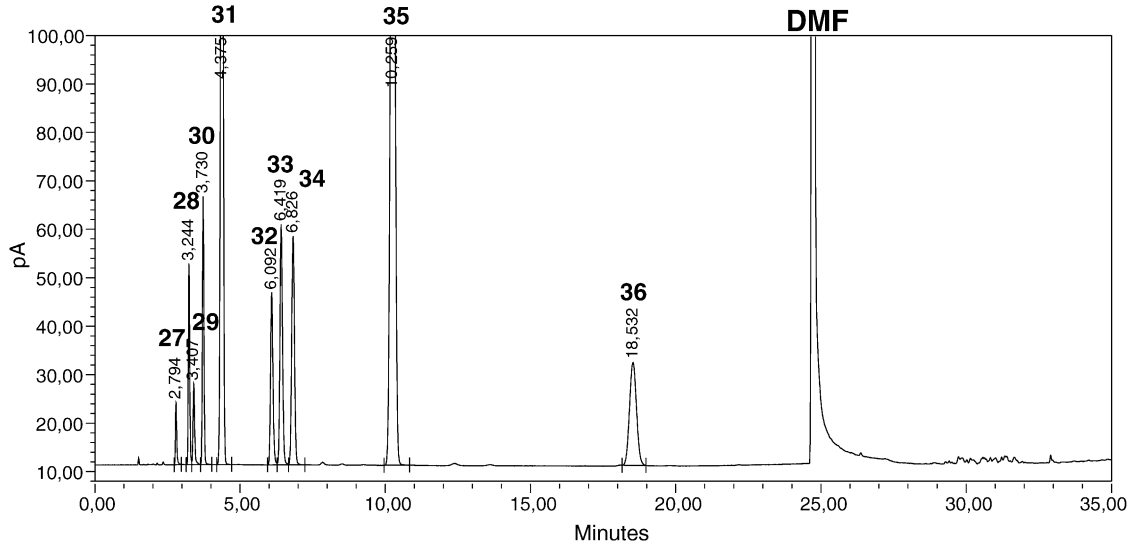

Fig. 4. Chromatogram of *Reference Solution C* (Class 3 solvents, THF Class 2 solvent). Ethanol (27), acetone (28), 2-propanol (29), methyl acetate (30), *tert*.-butyl methyl ether (31), methyl ethyl ketone (32), ethyl acetate (33), tetrahydrofuran (34), heptane (35), methyl isobutyl ketone (36).

Table 2
Linearity results on sample

Solvent	Nominal range (μg)	Experimental range (μg)	Equation standard addition	*R*
Ethanol	3.9–1726	3.9–1754	R.A. = −0.0015 + 0.0010 M	0.9999
Toluene	3.5–330	3.5–347	R.A. = −0.0648 + 0.0384 M	0.9989
THF	7.1–766	7.1–889	R.A = −0.0272 + 0.0058 M	0.9996

R.A.: relative area; M: added solvent (in μg).

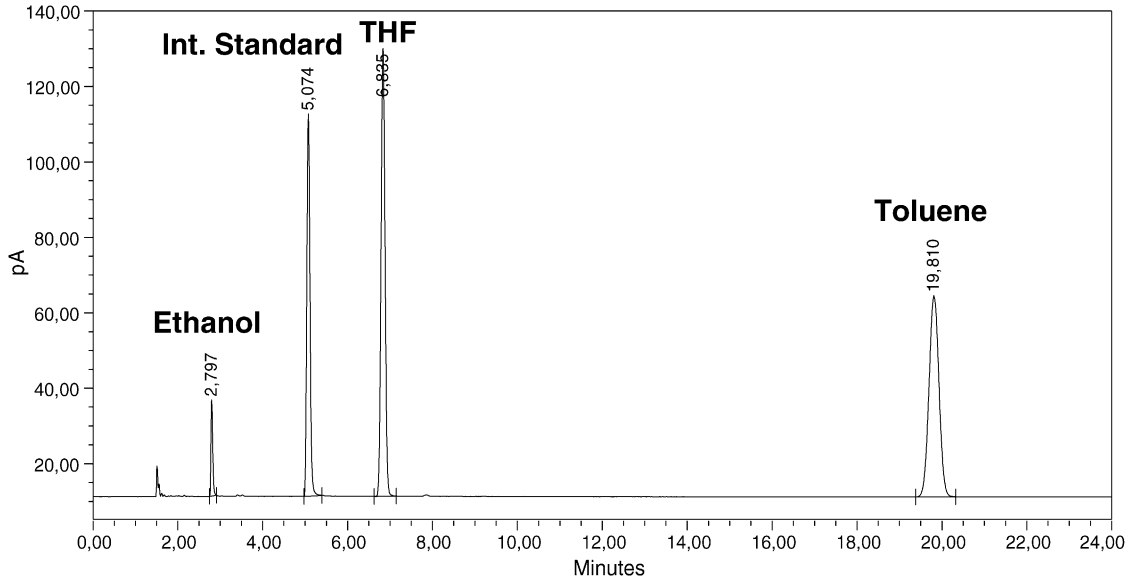

Fig. 5. Chromatogram of a spiked sample *(Reference Solution P1)*.

3.5. Precision

3.5.1. System repeatability

The relative peak areas of ethanol, toluene and THF obtained for the seven replicated preparations at working levels and at LOQs are shown in Table 4. The relative standard deviations of relative peak areas at working values were 0.1%, 1.7% and 3.3% for ethanol, THF and toluene, respectively. The relative standard deviations of the relative areas at LOQs were 0.2% for ethanol, 2.0% for THF and 2.4% for toluene, all lower than three times system repeatability. These criteria are accepted worldwide.

3.5.2. Method precision

The method precision was evaluated by the R.S.D. calculated from the nine recoveries obtained for accuracy (Table 3). The relative standard deviations of the nine determinations were 7.7% for ethanol, 4.2 % for THF and 5.6 % for toluene. These relative standard deviations were lower than 10%.

3.5.3. Intermediate precision

Six replicated determinations of a representative batch were analysed by two analysts using different instruments and different batches of capillary columns. Toluene and ethanol contents in this batch of drug substance were lower than LOQs (were lower than their LOQs values of 18 ppm and 20 ppm, respectively).

THF concentrations obtained by both analysts are shown in Table 5. It can be concluded that there are no significant differences between the standard deviations of both populations of results [17] indicating that the results were reproducible between analysts and instruments.

Table 3
Accuracy at three spiking levels (in triplicate)

Ethanol		THF		Toluene	
Real concentration (%)	*R* (%)	Real concentration (ppm)	*R* (%)	Real concentration (ppm)	*R* (%)
0.1	91.1	356	103.7	433	113.5
	94.2		105.9		116.9
	92.9		104.7		117.2
0.5	93.2	711	104.7	867	119.3
	94.5		105.6		120.1
	93.5		105.7		117.2
1.1	108.5	889	97.3	1084	119.2
	107.5		99.4		126.8
	108.5		94.4		102.5
Mean (%) ($n = 9$)	98	–	102	–	117
R.S.D. (%) ($n = 9$)	7.7	–	4.2	–	5.6

Table 4
System repeatability at working concentrations and at the limit of quantitation

Solution	Ethanol		THF		Toluene	
	R.A. (WA)	R.A. (LOQ)	R.A. (WA)	R.A. (LOQ)	R.A. (WA)	R.A. (LOQ)
	263 μg	3.9 μg	593 μg	7.1 μg	116 μg	3.5 μg
Solution 1	0.241181	0.003424	3.851564	0.044774	5.381173	0.159929
Solution 2	0.241354	0.003426	3.910553	0.044662	5.567668	0.160905
Solution 3	0.240998	0.003436	3.895616	0.044795	5.555805	0.158739
Solution 4	0.240784	0.003428	3.802863	0.044681	5.315442	0.158886
Solution 5	0.241737	0.003430	3.918466	0.046063	5.570908	0.166252
Solution 6	0.241263	0.003425	3.893280	0.046013	5.482452	0.165286
Solution 7	0.241231	0.003439	4.013661	0.043510	5.876359	0.155054
Mean	0.241221	0.003430	3.898001	0.044928	5.535687	0.160722
R.S.D. (%)	0.1	0.2	1.7	2.0	3.3	2.4

R.A. (WA): relative areas at working amounts; R.A. (LOQ): relative areas at limit of quantitation.

Table 5
Intermediate precision for THF

Determination (ppm)	Analyst 1	Analyst 2
Result 1	313.1	273.7
Result 2	308.4	436.3
Result 3	309.9	295.4
Result 4	320.1	317.1
Result 5	474.1	392.9
Result 6	396.5	306.6
Mean	353.7	337.0

Six replicated determinations of the same batch of the drug substance obtained by two analysts using different columns and different instruments.

3.6. Robustness

3.6.1. Changes in HS and GC conditions

The method was robust to the changes studied in HS and GC conditions. Variations of ±10% on the carrier flow, ±5 °C on the initial oven temperature, ±1 °C/min on the temperature rate and changes of instrument and column batch did not affect selectivity, which still complied with the Eur. Ph. requirements (Table 6). System sensitivity also complied with Eur. Ph. requirements for changes of instrument, column batch and simultaneous variations of ±4 °C on equilibrium temperature and ±10 min on headspace equilibrium time.

Variations of the relative areas of ethanol, toluene and THF in a standards solution *(Reference Solution P3)* due to simultaneous variations on the equilibrium temperature (±4 °C) and equilibrium time (±10 min) were between 90% and 105% of the relative areas obtained using method conditions.

3.6.2. Solutions stability

The sample solutions stability was tested at 0 h, 12 h, 18 h and 24 h after sample preparation. The percentage of the relative peak area variations of ethanol, THF and toluene from time 0 h up to 24 h were between 95% and 105%.

Table 6
Effect of changes of GC and HS conditions on selectivity and system sensitivity: the Eur. Ph. requirement for *Reference Solution B* (resolution acetonitrile–dichloromethane) and critical resolution in *Reference Solution C* (acetone-2-propanol)

Selectivity: *Reference Solutions B* (Class 2) and *C* (Class 3)

Different instruments	Different columns	Flow ± 10% (ml/min)	Initial *T* ± 5 (°C)	*T* rate ± 1 (°C/min)	Resolution ACN/DCM	Critical resolution acetone/2-propanol
GC A	Batch A	5.0	40	10	3.7 ≥ 1.0	1.7 ≥ 1.0
GC B	Batch B	5.0	40	10	2.1 ≥ 1.0	1.8 ≥ 1.0
GC A	Batch A	4.5	40	10	3.7 ≥ 1.0	1.7 ≥ 1.0
GC A	Batch A	5.5	40	10	3.7 ≥ 1.0	1.7 ≥ 1.0
GC A	Batch A	5.0	35	9	3.9 ≥ 1.0	2.1 ≥ 1.0
GC A	Batch A	5.0	45	11	3.3 ≥ 1.0	1.3 ≥ 1.0

Sensitivity: *Reference Solutions A* and *A1* (Class 1 solvents)

Different instruments	Different columns	HS equilibrium *T*	HS equilibrium time	GC conditions	S/N 1,1,1-trichloroethane	Class 1 solvents detection in *Reference Solution A1*
GC A	Batch A	Method	Method	Method	46 ≥ 5	All Class 1 solvents detected.
GC B	Batch B	Method	Method	Method	41 ≥ 5	All Class 1 solvents detected.
GC A	Batch A	84 °C	70 min	Method	51 ≥ 5	All Class 1 solvents detected.
GC A	Batch A	76 °C	50 min	Method	52 ≥ 5	All Class 1 solvents detected.

4. Conclusions

In this study, a HS-GC analytical method was developed and validated for the qualitative determination of residual solvents and the quantitative determination of ethanol, tetrahydrofurane and toluene in a drug substance. Development was carried out according to requirements of the Eur. Ph. general method [10]. Sample solvent water–DMF (3:2) was selected to obtain good recoveries for ethanol, tetrahydrofuran and toluene, and the sample dilution factor was adapted to detect all Classes 1 and 2 solvents at ICH levels by FID (except 1,1,1-trichloroethane that was evaluated at 10 ppm instead of 1500 ppm, ICH limit). The proposed method uses the standard addition technique with internal standard quantitation for ethanol, tetrahydrofuran and toluene determination. The method was validated within ICH guidelines Q2A and Q2B [15,16]. Selectivity, limits of detection and quantitation, linearity, accuracy, precision (system repeatability, method precision and intermediate precision) and robustness (changes in HS and GC conditions and solutions stability) were determined. Excellent results were obtained within the worldwide-accepted validation reference values, and particularly taking into account the low concentration levels investigated.

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