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SIMULTANEOUS DETERMINATION OF CLASS 1 RESIDUAL SOLVENTS IN ORGANIC DILUENTS BY CHROMATOGRAPHIC METHODS

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ABSTRACT

Residual solvents (RS) are volatile organic chemicals that are used during the manufacturing process of pharmaceuticals. They are classified into three categories according to their toxicity potential. The Class 1 residual solvents include the compounds considered to be the most toxic. A simple chromatographic method has been developed for the determination of tested Class 1 residual solvents (1,1-dichloroethene, 1,2-dichloroethane, 1,1,1-trichloroethane, carbon tetrachloride and benzene) in typical organic diluents like DMSO, DMF, DMA. The analytes separation was obtained on DB-624 and DB-WAX columns. The developed gas chromatographic method offers peak shape, good resolution and reasonable retention times for all the solvents.

KEY WORDS

Class 1 of residual solvents, HS-GC-FID, DMSO, DMA and DMF.

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INTRODUCTION

The volatile organic solvents which are used in the manufacturing of pharmaceutical formulations need to be removed from the finished product because of their possible health risk and toxicity to the consumers.

The acceptable maximum levels of residual solvents that can be left behind according to the worldwide regulatory standards were originally derived from patient safety considerations[1-4]. The International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceutical for

Human Use (July 1997) formulated guidelines for residual solvents control. The residual solvents determination has been adopted by Pharmacopoeias. While solvents play a key role in the production of pharmaceuticals, there is also a negative aspect of these substances. Each of these solvents used in the drugs production has different toxic or environmentally hazardous properties[5]. Since a class 1 residual solvent includes the solvents that are considered to be the most toxic, their use should be avoided in the production of the pharmaceutical products. These chemicals are: 1, 1-dichloroethene, 1, 2-dichloroethane, 1, 1, 1-trichloroethane, carbon tetrachloride and benzene. Class 2 and 3 residual solvents are considered to be of lower risk. Releases of benzene to the environment are largely to air, which is due to its volatile nature. Major sources of releases to air include gasoline vapor, auto exhaust and manufacturing industries. In case of carbon tetrachloride exposure can cause liver, kidney, and central nervous system damage. These effects can occur after ingestion or breathing carbon tetrachloride, and possibly from exposure to the skin.

Gas chromatography with different detectors is strongly popular in the determination and identification of numerous volatile substances. A new type of volatile substances detection such as electroantennographic detection coupled with gas chromatography was employed in recent studies[6]. These gas chromatography methods are generally used to determine residual solvents because of the excellent separation properties and low limit detection. Headspace sampling is preferred because of its ability to avoid direct liquid or solid probing. In the headspace sampling complex matrix in a solid or liquid sample matrix in liquid or solid sample can be simplified or even eliminated in its vapor phase. A modern GC capillary column can separate a large number of volatile components, facilitating identification through retention characteristics and detection at ppm levels. The most popular type of GC detectors – flame ionization detector (FID) constitutes universal detector for organic volatile. FID is the most preferred analytical detector for release-related tasks because of its low limit detection, linear range and robustness for organic compounds. There are abundant literature data concerning the analysis of the organic solvents, either in the GC method or in the headspace gas chromatography method[7-15]. Available scientific data on testing of residual solvent in pharmaceutical products reports that there is over 80% of literature citations on GC with FID for residual solvents detection [16-18].

The Ph.Eur. (European Pharmacopeia) general method for Identification and Control of Residual Solvents in drug substance defines a general procedure and describes two complementary GC conditions for the identification of unknown solvents. The first of these procedures - method A is recommended for general use. Another one, method B, is applied to confirm identification and to solve coelutions. Implementation of this general method is a subject of major concern in the pharmaceutical industry. In this study, the general Ph.Eur. method is applied for qualititative analysis of Class 1 residual solvents in a typical organic diluents like dimethyl sulphoxide (DMSO), N, N-dimethyl formamide (DMF), N,N-dimethyl acetamide (DMA). These solvents are used as diluents for analysis of residual solvents in drug substances, excipients or drug products. Determination and control of solvents class 1 in typical organic diluents are important. Waste of used organic diluents with high level of solvents class 1 can negative influence for environmental, human life. Residual solvents class 1 was determined by limit test.

Boiling points, maximum permitted limits of concentration for each compound and concerns in class 1 residual solvents are shown in Table No.1. Method A determination of class 1 residual solvents, recommended by Ph.Eur. was used correctly. DMSO, DMF and DMA were prepared as samples at the concentration of ~10 mg/mL. The concentration of compounds for Class 1 residual solvents are shown in Table No.1. Peak areas and R.S.D for class 1 residual solvents according to Ph.Eur. Method A of determination of residual solvent is shown in Table No.2. Peak areas for

each solvent were not satisfying to determine the levels of class 1 residual solvents. The procedure and concentration of sample solutions and standard solution were modified to achieve adequate results. Peak areas and R.S.D. for class 1 residual solvents after modification procedure are shown in Table No.2.

EXPERIMENTAL

Instrumentation and Reagents

Chemicals of high purity level and analytical grade were used. As diluents, filtrated purified water and dimethylsulphoxide (DMSO) from Merck (Germany) were applied. Residual solvents mixture – Class 1 from Sigma-Aldrich (Germany) was used as standards. The concentration of each solvent in class 1 mixture is shown in Table No.4.

Analysis was performed using gas chromatograph (Agilent Technologies 6890N) equipped with electronic pressure control (EPC), a split/splitless injector and FID detector connected to Agilent G1888 Headspace sampler (HS). Data were acquired and processed using Empower 2 software.

Methods

Method A: 30m long DB-624 column, 0.530mm in inner diameter and 3.0µm in film thickness (manufactured by J&W Scientific, USA) was used. Injection port was heated at 140^{o}C while the temperature of detector was 250^{o}C. Helium was allowed to flow at a rate of 4.9 mL/min. Hydrogen gas and air supply to the detector was 30 mL/min and 300 mL/min, respectively. The sample was introduced in the column in a split mode with split ratio, 5.0:1. The column temperature was kept 40^{o}C for 20 min followed by an increase in the temperature at a rate of 10^{o}C/min to 240^{o}C. The 240^{o}C temperature was held up to 20 min.

Method B: 30 m long DB-WAX column, 0.320mm in inner diameter and 0.25µm in film thickness (manufactured by J&W Scientific, USA) was used. Injection port was heated at 140^{o}C while the temperature of detector was 250^{o}C. Helium gas was allowed to flow at a rate of 2.1 mL/min. Hydrogen gas and air supply to the detector was 30 mL/min and 300 mL/min, respectively. The sample was introduced in the column in a split mode with split ratio, 5.0:1. The column temperature was kept 50^{o}C for 20 min followed by an increase in the temperature at a rate of 6^{o}C/min to 165^{o}C. The 165^{o}C temperature was held up to 20 min. The headspace conditions are shown in Table No.3.

Standard solutions Method A and Method B

Class I Standard Stock solution - 1.0 mL of Class I residual solvents mixture was transferred to a 100 mL volumetric flask, 9.0 mL of DMSO was added and diluted with water to final volume of 100 mL and mixed. 1.0 mL of prepared solution was transferred to a 10 mL volumetric flask and diluted with water to volume, then the solution was mixed.

Class 1 Standard solution – 1.0 mL of Class 1 Standard Stock solution was transferred to an appropriate headspace vial, 5.0 mLof water was added, the stopper and the cap was applied, and mixed. The concentration of analytes in Class I Standard Stock solution, Class 1 Standard solution are shown in Table No. 4.

Test Solution – 5.0 mL of the test sample (DMSO, DMF or DMA) was transferred to an appropriate headspace vial, 1.0 mL of water was added, the stopper and the cap was applied, then the solution was mixed. Density of the organic diluents and the concentration of the test solution are shown in Table No. 5.

Class 1 System Suitability Solution – 1.0 mL of Class 1 Standard Stock Solution was transferred to an appropriate headspace vial, 5.0 mL of Test Solution was added, the stopper and the cap was applied and then the solution was mixed.

Blank solution - 9.0 mL of DMSO was transferred to a 100 mL volumetric flask, diluted with water to volume and mixed. 1.0 mL of this solution was transferred to a 10 mL volumetric flask, diluted with water to volume, and then the solution was mixed.

Procedure

Equal volumes of the Class 1 Standard Solution and the Test Solution were separately injected, data were recorded, and responses for the major peaks were measured. If a peak responses to any peak, other than a peak for 1,1,1-Trichloroethane, and in the Test Solution the peak was greater than or equal to a

corresponding peak in the Class 1 Standard Solution, Method B was proceeded to verify the identification of the peak; otherwise the substance met the requirements of the test. The signal to noise of 1,1,1-Trichloroethane in Class 1 Standard Solution was not allowed to be less than 5, the signal to noise of each peak in the Class 1 System Suitability Solution was not less than 3.

RESULTS AND DISCUSSION

The DB-624 column (6% cyanopropylphenyl / 94% dimethylsiloxane) is a standard stationary phase, which is adopted as a first choice by the Ph.Eur. (Method A) and also recommended by the USP compendial method. Figure No. 1 demonstrates a typical chromatogram of Class 1 Standard solution on DB-624 column.

If a coeluting pair can be expected, a column with a different stationary phase had to be used to control of these solvents. The coelution of common solvents was reported but the coelutions may differ depending on specific laboratory conditions. The Ph.Eur. proposes a second column (DB-WAX, method B) to verify identity. Figure No. 2 demonstrates a typical chromatogram of Class 1 Standard solution on DB-WAX column. System suitability has been demonstrated by analyzed class 1 system suitability solution during validation. The system was checked by tailing factor, symmetry factor and number of theoretical plates. The list of the tailing factor, number of theoretical plates and the retention times in Table No.6. Fast separation of a limited number of the residual solvents by GC static headspace has been reported [11,19].

All experiments were performed according to the described general procedure without using an internal standard. The internal standards are not necessary for HS analysis but may be used to improve the precision. The precision of the analysis should be confirmed on a regular basis, either as an integrated part of the method (system suitability test) or by other regular controls of equipment performance. The repeatability at the levels was acceptable, with a R.S.D below 10%. Relative standard deviations of the peak areas for six injections of Class 1 Standard solution are shown in Table No. 7.

Specificity has been established by injections of class 1 standard solution. No peaks were observed in injections of blank solution. Chromatogram of blank solution and class 1 standard solution are presented in Figure No. 1 and Figure No. 2.

Limit of detection of an analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessary, quantified as an exact value. To define a limit of detection, the analyst must determine the minimum concentration of an analyte. Limit of quantification is the lowest amount of an analyte in a sample, which can be quantitately determined. LOD and LOQ have been established by six injections at LOD level and six injections at LOQ level. Table No.8 shows the limit of detection and limit of quantification for class 1, relative standard deviations of the peak areas for six injections (LOQ concentrations) of are shown in Table No.9.

The sample stability was tested at 0h, 12h, 24h after the sample preparation. The percentage of the peak areas for analystes from time 0 h up to 24h were between 97,8% and 101,4%.

Robustness has been establish by analyzing sample in triplicate as per proposed method and by changing the vial temperature in headspace sampler by +10% of the original value. The robustness of the purposes method is expressed in the term of %R.S.D. of the all data. %R.S.D. calculated for residual solvents class 1 were found to be less than 15%.

The signal to noise ratio (S/N) for 1,1,1-trichloroethane in the chromatogram of Class 1 Standard solution was 10.The signal obtained from a solvent analyzed by GC-HS using flame ionization detector is a combination of a detector response of the solvent and its concentration in the gaseous phase of the headspace vial. Selectivity and system sensitivity requirements defined in the Ph.Eur. for Method A conditions were checked for Class 1 residual solvents. The solvents were detected and quantified at the levels or below the ppm limits appointed by the Ph.Eur. guidelines.

Table No.1: Maximum permitted concentrations, Concentrations of compounds in Method A by Ph.Eur., concerns and the boiling points for the 5 solvents in Class 1 residual solvents

Solvent	ICH Limit (ppm)	Concern	Concentration of compound [µg mL^{-1}]	Concentration of compound in headspace vial [µg mL^{-1}]	Boiling point (^{o}C)
Benzene	2	Carcinogen	0.10	0.02	80
Carbon Tetrachloride	4	Toxic and environmental hazard	0.21	0.03	77
1,2-Dichloroethane	5	Toxic	0.27	0.04	83
1,1-Dichloroethene	8	Toxic	0.40	0.07	32
1,1,1-Trichloroethane	1500	Environmental hazard	0.51	0.08	72

Table No.2: Peak areas and R.S.D for Class 1 residual solvents according to Ph.Eur. and after modification procedure method A of the determination of the residual solvent (n=6)

Solvent	Ph.Eur. (method A)		After modification procedure	
	Peak area	R.S.D [%]	Peak area	R.S.D [%]
Benzene / 1,2-Dichloroethane	8.9	7.0	1191.2	1.6
Carbon Tetrachloride	0.3	10.1	73.3	2,3
1,1-Dichloroethene	4.6	10.7	887.6	1.8
1,1,1-Trichloroethane	5.3	10.8	968.5	0.9

Table No.3: Headspace conditions for the residual solvents determination

S. No	Headspace conditions	
1	Vial temperature	$80^{o}C$
2	Loop temperature	$80^{o}C$
3	Transfer line temperature	$85^{o}C$
4	Vial equilibration time	60 min
5	Vial pressurize time	0.50 min

6	Loop fill time	0.20 min
7	Loop equilibration time	0.1 min
8	Injection time	0.1 min
9	GC cycling time	75 min
10	Inject volume	1.0 mL

Table No.4: Concentration of solvents in Class 1 mixture, Standard Stock solution, concentration of Class 1 Standard solution and concentration of Class 1in headspace vial

Solvent	**Concentration of Class 1 mixture [mg mL^{-1}]**	**Concentration of Class 1 Standard Stock solution [mg mL^{-1}]**	**Concentration of Class 1 Standard solution [mg mL^{-1}]**
Benzene	10.17	0.01017	0.00170
Carbon Tetrachloride	20.86	0.02086	0.00348
1,2-Dichloroethane	26.58	0.02658	0.00443
1,1-Dichloroethene	40.32	0.04032	0.00672
1,1,1-Trichloroethane	50.78	0.05078	0.00846

Table No.5: Organic diluents density and the test solution concentrations

S. No	**Organic diluent**	**Density [mg mL^{-1}]**	**Concentration of test solutions [mg mL^{-1}]**
1	Dimethylsulphoxide	1095.8	913.2
2	N,N-dimethylformamide	944.0	786.7
3	N,N-dimethylacetamide	936.6	780.5

Table No.6: System suitability of the residual solvents (DB-624 and DB-WAX columns)

Solvent	DB-624		
	Retention time [min]	**Tailing factor**	**No. of theoretical plates**
Benzene / 1,2-Dichloroethane	10.6	1.30	13427
Carbon Tetrachloride	9.7	0.99	17003
1,1-Dichloroethene	3.9	1.05	17511
1,1,1-Trichloroethane	9.2	1.03	15694
Solvent	**DB-WAX**		
	Retention time [min]	**Tailing factor**	**No. of theoretical plates**
Benzene	3.0	1.02	15810
Carbon Tetrachloride / 1,1,1-Trichloroethane	2.6	0.98	10899
1,1-Dichloroethene	2.0	1.03	6559
1,2-Dichloroethane	5.0	1.03	57804

Table No.7: Precision of A and B methods for the determination of the residual solvents (n=6)

Solvent	Method A (DB-624)	Method B (DB-WAX)
	R.S.D [%]	**R.S.D [%]**
Benzene / 1,2-Dichloroethane	1.7	3.7
Carbon Tetrachloride	2.6	4.6
1,1-Dichloroethene	1.5	5.1
1,1,1-Trichloroethane	1.8	1.4

Table No.8: The limit of detection and limit of quantification for class 1residual solvents

Solvent	LOD [µg mL-1]	LOQ [µg mL-1]	LOD [µg mL^{-1}]	LOQ [µg mL-1]
	DB-624		DB-WAX	
Benzene	0.09	0.32	0.08	0.26
Carbon Tetrachloride	0.19	0.52	0.17	0.40
1,2-Dichloroethane	0.25	0.68	0.22	0.58
1,1-Dichloroethene	0.38	0.97	0.34	0.87
1,1,1-Trichloroethane	0.47	1.14	0.42	1.03

Table No.9: Repeatability and peak areas of A and B methods for the determination for class 1 (n=6)

Solvent	R.S.D [%]	Peak area in DMF	Peak area in DMA	Peak area in DMSO
Method A				
Benzene / 1,2-Dichloroethane	1.6	3.0	3.4	1.6
Carbon Tetrachloride	2.0	0.3	0.2	0.2
1,1-Dichloroethene	1.3	8.0	6.4	2.2
1,1,1-Trichloroethane	1.9	4.3	3.2	1.7
Method B				
Benzene	3.3	11.2	12.9	12.0
Carbon Tetrachloride / 1,1,1-Trichloroethane	2.2	19.7	21.9	18.7
1,1-Dichloroethene	4.3	37.5	44.7	35.2
1,2-Dichloroethane	1.6	3.5	4.1	3.2

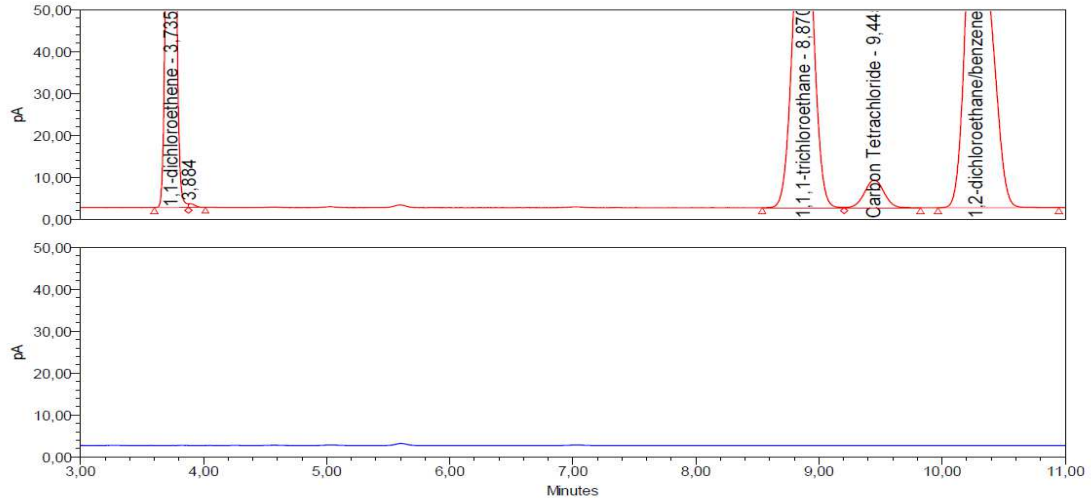

Figure No.1: Typical chromatogram of blank and Class 1 standard solution; Column: DB-624, 30m x 0.53mm x 3.0µm

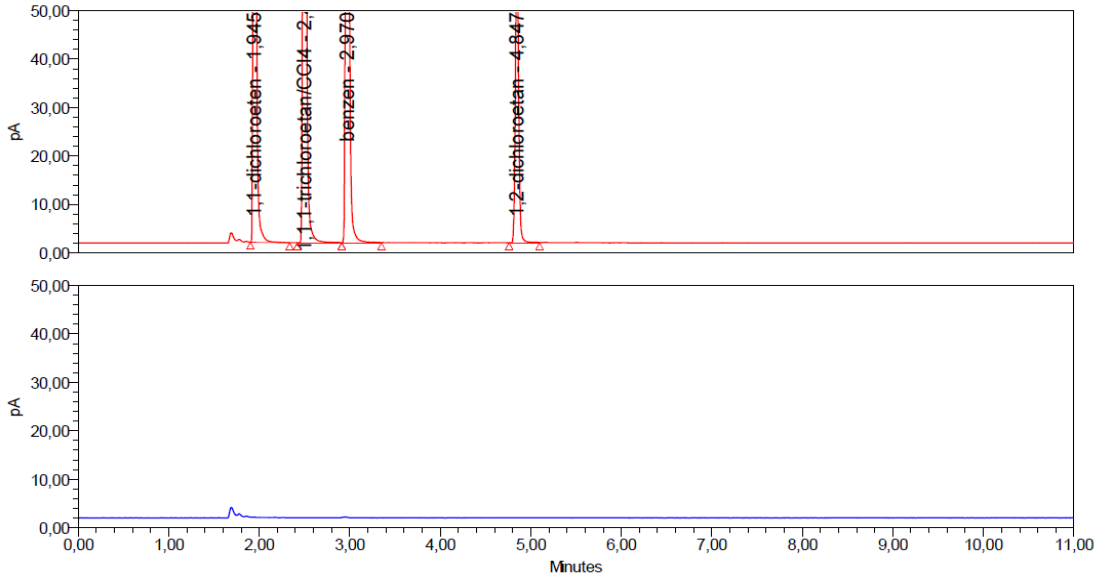

Figure No. 2 Typical chromatogram of blank and Class 1 standard solution; Column: DB-WAX, 30m x 0.32mm x 0.25µm.

CONCLUSIONS

A sensitive, specificity and precise GC analytical method was developed for the determination of Class I residual solvents in these diluents. We have modified this method to gain its universality in the quality control of Class 1 residual solvents at lower levels of concentration in typical organic diluents. Accordingly proceeded Ph.Eur. general method does not allow to test the residual solvents concentrations at the pharmacopeia lowest levels and to achieve satisfying results (resolution, peak area, tailing factor). Our method we have established meets ICH guidelines requirements and may be used for any routine control of pharmaceuticals. Our procedure simplifies the test solution preparation in advance and allows to achieve good peaks response for these

tested residual solvents in any of the most popular organic diluents (DMSO, DMF and DMA) used in routine quality control of drug substances, excipients or drug products.

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