Research Article

A New HSGC-FID Method Development and Validation for the Simultaneous Estimation of Residual Solvents in Ifosfamide

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ABSTRACT

The purpose of this study was to develop a novel, sensitive and accurate analytical method for quantification of 4 residual solvents in ifosfamide by using a headspace gas chromatography (HSGC) coupled with flame ionization detector (FID). Methanol, Diethyl ether, Dichloromethane, Tetrahydrofuran as residual solvents were determined in Ifosfamide API through an understanding of the synthetic process, nature of solvents and nature of stationary phases of columns. The separations of 4 residual solvents were achieved on DB-624 (30 meters ×0.32 mm I.D, 1.8µm) column. Nitrogen was used as a carrier gas with constant pressure 7.0 psi. As a sample diluent in a headspace sampling Dimethyl sulfoxide was selected owing to its high capacity for dissolving ifosfamide sample. The headspace condition was optimized with the vial temperature of 80°C and time at 45 min. The injection was administered in split mode, with a split ratio of 20:1. Excellent correlation coefficient between peak responses and concentrations were > 0.99. The recoveries of all 4 solvents spiked in ifosfamide were in the range from 99.5% to 100.07 %. Limit of quantitation for all 4 solvents were sufficiently lower than limits specified by ICH. The method has validated as per International Conference on Harmonization (ICH) guidelines. The developed gas chromatographic method offers a symmetric peak shape, good resolution and reasonable retention time for all the solvents. A precise, accurate, linear and robust Headspace Gas Chromatography method was developed for the quantification of 4 residual solvents in Ifosfamide. A single, rapid and highly selective HSGC method was developed and validated for the quantification of residual solvents present in ifosfamide API through an understanding of the synthetic process, nature of solvents and nature of stationary phases of columns.

Keywords: Headspace, GC-FID, Ifosfamide, residual solvents.

INTRODUCTION

Residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or with in the preparation of drug products. The residual solvents aren't completely removed by practical manufacturing techniques. Appropriate selection of the solvent for the synthesis of a drug substance or an excipient might enhance the yield, or determine characteristics such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical element in the synthetic process. Analysis of a residual solvent in pharmaceuticals is an important issue because of the potential risk to human health from the toxicity of many of these solvents. The amount of such solvents is therefore limited by international conference on harmonization (ICH) guidelines (ICH guidelines, 1997). The international conference on harmonization recommends and limits the amount of residual solvents considered safe in pharmaceutically finished goods and for human use. The ICH has published guidelines and daily exposure limit of many solvents. It has classified these solvents in three categories depending upon their toxicity. Class I solvents are known human carcinogens and environmental hazards, the use of these solvents should be avoided if at all possible. Class II solvents are non-genotoxic animal carcinogens or possible causative agents of different irreversible toxicity like neurotoxicity or teratogenicity. Use of these solvents should be limited. Class III solvents are solvents with low toxic potential to man; no health-based exposure limit is needed. In the pharmaceutical industries, all the pharmaceutical products must be analyzed for residual solvent content, regardless of the matrix. Because residual solvents don't offer therapeutic profit, they need to be compelled to be removed, to the extent possible, to satisfy ingredient and products specifications, good manufacturing practices, or other quality- based requirements. Drug product ought to contain no higher levels of residual solvents than will be supported by safety information. In the synthesis of drug substances numerous organic solvents are used at numerous stages. These solvents may be used as a medium for the reaction or purification of intermediates or drug substances. Intermediates and drug substances are dried at a specific temperature to remove the used solvents, but traces of them may carry forward to drug substance as impurities which are called as residual solvents.[1]

Gas chromatography is generally used to determine residual solvents because of its excellent separation abilities and low limit of detection. In gas chromatography the sample is either dissolved in a suitable solvent than injected directly (DI) or by headspace sampling. Headspace sampling is preferred because of its ability to avoid direct liquid or solid probing. In the headspace sampling complex sample matrix in a solid or liquid sample matrix in liquid or solid sample can be simplified or even eliminated in its

vapor phase. Headspace gas chromatography (HSGC) is a technique where the liquid or solid sample is set in a closed vessel till the volatile parts reach equilibrium between the sample and the gas volume on the top of, i.e., the so called "headspace". The advantage of the headspace sampling is that direct liquid or solid probing is avoided and complex sample matrix during a liquid or solid sample will be simplified or even eliminated in its vapor phase.[2]

Figure 1: Structure of Ifosfamide

Ifosfamide is an antineoplastic agent and immunomodulating agent. The chemical name for ifosfamide is 3-(2-chloroethyl)-2-((2-chloroethyl)amino) tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide with a Molecular Formula: $C_7H_{15}CL_2N_2O_2P$ and Molecular Weight: 261.1 g /mol. It is highly lipophilic and practically insoluble in water. The structural formula of ifosfamide is shown in Fig. 1.

The exact mechanism of ifosfamide has not been determined, however seems to be the same as alternative alkylating agents. Ifosfamide needs biotransformation with in the liver by mixed-function oxidases (cytochrome P450 system) before it becomes active. Once metabolic activation, active metabolites of ifosfamide alkylate or bind with several intracellular molecular structures, as well as nucleic acids. The cytotoxic action is essentially through the alkylation of DNA, done by attaching the N-7 position of guanine to its reactive electrophilic groups. The formation of inter and intra strand cross-links in the DNA ends up in cell death.

The metabolism is Primarily hepatic. Ifosfamide is metabolized through a couple of metabolic pathways: ring oxidation for the formation of active metabolite, 4-hydroxy-ifosfamide and side- chain oxidation to form the inactive metabolites, 3-dechloro-ethyl-ifosfamide or 2-dechloroethyl-ifosfamide with liberation of the poisonous metabolite, chloroacetaldehyde. Small quantities (nmol/mL) of ifosfamide mustard and 4-hydroxyifosfamide are detectable in human plasma. Metabolism of ifosfamide is needed for the generation of the biologically active species and where as metabolism is in depth, it is also quite variable among patients.

Ifosfamide is excreted primarily in the urine. The terminal half-life is about 7 hours at doses of 1.6 to 2.4 g/m daily and about 15 hours at a single dose of 3.8 to 5 g/m^2 with a half life of 7-15 hours. Ifosfamide shows little plasma protein binding.

Ifosfamide is clinically well established chemotheraphy medication mainly used for the management of recurrent testicular cancer and germ cell tumors, Sarcomas (soft-tissue, osteogenic sarcoma, Ewing's sarcoma), Non-Hodgkin's lymphoma, Hodgkin's disease, Non-small cell and small cell lung cancer, Bladder cancer, Head and neck cancer. Used as a part of varied chemotherapeutical regimens as third-line therapy for recurrent or refractory germ cell testicular cancer.[3,4]

During the development of different synthetic routes, a number of solvents were used for reactions, washes, recrystallizations etc. aimed at obtaining high-purity products. The residues should be determined in order to insure the quality of ifosfamide APIs, thus four organic solvents had been taken into consideration, which were named methanol, tetrahydrofuran (THF), diethylether, dichloromethane. However, test methods for the quality control of residual solvents in ifosfamide have not been reported. Therefore, a new GC method for simultaneous determination of the four organic solvents in ifosfamide bulk drug was developed and validated in this paper.

From the literature review there were few analytical methods have been reported for ifosfamide such as HPLC and GC-MS methods.[7-14] There was no reported method for the determination of Residual solvents in ifosfamide by headspace Gas chromatographic method. The major objective of the present work is to develop a simple and robust GC method for determination of 4 Residual solvents in ifosfamide. Hence, a reproducible Gas Chromatography with static headspace method was developed for the quantitative determination of 4 Residual solvents in ifosfamide. This method was successfully validated according to the International Conference Harmonization (ICH) guidelines. Based on ICH guidelines, methanol, THF and dichloromethane are Class 2 solvents with the maximum limit of 3000, 720 and 600 p.p.m., respectively. Additionally, diethyl ether belongs to Class 3.

MATERIALS AND METHODS

Reagents and chemicals

Ifosfamide raw material was procured from celon laboratories Pvt limited, India, diethylether was obtained from molychem, while rest of the solvents namely dimethyl sulfoxide, methanol, dichloromethane and tetrahydrofuran of GC grade was obtained from Qualigens. The drug formulation ifomid-M(1G) Mfg by: united biotech(p) ltd was obtained from a local pharmacy.

Method Development

Instrumentation and conditions

A gas chromatograph (Agilent Technologies 7890 B) equipped with FID (Flame ionization detectors) connected to Agilent 7697A Headspace sampler and a data processor Agilent technologies Open Labs software version 3.2.1 was employed. Under the standard conditions, capillary column DB-624 of length 30 m having internal diameter 0.530 mm and 3 µm film thickness (manufactured by J & W Scientific, Agilent Technologies, USA) was used. Both the injection ports were heated at 220°C while the temperature of detector was maintained at 230°C. Nitrogen, the carrier gas was allowed to flow at a rate of 1

ml per minute. The supply of the Hydrogen gas and air to the detector was 40 and 400 ml, respectively. The sample was introduced in the column in a split mode with split ratio, 20:1. The column temperature was kept at 40°C for 5 min followed with an increase in the temperature at a rate of 10°C per minute to 150°C. This temperature was held up to 5 min. The column was then cooled down to the original temperature of 40°C, so that one analysis was terminated in 40 mins of injection. The temperature of the detector was set at 230°C. The detailed GC, head space, detector and the oven conditions are mentioned in Table 1,2 and 3.

Table 1: Optimized Chromatographic Conditions

Column	DB-624 (30 m x 0.32 mm x1.8 µm)
Injector temperature	220°C
Carrier gas (N2)	1ml/min
Run time	20.5 mins
Split ratio	20:1
Detector temperature	230°C
Hydrogen flow	40ml/min
Air flow	400ml/min

Table 2: Optimized Headspace Conditions

Vial temperature	80°C
Loop temperature	95°C
Transfer line temperature	105°C
Vial equilibration temperature	45°C
Vial pressurize time	0.5min
Loop fill time	0.2min
Loop equilibration time	0.05min
Injection time	1.0min
GC cycle time	40min

Table 3: Column Oven Temperature Programme

Rate (°C/min)	Temperature (°C)	Hold time (min)
-	40	5
10	150	5

Standard solutions and sample preparation

DMSO was selected as the sample and standard diluent due to its low vapor pressure and high solubility for organic compounds, is usually used as the diluent in headspace GC.

Preparation of Standard Stock solution

Weigh accurately about 500 mg of Methanol, 500 mg of Diethylether, 500 mg of Dichloromethane 500mg of mg Tetrahydrofuran in 250ml Volumetric flask containing about 180 ml of dimethyl sulfoxide (diluent), make up to volume with dimethyl sulfoxide and mix.

Preparation of Standard solution

Pipette out 10 ml of above solution in 200 ml volumetric flask containing about 20 ml diluent, make up to volume with diluent. Pipette 1 ml of above prepared solution in headspace vial & seal the vial properly fitted with a septum and crimp cap. Dimethyl sulfoxide was used as a blank.

Test sample preparation

Weigh accurately about 500 mg of test sample (Ifosfamide API) and transfer in to 50mL volumetric flask add 35mL of Dimethyl sulfoxide, vortex it for 5min. Then make up the volume with diluent and mix well. Pipette 1 ml of above prepared solution into an agilent technologies manufactured flat bottomed headspace GC vial crimp cap and seal the vial.

RESULTS AND DISCUSSIONS

Specificity

Capacity of the method to measure the analyte peak (solvent) response in the presence of other components is termed as specificity. For this blank and spiked test sample solutions were injected and observed the chromatogram for any interference from blank and test sample peaks at the retention of solvent peaks.it was observed that there was no interference at the retention time of solvent peaks. The chromatograms of blank, and sample spiked with solvents standard are shown in Figure-I and II. The values are given in table -1.

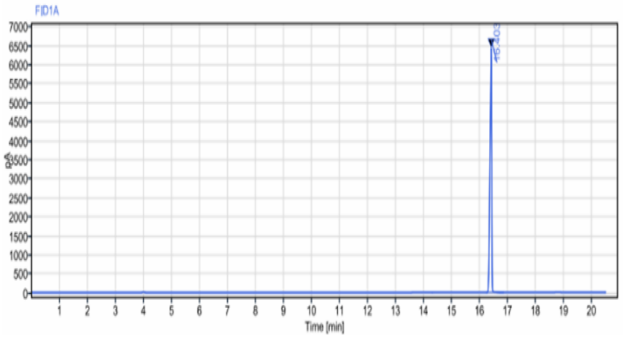

Figure 2: Chromatogram of blank for specificity

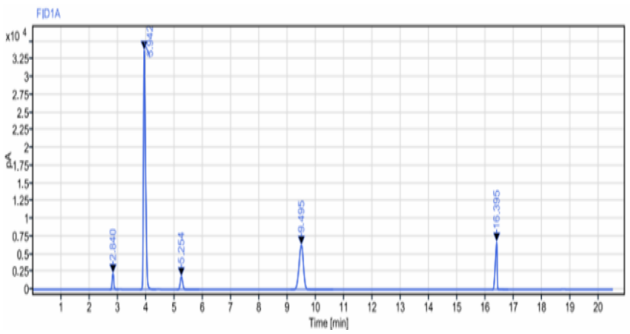

Figure 3: Chromatogram of mixed residual solvents for specificity

Linearity

The linearity of the method was determined by making injections of each residual solvent over the range 50-150%

correlation coefficient (R^2), slope and y-intercept were calculated from linearity data A plot between the concentrations vs. the average peak responses of each residual solvent peak for all linearity solution

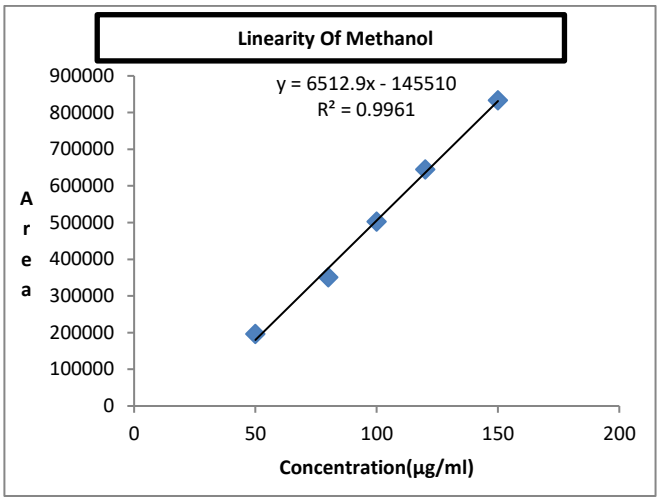

Figure 4: linearity graph of methanol

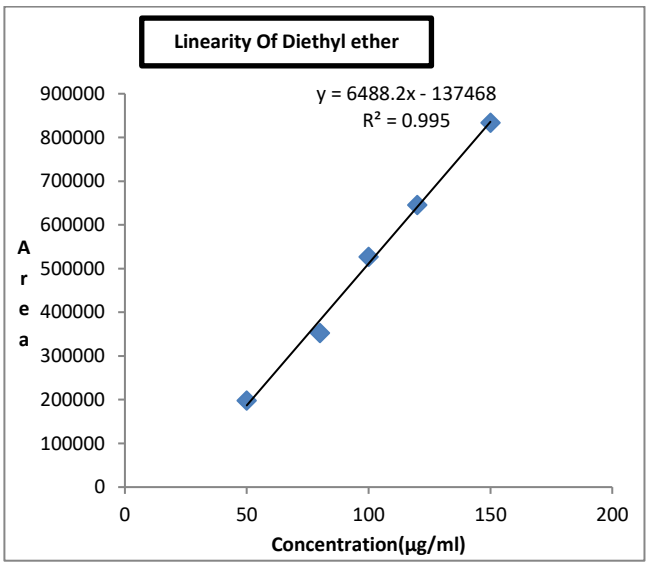

Figure 5: linearity graph of Diethyl ether

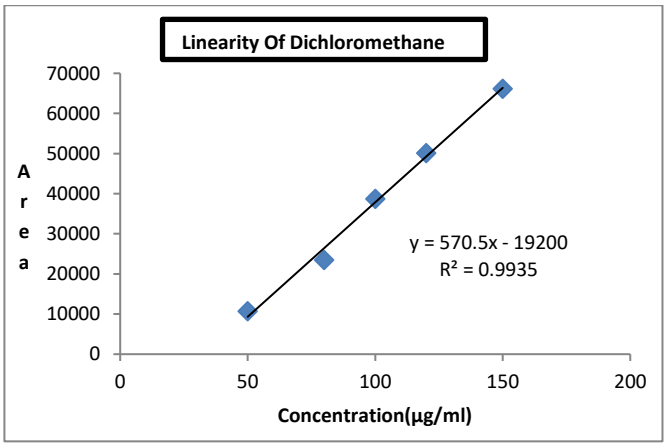

Figure 6: linearity graph of Dichloromethane

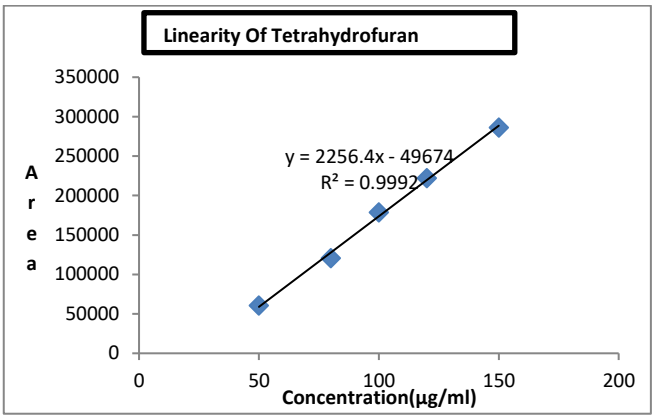

Figure 7: linearity graph of Tetrahydrofuran

Table 4: Validation Results for Residual Solvents.

Solvent	Specificity (Retention time)	LOD (µg/ml)	LOQ (µg/ml)
Methanol	2.840	0.0261	0.079
Diethyl ether	3.942	0.040	0.124
Dichloromethane	5.254	0.171	0.520
Tetrahydrofuran	9.495	0.010	0.0312

Accuracy

Accuracy of the method was determined by Recovery studies. To the API (pre analyzed sample), The solvents were added at the level of 50%, 100%, 150%. The average recoveries were calculated to be 99.870%, 99.877%, 101.1% and 100.047% for methanol, diethyl ether, dichloromethane and tetrahydrofuran respectively.so it may be concluded that the method is accurate. Recovery of each solvent was between 80-120%. All the values are listed in table 5.

System precision

System precision has been demonstrated by 6 replicate injections of standard solutions at the working concentration. The standard solution is prepared at the working concentration and analysed as per method. The system precision is expressed in terms of %RSD of the data. The RSD was found to be less than 10%. All values are listed in Table-6.

Method precision

Method precision has been demonstrated by separately analysing one batch of sample 6 times (as per the method). The method precision of the proposed method is expressed in terms of %RSD of the data. RSD was found to be less than 15% (calculated only for residual solvent). All values are listed in table-7.

Ruggedness

Ruggedness has been established by separate 6 analyses of single batch of sample, prepared by 2 different analysts on different days. The ruggedness of the proposed method is

expressed in terms of %RSD of all the data. Overall RSD of residual solvents were found out to be less than 10%. All values are listed in table-8.

Robustness

Robustness has been established by analysing sample in triplicate as per the proposed method and by changing the carrier gas flow rate and temperature by +/-20% and +/-40% of the original value. The robustness of the proposed method is expressed in terms of %RSD of the all data. Overall RSD calculated for residual solvents were found to be less than 15%. All values are listed in table-9.

Table 5: Results of Accuracy

Range	Methanol	Diethyl ether	Tetrahydrofuran	Dichloromethane
50% recovery	99.6	100.01	99.51	100.06
100% recovery	100.01	99.62	99.95	100.04
150% recovery	100	100	100.07	100.04
Avg % Recovery	99.870	99.877	101.1	100.047

Table 6: Results of System Precision.

Solvent Name	Methanol	Diethylether	Dichloromethane	Tetrahydrofuran
1	60707	1121167	94203	450861
2	59990	1148409	91903	456116
3	59336	1147301	95485	456323
4	60716	1121137	94204	451646
5	60005	1142514	94251	451251
6	62141	1125141	94525	451362
Avg	60482.500	1134278.17	94095.17	452926.417
Stdev	963.387	13154.348	1077.787	2563.851
%RSD	1.59	1.16	1.14	0.57

Table 7: Results for Method Precision of Solvents

Solvent Name	Methanol	Diethylether	Dichloromethane	Tetrahydrofuran
1	61723	1121210	94213	450843
2	59438	1148427	91929	456137
3	59322	1147329	95473	456343
4	60729	1121155	94262	451611
5	60122	1142575	94277	451242
6	62164	1125175	94601	451353
Avg	**60583.00**	**1134311.83**	**94125.83**	**452921.50**
St. dev	**1177.64**	**13154.52**	**1176.07**	**2583.20**
%RSD	**1.94**	**1.16**	**1.25**	**0.57**

Table 8: Results for Ruggedness

Solvent Name	Methanol	Diethylether	Dichloromethane	Tetrahydrofuran
1	60685	1121267	94213	450756
2	59856	1148581	91927	456128
3	59348	1147357	92522	456348
4	60851	1121185	94227	451725
5	60125	1142472	94267	451277
6	62285	1125159	94548	451422
Avg	60525.000	1134336.833	93617.333	452942.667
Stdev	1021.995	13165.302	1101.971	2572.693
%RSD	1.689	1.161	1.177	0.568

Table 9: Results for Robustness

Conditions	Methanol	Diethylether	Dichloromethane	Tetrahydrofuran
Low temp-1 (-20°C)	39302.99	894973.2	74930.79	292665.07
Low temp-2 (-40°C)	32378.9	700079.75	59232.51	23191.31
High temp-1 (+20°C)	31567.18	800621.57	60577.24	232611.72
High temp-2 (+40°C)	35875.41	682081.12	65012.12	248215.118
Avg	34781.12	769438.9	64938.17	251352.8
SD	3071.842	85400.96	6152.849	24725.68
%RSD	8.8	11.09	9.47	9.83

Limit of Detection (LOD) and Quantitation (LOQ)

The LOD and LOQ were calculated by instrumental and statistical methods. For the instrumental method, LOD is determined as the lowest amount to detect, and LOQ is the lowest amount to quantify, by the detector. The LODs of residual solvents in ifosfamide API were determined based on a signal-to-noise ratio of 3:1. The LOQs of residual solvents were determined based on a signal-to-noise of ratio 10:1. The values for the LOD and LOQ for methanol, diethyl ether, dichloromethane, and tetrahydrofuran are shown in Table 1.

System suitability

The system suitability criterion was taken to be the resolution between the critical pairs, i.e: diethyl ether and dichloromethane. The system suitability was evaluated by injecting the standard solutions on various days. The criterion for system suitability was that the resolution between the critical pair should not be less than 1.5 and it was found to be well above the minimum passing limit. The values are given in table -10

Table 10: Results for System Suitability

Validation parameter	Resolution between diethyl ethyl and dichloromethane
Specificity	8.82
Precision	7.97
Accuracy	8.31
Linearity	8.13

Application of the proposed method (Analysis of commercially available Ifosfamide)

The proposed method was evaluated by the assay of commercially available ifosfamide for quantification of residual solvents present in it. The results obtained for residual solvents were compared with the corresponding specifications limits of standard guidelines. The data is given in table 11.

Table 11: Application of the Proposed Method

Sample Source	Methanol	Diethyl Ether	Dichloromethane	Tetrahydrofuran
IFOMID-M-1g Mfg.by: United biotech(p) ltd Mfg.lic.no- MB/05/255 Batch no: FMDF8B4	ND	ND	ND	ND
ASSAY	99.5%			

*ND-Not detected

CONCLUSION

A single, rapid and highly selective HSGC method was developed and validated for the quantification of residual solvents present in ifosfamide API through an understanding of the synthetic process, nature of solvents and nature of stationary phases of columns. The residual solvents methanol, diethyl ether, dichloromethane, diethyl ether and tetrahydrofuran were well separated from each other and quantified by the proposed method. The above results clearly show that the current developed method is a good analytical technique for the quantitative analysis of residual solvents in ifosfamide. The present method has a wide linear dynamic range with good accuracy and precision. The RSD values obtained are accurate and within the limits. This method was also applied for the quantification of residual solvents in the marketed Ifosfamide. Finally, we conclude that the proposed method can be effectively applied for the quantification of residual solvents present in Ifosfamide. Thus, the present method is an attractive method for the determination of residual solvents in Ifosfamide in bulk and pharmaceutical formulations.

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REFERENCES

1.ICH Harmonised Guideline Impurities: Guideline for Residual Solvents Q3C(R6), Current Step 4 version, 2016.

2.Gurdeep R.Chatwal, Sham.K.Anand: Instrumental Analysis, Himalaya Publishing House, 2014, 2.673-2.700.

3. Drug profile, ifosfamide. www.drugbank.ca/drugs/DB01181.

4. Drug profile, ifosfamide pubchem.ncbi.nlm.nih.gov/compound/ifosfamide.

5. Dr.A.V.Kasture, Dr.K.R.Mahadik; Dr.S.G.Wadodkar. Dr.H.N.More, Pharmaceutical Analysis-Instrumental Method, Nirali prakashan, 2008; 8.1-8.17.

6. Dr.S.Ravi Sankar, Textbook of Pharmaceutical Analysis, Edition 4, 2010, 17.2-17.20.

7. Santosh Kumar Bhardwaj, K. Dwivedia and D.D.Agarwal, A Review: GC Method Development and validation, International Journal of Analytical and Bioanalytical Chemistry, 6, 2016, 1-7.

8.Panchumarthy Ravisankar, Ch.Naga Navya, D.Pravallika, D.Navya Sri, A Review on Step-by-Step Analytical Method Validation, IOSR Journal of Pharmacy, 5, 2015, 07-19.

9.J. Y. Zhu, and X.-S. Chai, Some Recent Developments in Headspace Gas Chromatography, Current Analytical Chemistry, 1, 2005, 79-83.

10.Hilde Lambrechts, Eric 0. Gheuens, Karel A. Van Cauwenberghe, Greet G.O. Pattyn, Allan T. Van Oosterom and Ernst.A. De Bruijn, Determination of ifosfamide by gas chromatography—mass spectrometry, Analytica Chemica Acta, 241, 1991, 229-233.

11.Kerbusch, T., Jeuken, M. J., Derraz, J., van Putten, J. W. G., Huitema, A. D. R. & Beijnen, J. H, Determination of ifosfamide, 2- and 3-dechloroethyifosfamide using gas chromatography with nitrogen-phosphorus or mass spectrometry detection, Therapeutic Drug Monitoring, 22, 2000, 613-620.

12.Noushin Rastkari, Reza Ahmadkhaniha, Maryam Alehashem, Shadi Baniasadi, Rapid Method for the Determination of Cyclophosphamide and Ifosphamide in Urine at Trace Levels by Gas Chromatography-Mass Spectrometry, International Journal of Pharmaceutical and Clinical Research, 8, 2016, 289-292.

13.Jeff J.H.Wang, Kenneth K Chan, Analysis of Ifosfamide, 4-hydroxyifosfamide, N2-dechloroethylifosfamide, N3-dechloroethylifosfamide and iphosphoramide mustard in plasma by gas chromatography-mass spectrometry, Journal of chromatography B, 674, 1995, 205-217.

14.Veeravan Lekskulchai, Quantitation of anticancer drugs –Cyclophosphamide and ifosfamide in urine and water sewage samples by gas Chromatography–mass spectrometry, International Journal of Occupational Medicine and Environmental Health, 29, 2016, 815–822.

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