DEVELOPMENT AND VALIDATION OF EDC RESIDUAL SOLVENT DETERMINATIONBY HEADSPACE GAS CHROMATOGRAPHY IN SAMPLE(ACTIVE PHARMACEUTICAL INGREDIENT, INTERMEDIATE AND KEY RAW MATERIAL)

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Abstract

Solvents are widely used during the manufacturing, purification and processing of pharmaceutical substances. The residues of these solvents must be removed to the extent possible, as they do not have any therapeutic effect but can cause undesirable effects in theconsumers. These solvent residues concentration should not exceed the limits prescribed in the ICH guidelines. This present review work is emphasized on various techniques are being used to The determination of residual solvents with their merits and demerits.An accurate, precise,linear and sensitive method was developed for residual solvents determination by fast static headspace gas chromatography (HSGC) with flame ionization detector in Sample. Residual solvents in drug substances are quantified using gas chromatography with headspace. As per regulatory guidelines, residual solvents must be controlled for release This paper includes the development and validation of HSGC method for the determination of residual solvents specifically EDC content in Sample. DB-624 capillary column, 30 m long × 0.53 mm internal diameter, the 3 µm film thickness was used for analysis with FID detector. The method can be readily used.

Keywords: Residual solvents · Headspace gas chromatography · Flame ionization detector · Method validation.

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DOI: 10.48047/ecb/2023.12.si5a.050

Introduction

Residual solvents or volatile organic solvents are used or formed during the manufacturing of pharmaceutical drug substances, intermediates, excipients or pharmaceutical drug product.The solvents are toxic, have no therapeutic importance and affect the quality and stability of drug substances and drug products so they are not desirablein the final product.

Although it is difficult toremove completely with the common techniques used in practical manufacturing process such as increased process temperature or/and decreased pressure, they need to be minimized. However, depending on the nature of the API, residual solvents and drying condition of the process, some amount of residual solvents traces can be retained in the final drug substances or drug product.

Thus, acceptable levels of many residual solvents are included in regulatory guideline; particularly in guideline Q3C issued by the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) ICH has also included daily exposure limit of many solvents it has classified these solvents into four classes on the basis of the toxicity level and the degree to which they can be considered an environmental hazard. Class I solvents (which covers 5 residual solvents) are known or suspected human carcinogens and environmental hazards, the use of these solvents should be avoided. Class I solvents should be identified and quantified.

Class II solvents (which covers 29 residual solvents) are non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity. Use of these solvents should be limited. Class II solvents have individual limits. Class III solvents (which covers 26 residual solvents) having low toxic potential to man; no health-based exposure limit isneeded. Class 3 solvents have PDEs of 50 mg or more perday. Finally, Class 4 solvents are those for which no adequate toxicological data have been found. Therefore determination of residual solvents becomes a necessary procedure for quality control of drug substances and drug product to meet regulatory guideline and ensure patient safety .Head space gas chromatography (HSGC) is generally used to determine residual solvents because of its high separation efficiency and sensitivity for organic volatile solvents. However head space bounds the analysis to those solvents being evaporated from HS only, it also requires larger sample load and analysis time should be longer due to sample equilibration. Headspace sampling is preferred because of its ability to avoid direct liquid or solid injection .

HSGC methods minimize any possible interference caused by non-volatile substances or by the degradation/decomposition products of the non-volatile components. While comparing to headspace, direct injection method requires relatively low sample concentration, but the high boiling/melting point components of the sample may not be eluted through GC Column and they may contaminate the GC injection port and leads to poor chromatography.

HSGC with FID detection has been mainly used for the analysis of organic volatile solvents present in the pharmaceutical drug substances and drug products. In this research article, we have been described a development and validation of an HS-GC analytical method for determination of EDC solvents used in the route of synthesis to produce the drug substances. Incorporated Residual solvents Permissible daily exposure limit and ICH class categories are described in class 1 solvents.

These solvents are known to cause unacceptable toxicities and should be avoided in the Manufacture of active pharmaceutical substances, excipients and medicinal products. However if their use is unavoidable, Restricted limits of Class I residual solvents.Solvent name-1, 2-Dichloromethane and Limit 5 ppm.

Experimental

Chemical, material, and reagents

Chemical, material, and reagents	Make	Grade
1-2 dichloromethane	Merck	AR Grade/GC Grade
Water	Merck	HPLC Grade

Table 1 Chemical, material, and reagents details

Instrumentation & column details

HSGC system of an Perkin Elmer Clarus 680 technologies equipped with a flame ionization detector with a headspace sampler turbmetrix 40 was used for method development and method validation studies. A split liner was used as an inlet liner and Total Chrom Navigator software was used for data acquisition and chromatographic data integration. A Mettler tolado analytical balance and glass pipette from Borosil were used.

Column :-30 m × 0.53 mm I.D., 3.0 µm film thickness DB-624 (bonded 6% cyanopropylphenyl-94% dimethylpolysiloxane) capillary GC column to achieve proper Separation in a developedmethod. DB-624 column was manufactured by J&W Scientific (Agilent Scientific Technologies)

Gas chromatographic conditions

Column	DB-624 Capillary column
Length	30 m
Internal dimeter	0.53 mm
Film thick ess	3.0 µm
Detector	FID
Carrier gas	Nitrogen
Injector temperature	220°C
Detector temperature	250°C
Split ratio	1:5
Column Flow	2.0 ml/min
Attenuation	-4
Range	1
Total run time	26.33 min

Oven Temperature

Rate (°C/min.)	Temperature (°C)	Hold time (min.)
0.0	40	5.0
15	240	8.0

Headspace Parameters

Vial Oven temperature	95°C
Needle temperature	100°C
Transfer line temperature	105°C
Head space carrier Pressure	10 psi
Thermo state time	15 min.
Pressurize time	3.0 min.
Withdrawal time	0.50 min.
Injection time	0.20 min
GC Cycle time	30 min.
Injection mode	Time
Operating Mode	Constant

Table 2 Gas chromatographic conditions and Headspace Parameters

Preparation of standard and sample solution

Diluent preparation:

Diluent: Water

Blank: - Pipette out 5.0 mL of Diluent in to 20 mL headspace vial, and crimped it with septa

Standard and Sample preparation:

Standard stock solution A:

Weigh accurately about 50 mg of EDC, in to 100 mL of volumetric flask containing about 10 mL of diluent, mix well and dilute up to mark with diluent.

Standard stock solution B:

Pipette out 1.0 mL of Standard stock solution A in to 50 mL of volumetric flask containing about 10 mL of diluent, mix well and dilute up to mark with diluent.

Standard solution:
Pipette out 2.5 mL of Standard stock solution B in to 100 mL of volumetric flask containing about 10 mL of diluent, mix well and dilute up to mark with diluent. (i.e. concentration of EDC is 5 ppm) Pipette out 5.0 mL of Standard solution in to 20 mL headspace vial, and crimped it with septa.

Sample preparation -1: Weigh accurately about 250 mg of sample in dry headspace vial add 5 ml of diluent in to 20 mL headspace vial, and crimped it with septa.

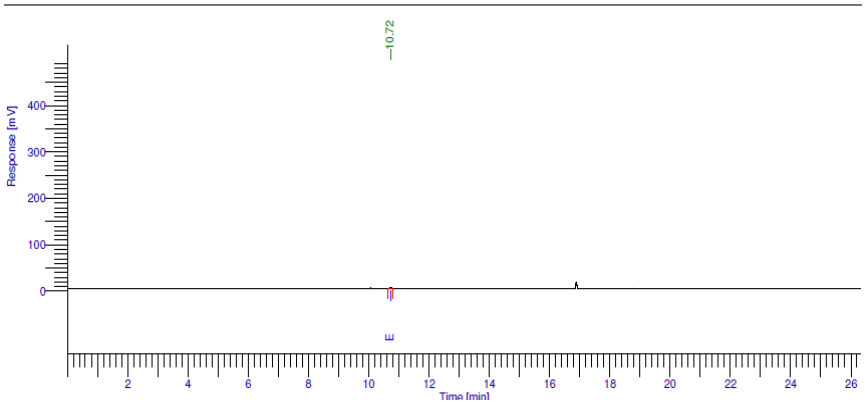
Sample preparation -2: Weigh accurately about 250 mg of sample in dry headspace vial add 5 ml of diluent in to 20 mL headspace vial, and crimped it with septa

System suitability parameter:
Relative standard deviation (%RSD) of the peak area of EDC in six replicate injections of Standard solution should not be more than 15.00%

Calculation formula:

$$\text{ppm of EDC Residual solvents} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{50} \times \frac{2.5}{100} \times \frac{5}{WT} \times 10^6$$

AT = Peak area response of Residual Solvent obtained from the Test solution
AS = Mean peak area response of Residual Solvent obtained from the Standard Solution
P = Purity of Residual Solvents.
WT = Weight of Test Sample in mg.
WS = Weight of Residual Solvent in Standard in mg.



Typical chromatogram of standard solution fig-1

Result and discussion
Gas chromatographic analytical method development
Method development by HSGC involves critical parameter such as selection of sample solvent, detector, column, carrier gas, optimization of headspace condition andchromato graphic conditions. Developed method should be specific, sensitive, precise and. Linear. The critical parameter of the developed method is discussed below.

Selection of detector and carrier gas
A flame ionization detector (FID) was used for this method because FID has good sensitivity. The carrier gas wasselected as nitrogen because it is economical as compared to helium.

Selection of column
The GC Column is a crucial parameter for developing an efficient and sensitive HSGC method. The residual solvents were commonly determined by bonded 6% cyanopropylphenyl-94% dimethylpoly siloxane (624) column due to that the DB-624 column is the best choice for the separation of EDC.

Selection of sample solvent
Several solvents were tried mainly DMF, DMSO and NMP for sample solvents and it was observed that Water gave smooth baseline with no interference at the retention times of the EDC solvents and ihance trhe peak resposnce of EDC peak.

Chromatographic conditions

To develop an HSGC method, there are two strategies for selecting oven programs. The first strategy was to keep initial oven temperature low and then gradient elution and in second strategy isothermal elution at relatively high oven temperatures. We wanted to increase the retention time of solvent, so we choose the first strategy to start our method development and finalized 40 °C Temp as initial oven temp with Hold time for 5 min. After that in ramp 1, a slow gradient was applied i.e. 15 °C/min to 240 °C to resolve the critical pairs with 15 min Final hold time. The flow rate of nitrogen was finalized at 2.0 mL/min.. Finally, the method was developed with a total run time of about 26.33 min.

Optimization of headspace parameters

The sensitivity of the HSGC method was directly impacted by headspace oven temperature, Headspace oven Temperature should be kept same or above the boiling point of the residual solvents To minimize the carryover problems Needle temperature has been kept 5 °C higher than oven temperature and the transfer line temperature also has been kept 5 °C higher than the Needle temperature . Headspace oven temperature was kept at 95 °C. Therefore, the headspace oven, Needle , and transfer line temperatures were selected at 95 °C, 100 °C and 105 °C, respectively. The vial equilibration time was set to 30 min. Other headspace parameter has been described in above table.

Method validation:-

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, strength, quality and purity.

The method validation was performed by evaluating specificity, limit of detection (LOD), limit of quantitation (LOQ),linearity, accuracy, intermediate precision, system suitability and method precision of residual solvents as specified in the ICH harmonized tripartite guideline.

Specificity

The method specificity was demonstrated by injecting the Blank, individual residual solvents standard solution (chromatogram has been attached in Fig. 1) and specificity solution In the developed chromatographic method, no interference was observed at the retention time of targeted solvents from diluent or other unknown peaks. The retention time of EDC was found 10.72 min. A typical chromatogram of the standard Solution is shown in fig.-1

Linearity and range

The linearity of the method was determined using 5 concentration level over the range 10–150% of ICH Limit Level. The calibration curve was found to be linear within the range and correlation coefficient (r2) values for EDC solvents were found to be higher than 0.99. Linearity curve and values for the residual solvents have been provided in Fig. 2 and Table 2. residual solvent was easily passed acceptance criteria for accuracy, system precision, method precision and linearity from the low concentration to high concentration, therefore the range of the method was 0.5–7.5 ppm for EDC.

Sample ID	Area	Concentration in ppm
LOQ Conc. in ppm	1052	0.5058
50% Conc. in ppm	5862	2.5900
80% Conc. in ppm	9147	4.0464
100% Conc. in ppm	11475	5.0580
120% Conc. in ppm	14132	6.0696
150% Conc. in ppm	17136	7.5870
Correlation coefficient		0.9992

Table 3 Linearity sample concentration and results

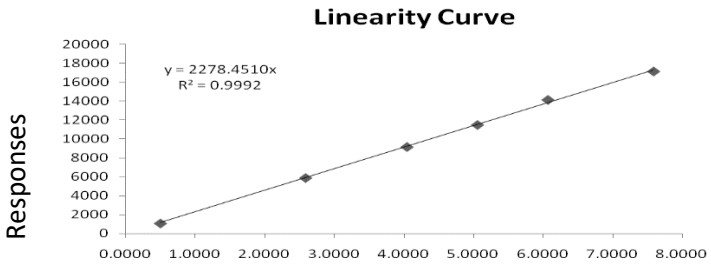


Fig. 2 Linearity plot of EDC solvents

Method sensitivity

The LODand LOQ were determined based on a signal-to-noise ratio of 3:1 and 10:1 respectively. Based on validation results, LOD and LOQ limit was found to be 0.25 and 0.5ppm for EDC respectively, Precise at LOQ level was confirmed. The LOQ concentration was found 0.5 ppm and %rsd was 2.42%.

Level	Solvent name	Concentration in ppm	Signal-to-noise ratio
LOQ Level	EDC (1,2-Dichloroethane)	0.5 ppm	24
LOD Level		0.25 ppm	7

Table 4 LOD and LOQ concentration and results

Accuracy (recovery)

The accuracy of the method was determined by spiking of all solvents at four different level i.e.,LOQ Level 50% level, 100% level, and 150% level of ICH limit in a triplicate analysis. Recovery of EDC solvent was found within the range of 80–120%. Recovery study and method precision results were reported in Table 4 and indicate that the method was accurate.

Accuracy and method precision data

Spiked sample No.	Sample Results	Added amount in ppm	Observed amount in ppm	% Recovery (D/CX100)
LOQ level % Recovery				
LOQ spiked sample per.-1	Not detected	0.5058	0.4792	94.74
LOQ spiked sample per.-2		0.5058	0.4912	97.11
LOQ spiked sample per.-3		0.5058	0.4818	95.26
%RSD				1.30%
50% level %Recovery				
50% spiked sample per.-1	Not detected	2.5900	2.5011	96.57
50% spiked sample per.-2		2.5900	2.5112	96.96
50% spiked sample per.-3		2.5900	2.5082	96.84
%RSD				0.21%
100% level %Recovery (Method precison)				
100% spiked sample per.-1	Not detected	5.0580	5.0112	99.07
100% spiked sample per.-2		5.0580	4.8852	96.58
100% spiked sample per.-3		5.0580	4.9518	97.90
100% spiked sample per.-4		5.0580	4.9253	97.38
100% spiked sample per.-5		5.0580	4.8954	96.79
100% spiked sample per.-6		5.0580	4.8754	96.39
%RSD				1.04%

Spiked sample No.	Sample Results	Added amount in ppm	Observed amount in ppm	% Recovery (D/CX100)
150% level %Recovery				
150% spiked sample per.-1	Not detected	7.5870	7.4581	98.30
150% spiked sample per.-2		7.5870	7.3589	96.99
150% spiked sample per.-3		7.5870	7.5048	98.92
%RSD				1.00%

Table 5 Accuracy and method precision data

Precision

The precision of the method was determined by system precision (six replicate injection of standard solution) and method precision (six different preparation of spike solution) studies. In both the studies %Relative Standard Deviation of peak areas for the solvents were less than 5.0%. The results were provided in Table 5. These results proved that the system suitability was passed and method is precise (Table 5).

Name of solution	Retention time (min.)	%RSD (n = 6) of peak area
Standard solution	10.72	1.36

Table 6 System precision and system suitability parameter

System suitability

Relative standard deviation (%RSD) of the peak area of EDC in six replicate injections of Standard solution within the limit (NMT 15.0%).

Result

Residual solvents analysis was performed on developed and validated method for EDC content determination and quantification. Results were reported in Table 5 and chromatogram has been attached in Fig.1.

Conclusion

A selective and sensitive fast static HSGC method has been successfully developed for the determination of EDC in sample. The developed method was successfully validated as per regulatory guideline and found to be precise, accurate, linear, and specific. Additionally, our method is suitable for analysis of EDC and other solvents in one single method, which is accurate, precise and linear in presence of sample matrix. However only a limited number of solvents are used, this method may be used to separate the residual solvents present in other drug substances and can be used for routine analysis to monitor in-process drying and in quality control for bulk drug manufacturing. Taken together, our developed HSGC method demonstrated precise, economical and commercially able quantitative technique for residual solvents determination which will also be advantageous for industrial scale manufacturing.

Acknowledgement

I would like to express my special thanks and gratitude to my guide Dr. Rashmi Shrivastwa as well as our supervisor who give me golden opportunity to this wonderful project on the Development and validation of EDC residual solvent determination by headspace gas chromatography in Sample. Which also helped me doing lot of research and I came to know about so many new things. I really thankful to team.

Secondly I would also like to thank my friends who helped me in finishing this topic in limited time. It really helped me increase my knowledge and skills.

THANKS TO EVERYONE WHO HELPED ME

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