Method Development For Residual Solvents, Separation Of Isopropyl Alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene, And Chlorobenzene, And Its Validation By Headspace Gas Chromatography. (For Active Pharmaceutical Ingredients, Intermediate And Raw Material Analysis)

Surendra chourasiya[1*], Dr. Rashmi Shrivastwa[1]

[1]Department of Chemistry, IES University, Bhopal, Madhya Pradesh, India, Tel: +917303858589, +917048494980

[*]**Corresponding Author:** Surendra chourasiya
chourasia.surendra@yahoo.co.in

Abstract
Solvents are widely used during the manufacturing, purification, and processing of Pharmaceutical substances like active pharmaceutical ingredients and intermediate and Key raw materials. 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 on the consumers. These solvent residue concentrations should not exceed the limits prescribed in the ICH guidelines. This present review work emphasizes GC-HS techniques being used for the separation method of residual solvents Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene, and Chlorobenzene separation method developed and validated. An accurate, precise, linear, and sensitive method was developed for residual solvent determination by headspace gas chromatography (HSGC) with a flame ionization detector in the Sample. All residual solvents are quantified using gas chromatography with headspace. As per regulatory guidelines, residual solvents must be controlled for release and all solvent peak well-separated methods developed and validated. CP-Sil 5 CB capillary column, 50 m long × 0.53 mm internal diameter, the 5 μm film thickness was used for analysis with FID detector. The method can be readily used.

Keywords: Separation of Residual solvents Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene and Chlorobenzene. Headspace gas chromatography, Flame ionization detector, Method validation.

Introduction

Residual solvents or volatile organic solvents are used or formed during the manufacturing of pharmaceutical drug substances, intermediates, excipients, or pharmaceutical drug products. The solvents are toxic, have no therapeutic importance, and affect the quality and stability of drug substances and drug products so they are not desirable in the final product. Although it is difficult to remove completely with the common techniques used in practical manufacturing processes such as increased process temperature and/or 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 solvent traces can be retained in the final drug substances or drug product. Thus, acceptable levels of many residual solvents are included in regulatory guidelines; 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 based on the toxicity level and the degree to which they can be considered an environmental hazard. Class I solvents (which cover 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 cover 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) have low toxic potential to man; no health-based exposure limit is needed. Class 3 solvents have PDEs of 50 mg or more per day. Finally, Class 4 solvents are those for which no adequate toxicological data have been found. Therefore, the determination of residual solvents becomes a necessary procedure for quality control of drug substances and drug product to meet regulatory guidelines and ensure patient safety. Headspace gas chromatography (HSGC) is generally used to determine residual solvents because of its high separation efficiency and sensitivity for organic volatile solvents. However, headspace bounds the analysis to those solvents being evaporated from HS only, it also requires a 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. Compared to headspace, the direct injection method requires a relatively low sample concentration, but the high boiling/melting point components of the sample may not be eluted through the GC Column and they may contaminate the GC injection port and lead to poor chromatography. HSGC with FID detection has been mainly used for the analysis of organic volatile solvents present in pharmaceutical drug

substances and drug products. In this research article, we have been described a solvent separation method development and validation for Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene, and Chlorobenzene solvents by HS-GC. Incorporated Residual solvents Permissible daily exposure limit and ICH class categories are described as 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 residual solvents Isopropyl alcohol Limit-5000ppm, Dichloromethane Limit-600ppm, Hexanes Limit-290ppm, Ethyl Acetate Limit-5000ppm, Toluene Limit-890ppm and Chlorobenzene Limit-360ppm

Experimental

Chemical, material, and reagents

Chemical, material, and reagents	Make	Grade
Isopropyl alcohol	Spectrochem	AR Grade/GC Grade
Dichloromethane	Spectrochem	AR Grade/GC Grade
Hexanes	Spectrochem	AR Grade/GC Grade
Ethyl Acetate	Spectrochem	AR Grade/GC Grade
Toluene	Spectrochem	AR Grade/GC Grade
Chlorobenzene	Spectrochem	AR Grade/GC Grade
N,-Mthyl-2-pyrrolidone	Spectrochem	AR Grade/GC Grade

Table 1 Chemical, material, and reagents details

Instrumentation & and column details

HSGC system of a 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 Mettlertolado analytical balance and glass pipette from Borosil were used.

Column:- CP-Sil 5 CB capillary column, 50 m long × 0.53 mm internal diameter, the 5 µm film thickness. CP-Sil 5 CB bonded 100% dimethylpolysiloxane (PDMS) capillary GC column to achieve proper Separation in a developed method. CP-Sil 5 CB column was manufactured by J&W Scientific (Agilent Scientific Technologies)

Gas chromatographic conditions

Column	CP-Sil 5 CB Capillary column
Length	50 m
Internal diameter	0.53 mm
Film thick ess	5.0 µm
Detector	FID
Carrier gas	Nitrogen
Injector temperature	240°C
Detector temperature	290°C
Split ratio	1:5
Column Flow	2.0 ml/min
Attenuation	-5
Range	1
Total run time	32.60 min

Oven Temperature

Rate (°C/min.)	Temperature (°C)	Hold time (min.)
0.0	60	2.0
15	120	10.0
25	285	10.0

Headspace Parameters

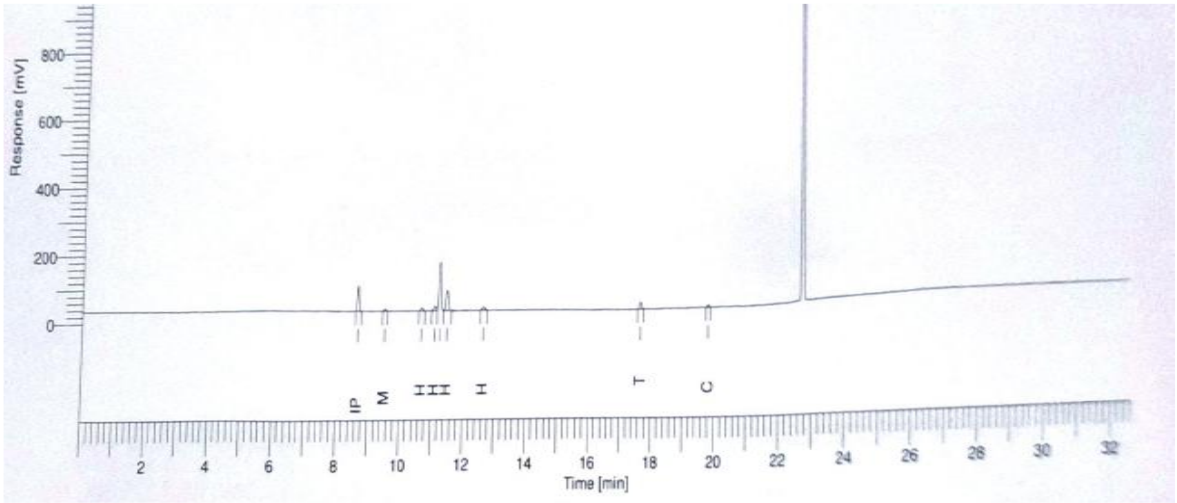
Vial Oven temperature	100°C
Needle temperature	105°C
Transfer line temperature	110°C
Headspace carrier Pressure	10 psi
Thermo state time	15 min.
Pressurize time	3.0 min.
Withdrawal time	0.20 min.
Injection time	0.10 min
GC Cycle time	40 min.
Injection mode	Time
Operating Mode	Constant

Table 2 Gas chromatographic conditions and Headspace Parameters

Preparation of standard and sample solution
Diluent preparation:
Diluent: N-Mthyl-2-pyrrolidone
Blank: - Pipette out 5.0 mL of Diluent in to 20 mL headspace vial, and crimp it with septa **Standard and Sample preparation:**
Standard stock solution:
Weigh accurately about 500 mg of Isopropyl alcohol, 60 mg of Dichloromethane, 290 mg of Hexanes, 500 mg of Ethyl Acetate, 89 mg of Toluene, and 36 mg of Chlorobenzene, into 100 mL of volumetric flask containing about 10 mL of diluent mix well and dilute up to mark with diluent.
Standard solution:
Pipette out 5.0 mL of Standard stock solution into 50 mL of volumetric flask containing about 10 mL of diluent, mix well, and dilute up to mark with diluent. Pipette out 5.0 mL of Standard solution into a 20 mL headspace vial, and crimped it with septa. (i.e., Isopropyl alcohol is 5000 ppm, Dichloromethane concentration is 60 ppm, Hexanes concentration is 290 ppm, Ethyl Acetate concentration is 5000 ppm, Toluene concentration is 890 ppm and Chlorobenzene concentration is 360 ppm.
Sample preparation -1: Weigh accurately about 500 mg of sample in a dry headspace vial add 5 ml of diluent into 20 mL headspace vial, and crimp it with septa.
Sample preparation -2: Weigh accurately about 500 mg of sample in a dry headspace vial add 5 ml of diluent into a 20 mL headspace vial, and crimp it with septa
System suitability parameter:
Relative standard deviation (%RSD) of the peak area of Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene, and Chlorobenzene in six replicate injections of Standard solution should not be more than 15.00%
Calculation formula :(This formula is used for all solvents)

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

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 parameters such as the selection of sample solvent, detector, column, carrier gas, optimization of headspace condition, and chromatographic conditions. The 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 was selected 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 CP-Sil 5 CB capillary column, 50 m long
× 0.53 mm internal diameter, the 5 µm film thickness. CP-Sil 5 CB bonded 100% dimethyl polysiloxane (PDMS) is the best choice for the separation of Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene and Chlorobenzene.

Selection of sample solvent

Several solvents were tried mainly DMF NMP and DMSO for sample solvents and it was observed that NMP gave a smooth baseline with no interference at the retention times of the Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene, and Chlorobenzene solvents and enhance the peak response of Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene, and Chlorobenzene solvents.

Chromatographic conditions

To develop an HSGC method, there are two strategies for selecting oven programs. The first strategy was to keep the initial oven temperature low and then gradient elution and in the second strategy isothermal elution at relatively high oven temperatures. We wanted to increase the retention time of solvent, so we chose the first strategy to start our method development and finalized 60 °C Temp as the initial oven temp with a Hold time of 2 min. After that in ramp 15, a slow gradient was applied i.e., 15 °C/min to 120 °C oven temp with Hold time for 10 min. After that in ramp 25, a slow gradient was applied i.e., 25 °C/min to 285 °C oven temp with Hold time for 10 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 32.60 min.

Optimization of headspace parameters

The sensitivity of the HSGC method was directly impacted by headspace oven temperature, Headspace oven Temperature should be kept the 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. The headspace oven temperature was kept at 100 °C. Therefore, the headspace oven, Needle, and transfer line temperatures were selected at 100 °C, 105 °C and 110 °C, respectively. The vial equilibration time was set to 15 min. Another headspace parameter has been described in the 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, the 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 Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene, and Chlorobenzene is below table.

Solvents Name	Retention time	Area Response
Isopropyl alcohol	8.677	296798
Dichloromethane	9.519	23505
Hexanes	10.686	46914
Hexanes	11.096	68771
Ethyl Acetate	11.251	691536
Hexanes	11.4286	313060
Hexanes	12.641	50851
Toluene	17.686	96409
Chlorobenzene	19.827	26807
N,-Mthyl-2-pyrrolidone	22.854	Not applicable

Table 3 Retention time and area response of solvent

Linearity and range

The linearity of the method was determined using 5 concentration levels over the range 20–150% of the ICH Limit Level. The calibration curve was found to be linear within the range and correlation coefficient (r^2) values for Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene, and Chlorobenzene solvents were found to be higher than 0.99. The linearity curve and values for the residual solvents have been provided in Fig. 2 and Table 3. Residual solvent 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 20–150% of the targeted concentration.

Linearity of Isopropyl alcohol:

Sample ID	Area of (Isopropyl alcohol)	Concentration in ppm (Isopropyl alcohol)	Area of (Dichloromethane)	Concentration in ppm (Dichloromethane)
LOQ Conc. in ppm	29574	500	2348	60
30% Conc. in ppm	89050	1501	7051	120
50% Conc. in ppm	148311	2502	11751	301
80% Conc. in ppm	237412	4002	18825	480
100% Conc. in ppm	296798	5005	23505	601
150% Conc. in ppm	445188	7515	35265	903
Correlation coefficient of Isopropyl alcohol		1.0000	Dichloromethane	0.9923

Linearity of Dichloromethane:

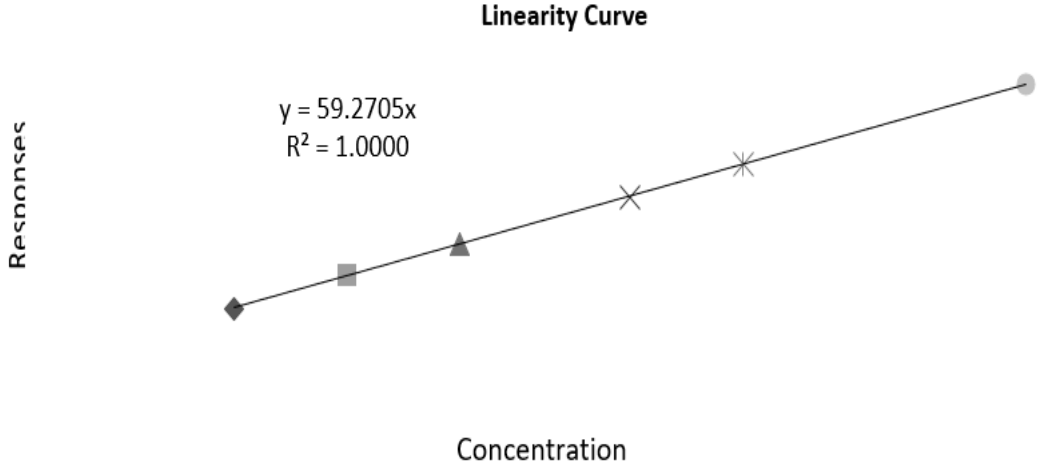
Sample ID	Sum Area of (Hexanes)	Concentration in ppm (Hexanes)	Area of (Ethyl Acetate)	Concentration in ppm (Ethyl Acetate)
LOQ Conc. in ppm	47958	29	69151	500
30% Conc. in ppm	143885	87	207458	1502
50% Conc. in ppm	239745	146	345746	2505
80% Conc. in ppm	383664	232	553214	4003
100% Conc. in ppm	479596	292	691536	5010
150% Conc. in ppm	719382	438	1037315	7530
Correlation coefficient of Hexanes		1.0000	Ethyl Acetate	1.0000

Linearity of Hexanes:

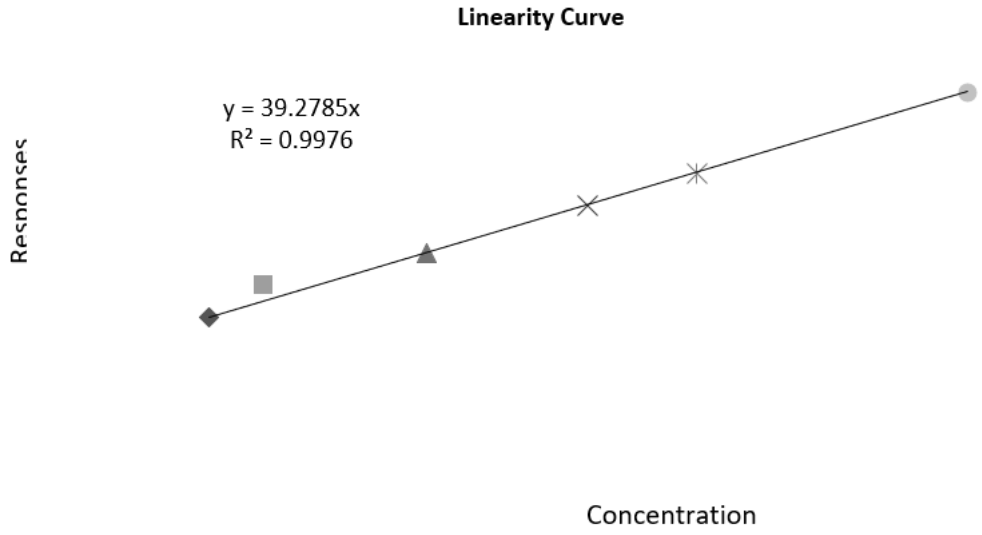
Sample ID	Area of (Toluene)	Concentration in ppm (Toluene)	Area of (Chlorobenzene)	Concentration in ppm (Chlorobenzene)
LOQ Conc. in ppm	9645	89	2675	36
30% Conc. in ppm	28919	267	8022	108
50% Conc. in ppm	48211	446	13414	181
80% Conc. in ppm	77145	712	21441	288
100% Conc. in ppm	96409	891	26847	362
150% Conc. in ppm	144652	1337	40219	543
Correlation coefficient of Toluene		1.0000	Chlorobenzene	1.0000

Table 4 Linearity sample concentration and results of Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene, and Chlorobenzene

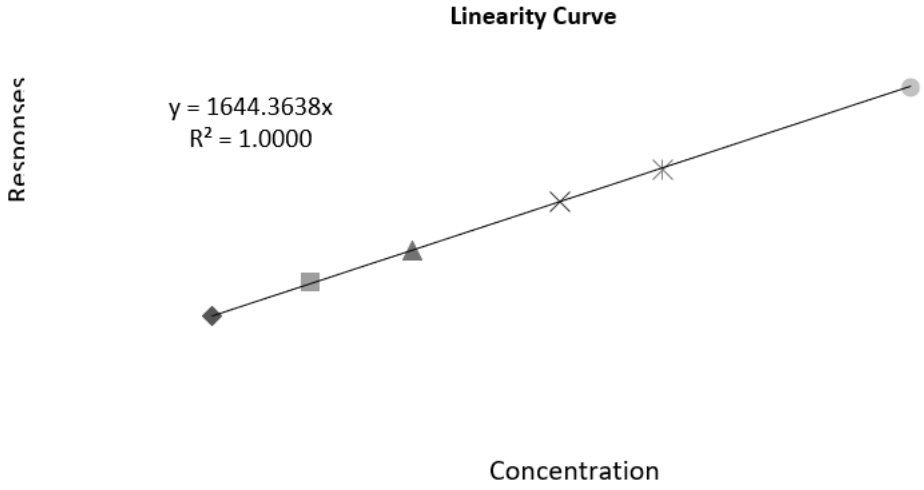
Linearity Curve of Isopropyl alcohol



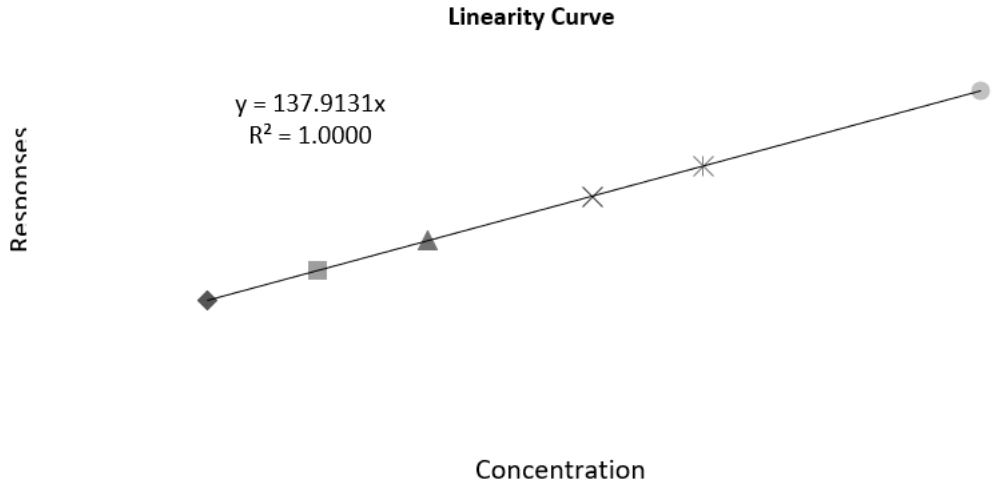
Linearity Curve of Dichloromethane



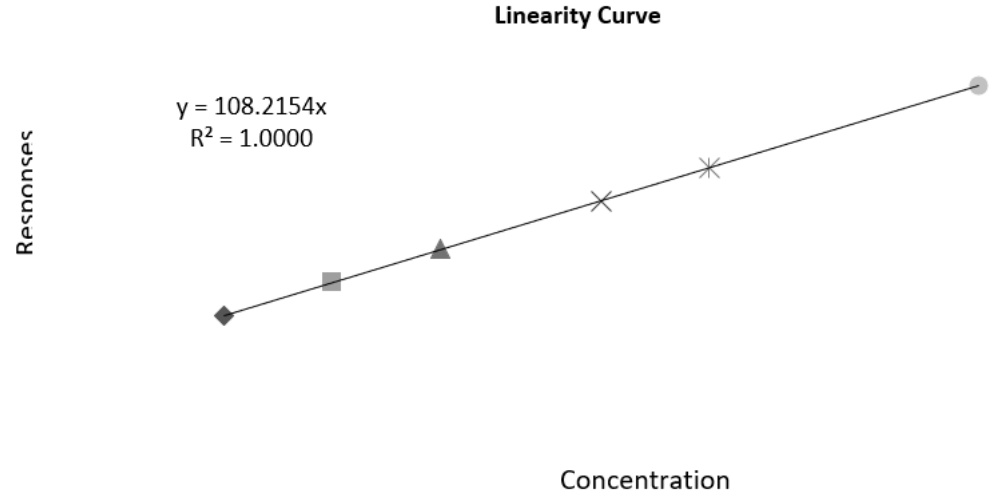
Linearity Curve of Hexanes



Linearity Curve of Ethyl acetate



Linearity Curve of Toluene



Linearity Curve of Chlorobenzene

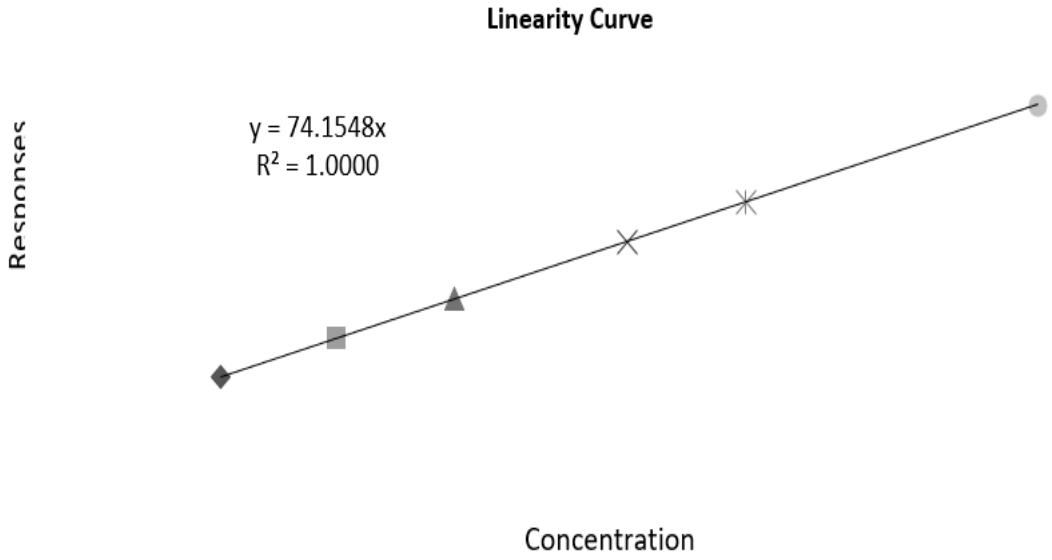


Fig. 2: Linearity plot of Residual solvents Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene and Chlorobenzene.

Method sensitivity

The LOD and LOQ were determined based on a signal-to-noise ratio of 3:1 and 10:1 respectively. Based on validation results, LOD and LOQ limits were determined respectively, and Precise at the LOQ level was confirmed.

Solvent name	LOD Level	Signal-to-noise ratio	Conc. in ppm	LOQ Level	Signal-to-noise ratio	Conc. in ppm
Isopropyl alcohol	8745	8	150	29574	35	501
Dichloromethane	1010	5	25	2348	25	62
Hexanes	14375	9	10	47958	38	30
Ethyl Acetate	19850	9	150	69151	37	502
Toluene	2815	7	30	9645	27	90
Chlorobenzene	1020	4	15	2675	22	38

Table 5 LOD and LOQ concentration and results

Accuracy (recovery)

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

Accuracy and method precision data of Isopropyl alcohol

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	500.13	498.32	99.64
LOQ spiked sample per.-2		500.13	491.58	98.29
LOQ spiked sample per.-3		500.13	493.52	98.68
%RSD				0.70
50% level %Recovery				
50% spiked sample per.-1	Not detected	2500.66	2481.25	99.22
50% spiked sample per.-2		2500.66	2471.65	98.84
50% spiked sample per.-3		2500.66	2450.89	98.01
%RSD				0.63
100% level of recovery (Method precision)				
100% spiked sample per.-1	Not detected	5001.32	4985.25	99.68
100% spiked sample per.-2		5001.32	4975.65	99.49
100% spiked sample per.-3		5001.32	4988.12	99.74
100% spiked sample per.-4		5001.32	4977.98	99.53
100% spiked sample per.-5		5001.32	4984.32	99.66
100% spiked sample per.-6		5001.32	4984.99	99.67
%RSD				0.10%
150% level %Recovery				
150% spiked sample per.-1	Not detected	7501.9800	7458.23	99.42
150% spiked sample per.-2		7501.9800	7421.45	98.93
150% spiked sample per.-3		7501.9800	7485.28	99.78
%RSD				0.43%

Accuracy and method precision data of Dichloromethane

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	60.12	58.25	96.89
LOQ spiked sample per.-2		60.12	57.82	96.17
LOQ spiked sample per.-3		60.12	59.02	98.17
%RSD				1.04%
50% level %Recovery				
50% spiked sample per.-1	Not detected	300.62	297.42	98.94
50% spiked sample per.-2		300.62	295.31	98.23
50% spiked sample per.-3		300.62	296.12	98.50
%RSD				0.36%
100% level of recovery (Method precision)				
100% spiked sample per.-1	Not detected	601.23	592.12	98.48
100% spiked sample per.-2		601.23	596.22	99.17
100% spiked sample per.-3		601.23	593.85	98.77
100% spiked sample per.-4		601.23	588.92	97.95
100% spiked sample per.-5		601.23	598.02	99.47
100% spiked sample per.-6		601.23	584.98	97.30
%RSD				0.81%
150% level %Recovery				
150% spiked sample per.-1	Not detected	901.8500	888.52	98.52
150% spiked sample per.-2		901.8500	875.95	97.13
150% spiked sample per.-3		901.8500	890.12	98.70
%RSD				0.88%

Accuracy and method precision data Hexanes

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	29.15	28.54	97.91
LOQ spiked sample per.-2		29.15	29.01	99.52
LOQ spiked sample per.-3		29.15	28.35	97.26
%RSD				1.19%
50% level %Recovery				
50% spiked sample per.-1	Not detected	145.76	142.58	97.82
50% spiked sample per.-2		145.76	143.62	98.53
50% spiked sample per.-3		145.76	144.87	99.39
%RSD				0.80%
100% level of recovery (Method precision)				
100% spiked sample per.-1	Not detected	291.52	285.65	97.99
100% spiked sample per.-2		291.52	289.42	99.28
100% spiked sample per.-3		291.52	290.32	99.59
100% spiked sample per.-4		291.52	288.21	98.86
100% spiked sample per.-5		291.52	287.45	98.60
100% spiked sample per.-6		291.52	289.03	99.15
%RSD				0.57%
150% level %Recovery				
150% spiked sample per.-1	Not detected	437.2800	434.58	99.38
150% spiked sample per.-2		437.2800	436.25	99.76
150% spiked sample per.-3		437.2800	435.88	99.68
%RSD				0.20%

Accuracy and method precision data Ethyl Acetate

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	500.25	489.95	97.94
LOQ spiked sample per.-2		500.25	485.63	97.08
LOQ spiked sample per.-3		500.25	487.12	97.38
%RSD				0.45%
50% level %Recovery				
50% spiked sample per.-1	Not detected	2501.23	2475.32	98.96
50% spiked sample per.-2		2501.23	2484.12	99.32
50% spiked sample per.-3		2501.23	2479.22	99.12
%RSD				0.18%
100% level of recovery (Method precision)				
100% spiked sample per.-1	Not detected	5002.45	4987.25	99.70
100% spiked sample per.-2		5002.45	4979.85	99.55
100% spiked sample per.-3		5002.45	4972.55	99.40
100% spiked sample per.-4		5002.45	4968.44	99.32
100% spiked sample per.-5		5002.45	4964.87	99.25
100% spiked sample per.-6		5002.45	4991.24	99.78
%RSD				0.21%
150% level %Recovery				
150% spiked sample per.-1	Not detected	7003.68	6984.25	99.72
150% spiked sample per.-2		7003.68	6972.24	99.55
150% spiked sample per.-3		7003.68	6966.81	99.47
%RSD				0.13%

Accuracy and method precision data of Toluene

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	89.25	87.15	97.65
LOQ spiked sample per.-2		89.25	86.91	97.38
LOQ spiked sample per.-3		89.25	86.64	97.08
%RSD				0.29%
50% level %Recovery				
50% spiked sample per.-1	Not detected	446.26	440.35	98.68
50% spiked sample per.-2		446.26	437.12	97.95
50% spiked sample per.-3		446.26	439.25	98.43
%RSD				0.37%
100% level of recovery (Method precision)				
100% spiked sample per.-1	Not detected	892.51	875.32	98.07
100% spiked sample per.-2		892.51	879.24	98.51
100% spiked sample per.-3		892.51	881.42	98.76
100% spiked sample per.-4		892.51	884.01	99.05
100% spiked sample per.-5		892.51	883.17	98.95
100% spiked sample per.-6		892.51	874.85	98.02
%RSD				0.44%
150% level of Recovery				
150% spiked sample per.-1	Not detected	1338.77	1323.54	98.86
150% spiked sample per.-2		1338.77	1318.87	98.51
150% spiked sample per.-3		1338.77	1315.15	98.24
%RSD				0.32%

Accuracy and method precision data of Chlorobenzene

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	36.32	35.11	96.67
LOQ spiked sample per.-2		36.32	34.98	96.31
LOQ spiked sample per.-3		36.32	35.12	96.70
%RSD				0.22%
50% level %Recovery				
50% spiked sample per.-1	Not detected	181.61	178.24	98.14
50% spiked sample per.-2		181.61	177.95	97.98
50% spiked sample per.-3		181.61	179.23	98.69
%RSD				0.38%
100% level of recovery (Method precision)				
100% spiked sample per.-1	Not detected	363.22	356.55	98.16
100% spiked sample per.-2		363.22	357.21	98.35
100% spiked sample per.-3		363.22	354.98	97.73
100% spiked sample per.-4		363.22	358.12	98.60
100% spiked sample per.-5		363.22	355.32	97.83
100% spiked sample per.-6		363.22	359.11	98.87
%RSD				0.45%
150% level %Recovery				
150% spiked sample per.-1	Not detected	544.83	541.28	99.35
150% spiked sample per.-2		544.83	538.84	98.90
150% spiked sample per.-3		544.83	539.21	98.97
%RSD				0.24%

Table 6: Accuracy and method precision data

Precision

The precision of the method was determined by system precision (six replicate injections of standard solution) and method precision (six different preparations of spike solution) studies. In both studies, the relative Standard Deviation of peak areas for the solvents was less than 15.0%. The results are provided in Table 6. These results proved that the system suitability was passed and the method is precise (Table 7).

Name of Solvent	The average area of Standard solution	%RSD (n = 6) of peak area
Isopropyl alcohol	296685	0.54%
Dichloromethane	23550	1.21%
Hexanes	479523	1.18%
Ethyl Acetate	691496	0.85%
Toluene	96415	1.11%
Chlorobenzene	26778	1.45%

Table 7 System precision and system suitability parameter

System suitability

Relative standard deviation (%RSD) of the peak area of Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene, and Chlorobenzene in six replicate injections of Standard solution within the limit (NMT 15.0%).

Result

Residual solvent analysis was performed on a developed and validated method for Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene, and Chlorobenzene content determination and quantification. Results were reported in Table 6 and a chromatogram has been attached in Fig.1.

Conclusion

A selective and sensitive fast static HSGC method has been successfully developed and the separation method of Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene, and Chlorobenzene solvents in the sample. The developed method was successfully validated as per regulatory guidelines and found to be precise, accurate, linear, and specific. Additionally, our method is suitable for the analysis of Isopropyl alcohol, Dichloromethane, Hexanes, Ethyl Acetate, Toluene Chlorobenzene, and other solvents in one single method, which is accurate, precise, and linear in the presence of a 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 a precise, economical, and commercially able quantitative technique for residual solvent determination which will also be advantageous for industrial-scale manufacturing.

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References

1. Government of India. Ministry of health and family welfare. Indian Pharmacopoeia Vol. I. The Controller of Publication, New Delhi; 2010: 650-656.
2. Government of Great Britain. British Pharmacopeia Commission, London. Vol. 1. London 2009: 913-915.
3. Nogueira R, Suzane M, Gisele E, Silva B, Werickson F, Rocha C. Determination of Volatiles in pharmaceutical-certified reference materials. J. Braz Chem Soc. 2012; 23: 1636-1646.
4. Katarzyna G, Andrzej P. Analytical methods for residual solvents determination in Pharmaceutical products. Polish Pharmaceutical Society Drug Research. 2010; 67: 13-26.
5. Reddy PB, Reddy MS. Residual solvents determination by HS-GC with flame ionization detector in Omeprazole pharmaceutical formulations. Int J. Pharma Tech Research. 2009; 1: 230-234.
6. Sitaramaraju Y, Van Hul A, Wolfs K, Schepdael AV, Hoogmartens J, Adams E (2008) Static headspace gas chromatography of semi-volatile drugs in pharmaceuticals for topical use. J Pharm Biomed Anal 47:834–840
7. Antolín EM, Quinonez YB, Canavaciolo VG, Cruz ER (2008) Validation of an analytical method for quality control of residual solvents (n-hexane and acetone) in D-002: new active ingredient from beeswax. J Pharm Biomed Anal 47:646–650 3. Nojavan S, Ghassempour A, Bashour Y, Darbandi MK, Ahmadi SH (2005) Determination of residual solvents and investigation of their effect on ampicillin trihydrate crystal structure. J Pharm Biomed Anal 36:983

8. Proceedings of the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH-2016), tripartite harmonized guideline (Q3C) impurities: residual solvents
9. Gomes PCFL, D'Andrea ED, Mendes CB, Siqueira MEPB (2010) Determination of benzene, toluene, and N-hexane in urine and blood by headspace solid-phase microextraction/gas-chromatography for the biomonitoring of occupational exposure. J Braz Chem Soc 21:119
10. Harmonized Tripartite Guideline on Maintenance of Note for Guidance on Impurities: Residual Solvents (Q3C (M)) (2016) International conference on harmonization of technical requirements for registrations of pharmaceuticals for human use (ICH), Geneva
11. Residual solvents <467>, the United States Pharmacopoeia, USP38/NF33 (2012) the United States Pharmacopeial Convention, Rockville, MD, USA
12. Identification and control of residual solvents (2.4.24). In: European Pharmacopoeia (2013), 8th ed. European Directorate for the Quality of Medicines & HealthCare, Strasbourg, France
13. Kolb B, Ettre LS (1991) Theory and practice of multiple headspace extraction. Chromatographia 32:505–513
14. B'Hymer C (2003) Residual solvent testing: a review of gas-chromatographic and alternative techniques. Pharm Res 20:337–344
15. Camarasu CC, Mezei-Szuts M, Varga GB (1998) Residual solvents determination in pharmaceutical products by GC-HD and GC– MS-SPME. J Pharm Biomed Anal 18:623–638
16. Yarramraju S, Akurathai V, Wolfs K, Van Schepdael A, Hoogmartens J, Adams E (2007) Investigation of sorbic acid volatile degradation products in pharmaceutical formulations using
1. static headspace gas chromatography. J Pharm Biomed Anal 44:456–463
17. Otero R, Carrera G, Dulsat JF, Fabregas JL, Claramunt J (2004) Static headspace gas chromatographic method for quantitative determination of residual solvents in pharmaceutical drug substances according to European Pharmacopoeia requirements. J Chromatogr A 1057:193–201
18. Adepu S, Valli Kumari RV, Tulja Rani G (2015) A new validated GC-HS method for the determination of residual solvents in famotidine using FID. Int J Pharm Sci Rev Res 31:63–67
19. Puranik SB, Sharath S, Sanjay Pai PN (2012) Headspace gas chromatography analysis of residual solvents by using EC-5 column. Int J Pharm Chem Res 1(1):22–27
20. Mistry NPK, Chetwyn L, Yazzie Dai DT, Quiroga KAC, Zhang HB, Dong Runes MW (2010) A generic headspace GC method for residual solvents in pharmaceutical: benefits, rationale and
2. adaptations for new chemical entities. LCGC N Am 28:54–66
21. Cheng C, Liu S, Mueller BJ, Yan Z (2010) A generic static headspace gas chromatography method for determination of residual solvents in the drug substance. J Chromatogr A 1217:6413–6421
22. Validation of Analytical Procedures Q2R1 (2005) Text and methodology, ICH harmonized tripartite guideline.