CHAPTER 3:

DEVELOPMENT AND VALIDATION OF RESIDUAL SOLVENT DETERMINATION BY HEAD SPACE GAS CHROMATOGRAPHY FOR IMATINIB MESYLATE API

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3.1. Introduction

3.1.1. Introduction to Residual Solvent

The organic solvents used in the process of manufacturing requires monitoring and control as the solvents are toxic, have no therapeutic importance and affect the quality and stability of drug substances and drug products. Hence they are not desirable in the finished product (1-3). Residual solvents or volatile organic solvents in drug substance or formulation during the process of manufacture of its components are also well-known as Organic Volatile Impurities (OVI). However, the term Organic volatile impurities, covers a wide range of impurities including volatile solvent. Most of the organic solvents used or formed during the manufacturing process of the drug substance, intermediates, excipient or pharmaceutical drug product are volatile and can be determined with help of the headspace-gas chromatography (4). It is very difficult to remove the organic solvents completely with the commonly available techniques used in practical manufacturing process such as increased process temperature and/or decreased pressure. As they have no pharmacological importance and are of toxic nature, they require control in either the drug substance, intermediate or drug product.

The amount of traces of residual solvents in the finished drug substance or drug product depends on the characteristics of the drug substance or drug product or sample to be analyzed. It also depends on the condition of the drying process such as temperature and pressure. Thus, by increasing temperature or by decreasing pressure, residual solvents can be controlled in the finished product. Even after considering all aspects of controlling the residual solvents, some traces of residual solvent adhere to the finished drug substance or drug product. Hence the allowable or permitted exposure of the residual solvents through drug product are described in the regulatory guideline, particularly in guideline Q3C(R7) issued by the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) (5, 6). The guideline Q3C is specifically for the control of residual solvent in drug substance.

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In case of the drug product, excipient also need to be considered so the total exposure of the residual solvent should not be higher than the defined permitted dose exposure of the residual solvent (7). Therefore determination of residual solvents becomes a necessary procedure to control the quality of drug substances and drug product to meet regulatory guideline and ensure patient safety (8, 9).

3.1.2. Headspace Gas-chromatography

Head space gas chromatography (HSGC) is the coupling of head space technique with gas chromatography which increases the sensitivity and separation efficiency for the determination of the residual solvent. Headspace denotes the space above the sample matrix in the HSGC glass vial in which the solvents vaporize and saturate the space above the sample matrix. This saturated vapor of the solvent is injected into the column through headspace injection. In this case the injection time also requires to define with the injection volume. Injected sample is passed through the column with the help of carrier gas and ionized into the flame ionization detector which detects most of the residual solvents used in the manufacturing of the pharmaceutical drug substance and drug products. Hence, it is widely used in the pharmaceutical analysis by gas chromatography.

Head space consists of several modules, each has its own functions. First of all, there is sample holder which holds the N number of samples through HS glass vial with aluminum septa (10). Sample holding capacity differs with make of the instrument. Sample holder holds the sample and conveys the sample to the temperature,-controlled shaker or HS oven which is designed for the shaking of vial in controlled temperature to set the equilibration between gas and liquid. Here, the whole operation is controlled through two different parameters; one is equilibration time and second is the oven temperature. Instrument shakes the sample containing HS vial for defined equilibration time at defined temperature which converts the liquid sample matrix into its gas form and again gas form into liquid form. Thus, at end of equilibration time, ideally, vaporized sample matrix gets saturated in space above the sample matrix which comprises of the residual solvents. So during development of method, equilibration time and temperature of the HS oven requires to be optimized; which normally depends on the sample matrix and diluents of the sample preparation (11).

Next part of the Head space instrument is HS-injector which mainly comprises of the needle, loop and transfer line. Needle punctures the septa and sets inside the sample vial so the vaporized sample transfers into the loop and through loop sample passes to transfer line. Loop and transfer line temperature can be controlled and should be always in increasing order to retard the condensation of the sample vapor. Loop and transfer line temperature should be higher than the HS oven which are the part of HS chromatographic parameter and optimized during the development of the method for sample analysis. Loop holds the sample to avoid the back flow of the vaporized sample to sample vial through needle (12). Transfer line works as a bridge between Headspace and gas chromatogram which transfer the vaporized sample to the

column. Through the column, vapors of the residual solvents present in the sample are resolved as per the affinity with stationary phase present in the column and mobilize to the Flame ionization detector with the help of the carrier gas which act as mobile phase. Normally, nitrogen or helium is used as a carrier gas whereas hydrogen and zero air are used for the flame in flame ionization detector. Argon or xenon is also used as a carrier gas in place of nitrogen or helium.

In flame ionization detector, vaporized sample gets ionized and imparts on the ion collector. Ion collector exists with anode and cathode which attracts the positive and negative charged particles respectively. This creates the potential difference which converts into signals on chromatogram through modular and amplification of the signal is done by amplifier. Detector is selective and most sensitive for the volatile organic solvents but hardly useful for the nonvolatile matters. HSGC with FID detection has been mainly used for the analysis of organic volatile solvents present in the pharmaceutical drug substances and drug products (13-16). 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 (17,18). 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 lead to poor chromatography (19).

Headspace has multiple uses now a days in gas chromatography. It is not only limited to determination of organic volatile solvents but also useful for the single content method determination for the selective impurity which cannot be analyzed through other sophisticated techniques (20). It is also useful in derivatization technique as it provides the controlled temperature with shaking during equilibration which could be a suitable condition for derivatization of the analyte. Though Headspace has multiple usage it has limitation like lower choice for the diluent selection because the HS vial air tightens with aluminum septa. Hence, solvent with low boiling point may burst the glass vial in HS oven and cannot be used as diluents (21). For the diluents with high boiling point, solvents like tetrahydrofuran, dimethyl sulphoxide, N-methyl pyrrolidone can be used as diluents.

Thus, Headspace technique is useful for the determination of residual solvent as it is selective and sensitive for the analysis of volatile organic impurities.

3.2 Literature Survey

Several analytical methods has been reported based on Headspace gas chromatography for quantification of residual solvents (22-23) but none of them are suitable for the quantification of each of the solvents used in the synthesis process of the Imatinib mesylate.

Battu et al. have developed the residual solvent method using head space chromatography with column DB-624 having 100% dimethylpolysiloxane 30.0 m × o.53 mm ID, 3.0 µm for the Imatinib mesylate but this method has poor establishment of resolution between ethyl acetate and hexane. (22).Second, Adepuetal. have reported an analytical method using GC-HS and ZB-624 (30m × 0.53mm, 0.5µ) as column along with FID as detector for the determination of residual solvent in Imatinib mesylate but the analytical method has limitation of resolution between acetone and dichloromethane. Hence difficulty might arise during the identification of the acetone and dichloromethane solvent (3).

In this chapter, we have described a development and validation of an HS-GC analytical method for determination of six residual solvents used during synthesis of Imatinib Mesylate drug substance.

3.3 Chemical, material, and reagents

Imatinib Mesylate API was synthesized in Cadila Healthcare Ltd. (Ahmedabad, Gujarat, India) (24). Methanol, acetone, dichloromethane (DCM),n-hexane, ethyl acetate and pyridine were purchased from Merck (India). N-Methyl-2-Pyrrolidinone (NMP) was purchased from Spectrochem (Mumbai, India).

3.4 Instrumentation

HSGC system model Agilent technologies 6890N equipped with flame ionization detector with a headspace sampler (Agilent technologies G1888) was used for method development and method validation studies. Deactivated direct split liner having 2mm internal diameter was used as an inlet liner and chemstation software was used for data acquisition and chromatographic data integration. A Sartorius micro balance CP225D (Germany) and micropipette (100-1000µL from Borosil) were used.

3.4.1 Gas chromatographic conditions

GC column having dimension of 30m length, 0.53mm Internal Diameter, 3.0µm film thickness DB-624with stationary bonded 6% cyanopropyl-phenyl- 94% dimethylpolysiloxane capillary

GC column was able to achieve proper separation in a developed method. DB-624 column was manufactured by J&W Scientific (Agilent Scientific Technologies, Wilmington, DE, USA). The GC method parameters, headspace sampler conditions, and oven temperature programming of the method have been mentioned in Table 3.1.

3.4.2 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.

3.4.3 Selection of column

The GC Column is a crucial parameter for developing an efficient and sensitive HSGC method. The residual solvents were usually determined by bonded 6% cyanopropyl-phenyl- 94% dimethylpolysiloxane (624) column due to its moderate polarity, higher temperature limit than other polar columns (DB-Wax), and higher retention of polar solvents compared to non-polar column (DB-1). The retention of polar solvents on a DB-624 column is relatively stronger at high temperatures and could provide efficient separation between solvents which has minor differences in boiling points. Since the objective was to develop an efficient HSGC method, a DB-624 column (30 m × 0.53 mm, 3.0 µm film thickness) was selected, which is commonly used for residual solvent determination. All residual solvents present in Imatinib Mesylate API were well separated from each other as well as from diluent using DB-624 columns shown in representative chromatogram (Fig. 3.1).

To separate dichloromethane and acetone was highly critical by HSGC due to their close boiling points and elution order. Resolutions of this critical pair were 6.3 in the present developed method. To define the effect of the stationary phase on the resolution, a non-polar GC column DB-1 (100% dimethyl polysiloxane) was used with the same chromatographic conditions of the developed method. Resolution of a critical pair was decreased to 1.2 from 6.3 with DB-1 Column. These results clearly indicated that the DB-624 column is the best choice for the separation of all six residual solvents in Imatinib Mesylate API.

3.4.4 Selection of sample solvent (Diluent)

Several solvents were investigated as diluents such as DMF, DMSO and NMP wherein it was observed that NMP gave smooth baseline with no interference at the retention times of the targeted solvents. When DMSO was used as sample solvent, a peak was observed during heating in oven. DMF gave baseline interference at the retention time of pyridine. It was

decided to use NMP as the standard and sample solvent because of its ability to dissolve a wide variety of drug substances. It has a high boiling point (202°C) that does not interfere with the analysis of volatile solvents.

3.4.5 Chromatographic conditions

To develop an HSGC method, there are two strategies for selecting oven programs. The first strategy is to keep initial oven temperature low and perform gradient elution while in second strategy isothermal elution at relatively high oven temperatures is done. To increase the retention time of methanol, the first strategy was chosen with initial oven temp at 35°C and hold time of 2min. Further, a temperature ramp was applied at a rate of 4°C/min up to 80°C to resolve the critical pair, dichloromethane and acetone. To elute other high boiling unknown impurities and NMP, which is having 202°C boiling point, oven temp was set at 40°C/min with increment up to 230°C with 12 min final hold time. The flow rate of nitrogen was analyzed at 3.3mL/min which is equivalent to 24cm/s of linear velocity. Finally, all residual solvents are well separated with sufficient theoretical plates (>2000), USP tailing (<2.0) with a total run time of about 29min.

3.4.6 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 but below the boiling point of the sample solvents. To minimize the carryover problems Loop temperature was kept 10-15°C higher than oven temperature and the transfer line temperature was also kept 10-15°C higher than the loop temperature. Headspace oven temperature was kept at 80°C because among all residual solvents, pyridine has a highest boiling point of 80°C. Therefore, the headspace oven, loop, and transfer line temperatures were selected at 80°C, 90°C and 110°C, respectively. The vial equilibration time was set to 30 min. Other headspace parameters are described in Table 3.1.

Components	Parameters	Requirements
Headspace condition	Injection volume	1mL
	G.C cycle time	40 min
	Oven temperature	80°C
	Loop temperature	90°C
	Transfer line temperature	110°C
	Sample equilibration time	30 min
	Loop equilibration time	0.20 min
	Loop fill time	0.10 min
	Inject time	1 min
Injector	Carrier gas	Nitrogen
	Injector Temperature	200°C
	Gas flow (Constant Pressure)	2.3 psi
	Injection mode	Split 1:2
	Liner	Glass liner
Column	DB-624 (30m long x 0.53mm I.D x 3µm film thickness) 6% Cyanopropylphenyl and 94% Dimethylpolysiloxane	
	Oven temperature program	Increment rate (°C/min) / Temperature (°C) / Hold time (min)
		-- / 35 / 2
		4 / 80 / 0
		40 / 230 / 12
Detector	Total program time	29.0 min
	Type	FID
	Temperature	260°C
	Hydrogen flow	40 ml/min
	Airflow	400 ml/min
	Make up flow (N2)	25 ml/min

Table 3.1 Experimental condition for determination of residual solvents of Imatinib Mesylate API

3.5 Preparation of standard and sample solution

Standard solution of residual solvent was prepared according to respective ICH limit. A composite standard stock solution of all the known residual solvents was prepared in such a way that it contains a final concentration of 300 ppm for methanol, 500 ppm for acetone, 60ppm for dichloromethane, 29 ppm of n-hexane, 500 ppm for ethyl acetate and 20 ppm for pyridine in NMP.

The blank vial was prepared with 1.0 mL of NMP, the standard vial was prepared with 1.0mL of the standard solution and the sample vials were prepared with 100 mg per 1.0mL NMP.

3.6 Method validation

The final method has been validated as per requirements prescribed in ICH guidelines (25). 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 (2005).

3.6.1 Specificity

The method specificity was demonstrated by injecting the Blank, individual residual solvents, standard solution and specificity solution (composite standard solution of all residual solvents). In the developed chromatographic method, no interference was observed at the retention time of targeted residual solvents from each other and from sample solvents or other unknown peaks. The retention times of methanol, acetone, dichloromethane (DCM), n-hexane, ethyl acetate and pyridine were found to be 4.10, 6.07, 6.92, 7.99, 9.61 and 14.69min, respectively. The retention time of sample solvent (NMP) was found to be 18.0min. A typical chromatogram of the standard solution is shown in Fig.3.1.

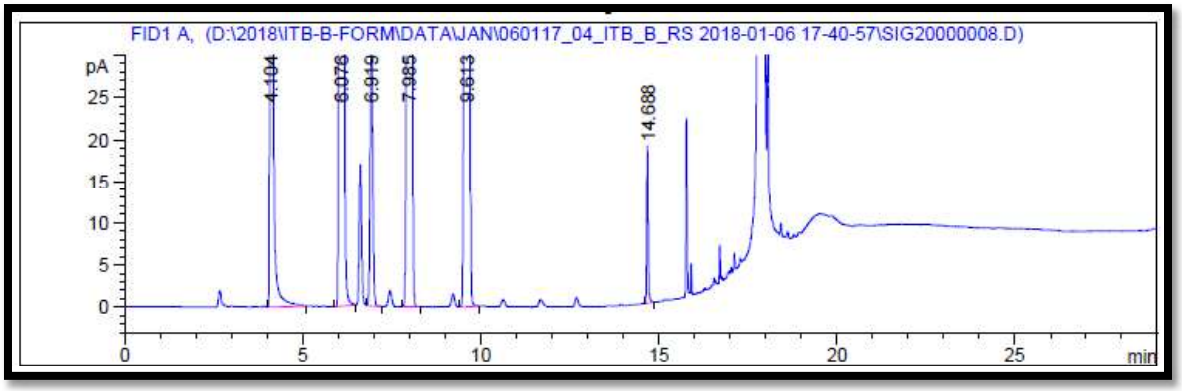

Fig 3.1 Typical chromatogram of the six composite standard solution.

3.6.2 Linearity and range

The linearity of the method was determined using 7 concentration levels over the range 20–150% of ICH Limit Level. Table 3.2a represents linearity stock preparation and Table 3.2b represents linearity and range sample preparation and level. The calibration curve was found to be linear within the range and correlation coefficient (r^2) values for all six residual solvents were found to be higher than 0.99. Linearity curve and values for the residual solvents are provided in Fig.3.2 to 3.8 and Table3.3 to 3.8. Each residual solvent easily passed acceptance criteria for accuracy, system precision, method precision and linearity from low concentration to high concentration, with a range 60-450 ppm for methanol, 100-750 ppm for acetone, 15-100ppm for dichloromethane, 6-50 ppm for n-hexane, 100-750 ppm for ethyl acetate and 5-35 ppm for pyridine.

Table 3.2a Linearity stock preparation

Standard Details						
	Methanol	**Acetone**	**DCM**	**n-Hexane**	**Ethyl Acetate**	**Pyridine**
Potency (%)	99.9	99.9	99.9	99.9	99.9	99.9
Wt. Taken (mg)	301.8	503.4	75.5	33.3	498.6	24.1
Dilution (ml) Stock-1	100	100	100	100	100	100
Volume taken (ml)	5	5	5	5	5	5
Dilution (ml)	50	50	50	50	50	50

Table 3.2b Linearity and range sample preparation and level

Sample ID	**Level**	**Volume taken from Stock-1 (mL)**	**Dilution (mL)**	**Volume taken (mL)**	**Dilution (mL)**
Linearity solution-1	20%	1.0	50	1	1
Linearity solution-2	30%	1.5	50	1	1
Linearity solution-3	50%	2.5	50	1	1
Linearity solution-4	80%	4.0	50	1	1
Linearity solution-5	100%	5.0	50	1	1
Linearity solution-6	120%	6.0	50	1	1
Linearity solution-7	150%	7.5	50	1	1

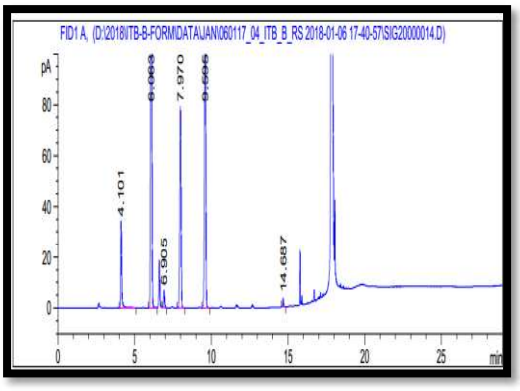

Fig 3.2 Linearity Level-1

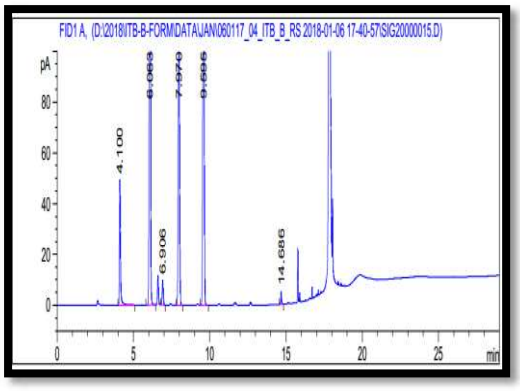

Fig 3.3 Linearity Level-2

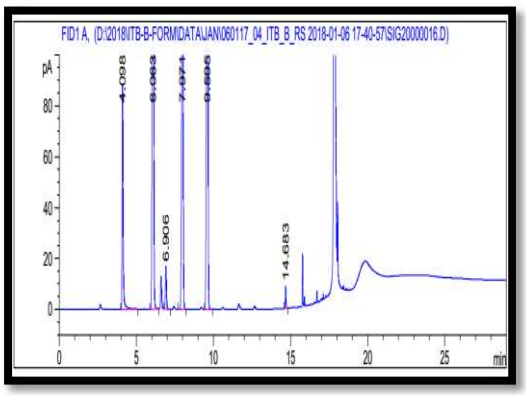

Fig 3.4 Linearity Level-3

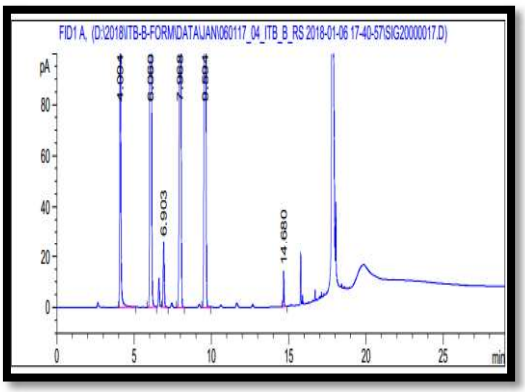

Fig 3.5 Linearity Level-4

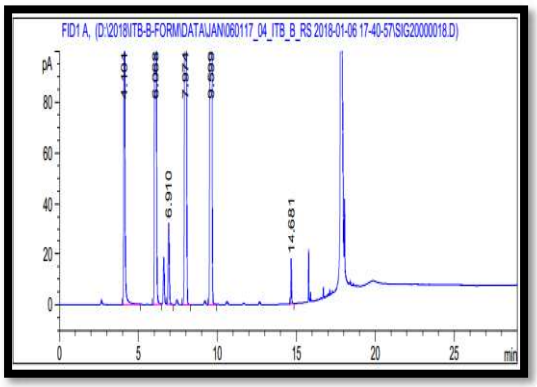

Fig 3.6 Linearity Level-5

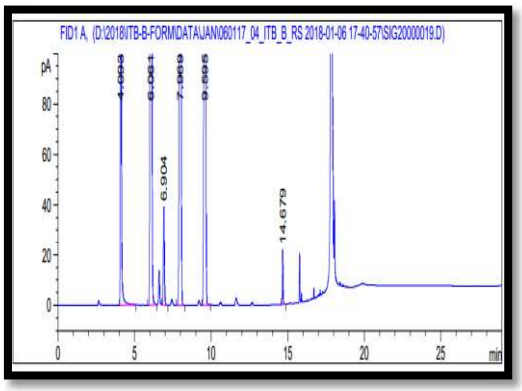

Fig 3.7 Linearity Level-6

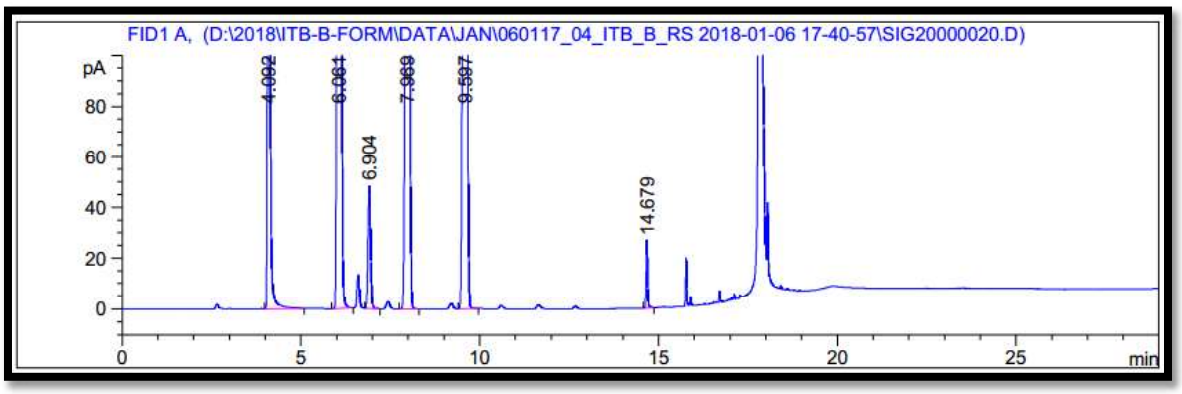

Fig 3.8 Linearity Level-7

Table 3.3 Linearity sample concentration and results

Solvent name	Conc. range (µg/ml)							Regression equation (Y=ax+b)	Correlation Co-efficient
	LOQ	10%	30%	50%	80%	120%	150%		
Methanol	60.3	90.4	150.7	241.2	301.5	361.8	452.2	Y=2.8677x−12.273	0.9996
Acetone	100.6	150.9	251.4	102.3	502.9	303.5	754.3	Y=12.01x+57.252	0.9997
DCM	15.1	22.6	37.7	60.3	75.4	90.5	113.1	Y=2.163x+1.6003	0.9997
n-Hexane	6.7	10.0	16.6	26.6	33.3	39.9	49.9	Y=72.831x+4.9266	0.9994
Ethyl acetate	99.6	149.4	249.1	398.5	498.1	597.7	747.2	Y=7.6815x+41.985	0.9997
Pyridine	4.8	7.2	12.0	19.3	24.1	28.9	36.1	Y=2.1041x+0.9255	0.9996

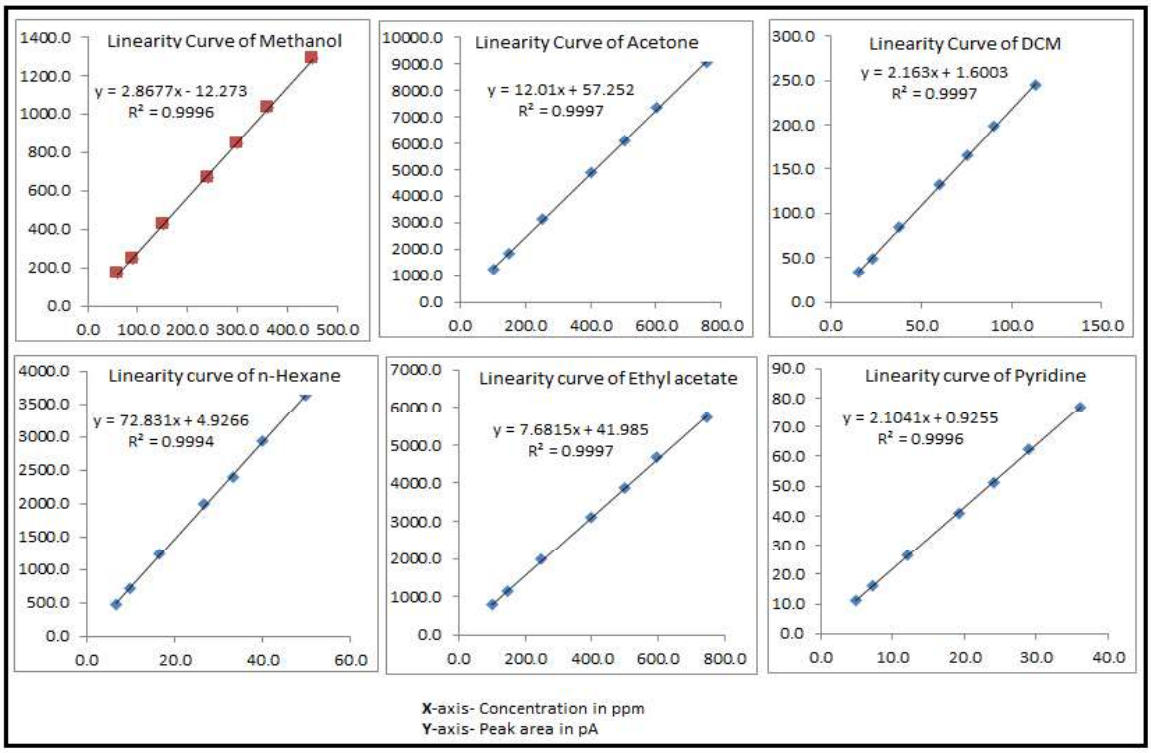

Fig 3.9 Linearity plot of residual solvents.

3.6.3 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, LOQ limit was found to be 60.3ppm for methanol, 100.6ppm for acetone, 15.1ppm for dichloromethane, 6.7ppm for n-hexane,99.6ppm for ethyl acetate and 4.8ppm for pyridine.

3.6.4 Accuracy (recovery)

The accuracy of the method was determined by spiking of all six solvents at four different levels i.e. LOQ Level, 50% level, 100% level, and 150% level of ICH limit in a triplicate analysis. Recovery for all six solvents was found within the range of 80-120%. The recovery for each solvent from the lowest concentration to its highest concentration determined and results of the same are reported in unit of percentage in Table 3.5 to 3.10and indicated that the method was accurate.

Sample Preparation					
Sr. No	**Level**	**Wt of sample (mg)**	**Sample Dilution**	**Vol. Of stock-1 (mL)**	**Dilution (mL)-accuracy**
1	Control sample	99.70	1	1	1
2	Level-(150%) Sample Prep.-1	100.65	1	7.5	50
3	Level-(150%) Sample Prep.-2	99.60	1	7.5	50
4	Level-(150%) Sample Prep.-3	101.20	1	7.5	50
5	Level-(50%) Sample Prep.-1	100.50	1	2.5	50
6	Level-(50%) Sample Prep.-2	101.20	1	2.5	50
7	Level-(50%) Sample Prep.-3	100.10	1	2.5	50
8	Level-(20%) Sample Prep.-1	100.10	1	1.0	50
9	Level-(20%) Sample Prep.-2	99.60	1	1.0	50
10	Level-(20%) Sample Prep.-3	99.52	1	1.0	50

Table 3.4 Sample Preparation for accuracy

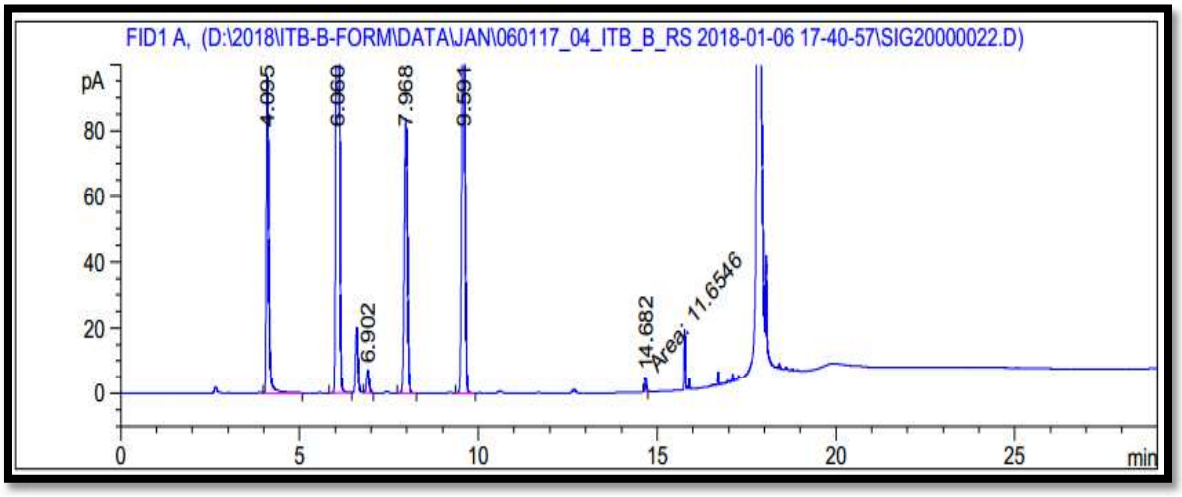

Fig 3.10 LOQ recovery chromatogram-1

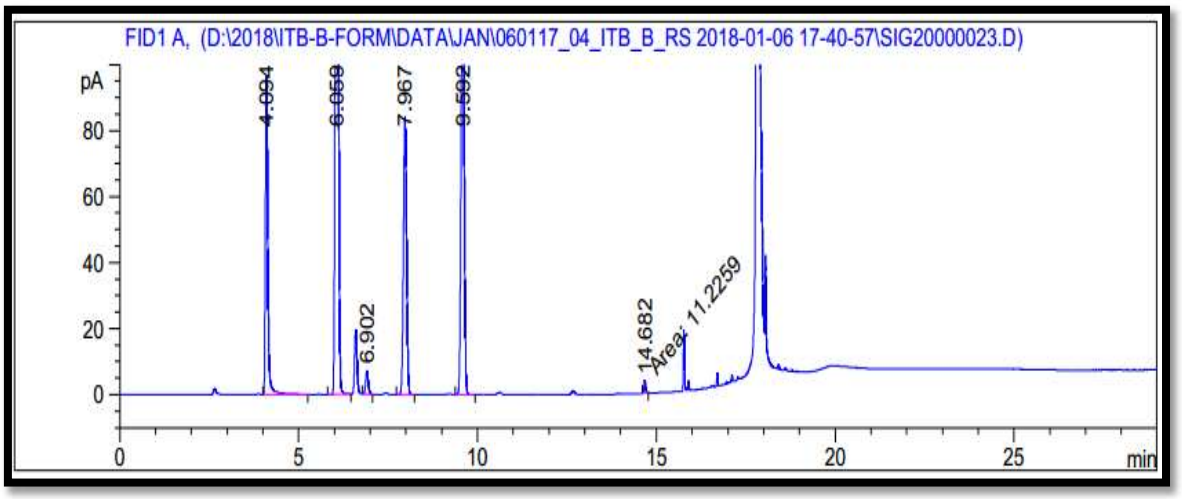

Fig 3.11 LOQ recovery chromatogram-2

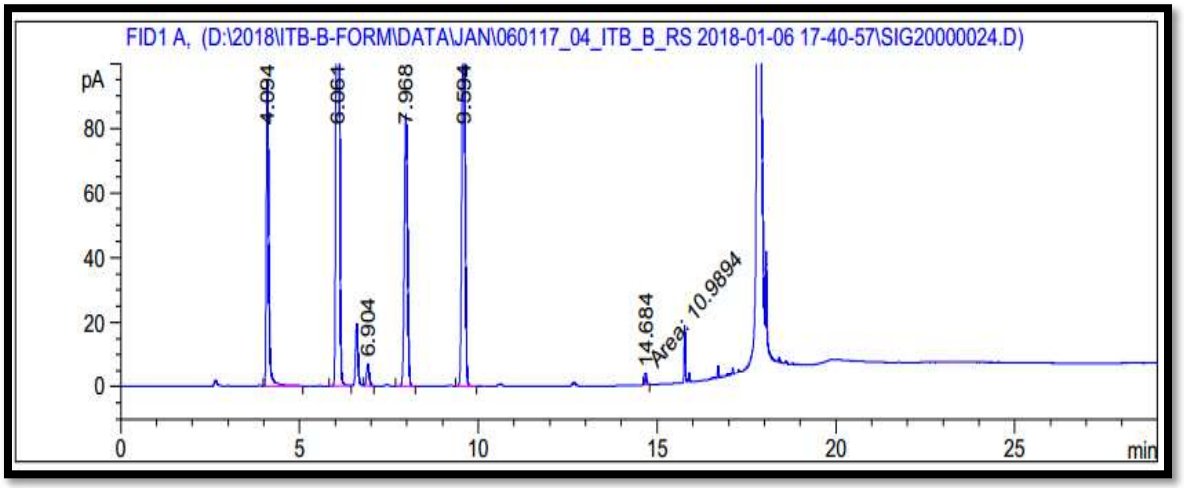

Fig 3.12 LOQ recovery chromatogram-3

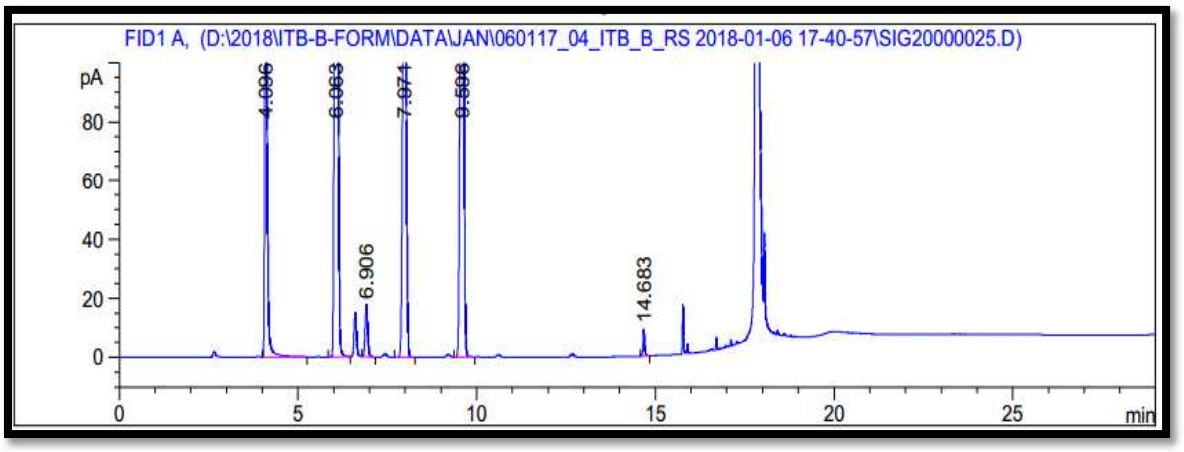

Fig 3.13 50% Level recovery chromatogram-1

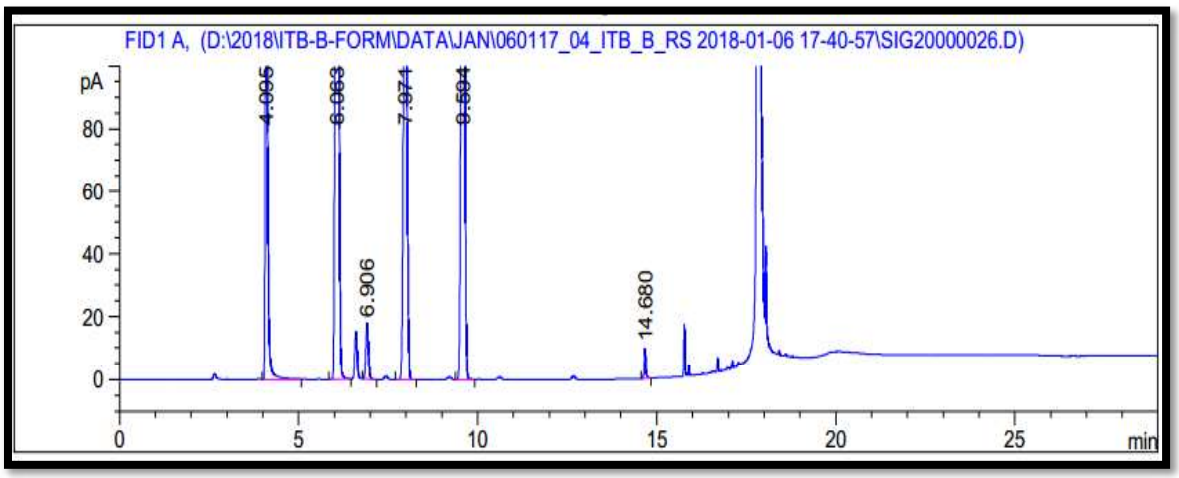

Fig 3.14 50% Level recovery chromatogram-2

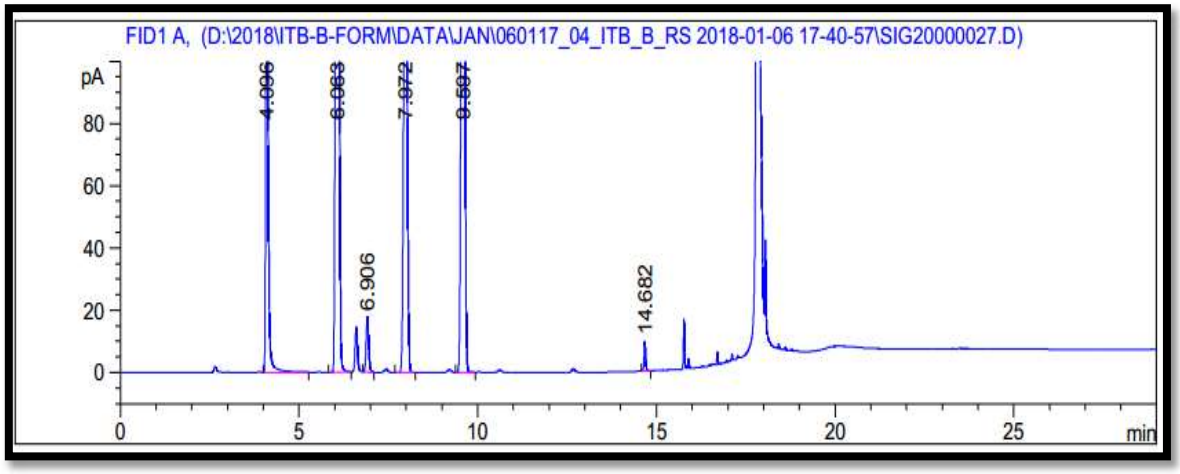

Fig 3.15 50% Level recovery chromatogram-3

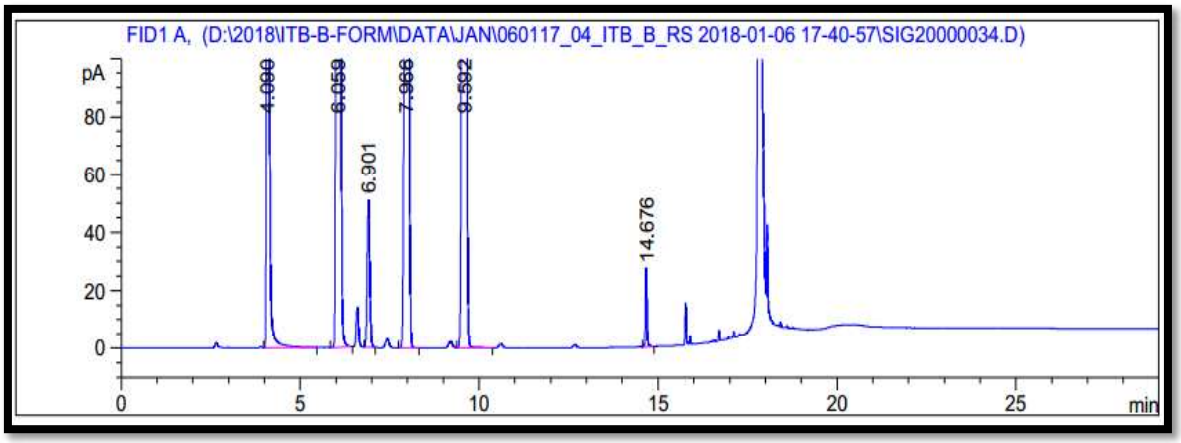

Fig 3.16 150% Level recovery chromatogram-1

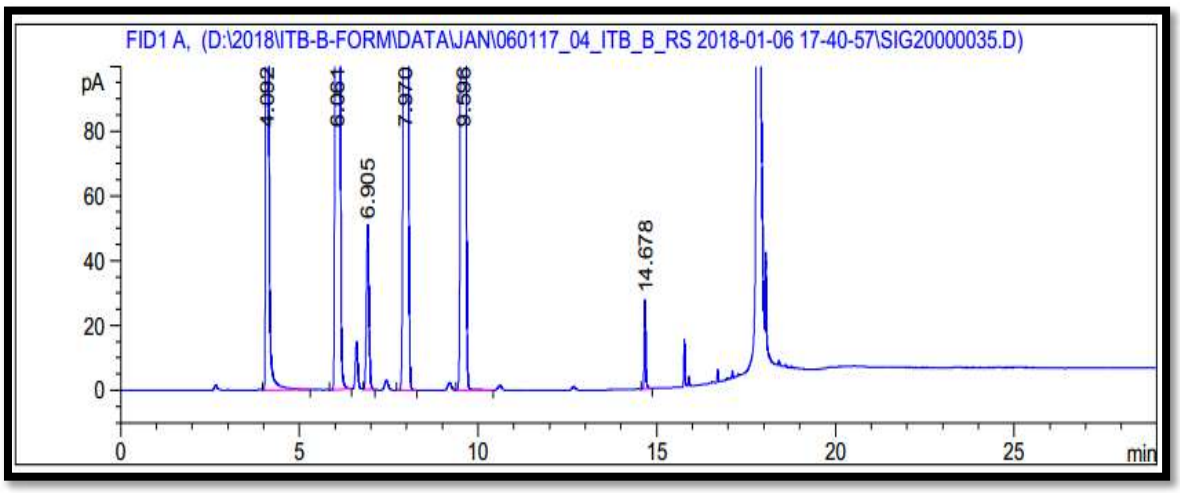

Fig 3.17 150% Level recovery chromatogram-2

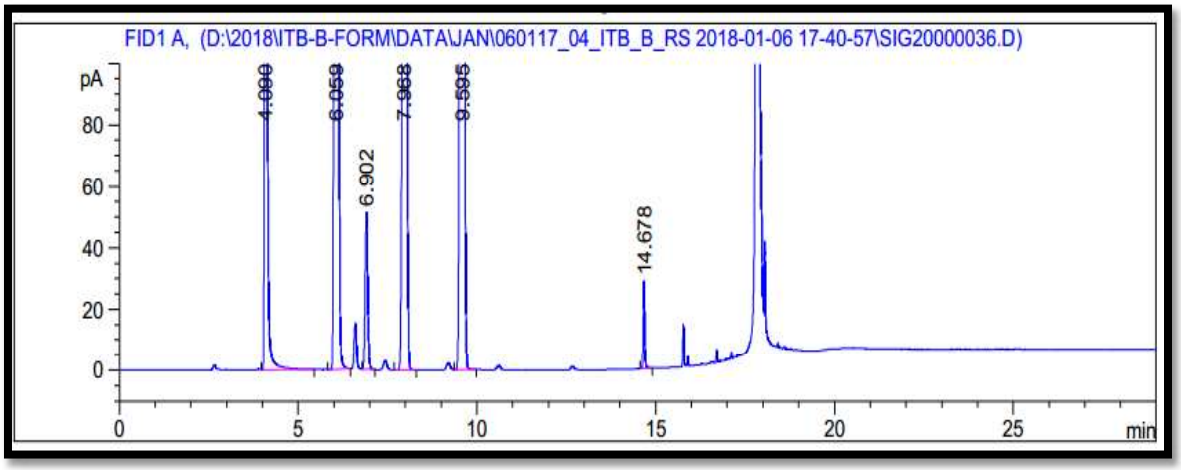

Fig 3.18 150% Level recovery chromatogram-3

Sr. No.	Levels	Area	Amount Added (ppm)	Amount Found (ppm)	Amount recovered (ppm)	Recovery (%)	Mean recovery (%)
1	Control sample	285.7	0.0	988.5	-988.5	NA	NA
2	Level-(150%) Sample Prep.-1	1595.2	4522.5	5467.3	4478.8	99.0	99.0
3	Level-(150%) Sample Prep.-2	1581.2	4522.5	5476.5	4487.9	99.2	99.2
4	Level-(150%) Sample Prep.-3	1586.5	4522.5	5408.0	4419.4	97.7	97.7
5	Level-(50%) Sample Prep.-1	728.5	1507.5	2500.6	1512.0	100.3	100.3
6	Level-(50%) Sample Prep.-2	722.8	1507.5	2463.8	1475.3	97.9	97.9
7	Level-(50%) Sample Prep.-3	725.8	1507.5	2501.2	1512.7	100.3	100.3
8	Level-(20%) Sample Prep.-1	460.0	603.0	1585.2	596.7	99.0	99.0
9	Level-(20%) Sample Prep.-2	458.4	603.0	1587.7	599.1	99.4	99.4
10	Level-(20%) Sample Prep.-3	452.9	603.0	1569.9	581.3	96.4	96.4
						Overall Mean	98.8
						Overall SD	1.3
						Overall RSD (%)	1.3

Table 3.5 Accuracy Calculation for Methanol

Sr. No.	Levels	Area	Amount Added (ppm)	Amount Found (ppm)	Amount recovered (ppm)	Recovery (%)	Mean recovery (%)
1	Control sample	624.1	0.0	513.1	-513.1	NA	NA
2	Level-(150%) Sample Prep.-1	10048.9	7543.4	8184.1	7670.9	101.7	101.7
3	Level-(150%) Sample Prep.-2	9983.6	7543.4	8216.6	7703.5	102.1	102.1
4	Level-(150%) Sample Prep.-3	10004.7	7543.4	8103.8	7590.7	100.6	100.6
5	Level-(50%) Sample Prep.-1	3901.7	2514.5	3182.4	2669.3	106.2	106.2
6	Level-(50%) Sample Prep.-2	3891.7	2514.5	3152.3	2639.1	105.0	105.0
7	Level-(50%) Sample Prep.-3	3874.5	2514.5	3172.8	2659.7	105.8	105.8
8	Level-(20%) Sample Prep.-1	1899.1	1005.8	1555.2	1042.0	103.6	103.6
9	Level-(20%) Sample Prep.-2	1881.0	1005.8	1548.1	1035.0	102.9	102.9
10	Level-(20%) Sample Prep.-3	1895.6	1005.8	1561.4	1048.2	104.2	104.2
						Overall Mean	103.6
						Overall SD	1.9
						Overall RSD (%)	1.8

Table 3.6 Accuracy Calculation for Acetone

Sr. No.	Levels	Area	Amount Added (ppm)	Amount Found (ppm)	Amount recovered (ppm)	Recovery (%)	Mean recovery (%)
1	Control sample	0	0.0	0.0	NA	NA	NA
2	Level-(150% \) Sample Prep.-1	260.1	1131.4	1160.2	1160.2	102.5	102.5
3	Level-(150%) Sample Prep.-2	257.7	1131.4	1161.6	1161.6	102.7	102.7
4	Level-(150%) Sample Prep.-3	258.4	1131.4	1146.3	1146.3	101.3	101.3
5	Level-(50%) Sample Prep.-1	89.8	377.1	401.2	401.2	106.4	106.4
6	Level-(50%) Sample Prep.-2	89.9	377.1	398.8	398.8	105.8	105.8
7	Level-(50%) Sample Prep.-3	89.8	377.1	402.8	402.8	106.8	106.8
8	Level-(20%) Sample Prep.-1	35.1	150.8	157.4	157.4	104.4	104.4
9	Level-(20%) Sample Prep.-2	35.1	150.8	158.2	158.2	104.9	104.9
10	Level-(20%) Sample Prep.-3	35.4	150.8	159.7	159.7	105.9	105.9
						Overall Mean	104.5
						Overall SD	1.9
						Overall RSD (%)	1.8

Table 3.7 Accuracy Calculation for DCM

Sr. No.	Levels	Area	Amount Added	Amount Found	Amount recovered	Recovery	Mean recovery
			(ppm)	(ppm)	(ppm)	(%)	(%)
1	Control sample	3.6	0.0	0.5	-0.5	NA	NA
2	Level-(150%) Sample Prep.-1	3861.2	499.0	533.1	532.6	106.7	106.7
3	Level-(150%) Sample Prep.-2	3873.9	499.0	540.5	540.0	108.2	108.2
4	Level-(150%) Sample Prep.-3	3891.2	499.0	534.3	533.8	107.0	107.0
5	Level-(50%) Sample Prep.-1	1322.3	166.3	182.8	182.3	109.6	109.6
6	Level-(50%) Sample Prep.-2	1316.2	166.3	180.7	180.2	108.4	108.4
7	Level-(50%) Sample Prep.-3	1320.2	166.3	183.3	182.8	109.9	109.9
8	Level-(20%) Sample Prep.-1	513.1	66.5	71.2	70.7	106.3	106.3
9	Level-(20%) Sample Prep.-2	509.6	66.5	71.1	70.6	106.1	106.1
10	Level-(20%) Sample Prep.-3	513.0	66.5	71.6	71.1	106.9	106.9
						Overall Mean	107.7
						Overall SD	1.4
						Overall RSD (%)	1.3

Table 3.8 Accuracy Calculation for n-Hexane

Sr. No.	Levels	Area	Amount Added	Amount Found	Amount recovered	Recovery	Mean recovery
			(ppm)	(ppm)	(ppm)	(%)	(%)
1	Control sample	3.2	0.0	4.1	-4.1	NA	NA
2	Level-(150%) Sample Prep.-1	6161.3	7471.5	7766.5	7762.4	103.9	103.9
3	Level-(150%) Sample Prep.-2	6112.7	7471.5	7786.5	7782.4	104.2	104.2
4	Level-(150%) Sample Prep.-3	6137.9	7471.5	7695.0	7690.9	102.9	102.9
5	Level-(50%) Sample Prep.-1	2141.2	2490.5	2703.1	2699.0	108.4	108.4
6	Level-(50%) Sample Prep.-2	2137.9	2490.5	2680.2	2676.2	107.5	107.5
7	Level-(50%) Sample Prep.-3	2134.4	2490.5	2705.3	2701.2	108.5	108.5
8	Level-(20%) Sample Prep.-1	834.6	996.2	1057.8	1053.7	105.8	105.8
9	Level-(20%) Sample Prep.-2	833.8	996.2	1062.1	1058.0	106.2	106.2
10	Level-(20%) Sample Prep.-3	841.0	996.2	1072.1	1068.1	107.2	107.2
						Overall Mean	106.1
						Overall SD	2.0
						Overall RSD (%)	1.9

Table 3.9 Accuracy Calculation for Ethyl Acetate

Sr. No.	Levels	Area	Amount Added (ppm)	Amount Found (ppm)	Amount recovered (ppm)	Recovery (%)	Mean recovery (%)
1	Control sample	0.0	0.0	0.0	0.0	NA	NA
2	Level-(150%) Sample Prep.-1	81.9	361.1	362.8	358.7	99.3	99.3
3	Level-(150%) Sample Prep.-2	79.1	361.1	354.1	350.0	96.9	96.9
4	Level-(150%) Sample Prep.-3	77.9	361.1	343.2	339.1	93.9	93.9
5	Level-(50%) Sample Prep.1	27.7	120.4	122.9	118.8	98.7	98.7
6	Level-(50%) Sample Prep.-2	28.5	120.4	125.6	121.5	100.9	100.9
7	Level-(50%) Sample Prep.-3	28.7	120.4	127.8	123.8	102.8	102.8
8	Level-(20%) Sample Prep.-1	11.6	48.2	51.7	47.6	98.8	98.8
9	Level-(20%) Sample Prep.-2	11.2	48.2	50.1	46.1	95.7	95.7
10	Level-(20%) Sample Prep.-3	10.9	48.2	48.8	44.8	93.0	93.0
						Overall Mean	97.8
						Overall SD	3.2
						Overall RSD (%)	3.3

Table 3.10 Accuracy Calculation for Pyridine

Accuracy Level	Methanol	Acetone	DCM	n-Hexane	Ethyl acetate	Pyridine
LOQ Recovery						
LOQ Recovery -1	99.0	103.6	104.4	106.3	105.8	98.8
LOQ Recovery -2	99.4	102.9	104.9	106.1	106.2	95.7
LOQ Recovery -3	96.4	104.2	105.9	106.9	107.2	93.0
50% level Recovery						
50% Level Recovery-1	100.3	106.2	106.4	109.6	108.4	98.7
50% Level Recovery-2	97.9	105.0	105.8	108.4	107.5	100.9
50% Level Recovery-3	100.3	105.8	106.8	109.9	108.5	102.8
100% Level Recovery (Method Precision)						
Spike solution-1	97.4	93.1	103.7	108.2	105.2	98.0
Spike solution-2	98.9	93.3	103.5	107.7	104.9	100.0
Spike solution-3	99.5	93.8	104.2	108.9	105.5	101.4
Spike solution-4	97.5	93.2	103.6	108.6	105.1	99.9
Spike solution-5	99.1	93.8	104.0	109.5	105.6	100.8
Spike solution-6	100.1	94.2	104.5	109.6	106.0	101.3
150% Level Recovery						
150% Level Recovery-1	99.0	101.7	102.5	106.7	103.9	99.3
150% Level Recovery-2	99.2	102.1	102.7	108.2	104.2	96.9
150% Level Recovery-3	97.7	100.6	101.3	107.0	102.9	93.9
%RSD Overall	**1.3**	**1.9**	**1.8**	**1.3**	**1.9**	**3.3**

Table 3.11 Accuracy and method precision data

3.6.5 Precision

Precision of the method was determined by system precision (six replicate injections of standard solution-refer Table 3.12 and method precision (six different preparation of spike solution) studies.

Injection	Area of Standard solution					
	Methanol	**Acetone**	**DCM**	**n-Hexane**	**Ethyl Acetate**	**Pyridine**
1	845.9	6078.1	164.6	2379.5	3860.9	50.3
2	858.8	6026.9	164.2	2340.7	3845.5	52.8
3	877.6	6178.1	167.8	2427.9	3941.2	54.4
4	848.6	6031.8	164.1	2340.8	3844.8	52.5
5	864.5	6121.1	166.5	2385.8	3900.9	53.6
6	960.9	6373.9	176.1	2476.0	4100.0	57.7
Bkt	858.6	6101.1	165.4	2419.4	3877.7	54.0
Avg.	**874.0**	**6135.0**	**168.0**	**2394.0**	**3926.0**	**54.0**
SD	40	120	4	49	90	2
%RSD	**4.6**	**1.9**	**2.5**	**2.1**	**2.3**	**4.3**

Table 3.12 System precision data

In both the studies %Relative Standard Deviation (%RSD) of peak areas for all the solvents were less than 5.0%. The acceptance criteria for the %RSD for each solvent in six replicate injections of standard must be within 15% to conclude the method to be precise. The results proved that the system suitability was passed and method is precise (Table-3.14 to 3.19).

Sample Preparation					
Sr. No	**Level**	**Wt. of sample (mg)**	**Sample Dilution**	**Vol. Of stock-1 (mL)**	**Dilution (mL) Recovery**
1	Control sample	99.70	1	5	50
2	Precision Set-1	100.20	1	5	50
3	Precision Set-2	100.10	1	5	50
4	Precision Set-3	99.95	1	5	50
5	Precision Set-4	99.58	1	5	50
6	Precision Set-5	99.62	1	5	50
7	Precision Set-6	100.30	1	5	50

Table 3.13 Sample preparation for method precision

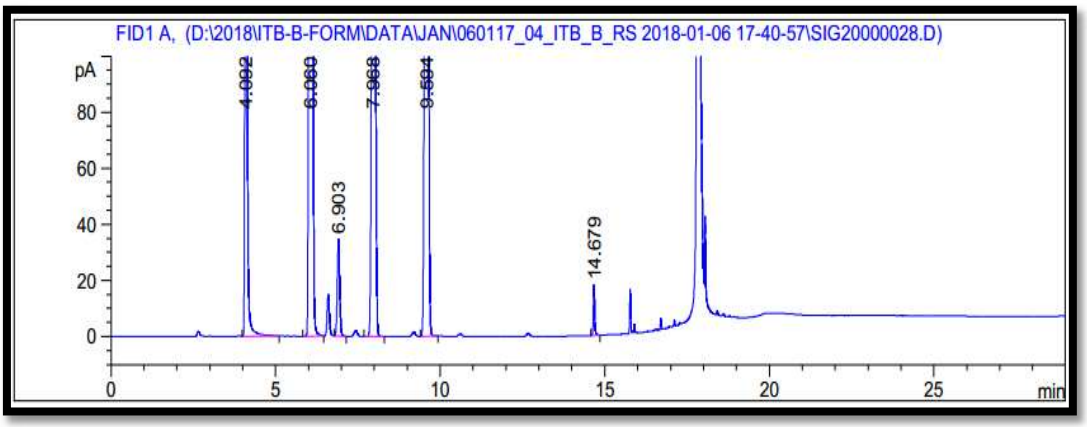

Fig 3.19 Method precision chromatogram-1

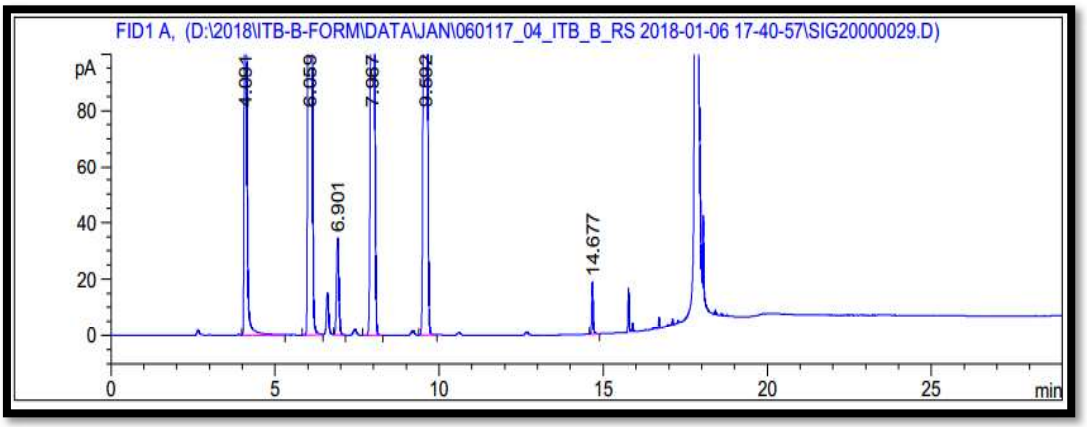

Fig 3.20 Method precision chromatogram -2

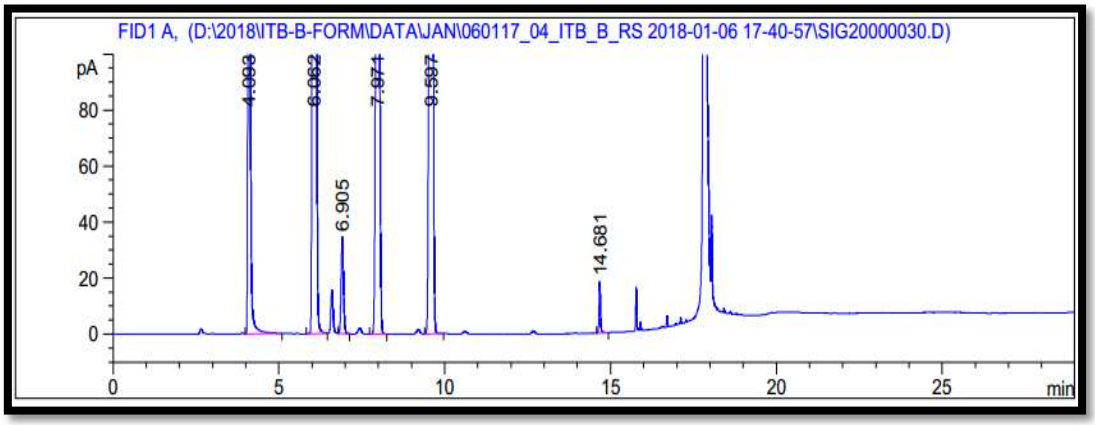

Fig 3.21 Method precision chromatogram-3

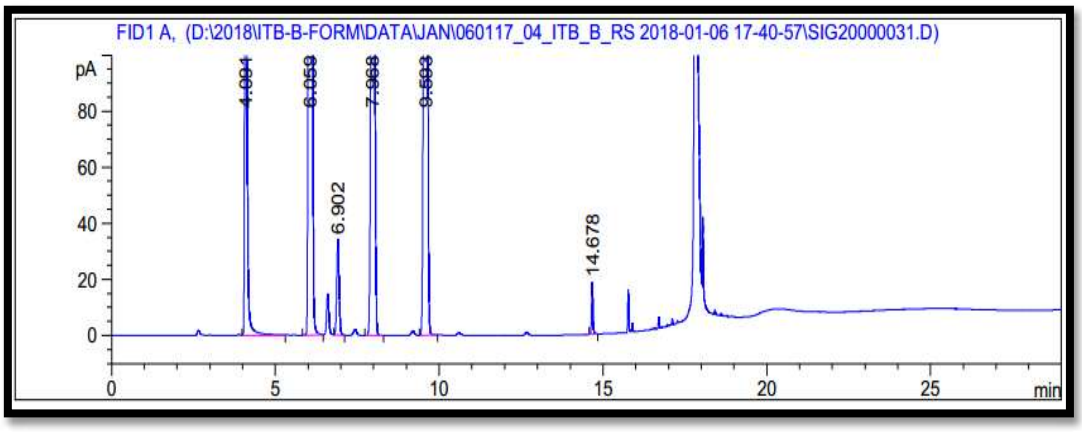

Fig 3.22 Method precision chromatogram -4

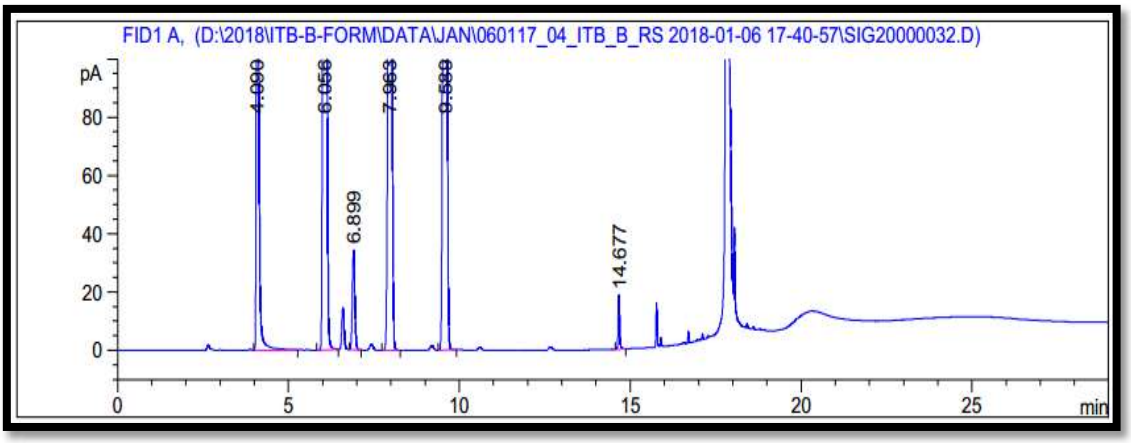

Fig 3.23 Method precision chromatogram -5

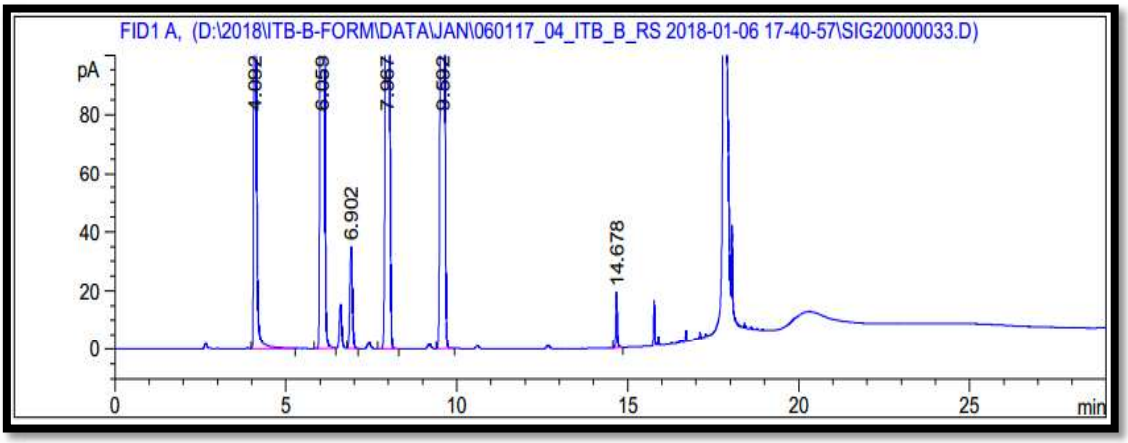

Fig 3.24 Method precision chromatogram-6

Sr. No.	Levels	Area	Amount Added	Amount Found	Amount recovered	Recovery
			(ppm)	(ppm)	(ppm)	(%)
1	Control sample	285.7	0.0	988.5	-988.5	0.0
2	Precision Set-1	1140.7	3018.0	3927.1	2938.6	97.4
3	Precision Set-2	1153.0	3018.0	3973.5	2984.9	98.9
4	Precision Set-3	1156.9	3018.0	3992.9	3004.4	99.5
5	Precision Set-4	1134.8	3018.0	3931.2	2942.6	97.5
6	Precision Set-5	1149.2	3018.0	3979.4	2990.9	99.1
7	Precision Set-6	1165.9	3018.0	4009.9	3021.4	100.1
					Overall Mean	**98.8**
					Overall SD	1.1
					Overall RSD (%)	**1.1**

Table 3.14 Method precision calculation for Methanol

Sr. No.	Levels	Area	Amount Added	Amount Found	Amount recovered	Recovery
			(ppm)	(ppm)	(ppm)	(%)
1	Control sample	624.1	0.0	307.6	-307.6	0.0
2	Precision Set-1	6939.9	5034.0	5677.4	4688.9	93.1
3	Precision Set-2	6940.6	5034.0	5683.6	4695.1	93.3
4	Precision Set-3	6960.0	5034.0	5708.1	4719.6	93.8
5	Precision Set-4	6901.5	5034.0	5681.1	4692.6	93.2
6	Precision Set-5	6940.4	5034.0	5710.9	4722.3	93.8
7	Precision Set-6	7014.6	5034.0	5732.8	4744.3	94.2
					Overall Mean	**93.6**
					Overall SD	0.4
					Overall RSD (%)	**0.5**

Table 3.15 Method precision calculation for Acetone

Sr. No.	Levels	Area	Amount Added	Amount Found	Amount recovered	Recovery
			(ppm)	(ppm)	(ppm)	(%)
1	Control sample	0	0.0	0.0	0.0	0.0
2	Precision Set-1	174.8	755.0	783.2	783.2	103.7
3	Precision Set-2	174.2	755.0	781.3	781.3	103.5
4	Precision Set-3	175.1	755.0	786.5	786.5	104.2
5	Precision Set-4	173.5	755.0	782.2	782.2	103.6
6	Precision Set-5	174.3	755.0	785.5	785.5	104.0
7	Precision Set-6	176.2	755.0	788.7	788.7	104.5
					Overall Mean	**103.9**
					Overall SD	0.4
					Overall RSD (%)	**0.4**

Table 3.16 Method precision calculation for DCM

Sr. No.	Levels	Area	Amount Added	Amount Found	Amount recovered	Recovery
			(ppm)	(ppm)	(ppm)	(%)
1	Control sample	3.6	0.0	0.5	-0.5	0.0
2	Precision Set-1	2602.6	333.0	360.9	360.4	108.2
3	Precision Set-2	2587.7	333.0	359.2	358.7	107.7
4	Precision Set-3	2611.7	333.0	363.1	362.6	108.9
5	Precision Set-4	2594.0	333.0	362.0	361.5	108.6
6	Precision Set-5	2617.6	333.0	365.1	364.6	109.5
7	Precision Set-6	2637.0	333.0	365.3	364.8	109.6
					Overall Mean	**108.7**
					Overall SD	0.7
					Overall RSD (%)	**0.7**

Table 3.17 Accuracy calculation for n-Hexane

Sr. No.	Levels	Area	Amount Added	Amount Found	Amount recovered	Recovery
			(ppm)	(ppm)	(ppm)	(%)
1	Control sample	3.2	0.0	4.0	-4.0	0.0
2	Precision Set-1	4147.6	4986.0	5251.7	5247.6	105.2
3	Precision Set-2	4131.5	4986.0	5236.5	5232.5	104.9
4	Precision Set-3	4145.8	4986.0	5262.5	5258.5	105.5
5	Precision Set-4	4115.4	4986.0	5243.3	5239.3	105.1
6	Precision Set-5	4136.3	4986.0	5267.8	5263.8	105.6
7	Precision Set-6	4179.8	4986.0	5287.2	5283.1	106.0
					Overall Mean	**105.4**
					Overall SD	0.4
					Overall RSD (%)	**0.4**

Table 3.18 Method precision calculation for Ethyl Acetate

Sr. No.	Levels	Area	Amount Added	Amount Found	Amount recovered	Recovery
			(ppm)	(ppm)	(ppm)	(%)
1	Control sample	0	0.0	0.0	0.0	0.0
2	Precision Set-1	53.1	241.0	236.3	236.3	98.0
3	Precision Set-2	54.1	241.0	241.0	241.0	100.0
4	Precision Set-3	54.8	241.0	244.4	244.4	101.4
5	Precision Set-4	53.8	241.0	240.9	240.9	99.9
6	Precision Set-5	54.3	241.0	243.0	243.0	100.8
7	Precision Set-6	54.9	241.0	244.0	244.0	101.3
					Overall Mean	**100.3**
					Overall SD	1.3
					Overall RSD (%)	**1.3**

Table 3.19 Method precision calculation for Pyridine

3.6.6 System suitability

The system suitability criterion was taken to be the resolution between the closely eluting pair, i.e. acetone and dichloromethane. The system suitability was checked by injecting the standard solution before starting any analysis of residual solvents. The criterion for system suitability was that the resolution between the closely eluting pair should not be less than 1.5 and it was found that optimum resolution 6.3 has been achieved (Table 3.20).

Solvent Name	RT (min)	USP Resolution	USP Tailing factor	USP Theoretical plate	%RSD (n=6) of Peak area
Methanol	4.10	--	1.292	20863	4.6
Acetone	6.07	16.0	1.028	33847	1.9
Dichloromethane	6.92	6.3	1.065	42971	2.5
n-Hexane	7.98	7.3	1.003	39627	2.1
Ethyl acetate	9.61	10.4	0.996	62912	2.3
Pyridine	14.69	45.0	1.162	652171	4.3

Table 3.20 System precision and System suitability parameter

3.6.7 Robustness

Robustness of the method has been performed by changing the parameter settings of carrier gas flow rate, oven temperature and split ratio. The following parameters were changed: column oven temperature ±3°C from the ideal conditions (initial column oven temperature at 32°C and 38°C), the flow rate ±5% from the ideal conditions (flow rate 2.0 psi and 2.5 psi), the split ratio ±10% from the ideal conditions (the split ratio of 1:1.8 and 1:.2.2. No significant difference in % RSD, resolution and elution order was observed which demonstrated robustness of the method. Results of robustness study are reported in Table 3.21.

Parameter/ variation	USP resolution					
	Methanol	Acetone	DCM	n-Hexane	Ethyl acetate	Pyridine
Conditions	NA	16.0	6.3	7.3	10.4	45.0
Flow rate (psi)						
a. 2.0	NA	16.4	6.4	7.4	10.6	48.0
b. 2.5	NA	15.2	6.1	6.9	96	38.2
Column oven Temp. (°C)						
a. 32	NA	16.5	6.5	7.5	10.5	45.8
b. 38	NA	12.6	5.9	6.8	10.1	44.2
Split ratio						
a. 1:1.8	NA	16.4	6.6	7.8	10.6	45.6
b. 1:2.2	NA	16.1	6.1	7.1	10.1	44.3

Table 3.21 Results of robustness study

3.7 Result

Residual solvents analysis was performed on developed and validated method for commercial batch of Imatinib Mesylate API in triplicate. Results are reported in Table 3.22.

Solvent Name	Batch set-1	Batch set-2	Batch set-3
Methanol	988 ppm	954 ppm	928 ppm
Acetone	513 ppm	535 ppm	522 ppm
Dichloromethane	ND	ND	ND
n-Hexane	0.5 ppm	0.4 ppm	0.5 ppm
Ethyl acetate	4.1 ppm	4.2 ppm	4.5 ppm
Pyridine	ND	ND	ND

Table 3.22 Residual solvents contents in the commercial batch of Imatinib Mesylate API

3.8 Conclusion

A selective and sensitive fast static HSGC method was successfully developed for the determination of methanol, acetone, dichloromethane, n-hexane, ethyl acetate and pyridine in Imatinib Mesylate API through consideration of route of synthesis and solvent nature. The developed method was successfully validated as per regulatory guideline and found to be precise, accurate, linear, and robust and specific. Additionally, the developed method is suitable for analysis of pyridine 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 during synthesis of Imatinib Mesylate API. This method may also 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, developed HSGC method demonstrated precise, economical and commercially viable quantitative technique for residual solvents determination in Imatinib Mesylate API which will also be advantageous for industrial scale manufacturing.

3.9 References:

1.Sitaramaraju Y, van Hul A, Wolfs K, Van Schepdael A, Hoogmartens J, Adams E. Static headspace gas chromatography of (semi-) volatile drugs in pharmaceuticals for topical use. Journal of pharmaceutical and biomedical analysis. 2008;47(4-5):834-40.

2.Antolín EM, Quiñónez YB, Canavaciolo VG, Cruz ER. Validation of an analytical method for quality control of residual solvents (n-hexane and acetone) in D-002: new active ingredient from beeswax. Journal of pharmaceutical and biomedical analysis. 2008;47(3):646-50.

3.Nojavan S, Ghassempour A, Bashour Y, Darbandi MK, Ahmadi SH Determination of residual solvents and investigation of their efect on ampicillin trihydrate crystal structure. Journal of Pharmaceutical and Biomedical Analysis. 2005; 36:983

4.Witschi C, Doelker E. Residual solvents in pharmaceutical products: acceptable limits, influences on physicochemical properties, analytical methods and documented values. European Journal of Pharmaceutics and Biopharmaceutics. 1997;43(3):215-42.

5.Committee IS, editor. Impurities: guideline for residual solvents. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use; 1997.

6.Gomes PC, D'Andrea ÉD, Mendes CB, de Siqueira MEP. Determination of benzene, toluene and n-hexane in urine and blood by headspace solid-phase microextration/gas-chromatography for the biomonitoring of occupational exposure. Journal of the Brazilian Chemical Society. 2010;21(1):119-26.

7.Guideline IHT. Impurities: Guideline for residual solvents Q3C (R5). Current Step. 2005;4:1-25.

8.Solvents R, editor. 467>. The United States Pharmacopoeia, USP38/NF33, The United States Pharmacopeial Convention, Rockville, MD, USA; 2012.

9.Kohl S. European directorate for the quality of medicines & healthcare (edqm): final report of the pharmaceutical care quality indicators project published. Bmj publishing group british med assoc house, tavistock square, london; 2018.

10.Kolb B, Ettre L. Theory and practice of multiple headspace extraction. Chromatographia. 1991;32(11-12):505-13.

11.Zhu J, Chai X-S. Some recent developments in headspace gas chromatography. Current Analytical Chemistry. 2005;1(1):79-83.

12.B'Hymer C. Residual solvent testing: a review of gas-chromatographic and alternative techniques. Pharmaceutical research. 2003;20(3):337-44.

13.Camarasu CC, Mezei-Szűts M, Varga GB. Residual solvents determination in pharmaceutical products by GC-HS and GC-MS–SPME. Journal of pharmaceutical and biomedical analysis. 1998;18(4-5):623-38.

14.Laus G, Andre M, Bentivoglio G, Schottenberger H. Ionic liquids as superior solvents for headspace gas chromatography of residual solvents with very low vapor pressure, relevant for pharmaceutical final dosage forms. Journal of Chromatography A. 2009;1216(32):6020-3.

15.Yarramraju S, Akurathi V, Wolfs K, Van Schepdael A, Hoogmartens J, Adams E. Investigation of sorbic acid volatile degradation products in pharmaceutical formulations using static headspace gas chromatography. Journal of pharmaceutical and biomedical analysis. 2007;44(2):456-63.

16.Otero R, Carrera G, Dulsat JF, Fábregas JL, Claramunt J. Static headspace gas chromatographic method for quantitative determination of residual solvents in pharmaceutical drug substances according to European Pharmacopoeia requirements. Journal of Chromatography A. 2004;1057(1-2):193-201.

17.Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. New England Journal of Medicine. 2002;346(9):645-52.

18.Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. New England Journal of Medicine. 2001;344(14):1038-42.

19.Ishikawa Y, Kiyoi H, Watanabe K, Miyamura K, Nakano Y, Kitamura K, et al. Trough plasma concentration of imatinib reflects BCR–ABL kinase inhibitory activity and clinical response in chronic- phase chronic myeloid leukemia: A report from the BINGO study. Cancer science. 2010;101(10):2186-92.

20.Dagher R, Cohen M, Williams G, Rothmann M, Gobburu J, Robbie G, et al. Approval summary: imatinib mesylate in the treatment of metastatic and/or unresectable malignant gastrointestinal stromal tumors. Clinical Cancer Research. 2002;8(10):3034-8.

21.Cohen MH, Williams G, Johnson JR, Duan J, Gobburu J, Rahman A, et al. Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. Clinical Cancer Research. 2002;8(5):935-42.

22.Battu PR, Reddy M. Residual Solvents Determination by HS-GC with Flame Ionization Detector in Omeprazole Pharmaceutical formulations. Int J PharmTech Res. 2009;1(2):230-4.

23.Puranik S, Sharath S, Sanjay Pai P. Headspace gas chromatography analysis of residual solvents by using EC-5 column. Int J Pharm Chem Res. 2012;1(1):22-7.

24.Dwivedi SD, Singh KK, Charan GDS, Vasava CJ. Process for the preparation of amorphous imatinib mesylate. US2014/0121215 A1; 2016.

25.Guideline IHT, editor. Validation of analytical procedures: text and methodology Q2 (R1). International conference on harmonization, Geneva, Switzerland; 2005.