IDENTIFICATION OF RESIDUAL SOLVENTS BY USING GC – HS METHOD FOR EZETIMIBE

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ABSTRACT: A Simple, Specific, sensitive, accurate, robust, precise GC -HS Method was developed and validated for stability indicating analytical method development and validation for identification of residual solvents for Ezetimibe drug. The method was developed by using DMSO (Dimethyl Sulphoxide) as a mobile phase. The Column used for separation was ZB-624 with dimensions as 30m x 0.53mm, 3.0 µm thickness with flow rate 2.0ml/min and Injector temperature is 200^0 c and 260 ^{0} c detector temperature with the split ratio is 1:5 and with run time 25 min and constant flow 2.0 ml/min. The validation was carried out on the optimised method and the obtained results were within the limits of acceptance criteria for parameters like System suitability, Specificity, Linearity, Accuracy, Precision, LOD, LOQ, Robustness and the stability of the method has been checked by solution stability studies. Based on the above information the method can be used for daily routine analysis.

Key words: GC – HS, Ezetimibe drug, Residual Solvents – Methanol, Acetone, IPA, DCM, n – Hexane, DMF, Toluene, Ethyl Acetate, THF

1. INTRODUCTION:

Analytical Chemistry: A branch of chemistry that deals with identification of mixtures (qualitative and quantitative analysis) or the determination of the proportions of the constituents. Analytical chemistry is important because it deals with the collection, classification, identification, and measurement of various molecular types. It covers methods for qualitative and quantitative analysis such as electrochemistry, titration, spectroscopy, and chromatography. (1)

The discipline focuses on developing new ideas and methods to address the complex problems, one of which is the use of in situ analytic techniques for the real-time evaluation of chemicals and biological materials. The aim of analytical chemistry is the dissemination of analytical technology and knowledge, together with the production of chemical information which is essential to many sectors.(12)

Pharmaceutical analysis: It is the process or series of processes that can be used for the identification, determination, separation, purification, and structure elucidation of the given compound used in the formulation of pharmaceutical products. Pharmaceutical analysis can be done using various analytical techniques.

Analysis involves the qualitative and quantitative identification of the individual components of substances, samples, or mixtures. There are two different kinds of analysis. qualitative analysis and quantitative analysis.(13)

Qualitative Analysis: It is the type of pharmaceutical analysis, in which non-quantifiable method of determination of what constituent or substance is present in an unknown sample or compound. The identification of mixture of sample's constituents or analytes is done by qualitative analysis.

Quantitative Analysis: It is the type of pharmaceutical analysis which is intended to measure the exact concentration of the substance of interest in a given sample. In quantitative analysis, as we intend to measure the exact concentration of substance of interest, therefore, it is also referred to as determination. Quantitative analysis involves calculating the quantity of each component or analyte in a mixture or sample.

Gas Chromatography:
Gas chromatography (GC) was developed by "Nobel Laurate, Martin, et. al." **Gas chromatography** is a column chromatography technique, in which the mobile phase is gas and the stationary phase is either an immobilized liquid or a solid packed in a closed tube. GC is used to separate thermally stable volatile components of a mixture. It is used for both qualification and quantification.GC is useful for a wide range of market analyses, including trace level environmental, petrochemical, and fine chemical industrial analysis as well as residual solvents and pesticide analysis in pharmaceutical and food safety sectors.

The main requirement for GC is thermal stability and volatile nature of compound. In Gas Chromatography, the carrier gas is mobile phase. The rate of flow of the carrier gas is carefully controlled to give the clearest separation of the components in the sample. The Gas Chromatography is carried out at suitable temperature in a glass or metal tubing known as "Column", which contains the liquid or solid stationary phase. Inert gases like helium or unreactive gases like nitrogen are used as a mobile phase which passed over stationary phase. Gas Chromatography is known as "Gas chromatogram or Gas separator or Aerograph."

Principle of Gas Chromatography: In this technique, the sample is first vaporized by heating and then it is injected into the head of the chromatographic column. The sample is transferred into the column by the flow of inert gaseous mobile phase. The column has a liquid stationary phase which is adsorbed on the surface of an inert solid. It has same principle as chromatography, separation of the components due to partition between stationary phase and mobile phase and components of sample will partition between two phases; i.e. stationary phase and mobile phase.

- Higher affinity for the stationary phase compounds spends longer time in the column, elute later, and have longer retention times (Rt) than samples with lower affinity.
- Intermolecular interactions are the primary factor driving affinity for the stationary phase; by selecting the stationary phase's polarity to optimize and separation occurs.
- In order to achieve the separation, the sample is divided into two sections: the gas and a thin layer of a non-volatile liquid supported by a solid medium.
- Band spreading and partition coefficients will determine the bands split into unique zones.
- Quantitative information can be obtained by measuring the height, width, area, and appearance time of these peaks.

Key parameters of the analytical method validation:
- Specificity
- Linearity
- Precision
- Accuracy (Recovery)
- Limit of Detection (LOD)
- Limit of Quantification (LOQ)
- Robustness
- System suitability
- Solution stability.

Specificity: Specificity of an analytical method as its ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. It may alternatively be described as the method's capacity to precisely detect an analyte in the presence of interference, which includes excipients, synthetic precursors, enantiomers, and known (or probably predicted) degradation products that might be present in the sample matrix.

Linearity: Linearity refers to the ability to produce test results that are directly proportional to the analyte concentration in samples within a given range. A linear relationship should be evaluated across the range of the analytical procedure. It may be established directly on the drug substance by dilution of a standard stock solution. Linearity should be evaluated by visual inspection of a plot a graph of concentration (on x - axis) Vs mean response (on Y - axis). Calculate the regression equation, Y- intercept and correlation coefficient. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity. For the determination of linearity, a minimum of 5 concentrations are recommended.

Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability: It expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intermediate precision: This expresses within-laboratories variations-different days, different analysts, different equipment, etc.

Reproducibility: It expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

It is expressed as SD/ RSD.

% RSD =Standard Deviation Mean x 100

Accuracy (Recovery): The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. It is determined by applying the method to samples to which known amounts of analyte have been added. It is recommended to compare these to both standard and blank solutions in order to rule out any interference. The accuracy is then computed as a percentage of the analyte recovered by the assay using the test findings. It is frequently expressed as the recovery by assaying known, additional analyte concentrations.

Solution stability: In order to ascertain if certain storage conditions, such as refrigeration or light protection, are required, the stability of standards and samples is determined during validation under normal settings, normal storage conditions, and sometimes in the instrument.

Limit of Detection (LOD): Limit of detection (LOD) of an individual procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. In analytical procedures that exhibit baseline noise, the LOD can be based on a signal-to-noise (S/N) ratio (3:1), which is usually expressed as the concentration of analyte in the sample.

The signal-to-noise ratio is determined by:

$$s = H/h$$

where H = height of the peak corresponding to the component.

h = absolute value of the largest noise fluctuation from the baseline of the chromatogram of a blank solution.

Limit of Quantification (LOQ): The limit of Quantitation (LOQ) or Quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. For analytical procedures such as HPLC that exhibit baseline noise, the LOQ is generally estimated from a determination of S/N ratio (10:1) and is usually confirmed by injecting standards which give this S/N ratio and have an acceptable percent relative standard deviation.

Robustness: It is defined as the measure of the ability of an analytical method to remain unaffected by small but deliberate variations in method parameters (e.g. carrier gas composition, column oven temperature and instrumental settings) and provides an indication of its reliability during normal usage. Determination of robustness is a systematic process of varying a parameter and measuring the effect on the method by monitoring system suitability and/or the analysis of samples.

System Suitability: System suitability tests are an integral part of liquid chromatographic methods. They are used to verify that the detection of sensitivity, resolution and reproducibility of the chromatographic system which are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. Factors, such as the peak resolution, number of theoretical plates, peak tailing and capacity have been measured to determine the suitability of the used method.(16)

DRUG PROFILE:

Description of the Ezetimibe drug substance (18):

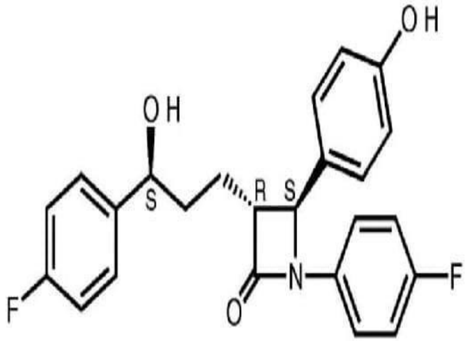

Fig no: 1: Chemical Structure of Ezetimibe Drug.

Chemical formula : $C_{24}H_{21}F_2NO_3$
Molecular Formula : 4.43 g/mol
IUPAC Name : 1-(4-Fluorophenyl)-(3R)-[3-(4-Flouropheny) -(3S)-hydroxypropyl] -(4S) -(4-hydroxyphenyl) -2-azetidinone

Category : Anti Hyperlipidemic drug
p Ka Value : 9.75
Log P Value : 4 52
Solubility :Very soluble in acetonitrile, methanol and ethanol. But, poorly soluble in water.

Mechanism of Action:1. Ezetimibe is an Azetidinone derivative and Anti cholesteremic agent that inhibits Intestinal Cholesterol absorption. It is used to reduce LDL and total Cholesterol and apoproteins B in treatment of Hyperlipidemia.Inhibits sterol transporters at brush borders of Intestine.

Storage Conditions :Store Ezetimibe at Room temperature (20^{0}c to 25 ^{0}c)
Melting Range :163 ^{0}c
Hygroscopicity : Hygroscopic in nature.

PLAN OF WORK:

To develop and validate an effective GC - HS method for identification of residual solvents by using GC-HS method for Ezetimibe.

The Plan of work for GC – HS method as follows:

- Selection of drug and study of physicochemical properties of drug and residual solvents in the drug substance.
- Literature survey for the selection of drug and to gain theoretical knowledge on analytical method development and validation.
- Development and optimization of analytical method which includes the following:

  - Selection of stationary phase (column parameters)
  - Selection of Carrier gas (mobile phase)
  - Optimization of column oven temperature program
  - Optimization of temp, injection volume, detector temp &type
  - Sample preparation and to develop the approach for analysis.
- Validation of proposed method as per ICH guidelines

Preparation of sample solution:

Weighed 200.15 mg of sample into a 20 ml head space vial, added 2.0 ml of standard solution then immediately crimped the vial with septum and a cap.

Preparation of selectivity solution:

Weighed 200.26 mg of sample into a 20 ml head space vial, added 2.0 ml of standard solution then, immediately crimped the vial with septum and a cap.

Preparation of LOQ solution:

Transfer 1.85 ml of methanol std solution, 0.15 ml of Acetone std solution, 1.8 ml of IPA std solution, 0.2 ml of Dichloromethane std solution, 0.3 ml of n- Hexane std solution, 0.08 ml of Ethyl Acetate std solution, 0.4 ml of Tetrahydro furan std solution, 0.25 ml of Toluene std solution, 0.2 ml of Dimethyl formamide std solution into 100 ml Volumetric flask containing 20 ml of diluent and diluted to volume with diluent and mixed well.

Preparation of LOD solution:

Transfer 0.56 ml of methanol std solution, 0.05 ml of Acetone std solution, 0.54 ml of IPA std solution, 0.06 ml of Dichloromethane std solution, 0.09 ml of n- Hexane std solution, 0.02 ml of Ethyl Acetate std solution, 0.12 ml of Tetrahydro furan std solution, 0.08 ml of Toluene std solution, 0.06 ml of Dimethyl formamide std solution into 100 ml Volumetric flask containing 20 ml of diluent and diluted to volume with diluent and mixed well.

2. METHOD DEVELOPMENT: Trial no: 01: Chromatographic

METHOD VALIDATION:

System Suitability: Verified that analytical system was working properly and can give accurate and precise results, for analysis was done. A standard solution of Ezetimibe and its impurities (residual solvents) were injected six times into the GC system. The system suitability parameters were obtained by calculating the resolution, retention times.
the results are shown in table.

Acceptance criteria:

- The % RSD for each solvent peak areas for six injections should not be more than 15.0
- The % RSD for each solvent peak areas for seven injections should not be more than 15.0.
- USP Resolution between Acetone and IPA is NLT 1.5.

Selectivity: In order to prove that the method chosen was specific and selective, the sample peak was compared to the retention time against the blank (diluent) chromatogram. the results are shown in fig and table.

Acceptance criteria:

- No peak from blank at methanol, Isopropyl alcohol, Acetone, Dichloromethane, n – Hexane, Ethyl acetate, THF, Toluene and DMF Retention times.
- Methanol, Isopropyl alcohol, Acetone, Dichloromethane, n - Hexane, Ethyl acetate, THF, Toluene and DMF should elute at different RT `s.
- Report resolution between all solvent peaks in selectivity solution.

Limit of Detection (LOD) and Limit of Quantification (LOQ): Limit of Detection and Limit of Quantification prediction data was considered from the linearity experiment and calculated the standard error as standard deviation from the linearity data the results are shown in table. To calculate the predicted LOD & LOQ values the following formulas were used.

$$\text{LOD} = \frac{\text{standard deviation}}{SLOPE} \times 3.3$$

$$\text{LOQ} = \frac{\text{standard deviation}}{SLOPE} \times 10.0$$

Acceptance criteria:

- S/N ratio should be greater than or equal to 3 for LOD of each solvent.
- S/N ratio should be greater than or equal to 10 for LOD of each solvent.

Linearity: The different levels like 50%, 80%, 100%, 120%, 150% levels were injected into the chromatographic system and peak area was measured and Plotted a graph of peak area versus concentration (on x-axis concentration and on y-axis peak area) and calculated the R 2 the results are shown in table**.**
Acceptance criteria: Correlation coefficient (r) should be more than 0.99 for each solvent.

Method Precision: The method precision was analysed by the six different solutions of same concentration and determined the % RSD values were obtained the results are shown in table.
Acceptance criteria: RSD for content of each solvent from six spiked sample preparations should be not more than 15.0 %.
Accuracy: Sample and spiked solutions of 50%, 100%, 150% levels were injected and accuracy was measured the results are shown in table.
Acceptance criteria: Recoveries should be in the range of 80% to 120% for each solvent.

Robustness: Robustness was analysed by subjecting all residual solvents in selectivity solution into GC by change in the column oven temperature, injector temperature, detector temperature. the results are shown in fig & table.
Acceptance criteria: Report the RT`s of all solvents in selectivity solution.

Solution stability: To analyze and to test whether solution is stable at room temperature after 12 hrs., 24 hrs., 48 hrs the results are shown in table.
Acceptance criteria: Variation in solvent content should be within ± 15.0% for all solvent

3. RESULTS AND DISCUSSION

METHOD VALIDATION:

System Suitability: The Validation of GC method for the identification and the determination of Residual solvents for Ezetimibe drug to demonstrate that the method is appropriate for its intended use and was studied for the following parameters.
Acceptance Criteria: The % RSD for each solvent peak areas for six injections should not be more than 15.0

- The % RSD for each solvent peak areas for seven injections should not be more than 15.0.
- USP Resolution between Acetone and IPA is NLT 1.5.

Table 1: Standard solution % RSD:

S. No:	Solvent Name	% RSD of Standard solution (n = 6)	% RSD of bracketing Standard & Standard solutions (n= 7)
1	Methanol	6.2	6.3
2	Acetone	5.8	5.9
3	IPA	6.8	6.9
4	DCM	5.8	6.0
5	n- Hexane	5.5	5.0
6	Ethyl Acetate	6.0	6.2
7	THF	5.9	6.1

8	Toluene	6.0	6.2
9	DMF	9 .3	9.3

Table 2 : Resolution of standard solution:

S. No	Solvent Name	Resolution
1	Acetone – IPA	1.9

Observation: The Resolution is 1.9 for acetone – IPA and the % RSD of standard solutions is not more than 15.0 and all meets the acceptance criteria limits. So, the System meets required system suitability criteria.

Selectivity:

Acceptance criteria:

- No peak from blank at methanol, Isopropyl alcohol, Acetone, Dichloromethane, n – Hexane, Ethyl acetate, THF, Toluene and DMF Retention times.
- Methanol, Isopropyl alcohol, Acetone, Dichloromethane, n - Hexane, Ethyl acetate, THF, Toluene and DMF should elute at different RT `s.
- Report resolution between all solvent peaks in selectivity solution.

Table 3: Resolution and comparison of RT`s:

S. No:	Solvent Name	Retention times for each individual solvent	Retention times of solvents in selectivity solution	Resolution between solvent peaks in selectivity solution
1	Methanol	5.52	5.53	-
2	Acetone	8.66	8.65	13.2
3	IPA	9.2	9.19	1.9
4	DCM	10.27	10.27	3.7
5	n- Hexane	11.98	11.98	6.8
6	Ethyl Acetate	13.78	13.78	10.4
7	THF	14.13	14.13	2.8
8	Toluene	17.13	17.13	29.4
9	DMF	18.17	18.18	14.1

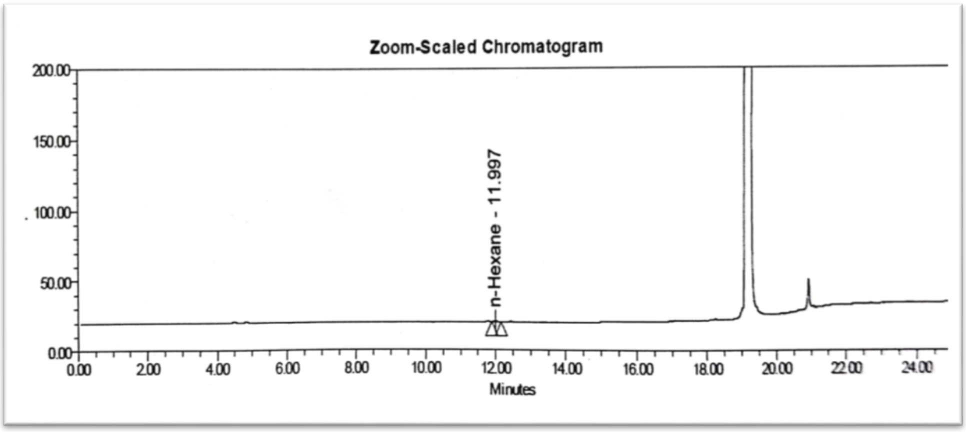

Fig. 2: Chromatogram of Blank

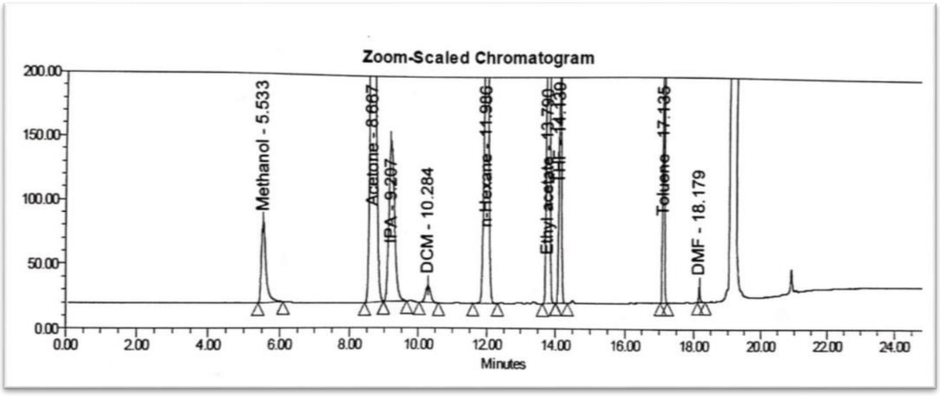

Fig. 3: Standard Chromatogram

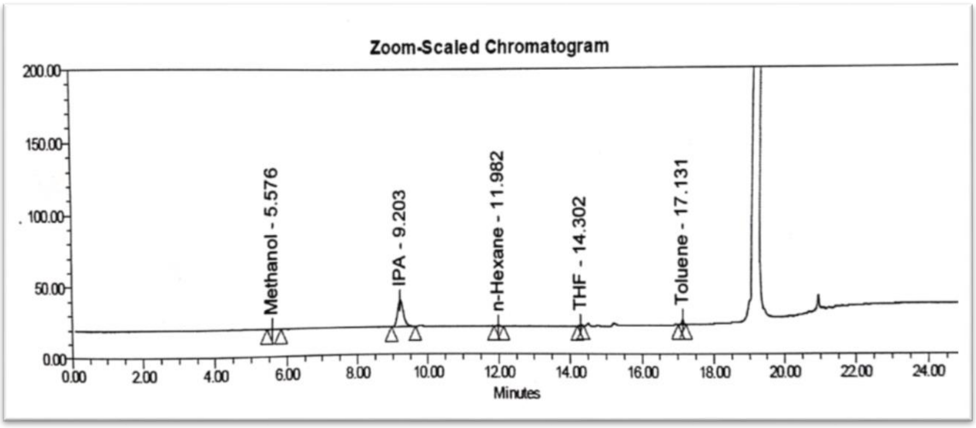

Fig. 4: Sample Chromatogram

Limit of Detection and Limit of Quantification: Acceptance criteria:

- S/N ratio should be greater than or equal to 3 for LOD of each solvent.
- S/N ratio should be greater than or equal to 10 for LOD of each solvent.

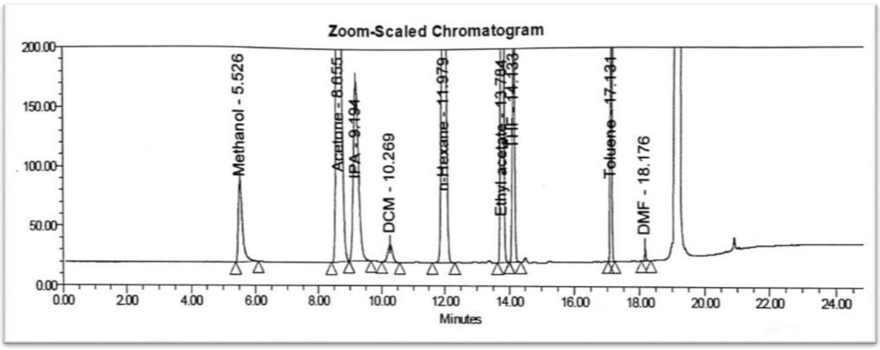

Fig. 5: Selectivity Chromatogram

Table 4: LOD Solution:

S. No:	Solvent Name	Conc. (ppm)	S/N ratio
1	Methanol	16.9	3
2	Acetone	2.5	3
3	IPA	27.0	4
4	DCM	19.0	5

5	n- Hexane	0.3	4
6	Ethyl Acetate	1.0	15
7	THF	0.9	3
8	Toluene	0.7	9
9	DMF	26.6	3

Table 5: LOQ Solution:

S. No:	Solvent Name	Conc. (ppm)	S/N ratio
1	Methanol	55.8	11
2	Acetone	7.5	10
3	IPA	90.1	14
4	DCM	63.3	19
5	n- Hexane	0.9	13
6	Ethyl Acetate	4.0	20
7	THF	3.0	11
8	Toluene	2.3	17
9	DMF	88.6	12

Result: S/N ratio was within the limit.

Linearity:

Acceptance criteria:

- Correlation coefficient (r) should be more than 0.99 for each solvent.
- Slope and Intercept will be reported for all solvents.

Table 6: Correlation coefficient:

S. No:	Solvent Name	Correlation coefficient
1	Methanol	1.00
2	Acetone	1.00
3	IPA	1.00
4	DCM	1.00
5	n- Hexane	1.00
6	Ethyl Acetate	1.00
7	THF	1.00
8	Toluene	1.00
9	DMF	1.00

Table 7: Methanol Linearity:

S. No:	Spiked Level (%)	Concentration (ppm)	Avg. Area
1	LOQ	55.8	9.7
2	50	1511	278.4
3	80	2417.6	454.9
4	100	3022	542.1
5	120	3626.4	653
6	150	4533	814
Correlation coefficient			0.9996
Slope			0.179
Y – Intercept			6.1882

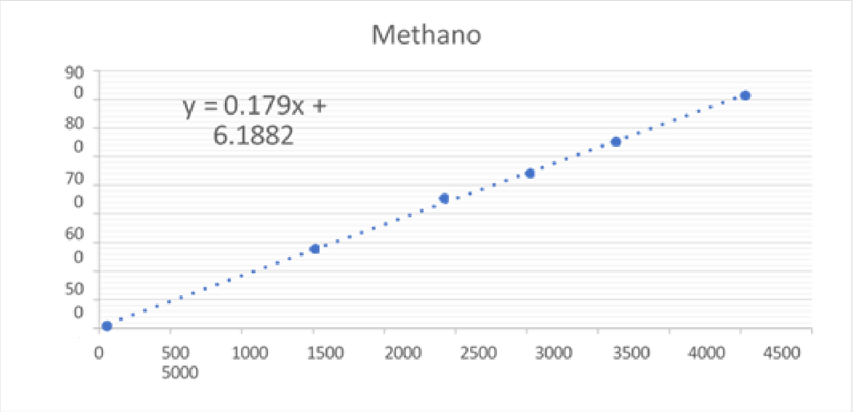

Fig no: 8.1 Methanol Linearity graph.

Table 8 : Acetone Linearity:

S. No:	Spiked Level (%)	Concentration (ppm)	Avg. Area
1	LOQ	7.5	6.8
2	50	2514	2392.7
3	80	4022.4	3906.6
4	100	5028	4611.3
5	120	6033.6	5541.9
6	150	7542	6881.3
Correlation coefficient			0.9993
Slope			0.9091
Y – Intercept			79.8701

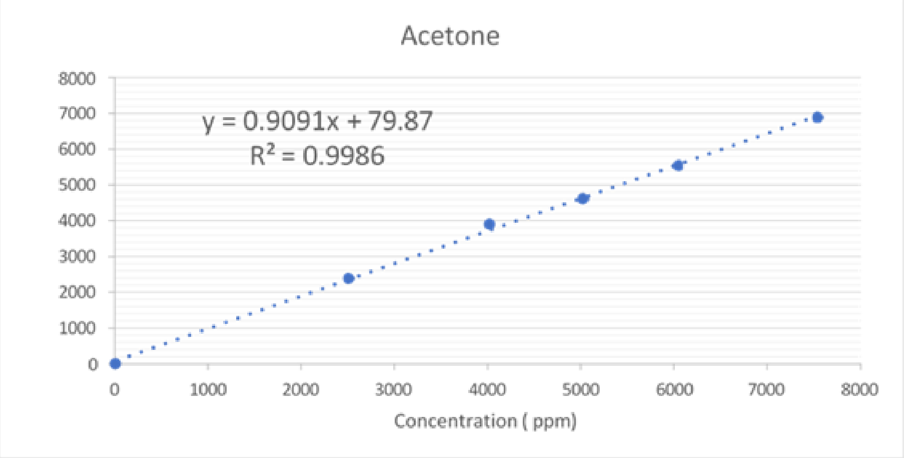

Fig. 6 : Acetone Linearity Graph

Table 9: IPA Linearity:

S. No:	Spiked Level (%)	Concentration (ppm)	Avg. Area
1	LOQ	90.1	11.9
2	50	2506	715.4
3	80	4009.6	1162.9
4	100	5012	1385.6
5	120	6014.6	1662.1
6	150	7518	2067.5

Correlation coefficient	0.9993
Slope	0.9091
Y – Intercept	79.8701

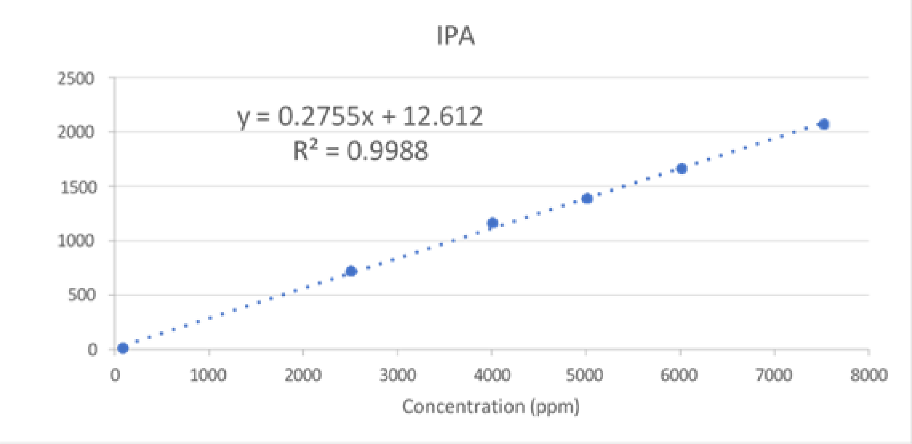

Fig. 7: IPA Linearity Graph

Table 10: DCM Linearity:

S. No:	Spiked Level (%)	Concentration (ppm)	Avg. Area
1	LOQ	63.3	14.1
2	50	303	70.4
3	80	484.8	114.7
4	100	606	135.7
5	120	727.2	163.1
6	150	909	202.4

Correlation coefficient	0.9993
Slope	0.2215
Y – Intercept	2.5184

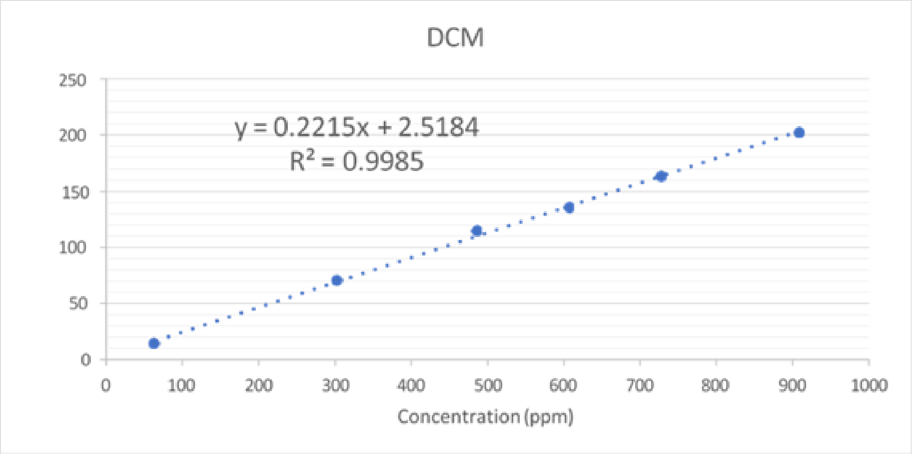

Fig. 8: DCM Linearity Graph

Table 11 : n – Hexane Linearity:

S. No:	Spiked Level (%)	Concentration (ppm)	Avg. Area
1	LOQ	0.9	8.9
2	50	144	1173.9
3	80	230.4	1983.2
4	100	288	2298.2
5	120	345.6	2847.7

6	150	432	3525.7
Correlation coefficient			0.9992
Slope			8.1538
Y – Intercept			14.8074

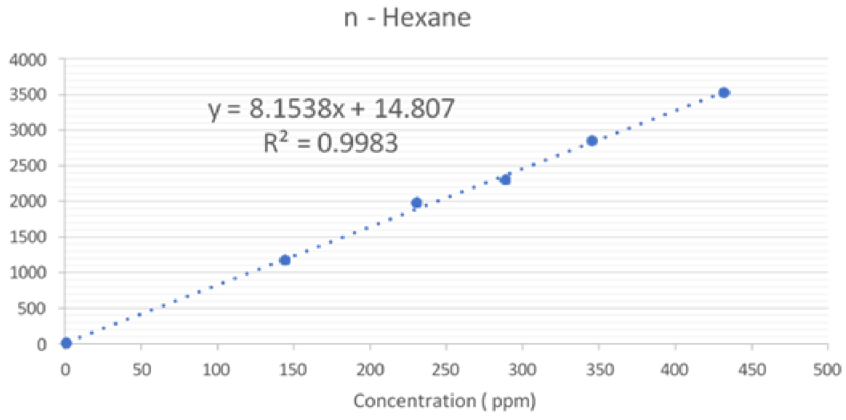

Fig. 9: n – Hexane Linearity Graph

Table 12: Ethyl Acetate Linearity:

S. No:	**Spiked Level (%)**	**Concentration (ppm)**	**Avg. Area**
1	LOQ	4	31
2	50	2501	1965.4
3	80	4001.6	3202.4
4	100	5002	3791.5
5	120	6002.4	4548.6
6	150	7503	5652.5
Correlation coefficient			0.9994
Slope			0.7473
Y – Intercept			83.208

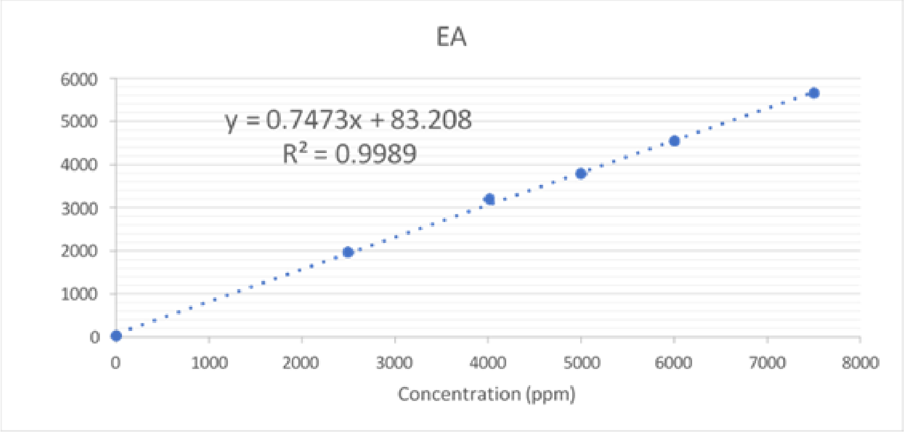

Fig. 10: Ethyl Acetate Linearity Graph

Table 13: THF Linearity:

S. No:	**Spiked Level (%)**	**Concentration (ppm)**	**Avg. Area**
1	LOQ	3	4.6
2	50	349	506.2
3	80	558.4	826.4
4	100	698	976.1

5	120	837.6	1172.8
6	150	1047	1456.1
Correlation coefficient			0.9993
Slope			1.3854
Y – Intercept			17.1572

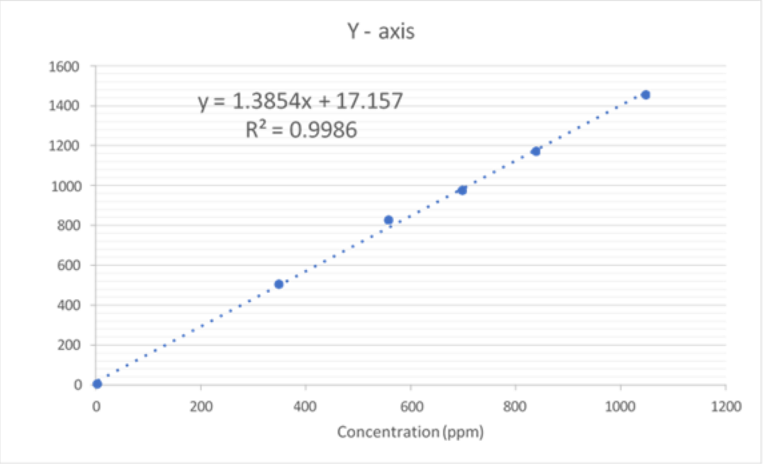

Fig. 11: THF Linearity Graph

Table 14: Toluene Linearity:

S. No:	Spiked Level (%)	Concentration (ppm)	Avg. Area
1	LOQ	2.3	9.1
2	50	449	517.8
3	80	718.4	839
4	100	898	996.9
5	120	1077.6	1193.7
6	150	1347	1482.4
Correlation coefficient			0.9994
Slope			1.0921
Y – Intercept			22.1753

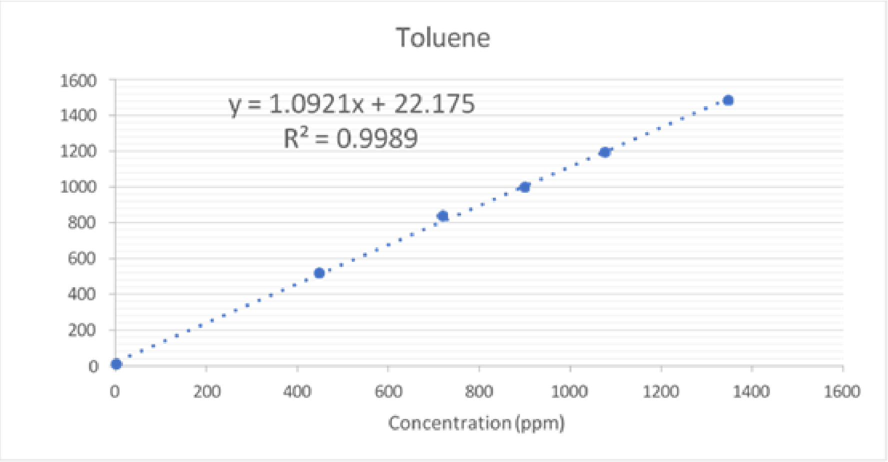

Fig. 12: Toluene Linearity Graph.

Table 15: DMF Linearity:

S. No:	Spiked Level (%)	Concentration (ppm)	Avg. Area
1	LOQ	88.6	2.3
2	50	439	14.2

3	80	702.4	22.9
4	100	898	27.9
5	120	1053.6	33.1
6	150	1317	41
Correlation coefficient			0.9993
Slope			0.0314
Y – Intercept			0.1294

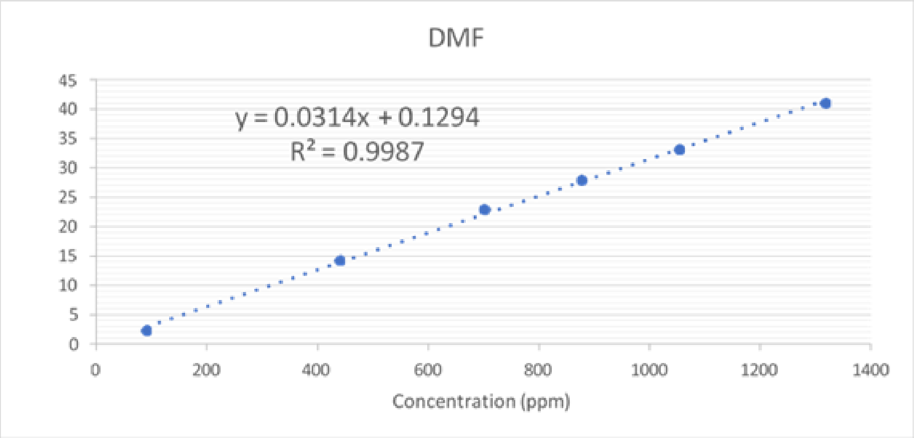

Fig. 13: DMF Linearity Graph

Table 16: Linearity plot for (impurity) residual solvents of Ezetimibe:

S. No:	**Solvent Name**	**Slope (m)**	**Y – Intercept (c)**	**Regression on equation**	**R^2**
1	Methanol	0.179	6.1882	y = 0.1790x+6.1882	0.992
2	Acetone	0.9091	79.8701	y = 0.9091x+79.870	0.9986
3	IPA	0.2755	12.612	y = 0.2755x+12.612	0.9988
4	DCM	0.2215	2.5184	y = 0.2215x+2.5184	0.9985
5	n- Hexane	8.1538	14.8074	y = 8.1538x+14.8074	0.9983
6	Ethyl Acetate	0.7473	83.2080	y= 0.7473 x+83.2080	0.9989
7	THF	1.3854	17.1572	y = 1.3854x+17.1572	0.9986
8	Toluene	1.0921	22.1753	y = 1.0921x+22.1753	0.9989
9	DMF	0.0314	0.1294	y = 0.0314x+0.1294	0.9987

Observation:

The method was linear from 50% level to 150% level with respect to the test concentration.

Method Precision: The Method Precision of the analytical method was studied by analysis of six different solutions of same concentration. The method precision is expressed as relative standard deviation.

Acceptance criteria:

- RSD for content of each solvent from six spiked sample preparations should not be more than 15.0 %.

Table 17: %RSD of 6 Precision solutions:

S. No:	**Solvent Name**	**%RSD**
1	Methanol	1.7
2	Acetone	1.3
3	IPA	2.1
4	DCM	1.4

5	n- Hexane	1.5
6	Ethyl Acetate	1.4
7	THF	1.5
8	Toluene	2
9	DMF	6.5

Table 18: Method Precision solution % of RSD: Solvents content in ppm

S. No	INJ	Methanol	Acetone	IPA	DCM	n - hexane
1	**1**	3175.08	4990.95	5489.22	617.42	277. 77
2	**2**	3229.05	4994.23	5481	615.13	273.75
3	**3**	3195.08	4982.51	5483.44	616.2	276.11
4	**4**	3269.96	5009.11	5595.35	619.35	270.52
5	**5**	3163.55	4916.19	5431.68	607.74	268.84
6	**6**	3322.96	5122.89	5548.13	625.81	319.12
7	**Avg**	**3225.9**	**5002.6**	**5504.8**	**616.9**	**281**
8	**STDEV**	**61.306**	**67.213**	**57.773**	**5.885**	**18.962**
9	**%RSD**	**1.9**	**1.3**	**1**	**1**	**6.7**

S. No	INJ	Ethyl Acetate	THF	Toluene	DMF
1	**1**	5006.22	707.53	869.94	803.83
2	**2**	4997.32	709.8	872.04	860.49
3	**3**	4990.82	706.22	867.73	820.48
4	**4**	5017.49	708.96	878.36	847.57
5	**5**	4945.26	697.81	861.15	823.97
6	**6**	5105.25	731.2	890.98	891.87
7	**Avg**	**5010.4**	**710.3**	**873.4**	**841.4**
8	**STDEV**	**52.653**	**11.128**	**10.293**	**31.946**
9	**%RSD**	**1.1**	**1.6**	**1.2**	**3.8**

Table 19: Intermediate Precision Results:

S. No	INJ	Methanol	Acetone	IPA	DCM	n - Hexane
1	**1**	2943.53	4843.08	5619.85	625.4	274.52
2	**2**	2964.29	4911.66	5657.26	634.73	277.55
3	**3**	2947.15	4875.47	5592.29	627.19	279.51
4	**4**	2916.48	4800.89	5662.35	620.24	271.42
5	**5**	2991.76	4878.97	5691	630.98	279.46
6	**6**	3063.51	4979.98	5917.85	646.21	270.08
7	**Avg**	**2971.1**	**4881.7**	**5690.1**	**630.8**	**275.4**
8	**STDEV**	**51.633**	**61.032**	**116.809**	**9.018**	**4.072**
9	**%RSD**	**1.7**	**1.3**	**2.1**	**1.4**	**1.5**

S. No	INJ	Ethyl Acetate	THF	Toluene	DMF
1	**1**	4834.65	676.82	860.38	827.89
2	**2**	4902.39	686.9	872.8	776.35
3	**3**	4864.91	682.33	864.94	805.06
4	**4**	4789.26	668.13	849.19	781.88
5	**5**	4869.34	682.89	870.33	895.68
6	**6**	4987.95	698.13	901.87	898.34
7	**Avg**	**4874.8**	**682.5**	**869.9**	**830.9**
8	**STDEV**	**67.262**	**10.026**	**17.747**	**54.406**
9	**%RSD**	**1.4**	**1.5**	**2**	**6.5**

Observation:
The %RSD values from the Method precision and % RSD for the residual solvents for Ezetimibe was within the acceptance criteria (Not more than 15.0).
Hence, it was proved that the method was precise for the determination of residual solvents in the Ezetimibe.

Accuracy:

Table 20: Accuracy results of Methanol: Methanol Concentration in sample – 4.47 ppm

Preparation	Theoretical Conc. (ppm)	Corrected found Conc. (ppm)	% Recovery	Mean Recovery
50% level - 1	1491.4	1433.6	96.1	**97.2**
50% level – 2	1492.2	1468.9	98.4	
50% level – 3	1492.1	1448.1	97.1	
100% level – 1	2985.5	2939.1	98.5	**98.8**
100% level – 2	2985.7	2959.8	99.1	
100% level – 3	2976.7	2942.7	98.9	
150% level - 1	4469.2	4344.5	97.2	**97.1**
150% level - 2	4471	4338.6	97	
150% level - 3	4473	4347.1	97.2	

Table 21: Accuracy results of Acetone: Acetone Concentration in sample – 2.48 ppm

Preparation	Theoretical Conc. (ppm)	Corrected found Conc. (ppm)	% Recovery	Mean Recovery
50% level - 1	2502.4	2442.8	96.1	**98.4**
50% level – 2	2503.8	2499.4	98.4	
50% level – 3	2503.7	2447	97.1	
100% level – 1	5009.1	4840.6	98.5	**97.4**
100% level – 2	5009.9	4909.2	99.1	
100% level – 3	4994.7	4873	98.9	
150% level - 1	7499.1	7039.7	97.2	**94.5**
150% level - 2	7502.1	7081.3	97	
150% level - 3	7505.5	7155	97.2	

Table 22: Accuracy results of IPA: IPA Concentration in sample – 618.78 ppm

Preparation	Theoretical Conc. (ppm)	Corrected found Conc. (ppm)	% Recovery	Mean Recovery
50% level - 1	2491.2	2461.3	98.8	**99.7**
50% level – 2	2492.6	2486.1	99.9	
50% level – 3	2492.5	2508.2	100.6	
100% level – 1	4986.7	5001.1	100.3	100.4
100% level – 2	4987.4	5038.5	101	
100% level – 3	4972.3	4973.5	100	
150% level - 1	7465.5	7314.9	98	**98.2**
150% level - 2	7468.5	7272.9	97.4	
150% level - 3	7471.8	7405.2	99.1	

Table 23: Accuracy results of DCM: DCM Concentration in sample – 0 ppm

Preparation	Theoretical Conc. (ppm)	Corrected found Conc. (ppm)	% Recovery	Mean Recovery
50% level - 1	312.9	316.2	101.1	**100.8**
50% level – 2	313.1	322.2	102.9	
50% level – 3	313	317.6	101.5	

100% level – 1	626.3	625.4	99.9	**100.5**
100% level – 2	626.4	634.7	101.3	
100% level – 3	624.5	627.2	100.4	
150% level - 1	937.6	909.5	97	**97.6**
150% level - 2	938	913.9	97.4	
150% level - 3	938.4	923.9	98.5	

Table 24:. Accuracy results of n -Hexane: n - Hexane Concentration in sample – 0 ppm

Preparation	Theoretical Conc. (ppm)	Corrected found Conc. (ppm)	% Recovery	Mean Recovery
50% level - 1	146.1	134.8	92.3	**93.0**
50% level – 2	146.2	140.4	96	
50% level – 3	146.1	132.5	90.7	
100% level – 1	292.4	274.5	93.9	**94.9**
100% level – 2	292.4	277.6	94.9	
100% level – 3	291.5	279.5	95.9	
150% level - 1	437.7	404.3	92.4	**92.9**
150% level - 2	437.9	409.5	93.5	
150% level - 3	438.1	406.9	92.9	

Table 25 : Accuracy results of Ethyl Acetate: Ethyl Acetate Concentration in sample-0 ppm

Preparation	Theoretical Conc. (ppm)	Corrected found Conc. (ppm)	% Recovery	Mean Recovery
50% level - 1	2493	2433	97.6	**98.6**
50% level – 2	2494.3	2499.9	100.2	
50% level – 3	2494.2	2444.5	98	
100% level – 1	4990.2	4834.7	96.9	**97.6**
100% level – 2	4990.9	4902.4	98.2	
100% level – 3	4975.8	4864.9	97.8	
150% level - 1	7470.7	7033.3	94.1	**94.9**
150% level - 2	7473.7	7069.8	94.6	
150% level - 3	7477	7174	95.9	

Table 26: Accuracy results of THF: THF Concentration in sample – 2.76 ppm

Preparation	Theoretical Conc. (ppm)	Corrected found Conc. (ppm)	% Recovery	Mean Recovery
50% level - 1	361.8	337	93.1	**94.2**
50% level – 2	362	348.1	96.2	
50% level – 3	362	337.8	93.3	
100% level – 1	724.2	674.1	93.1	**93.9**
100% level – 2	724.3	684.1	94.4	
100% level – 3	722.1	679.6	94.1	
150% level - 1	1084.2	987.3	91.1	**91.6**
150% level - 2	1084.7	994.2	91.7	
150% level - 3	1085.2	999.9	92.1	

Table 27: Accuracy results of Toluene: Toluene Concentration in sample – 6.44 ppm

Preparation	Theoretical Conc. (ppm)	Corrected found Conc. (ppm)	% Recovery	Mean Recovery
50% level - 1	449.1	428	95.3	

50% level – 2	449.3	442.2	98.4	**96.6**
50% level – 3	449.3	431.7	96.1	
100% level – 1	897.9	853.9	95	**95.7**
100% level – 2	899.1	866.4	96.4	
100% level – 3	896.3	858.5	95.8	
150% level - 1	1345.8	1244.7	92.5	**93.1**
150% level - 2	1346.3	1249.9	92.8	
150% level - 3	1346.9	1267.9	94.1	

Table 28:. Accuracy results of DMF: DMF Concentration in sample – 0 ppm

Preparation	Theoretical Conc. (ppm)	Corrected found Conc. (ppm)	% Recovery	Mean Recovery
50% level - 1	446	385.4	86.4	**90.4**
50% level – 2	446.2	413.4	92.6	
50% level – 3	446.2	410.9	92.1	
100% level – 1	892.7	827.9	92.7	**90.0**
100% level – 2	892.9	776.4	87.0	
100% level – 3	890.2	805.1	90.4	
150% level - 1	1336.6	1255.3	93.9	**90.4**
150% level - 2	1337	1194.4	89.3	
150% level - 3	1337	1178.6	88.1	

Table 29: % Mean Recovery of Residual solvents:

S. No.	Solvent Name	50%	100%	150%
1	Methanol	97.2	98.8	97.1
2	Acetone	98.4	97.4	94.5
3	IPA	99.7	100.4	98.2
4	DCM	101.8	100.5	97.6
5	n- Hexane	93.0	94.9	92.9
6	Ethyl Acetate	98.6	97.6	94.9
7	THF	94.2	93.9	91.6
8	Toluene	96.6	95.7	93.1
9	DMF	90.4	90.0	90.4

Acceptance criteria:

Recoveries should not be less than 80% and should not be more than 120 % for each solvent. **Observation:** The % recovery should not be less than 80.0 and should not be more than 120.0 **Result:** The method was accurate from 50% level to 150% level.

Robustness:

Table 30: All Conditions RT`s in selectivity solution:

S. No.	Solvent Name	As per method	Low column oven temperature (35^{0}c)	High column oven temperature (45^{0}c)	Low injector temperature (135^{0}c)
1	Methanol	5.40	5.90	5.16	5.39
2	Acetone	8.45	9.65	7.79	8.45
3	IPA	8.98	10.41	8.15	8.97
4	DCM	10.02	11.50	9.13	10.01
5	n- Hexane	11.79	12.85	10.81	11.78
6	Ethyl Acetate	13.65	14.43	13.02	13.65
7	THF	14.01	14.74	13.43	14.00
8	Toluene	17.03	17.51	16.71	17.02
9	DMF	18.10	18.49	17.82	18.09

S. No:	Solvent Name	High injector temperature (205^{0}c)	Low Detector emperature (255^{0}c)	High Detector emperature (265^{0}c)
1	Methanol	5.37	5.36	5.37
2	Acetone	8.42	8.40	8.43
3	IPA	8.95	8.93	8.96
4	DCM	9.99	9.97	10.00
5	n- Hexane	11.77	11.76	11.78
6	Ethyl Acetate	13.64	13.63	13.65
7	THF	14.00	13.99	14.00
8	Toluene	17.02	17.01	17.02
9	DMF	18.09	18.08	18.09

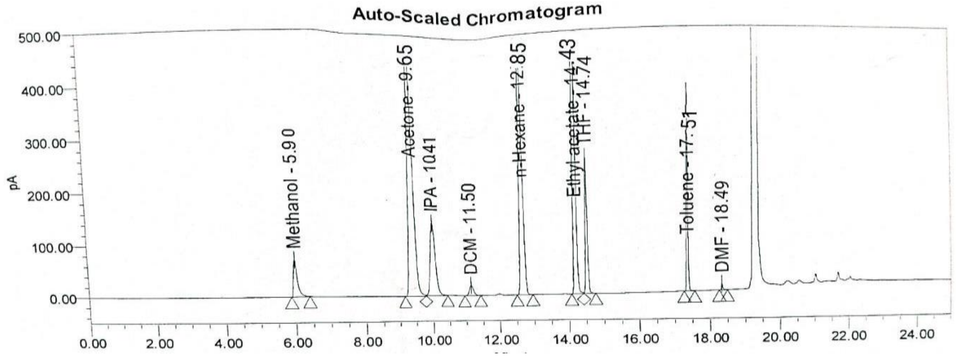

Fig. 14: Low Column temperature (-)

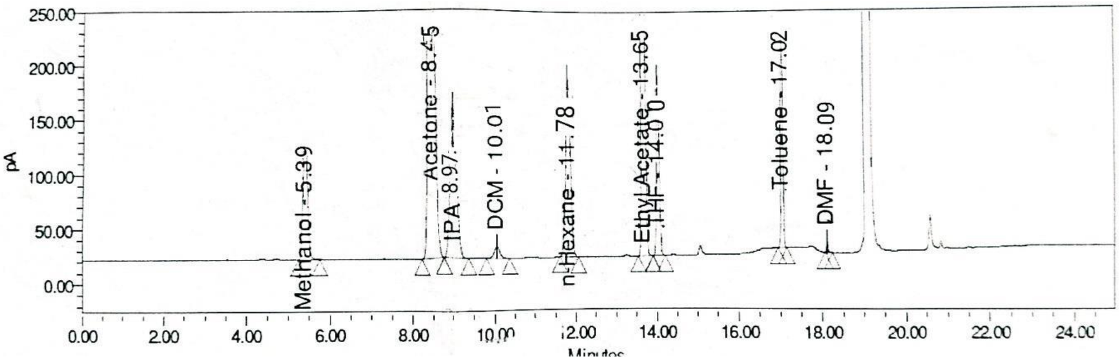

Fig. 15: High Column temperature (+)

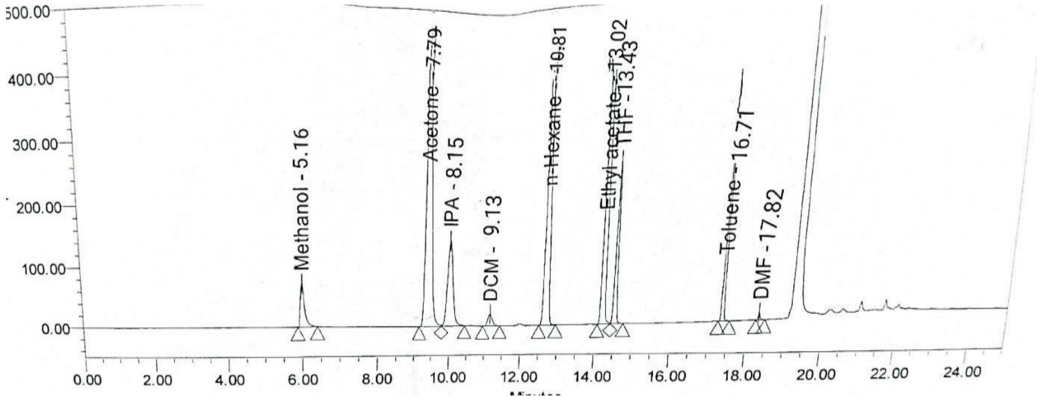

Fig. 16 : Low Injector temperature (-)

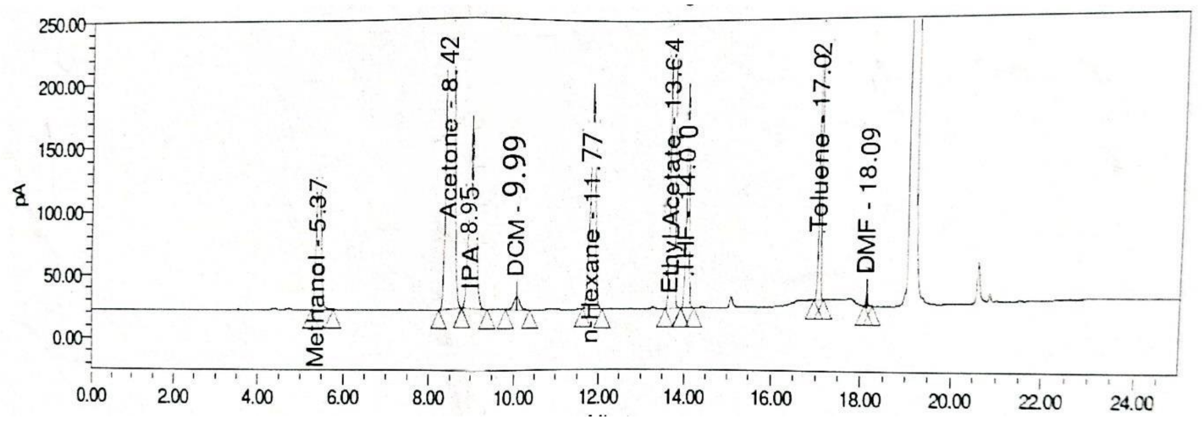

Fig. 17: High Injector temperature (+)

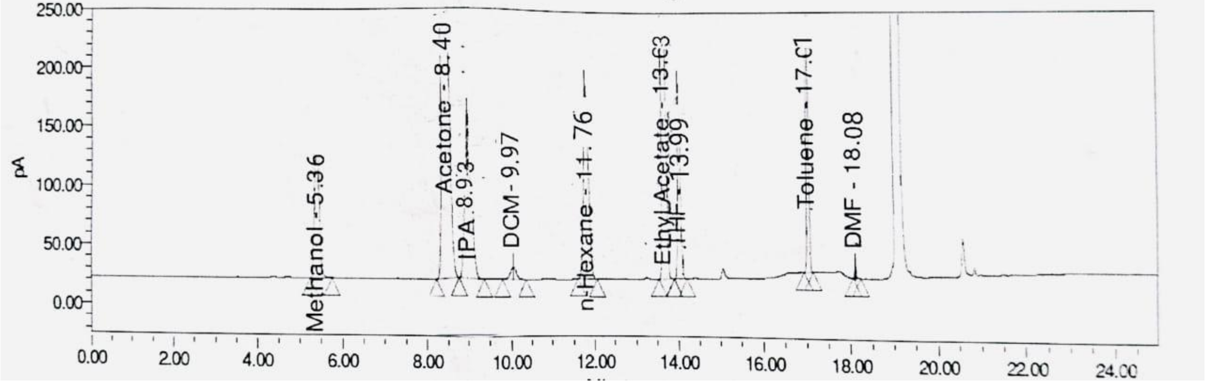

Fig. 18: Low Detector temperature (-)

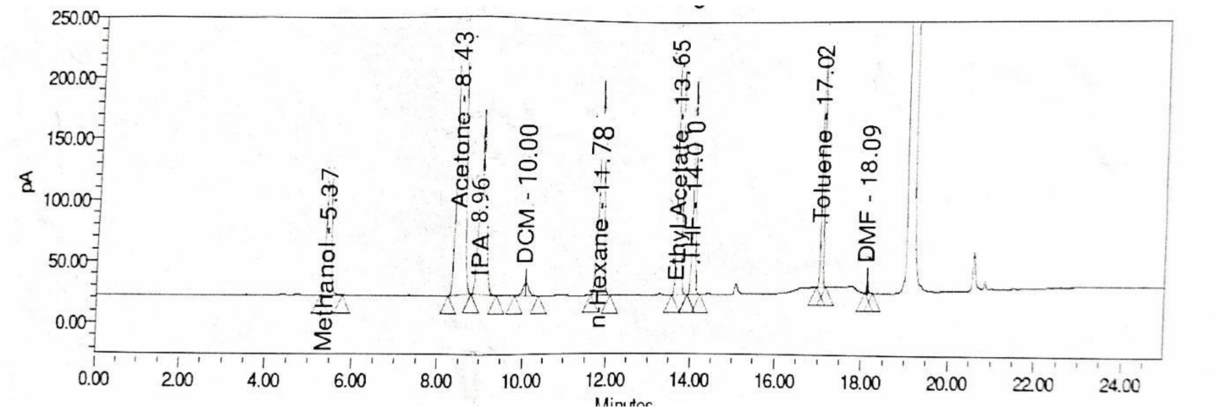

Fig. 19: High Detector temperature (+)

Acceptance criteria:

Report all RT`s of all solvents in selectivity solution

Result: The System meets required system suitability criteria. So, the method was found robust for all conditions.

Solution Stability:

Table 31:. All Conditions RT`s in selectivity solution:

S. No:	Solvent Name	After 12 hrs. % of variation	After 24 hrs. % of variation	After 48 hrs. % of variation
1	Methanol	-1.4	1.7	0.6
2	Acetone	0.3	2.8	1.8
3	IPA	-1.3	2.4	0.4
4	DCM	0.0	2.5	1.1

5	n- Hexane	5.2	4.6	4.3
6	Ethyl Acetate	-0.3	2.6	1.0
7	THF	-0.4	3.1	1.7
8	Toluene	-1.3	2.3	0.4
9	DMF	-3.9	1.1	-4.0

Acceptance Criteria: Variation in solvent content should be within ± 15.0 % for all solvents.
Result: The solution is stable up to 48 hours at room temperature.

Summary Report:
Table 32: Summary:

Para meters	methanol	Acetone	IPA	DCM	n-Hexane	EA	THF	Toluene	DM F	Limit
Specificity	specific	specific	specific	specific	specific	specific	specific	specific	specific	No interference peaks
Method Precision	1.7	1.3	2.1	1.4	1.5	1.4	1.5	2.0	6.5	NMT 15.0 %
Accuracy % Recovery	97.7	96.8	99.5	99.9	93.6	97.03	93.23	95.13	90.26	(80%-120 %)
Linearity Concentration (ppm)	55.8-4533 ppm	7.5-7542 ppm	90.1-7518 ppm	63.3-909 ppm	0.9-432 ppm	4-7503 ppm	3-1047 ppm	2.3-1347 ppm	88.6-1317 ppm	NLT 0.99
Regression Equation (y= mx +c)	y=0.1790x+6.1882	y=0.9091x+79.8701	y=0.2755x+12.6120	y=0.2155x+2.5184	y=8.1538x+14.8074	y=0.7473x+83.2080	y=1.3854x+17.1572	y=1.0921x+22.1753	y=0.0314x+0.1294	
R^2	0.9992	0.9986	0.9988	0.9985	0.9983	0.9989	0.9986	0.9989	0.9987	NLT 0.99
LOD (S/N)	3	3	4	5	4	15	3	9	3	S/N ratio ≥3
LOQ (S/N)	11	10	14	19	13	20	11	17	12	S/N ratio ≥ 10

Robustness Column (-) oven temp	5.90	9.65	10.41	11.50	12.85	14.43	14.74	17.51	18.49	Report RT`S for all solvents.
Column (+) oven temp	5.16	7.79	8.15	9.13	10.81	13.02	13.43	16.71	17.82	
Injector (-) temp	5.39	8.45	8.97	10.01	11.78	13.65	14.00	17.02	18.09	
Injector (+) temp	5.37	8.42	8.95	9.99	11.77	13.64	14.00	17.02	18.09	
Detector (-) Temp	5.36	8.40	8.93	9.97	11.76	13.63	13.99	17.01	18.08	Report RT`S For all solvents
Detector (+)										

Temp	5.37	8.43	8.96	10.00	11.78	13.65	14.00	17.02	18.09	
Solution stability After 12 hrs	-1.4	0.3	-1.3	0.0	5.2	-0.3	0.4	-1.3	-3.9	Variation in solvent content be within ±15.0 %
After 24 hrs	1.7	2.8	2.4	2.5	4.6	2.6	3.1	2.3	1.1	
After 48 hrs	0.6	1.8	0.4	1.1	4.3	1.0	1.7	0.4	-4.0	

4. CONCLUSION:

- A Simple, specific, sensitive, accurate, robust, precise GC -HS Method was developed and validated for analytical method development and validation for Identification of residual solvents for Ezetimibe drug.bThe method was optimised after many trials because in this all residual solvents present in the drug were well separated. For the Optimised method, the mobile phase used was DMSO (Dimethyl sulphoxide) and the Column used for separation was ZB-624 with 30m x 0.53mm,
- 3.0 µm thickness with flow rate was 2.0ml/min and Injector temperature is 200^0 c and 260 ^{0} c detector temperature and the split ratio is for 1:5 and with run time 25 min and constant flow 2.0 ml/min.
- The method was validated for all the parameters like System suitability, Specificity, Linearity, Precision, Accuracy, Limit of Detection, Limit of Quantification, Robustness and Solution stability. The results obtained in all parameters were within the acceptance criteria.
- The method was specific as there were no interference peaks found and the % RSD of all the solvent peaks were not more than 15.0 and meets the system suitability criteria.
- The method was linear for the determination of residual solvents as impurities R^2 values were in the range 0.9983-0.9992. The LOD, LOQ S/N values of methanol, Acetone, IPA, DCM, n-Hexane, EA, THF, Toluene, DMF were found to be 3 & 11, 3 & 10, 4 & 14, 5 & 19, 4 & 13, 15 & 20, 3& 11, 9 & 17, 3 & 12 (S/N) respectively.
- The method was accurate because the mean % recovery of the impurities was found to be 97.7%, 96.8%, 99.5%, 99.9%, 93.6%, 97.03%, 93.23%, 95.13%, 90.26 % which is between 80 and 120 according to specification. The method was precise for determination and identification of residual solvents because for all the precise conditions the % RSD was not more than 15.0 %.
- The Robustness was carried out by varying the conditions like Column oven temperature, Injector temperature, Detector temperature. No effect on the method was observed. Hence, it`s a robust method.
- The method was stable and it was confirmed by solution stability studies under different time intervals. In All the time intervals. The method was found to be stable up to 48 hrs stored at ambient temperature.
- Based on above information. It was concluded that this method can be used for routine analysis. Hence, this method can be used for the determination of residual solvents by GC for Ezetimibe samples for purpose of analysis.

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