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Identification of the Organic Volatile Impurities in Ezitamibe using GC-HS Technique

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Ezetimibe prevents intestinal cholesterol synthesis, which in turn reduces the quantity of cholesterol and thereby helps to reduce heart problems and strokes. In the production of ezetimibe, several organic chemical solvents such as methanol, acetone, isopropyl alcohol, dichloromethane, *n*-hexane, ethyl acetate, tetrahydrofuran, toluene and dimethyl formamide were used. The measurement of residual solvents is necessary for release checks of all drug substances, based on sound manufacturing processes. The analysis of above mentioned nine solvents in ezetimibe drug was investigated using gas chromatographic method employing detection with flame ionization mode. All analyses were performed using ZB-624 column (30 m length × 0.53 mm identification, 3.0 µm thickness film). The column oven temperature flux was managed to maintain for 11 min at 40 ºC and then continued to upsurge to a temperature close of 240 ºC at a rate of 20 ºC/min and retained for 4 min. The flame ionization detector and injector port were managed at 260 and 200 ºC, respectively. Results after the validation of the gas chromatographic method showed the satisfactory linearity, sensitivity, robustness, accuracy, selectivity and precision for the tested organic solvents. This gas chromatographic approach can therefore be exploited in the assessment of studied nine residual chemical solvents for periodic analysis by gas chromatography for samples of ezetimibe drug.

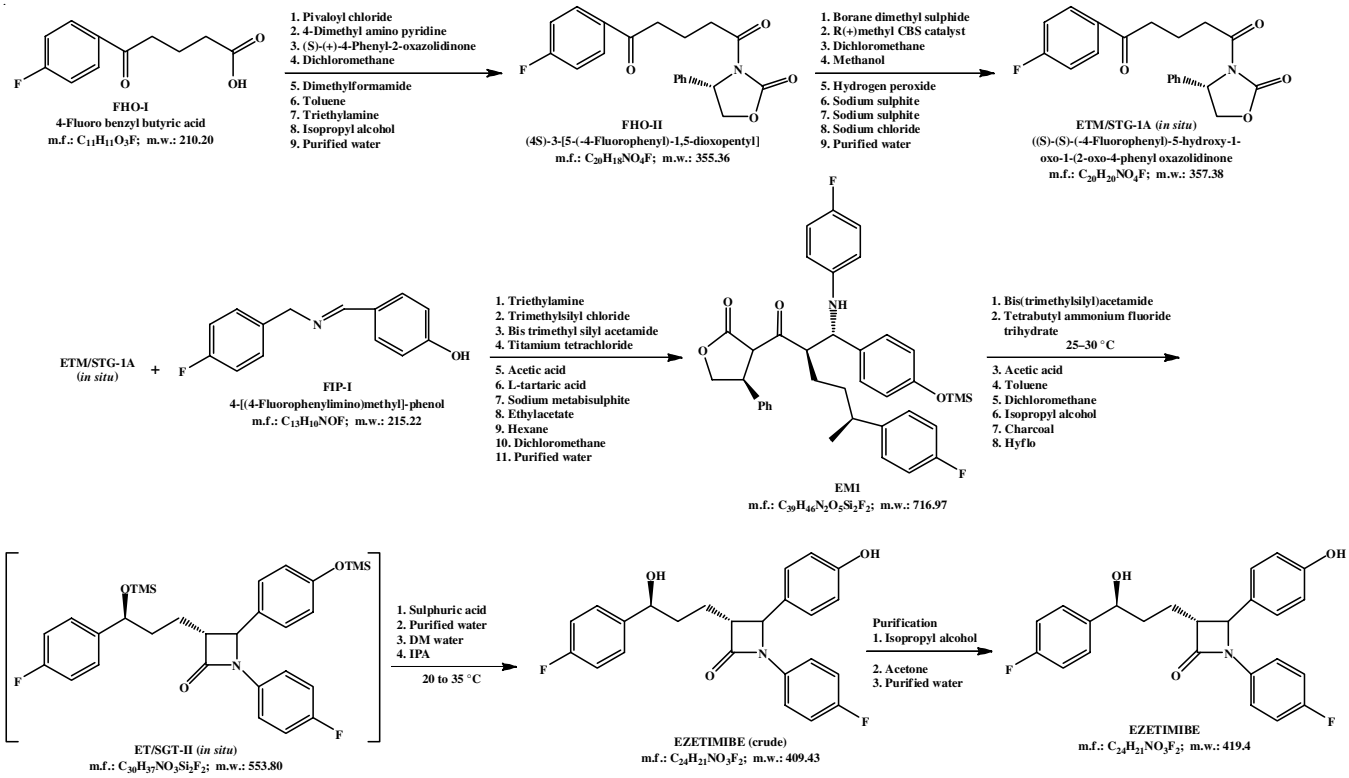
Keywords: Gas chromatography, Head space, Flame ionization, Ezetimibe, Validation, Residual chemical solvents.

INTRODUCTION

Organic solvents are generally used during drug material synthesis, excipients, as well as in the drug product formulation [1,2]. They are not acceptable in the finished product, mostly because of their harmfulness, their impact on the consistency of the drug substance's crystals and their taste or odour, which could be uncomfortable for patients [3]. Various processing technologies or procedures are being utilized to eliminate organic chemical solvents. Organic chemical solvents are typically removed under elevated temperature or/and reduced pressure [4,5]. Some organic chemical solvents do exist in limited numbers, in spite after several processes. Such small concentrations of organic chemical solvents are also referred to as residual chemical solvents. One of the most complex and challenging analytical activities in the pharma companies is the detection of residual chemical solvents in medicinal compounds including drug products.The processing of active medicinal ingredients and formulations of pharmaceutical compounds under conditions of good industrial practise requires sufficient quality control of the various ingredients expended in the synthesis. Therefore, before any good industrial practise synthesis, organic residual chemical solvents have to be regulated and purity ought to be established. Regulatory guidance reports contain the acceptable amounts of many organic solvents [6].

Ezetimibe, an azetidinone derivative, is intended to help reduce cholesterol quantity in the blood, along with a reduced cholesterol/fat diet and exercising [7,8]. Ezetimibe can be administered alone or with other medications belonging to the statins or fibrates groups [9,10]. Ezetimibe operates by dropping the cholesterol amount that the body captivates from the diet. Cholesterol reduction can help avoid heart attacks and strokes [11]. Several pharmaceutical firms are developing ezetimibe medication because of its therapeutic significance. The synthetic route of ezetimibe is outlined in **Scheme-I** [12,13].

The organic solvents like methanol, acetone, isopropyl alcohol, dichloromethane, *n*-hexane, ethyl acetate, toluene,



Scheme-I: Chemical scheme of preparation of ezetimibe

tetrahydrofuran and dimethyl formamide were used in the ezetimibe preparation. As per ICH Q3C (R6), methanol, dichloromethane, *n*-hexane, tetrahydrofuran, toluene and dimethyl formamide were grouped under class-2 organic solvents while acetone, isopropyl alcohol and ethyl acetate were grouped under class-3 organic solvents [6]. Organic solvents of class-2 have inherent toxicity to human well being and chemical solvents of class-3 are less harmful. Therefore, the organic solvents utilized in ezetimibe have to be regulated. The quantity level values are considered as 100% specification limit values for opted nine chemical solvents.

In general, validation is required for all approaches for the quantitative evaluation of residual chemical solvents collected from pharmacopoeia. For this cause, manufacturers are seeking to develop their own approaches that can be faster, simpler and resilient to their particular specimens and analytes [14]. No analytical approach was yet proposed to monitor and simultaneously quantify the investigated organic solvents, methanol, acetone, isopropyl alcohol, dichloromethane, *n*-hexane, ethyl acetate, tetrahydrofuran, toluene and dimethyl formamide in ezetimibe drug. In this work, a gas chromatography methodology employing flame ionization detection was developed and authorized ensuing policies of ICH to monitor and quantify all the organic solvents in ezetimibe drug.

EXPERIMENTAL

The solvents *viz*. methanol, acetone, isopropyl alcohol, dichloromethane, *n*-hexane, ethyl acetate, tetrahydrofuran, toluene, dimethyl sulfoxideand dimethyl formamide (all procured from Merck, India) and HPLC grade Milli Q water was employed all through the investigation. Ezetimibe was obtained from GVK Biosciences Pvt. Ltd. (Hyderabad, India).

AligentGC6890 N system, Aligent head space G1888 N system, Aligentflame ionization detector system, Waters (USA) Empower version 3 software, Millipore 0.45 microns filter paper were used during detection and evaluation of opted nine organic chemical solvents in ezetimibe.

Chromatographic conditions: All analyses were performed using ZB-624 column with 30 m length, 0.53 mm identification and 3.0 µm thickness film; 5 µL with split ratio of 1:5 injection at 200 ºC; inlet pressure of 14 psi, resulting in a nitrogen flow of 2 mL/min; column oven temperature flux was managed to maintain for 11 min at 40 ºC and then continued to upsurge to a temperature close of 240 ºC at a rate of 20 ºC/min and retained for 4 min; flame ionization detector managed at 260 ºC; air flow was 400 mL/min; and hydrogen flow was 40 mL/min. The head space parameters include; zone temperatures were managed at 100 ºC (at vial), 110 ºC (at loop), 120 ºC (at transfer line); and event times were managed at 35 min (for cycle time), 10 min (for equilibration time), 0.2 min (for vial pressuring time), 0.5 min (for injection time). Dimethyl sulfoxide was employed as diluent.

Stock and working solvent solutions: Weighed precisely 300 mg of methanol, 500 mg of acetone, 500 mg of isopropyl alcohol, 60 mg of dichloromethane, 29 mg of *n*-hexane, 500 mg of ethyl acetate, 72 mg of tetrahydrofuran, 89 mg of toluene and 88 mg of dimethyl formamide into 20 volumetric flask with 5 mL of diluent (DMSO), dissolved and then diluted to marked volume with DMSO. The concentration of stock solvent solution was 15000 ppm (methanol), 25000 ppm (acetone), 25000 ppm

(isopropyl alcohol), 3000 ppm (dichloromethane), 1450 ppm (*n*-hexane), 25000 ppm (ethyl acetate), 3600 ppm (THF), 4450 ppm (toluene) and 4400 ppm (DMF).

A series of working linearity solvent solutions having concentration in span of 55.8-4533 ppm (methanol), 7.5-7542 ppm (acetone), 90.1-7518 ppm (isopropyl alcohol), 63.3-909 ppm (dichloromethane), 0.9-432 ppm (*n*-hexane), 4-7503 ppm (ethyl acetate), 3-1047 ppm (THF), 2.3-1347 ppm (toluene) and 88.6-1317 ppm (DMF) were prepared by diluting appropriate aliquots of the mixed stock solvent solution with DMSO (diluent).

Working standard solvent solution of quantities 3000 ppm (methanol), 5000 ppm (acetone), 5000 ppm (isopropyl alcohol), 600 ppm (dichloromethane), 290 ppm (*n*-hexane), 5000 ppm (ethyl acetate), 720 ppm (THF), 890 ppm (toluene) and 880 ppm (DMF) was also made by diluting appropriate aliquot of mixed stock solvent solution with DMSO (diluent).

Ezetimibe sample solution: Precisely 200 mg of ezetimibe specimen was weighed into 20 mL head space vial and 2 mL of DMSO had been added, the septum was placed and the vial crimped.

Procedure to evaluatethe opted solvents in ezetimibe drug: After column equilibration for 60 min, blank dimethyl sulfoxide (diluent) solution (n = 2); working standard solvent solution (n = 6); and ezetimibe drug sample (n = 1) were infused (1 µL) and recorded the corresponding chromatograms by applying suggested gas chromatographic method. The response area of opted solvents in ezetimibe drug sample, in working standard solvent solution and blank DMSO solution were documented. The ppm concentration of opted solvents in ezetimibe drug sample was assessed using the following formula:

$$\text{Solvent concentration (ppm)} = \frac{\text{ASP} - \text{ABP}}{\text{ASS} - \text{ABP}} \times \frac{\text{Solvent wt. (mg)}}{20\ \text{mL}} \times \frac{1.0\ \text{mL}}{40\ \text{mL}} \times \frac{2}{\text{Sample wt. (mg)}} \times 10^6$$

where, ASP = response area of solvent in ezetimibe drug sample; ABP = response area of solvent in dimethyl sulfoxide (diluent) solution; ASS = response area of solvent in working standard solvent solution.

RESULTS AND DISCUSSION

Gas chromatographic methodology: Due of toxicity, quantification conferring to the established standards of residual organic solvents in the ultimate pharmaceutical formulation is mandatory for the launch of the market formulation. Any residual organic solvents may already be present in the finished substance, even after the last phase of the development procedure. These facts justify the need for certain attempts to measure the residual organic solvents in ezetimibe drug using gas chromatography separation and then followed by flame ionization detection.

For its ability to dissolve a broad range of organic solvents and will not impede with chosen solvents, analyzed by gas chromatography, DMSO was exploited as the standard as well as sample diluent. Four different column oven temperature fluxes were tried. (i) maintained for 11 min at 40 ºC and then continued to upsurge to a temperature close of 240 ºC at a rate of 20 ºC/min and retained for 30 min; (ii) maintained for 5 min at 40 ºC and then continued to upsurge to a temperature close of 240 ºC at a rate of 10 ºC/min and retained for 35 min; (iii) maintained for 11 min at 40 ºC and then continued to upsurge to a temperature close of 240 ºC at a rate of 15 ºC/min and retained for 30 min; and (iv) maintained for 11 min at 40 ºC and then continued to upsurge to a temperature close of 240 ºC at a rate of 20 ºC/min and retained for 4 min.

Better separation with good resolution were obtained with 4th column oven temperature flux. In 1st, 2nd and 3rd column oven temperature fluxes, the peaks of all the nine organic solvents were closely eluted. Nitrogen, as carrier gas, with flow stream of 1 mL per min and 2 mL per min were tested. A 2 mL per min flow stream was optimized. The remaining optimized parameters were 200 ºC temperature at injector port; 260 ºC temperature at detector port; split mode injection in 1:5 ratio; air flow and hydrogen flow were 400 mL/min and 40 mL/min, respectively. Fig. 1 displays the chromatogram acquired using configured parameters. The opted solvents were eluted at 5.533 min (methanol), 8.667 min (acetone), 9.207 min (isopropyl alcohol), 10.284 min (dichloromethane), 11.986 min (*n*-hexane), 13.790 min (ethyl acetate), 14.130 min (THF), 17.135 min (toluene) and 18.179 min (DMF).

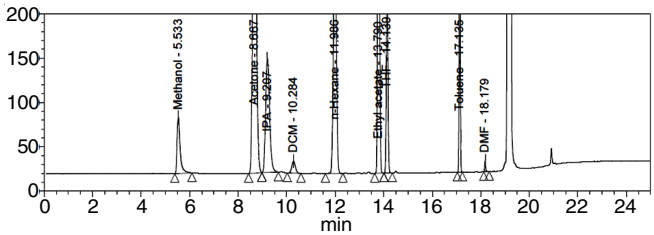


Fig. 1. Chromatogram of nine organic solvents acquired using configured parameters

Validation: The method for opted chemical solvents evaluation in ezetimibe drug was verified in harmony through ICH strategies [15].

System suitability: To verify suitability of gas chromatographic system, working standard solvent solution were analyzed six times by gas chromatographic method. The resolution for peaks of acetone and isopropyl alcohol and percent relative standard variation for each opted solvent peak area were observed (Table-1). The suitability of gas chromatographic method for the evaluation of studied organic solvents evaluation in ezetimibe drug sample was verified by the measured values. The resolution amid acetone peak and isopropyl alcohol peak was 1.9.

Selectivity: The selectivity of this procedure was checked to make sure the ezetimibe and diluent (DMSO) did not interfere with analysis of nine organic solvents. Ezetimibe drug sample, working standard solvent solution, solution of ezetimibe spiked with opted nine solvents (spiked concentration was same as standard solvent solution) and diluent (DMSO) blank were prepared and analyzed by way of suggested gas chromatographic method. The characteristic chromatograms for selectivity are shown in Fig. 2. Chromatograms exhibit that the retention times of nine organic solvents *viz*. methanol, acetone,

TABLE-1
SYSTEM SUITABILITY DATA

Solvent	Quantity of solvent (ppm)	Mean peak area from 5 values	Standard variation for 6 values	Percent standard variation (%)	Bracketing
Methanol	3000	587.0	36.507	6.2	547.17
Acetone	5000	4850.2	282.039	5.8	4527.55
Isopropyl alcohol	5000	1490.7	101.250	6.8	1374.10
Dichloromethane	600	145.9	8.508	5.8	135.43
n-Hexane	290	2825.7	155.416	5.5	2796.37
Ethyl acetate	5000	3970.8	239.753	6.0	3686.96
Tetrahydrofuran	720	1018.9	60.593	5.9	949.72
Toluene	890	1055.3	63.595	6.0	976.92
Dimethyl formamide	880	31.8	2.944	9.3	28.81

isopropyl alcohol, dichloromethane, *n*-hexane, ethyl acetate, tetrahydrofuran, toluene and dimethyl formamide are completely different. This also proved that ezetimibe drug has no effect on analysis of opted nine solvents. By comparison, blank peak did not overlap peaks of opted nine solvents. The resolution among the opted solvents were too acceptable (≥ 1.9). So it's highly selective method.

Method precision: The gas chromatographic method precision was verified by analyzing the ezetimibe drug sample spiked with opted nine different solvents at 100% specification limit values. The method precision was vented as mean concentration quantified and relative standard deviation of six quantified values of nine opted solvents (Table-2). The relative standard deviation calculated for opted nine solvents was noticed as ≤ 6.5%, which proved that gas chromatographic method was precise for evaluation of opted nine solvents in ezetimibe drug.

Quantification and detection limits: The quantification and detection limits were verified for all the nine organic solvents at the concentration that gives an S/N fraction ≥ 10 and ≥ 3, respectively. The quantification and detection limits for opted nine solvents are shown in Table-3. The quantification limit values for opted nine solvents were confirmed by precision examination. The determined percent relative standard deviation of six area responses of opted nine solvents at their quantification limit level were in span of 1.5-3.7%. This confirmed the quantification limit levels for opted nine solvents.

Linearity: The linear quantity range for nine organic solvents was verified in a quantity range from the quantification limit

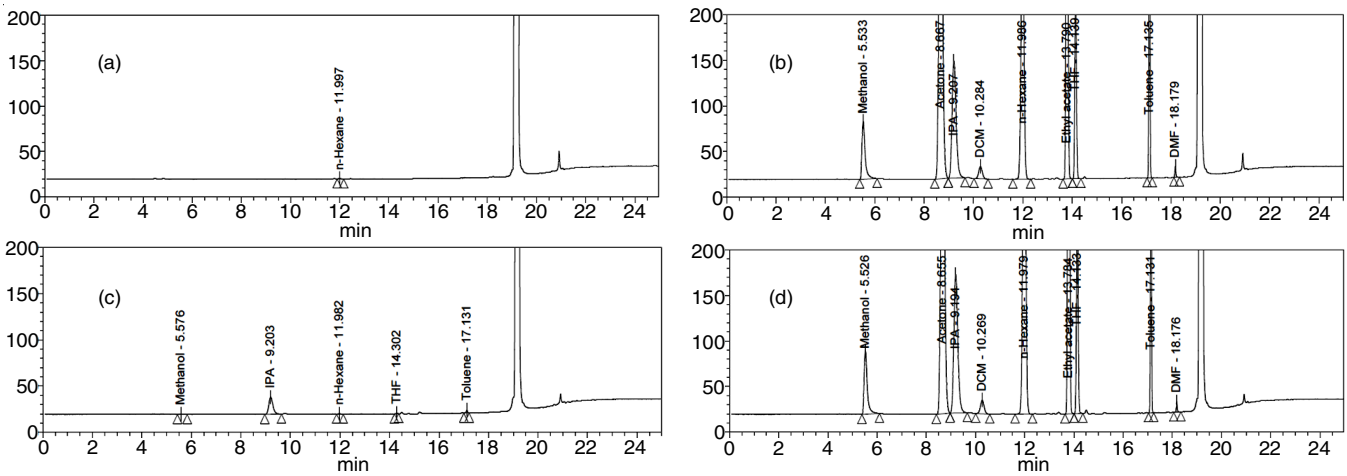


Fig. 2. (a) Diluent (dimethyl sulfoxide) blank chromatogram, (b) Working standard solvent solution chromatogram, (c) Ezetimibe drug sample solution chromatogram and (d) Ezetimibe drug sample spiked with opted nine solvents solution chromatogram

TABLE-2
METHOD PRECISION DATA

Solvent	Quantity of solvent (ppm)	Mean quantity quantified from 6 values (ppm)	Standard variation for 6 values	Percent standard variation (%)
Methanol	3000	2971.1	51.633	1.7
Acetone	5000	4881.7	61.032	1.3
Isopropyl alcohol	5000	5690.1	116.809	2.1
Dichloromethane	600	630.8	9.018	1.4
n-Hexane	290	275.4	4.072	1.5
Ethyl acetate	5000	4874.8	67.262	1.4
Tetrahydrofuran	720	682.5	10.026	1.5
Toluene	890	869.9	17.747	2.0
Dimethyl formamide	880	830.9	54.406	6.5

TABLE-3
METHOD SENSITIVITY DATA

Solvent	Detection limit		Quantification limit	
	Value (ppm)	S/N fraction	Value (ppm)	S/N fraction
Methanol	16.9	3	55.8	11
Acetone	2.5	3	7.5	10
Isopropyl alcohol	27.0	4	90.1	14
Dichloromethane	19.0	5	63.3	19
n-Hexane	0.3	4	0.9	13
Ethyl acetate	1.0	15	4.0	20
Tetrahydrofuran	0.9	3	3.0	11
Toluene	0.7	9	2.3	17
Dimethyl formamide	26.6	3	88.6	12

level to 150% of specification quantity limit. A linear correlation was detected between area responses and concentrations of studied nine solvents in the range (Table-4). The coefficient of correlation disclosed that the process was linear inside the concentration range studied (Table-4). The regression equation parameters for the opted nine solvents are shown in Table-4.

Accuracy: Appropriate amounts of opted nine solvents were spiked to ezetimibe drug sample with replicates (n = 3) at LOQ level, 50% level, 100% level and 150% level of specification quantity value. Theses spiked samples were analyzed by way of suggested gas chromatographic method and ascertained the recoveries of opted nine solvents at every level. The ascertained values of recoveries of opted nine solvents for the suggested gas chromatographic method used were in the range 72.5-105.2% (Table-5), which proved that the gas chromatographic method is accurate enough for evaluation of nine organic solvents in ezetimibe drug sample.

TABLE-5
ACCURACY DATA

Solvent	Mean recovery (%) values obtained from 3 values at			
	LOQ level	50% level	100% level	150% level
Methanol	73.5	97.2	98.8	97.1
Acetone	74.5	98.4	97.4	94.5
Isopropyl alcohol	–*	97.7	100.4	98.2
Dichloromethane	96.1	101.8	100.5	97.6
n-Hexane	105.2	93.0	94.9	92.9
Ethyl acetate	80.7	98.6	97.6	94.9
Tetrahydrofuran	77.5	94.2	93.9	91.6
Toluene	84.8	96.6	95.7	93.1
Dimethyl formamide	72.5	90.4	90.0	90.4

*Note: Recovery percentile for isopropyl alcohol is not determined due to its very high presence (680.45 ppm) in ezetimibe drug sample.

Robustness: To verify robustness, ezetimibe drug sample solution spiked with opted nine solvents at 100% level of specification quantity value were analyzed by way of suggested gas chromatographic method with slight variations (± 5 ºC) in column oven temperatures, injector port and detector port. The retention times of opted nine solvents under all varied and optimized gas chromatographic conditions were also recorded. The results (Table-6) had shown that the retention time values of opted nine solvents are not changed significantly, hence, method was considered as robust.

Solvent solution stability: The stability of the studied nine solvents stability in the ezetimibe drug sample was performed at periodic intervals (after 12, 24 and 48 h of storage) through the assay study. The variation percentage between

TABLE-4
LINEARITY AND REGRESSION DATA

Solvent	Linearity (ppm)	Correlation coefficient (R^2)	Slope (m)	Intercept (c)
Methanol	55.8–4533	0.9996	0.1790	6.1882
Acetone	7.5–7542	0.9993	0.9091	79.8701
Isopropyl alcohol	90.1–7518	0.9994	0.2755	12.6120
Dichloromethane	63.3–909	0.9993	0.2215	2.5184
n-Hexane	0.9–432	0.9992	8.1538	14.8074
Ethyl acetate	4–7503	0.9994	0.7473	83.2080
Tetrahydrofuran	3–1047	0.9993	1.3854	17.1572
Toluene	2.3–1347	0.9994	1.0921	22.1753
Dimethyl formamide	88.6–1317	0.9993	0.0314	0.1294

TABLE-6
ROBUSTNESS DATA

Solvent	Retention times of opted nine organic solvents						
	Optimized conditions	Column oven		Injector port		Detector port	
		30 °C	45 °C	195 °C	205 °C	255 °C	265 °C
Methanol	5.40	5.90	5.16	5.39	5.37	5.36	5.37
Acetone	8.45	9.65	7.79	8.45	8.42	8.40	8.43
Isopropyl alcohol	8.98	10.41	8.15	8.97	8.95	8.93	8.96
Dichloro methane	10.02	11.50	9.13	10.01	9.99	9.97	10.00
n-Hexane	11.79	12.85	10.81	11.78	11.77	11.76	11.78
Ethyl acetate	13.65	14.43	13.02	13.65	13.64	13.63	13.65
THF	14.01	14.74	13.43	14.00	14.00	13.99	14.00
Toluene	17.03	17.51	16.71	17.02	17.02	17.01	17.02
DMF	18.10	18.49	17.82	18.09	18.09	18.08	18.09

TABLE-8
METHOD RUGGEDNESS DATA

Solvent	Quantity of solvent (ppm)	Mean quantity quantified from 12 values (ppm)	Standard variation for 12 values	Percent standard variation (%)
Methanol	3000	3098.5	143.632	4.6
Acetone	5000	4942.2	87.964	1.8
Isopropyl alcohol	5000	5597.5	130.702	2.3
Dichloromethane	600	623.9	10.248	1.6
n-Hexane	290	278.2	13.398	4.8
Ethyl acetate	5000	4942.6	91.294	1.8
Tetrahydrofuran	720	696.4	17.651	2.5
Toluene	890	871.6	13.948	1.6
Dimethyl formamide	880	836.1	42.888	5.1

storage and freshly processed samples was estimated. The results (Table-7) show decent stability of the opted nine solvents in the drug sample of ezetimibe for 48 h.

TABLE-7
STABILITY DATA OF NINE ORGANIC SOLVENTS

Solvent	Variation (%) values obtained after		
	12 h	24 h	48 h
Methanol	-1.4	1.7	0.6
Acetone	0.3	2.8	1.5
Isopropyl alcohol	-1.3	2.4	0.4
Dichloromethane	0	2.5	1.1
n-Hexane	5.2	4.6	4.3
Ethyl acetate	-0.3	2.6	1.0
Tetrahydrofuran	0.4	3.1	1.7
Toluene	-1.3	2.3	0.4
Dimethyl formamide	-3.9	1.1	-4.0

Ruggedness: The ruggedness of gas chromatographic method was assessed by analyzing ezetimibe drug sample solution spiked with opted nine solvents at 100% level of specification quantity value, six times, with two different column, analyst, day and systems. The ruggedness was vented as mean concentration quantified and relative standard deviation of twelve quantified values of nine opted solvents. The relative standard deviation measured for the nine solvents opted was observed to be ≤ 5.1%, which revealed that gas chromatographic process for investigating the nine opted solvents in the ezetimibe drug was rugged (Table-8).

Conclusion

A reliable and effective gas chromatography coupled with flame ionization mode of detection dependent methodology to detect and evaluate residual chemical solvents methanol, acetone, isopropyl alcohol, dichloromethane, *n*-hexane, ethyl acetate, tetrahydrofuran, toluene and dimethyl formamide simultaneously in ezetimibe drug was developed and authenticated in this study. The validation parameters (linear regression, system suitability, quantification limit, detection limit, robustness, accuracy, precision, selectivity, ruggedness) for opted nine residual chemical solvents were in line with ICH requirement. Present results revealed that the quality of the ezetimibe drug sample can be evaluated using the methodology of gas chromatography proposed in this work.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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