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DOVELOPMENT AND VALIDATION OF A HEAD SPACE GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF NINE ORGANIC VOLATILE IMPURITIES IN TOPIRAMATE API

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ABSTRACT

Objective: Topiramate is an anticonvulsant drug. To provide quality control over the manufacture of any API, it is essential to develop highly selective analytical methods. In the current article we are reporting the development and validation of a rapid and specific head space gas chromatographic (HSGC) method for the determination of nine organic volatile impurities in topiramate API.

Methods: The method development and its validation were performed on Shimadzu GC-2010 Gas Chromatographic system equipped with flame ionization detector and teledyne tekmar HT3^{TM} head space analyzer. The method involved a thermal gradient elution of nine organic volatile impurities present in topiramate API. VF-624ms, 60 m × 0.32 mm, 1.0 µ column using nitrogen gas as a carrier. The flow rate was 2.5 ml/min and flame ionization detector (FID) was used.

Results: The method was linear for all nine solvents in topiramate LOQ to 200% µg/ml, respectively. The coefficient of correlation (r2) for the all solvents was not less than 0.99. The limit of detection obtained for acetone, IPA, acetonitrile, MDC,n-Hexane, EA, THF, pyridine and toluenewas 0.19, 0.36, 1.01, 0.18, 0.86, 0.22, 0.20, 1.17, 0.19% and the limit of quantification (LOQ) obtained was 0.59, 1.10, 3.05, 0.54, 2.61, 0.66, 0.61, 3.56, 0.58%. The method was fully validated, complying Food and Drug Administration, ICH and European Medicines Agency guidelines. Furthermore, verified precision, accuracy, LOQ precision, LOQ accuracy, ruggedness, and robustness.

Conclusion: The method has been successfully applied for the quantification of the amount of Organic Volatile Impurities present in Topiramate API. The method presents a simple and reliable solution for the routine quantitative analysis of Organic Volatile Impurities present in Topiramate API.

KEYWORDS: Acetone, IPA, Acetonitrile, Dichloromethane, n-Hexane, Ethyl acetate, Tetra Hydro Furan, Pyridine, Toluene, Topiramate, Method Validation.

INTRODUCTION

Topiramate is an anticonvulsantdrug (Fig. l). In late 2012, topiramate was approved by the United States Food and Drug Administration (FDA) in combination with phentermine for weight loss. The drug had previously been used off-label for this purpose. Topiramate was originally produced by Ortho-McNeil Neurologics and Noramco, Inc.Both divisions of the Johnson & Johnson Corporation. This medication was discovered in 1979 by Bruce E. Maryanoff and Joseph F. Gardocki during their research work at McNeil Pharmacitical.[1-3] Topiramate came into commercial use in 1996.[4] Generic versions are available in Canada and for generic topiramate by the FDA for sale in the United States.[5] The last patent for Topiramate in the U.S. was for pediatric use. This patent expired on February 28, 2009.[6]

Topiramate a derivative of fructose is a new anticonvulsant and antiepileptic drug under development for the treatment of human diseases.The drug is recrystallized from these nine solvents in the manufacturing process. Though the drug is dried and most of the organic solvents evaporated, there are residual organic solvents remaining in the API drug.

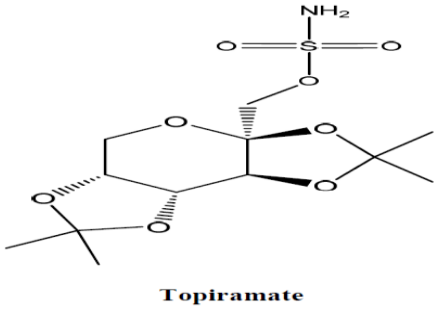


Fig. 1: Chemical Structure of Topiramate.

The level of these residual organic solvents has to be determined and controlled. There has not been any literature available to simultaneously determine its residual Acetone, IPA, Acetonitrile, Dichloromethane, n-Hexane, Ethyl acetate, Tetra Hydro Furan, Pyridine, and Toluene in this Topiramate drug substance. These structures are followed in Fig.2. Thus; a Headspace Gas Chromatographic method for the determination of residual solvents was developed and validated in this paper.

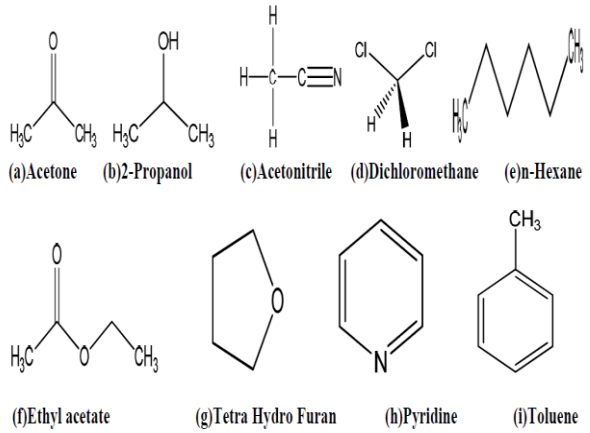


Fig. 1: Chemical Structures of Nine Organic Volatile Impurities.

Residual solvents are organic volatile chemicals used in synthesis and process chemistry of drug substances. There is an existing ICH (International Conference on Harmonization) guideline for residual solvents in pharmaceuticals (ICH 1997). The guideline groups residual solvents into three classes: class 1 solvents that should be avoided due to their high level of toxicity; class 2 solvents that should be limited; and class 3 solvents with low toxic potential.[7]

METHODS

Chemicals and reagents

Acetone(GC grade) ,Isopropyl alcohol (HPLC grade), Acetonitrile(HPLC grade),Dichloro methane(GC grade), n-Hexane(HPLC grade),Ethyl acetate (HPLC grade), Tetra hydro furan (HPLC grade),Pyridine(synthasis grade), Toluene(GC grade) and Dimethyl sulfoxide (GC grade)were provided by sigma-Aldrich. Topiramate API is from local research Laboratory. Di methyl sulfoxide is used as a diluents and blank.

Apparatus and chromatographic conditions

Chromatography was performed on Shimadzu chromatographic system equipped with Shimadzu GC-2010 system with FID, Samples were injected through a Teledyne tekmar HT3^{TM} Head space.10µl, 25 µl, 250 µl Micro syrenges. Data acquisition and integration was performed using GC solution software. The instrument parameters described below were set up to determine the residual solvents.

GC Conditions

Column was VF-624ms 1 µm film thickness 60 m 0.32 mm (maximum temperature 280°C). Column flow is 2.5ml/min. Injector temperature: 225°C. Split ration is 20:1 and Detector temperature is FID, 250°C. Column Initial temperature is 40°C, hold for 5 min; Increase the at rate, 20°C/ min, to final temperature 200°C ,hold for 12 min. Carrier gas is N_2.

Table. 1: Head space conditions.

G.C. Cycle Time	30.00 min.
Valve Oven Temperature	100°C
Transfer Line Temperature	110°C
Platen/Sample Temperature	80°C
Sample Equilibration Time	30.00 min.
Inject Time	1.00 min.

Preparation of Standard solution

158.0 µL of Acetone, 158.0 µL of 2-Propanol, 13.0 µL of Acetonitrile, 11.3 µL of Dichloromethane, 11.0 µL of n-Hexane, 139.0 µL of Ethyl acetate, 26.2 µL of Tetra hydro furan, 5.2 µL of Pyridine and 25.5 µL Toluene are accurately transferred by a Micro syringes into a 100 mL volumetric flask and diluted to volume with Di methyl sulfoxide. Then the standard Head space vials were prepared with 2 ml of the Standard solution and seal the vial with aluminum closure. (The standard solution concentration was prepared with respect to Sample concentration).

Preparation of Sample solution

About 0.5 gr of sample was accurately weighed into a Head Space vial and add 2.0 mL of DMSO was accurately pipetted in to the Sample vial. The vial was sealed with aluminum closure.

Calculation

Theresidual solvent Content was calculated from,

$$PPM(OVI)= \frac{\text{Solvent Area in Sample}}{\text{Solvent Area in Standard Solution}} \times \frac{\text{Standard Solution Concentration}}{\text{Sample Solution Concentration}} \times 10^6$$

Method validation

The method was validated according to the ICH guidelines and all validation parameters useful to evaluate the overall performance of analytical method were investigated including specificity, linearity, System suitability, accuracy, Ruggedness, Robustness, Limit of detection and Limit of quantification, LOQ-Precision and LOD-Accuracy.[8]

Specificity: Topiramate API sample was spiked with Acetone, IPA, Acetonitrile, Dichloromethane, n-Hexane, Ethyl acetate, Tetra Hydro Furan, Pyridine, and Toluene individually and each sample was chromatographed to examine interference, if any, of the residual solvents' peak with each other.

System suitability: A system suitability criterion was taken to be the %RSD. The system suitability was evaluated by injecting the standard solution on various days before starting the any exercise during validation study.

Linearity: The linearity of the method was determined by making injections of each residual solvent over the range of limit of quantification (LOQ) to 200% of the standard concentration as mentioned earlier. System precision six vials of standard solution were prepared and chromatographed in the GC system.

Detection and quantification limits

The LOD of residual solvents in Topiramate API were determined based on Linearity. The LOQ of residual solvents were determined based on Linearity. Six replicates were performed at each level.

Accuracy: Known amount of sample (about 500 mg) was taken separately in nine different vials and spiked with known quantities of Acetone, IPA, Acetonitrile, Dichloromethane, n-Hexane, Ethyl acetate, Tetra Hydro Furan, Pyridine and Toluene at three different levels, in triplicate.

RESULT AND DISCUSSION

Method development: An understanding of the nature of the various residual solvents present in API is the foremost prerequisite for successful method development in HSGC. In addition, successful method development should result in a fast, simple and time efficient method that is capable of being utilized in a manufacturing setting. Following were the stepwise strategies for the method development in our case.

Column selection: The primary goal of column selection was to resolve a total of 9 residual solvents (i.e., Acetone, IPA, Acetonitrile, Dichloromethane, n-Hexane, Ethyl acetate, Tetra Hydro Furan, Pyridine and Toluene.) which were used during the synthesis and manufacturing of Topiramate. Several columns were initially investigated to finalize a single column for the separation and quantitation of solvents. Wall-coated capillary columns of various brands with a variety of phases and dimensions have been investigated, e.g., ZB-624 (30 m length, 0.32 mm i.d. with a stationary phase of 6% cynopropyl phenyl and 94% dimethyl polysiloxane film of 3.0 µ), VF-624 ms (60 m length, 0.32 mm i.d. with a stationary phase of 6% cynopropyl phenyl and 94% dimethyl polysiloxane film of 1.0 µ). In the above column, the response was found to be comparatively lower and peak shapes were found to be unsatisfactory. Therefore, VF-624 ms with dimensions of 60 m × 0.32 mm, 1.0 µ proved to be the best column that could fulfill all the needs of the method, i.e., higher sensitivity, shorter runtime and higher resolution between the critical pairs.

Headspace method optimization: The headspace method was optimized in such a way that maximum amount of the solvents present in the sample get evaporated for the detection. For this the standard and sample Vials were heated at 60°C to 95ºC for 20 to 30min with constant shaking. A combination of sample vial heating at 80ºC with 30 min shaking was found to be suitable for getting a good response.Table 1 shows the complete headspace conditions.

Method Validation: The method validation was done by evaluating specificity, limit of detection (LOD) and limit of quantitation (LOQ), linearity, accuracy, repeatability, and LOQ- precision, LOQ –Accuracy, Ruggedness and Robustness of residual solvents as indicated in the ICH guideline.

Specificity: The relative retention time of the all-residual solvents indicated that they were well separated from each other [Table 2]. A typical chromatogram of standard solution is shown in Fig.3.

Table 2: Specificity data for Nine OVI'S

S. No.	Name of OVI	RT	Theoretical Plates	Tailing Factor
1	Acetone	7.03	146657	1.062
2	IPA	7.23	141688	1.011
3	Acetonitrile	7.52	201187	1.107
4	MDC	7.84	172977	1.110
5	n-Hexane	8.66	186425	1.035
6	Ethyl Acetate	9.67	292979	1.049
7	THF	10.03	305658	1.060
8	Pyridine	12.84	592291	1.282
9	Toluene	12.97	616620	1.068

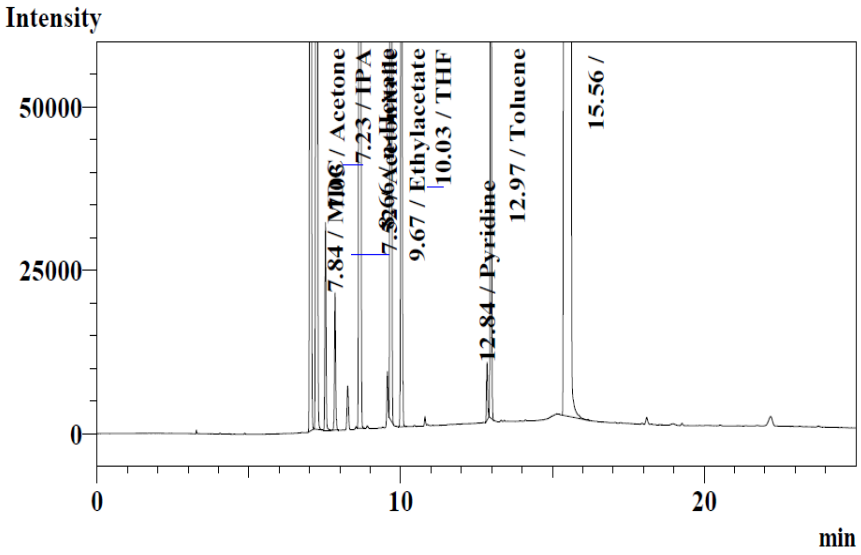


Fig. 3: Specificity Chromatogram of Nine OVI'S Standard Solution.

System Suitability: System suitability was evaluated by injecting six replicates of standard solution into the Chromatographic system as per the test method and solvents peak area was measured. % RSD was calculated and the results revealed that the system meets the required System suitability. Results are summarized in Table 3. %RSD of each Impurity is NMT 15%. A typical chromatogram of six standard solutions is shown Fig, 4.

Table. 3: System Suitability data for Nine OVI'S.

	Acetone	IPA	Acetonitrile	MDC	n-Hexane	EA	THF	Pyridine	Toluene
Run-1	1292853	1292772	81811	60825	1771101	1276409	671657	22515	479829
Run-2	1314148	1542205	86366	60947	1779548	1303589	667619	28347	497603
Run-3	1298894	1319779	82095	60004	1729443	1278824	656815	23008	481487
Run-4	1312228	1321420	82417	60534	1744782	1297204	665818	22886	487118
Run-5	1307744	1527017	84993	60067	1744829	1292496	661818	28000	493074
Run-6	1325277	1365575	84397	60963	1736595	1308227	670114	23886	496109
ACVG	**1308524**	**1394795**	**83680**	**60557**	**1751050**	**1292792**	**665640**	**24774**	**489203**
STDV	**11532**	**110893**	**1847**	**432**	**19839**	**12945**	**5526**	**2674**	**7550**
RSD	**0.88**	**7.95**	**2.21**	**0.71**	**1.13**	**1.00**	**0.83**	**10.79**	**1.54**

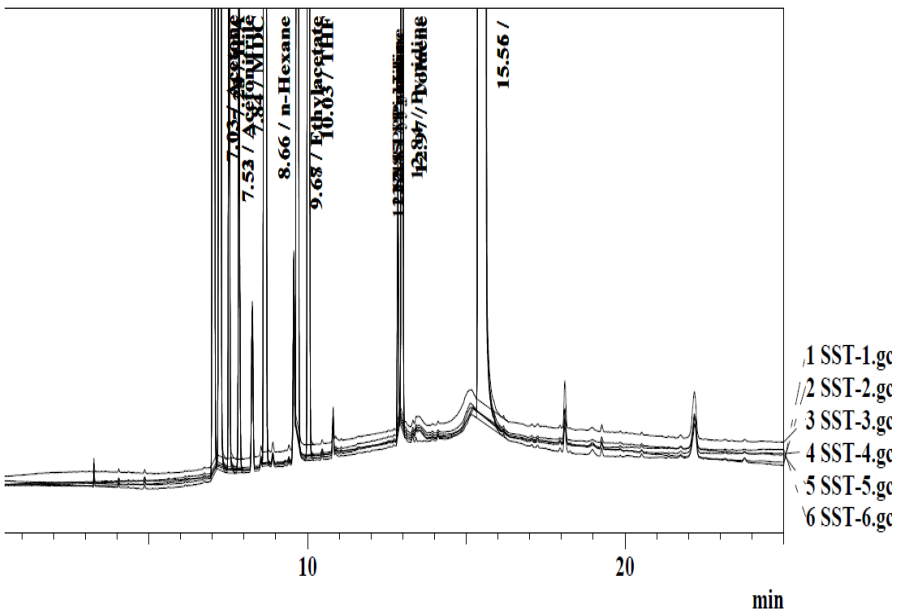


Fig. 4: System Suitability Chromatogram of Nine OVI'S Standard Solution.

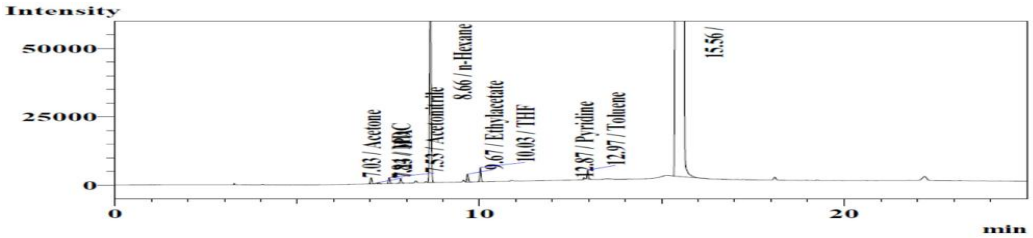
Linearity(Low level) for LOD and LOQ: Linearity of the method was determined over the concentration range of 1% 3% 5% 7% and 10%. Correlation coefficient (R^2), STEYX, SLOPE, LOD and LOQ were calculated from these linearity data and shown in Table 4.

Table. 4: Low level Linearity data for LOD and LOQ.

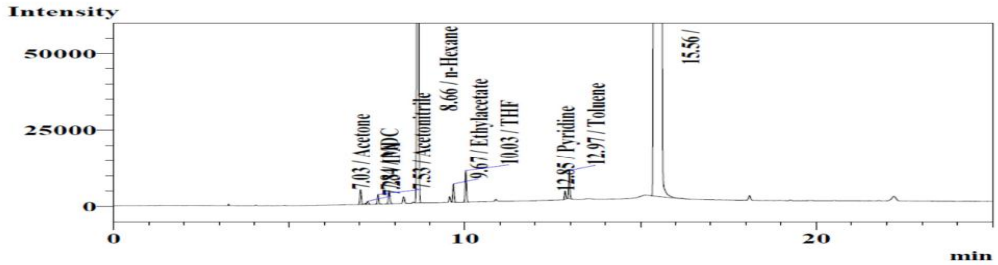
Con.(%)	Acetone	IPA	Acetonitrile	MDC	n-Hexane	EA	THF	Pyridine	Toluene
1	12277	14611	2362	718	11884	11991	6199	497	5905
3	35116	39575	3378	1789	35076	34447	17559	732	14739
5	60264	62091	5373	2982	73594	59167	30030	1082	24667
7	84181	86399	6922	4065	98033	82462	41981	1511	33748
10	121583	125727	9279	5773	142441	119622	60639	1923	48025
r2	**1.000**	**1.000**	**0.996**	**0.998**	**0.999**	**0.998**	**1.000**	**0.996**	**0.996**
STEYX	**714**	**1352**	**241**	**31**	**3837**	**791**	**368**	**59**	**271**
SLOPE	**12177**	**12257**	**790**	**563**	**14721**	**11980**	**6065**	**165**	**4695**
LOQ	**0.59**	**1.10**	**3.05**	**0.54**	**2.61**	**0.66**	**0.61**	**3.56**	**0.58**
LOD	**0.19**	**0.36**	**1.01**	**0.18**	**0.86**	**0.22**	**0.20**	**1.17**	**0.19**

Limit of detection (LOD) and limit of quantification (LOQ):

The LOD and LOQ for the proposed method were determined using calibration standards and calculated using 3.3 σ /s and 10 σ/s formulae respectively, where s is the slope of the calibration curve and σ is the standard deviation ofy- intercept of the regression equation. Chromatograms are shown in Fig 5.



(a) LOD



(b) LOQ

Fig. 5: (a) LOD and (b) LOQ Chromatogram of Nine OVI'S.

Linearity with LOQ

Linearity of the method was determined over the concentration range of LOQ, 25%. 50% 75% 100%, 150% and 200%. Correlation coefficient ($r^{2)}$ of each impurity is NLT 0.99. The linearity data and graphs as shown in Table 5 & Fig 6.

Table. 5: Linearity data with LOQ.

Con.(%)	Acetone	IPA	Acetonitrile	MDC	n-Hexane	EA	THF	Pyridine	Toluene
LOQ Con.	12375	3176	6695	13508	13435	14678	25274	728	22667
25	324314	496772	25635	60218	348142	569983	165453	5110	112235
50	654907	844772	45482	75516	686295	686295	295449	11145	226313
75	980655	1230375	63809	100449	1086989	1187744	426239	16952	338403
100	1347267	1621378	86188	106217	1877412	1526776	576207	23215	464731
150	1975696	2361513	129070	144891	2557748	2149387	818085	36589	733064
200	2629355	3107645	170474	184701	3778170	2725431	1073306	50119	962866
r2	**1.000**	**0.999**	**1.000**	**0.995**	**0.995**	**0.995**	**1.000**	**0.999**	**0.999**

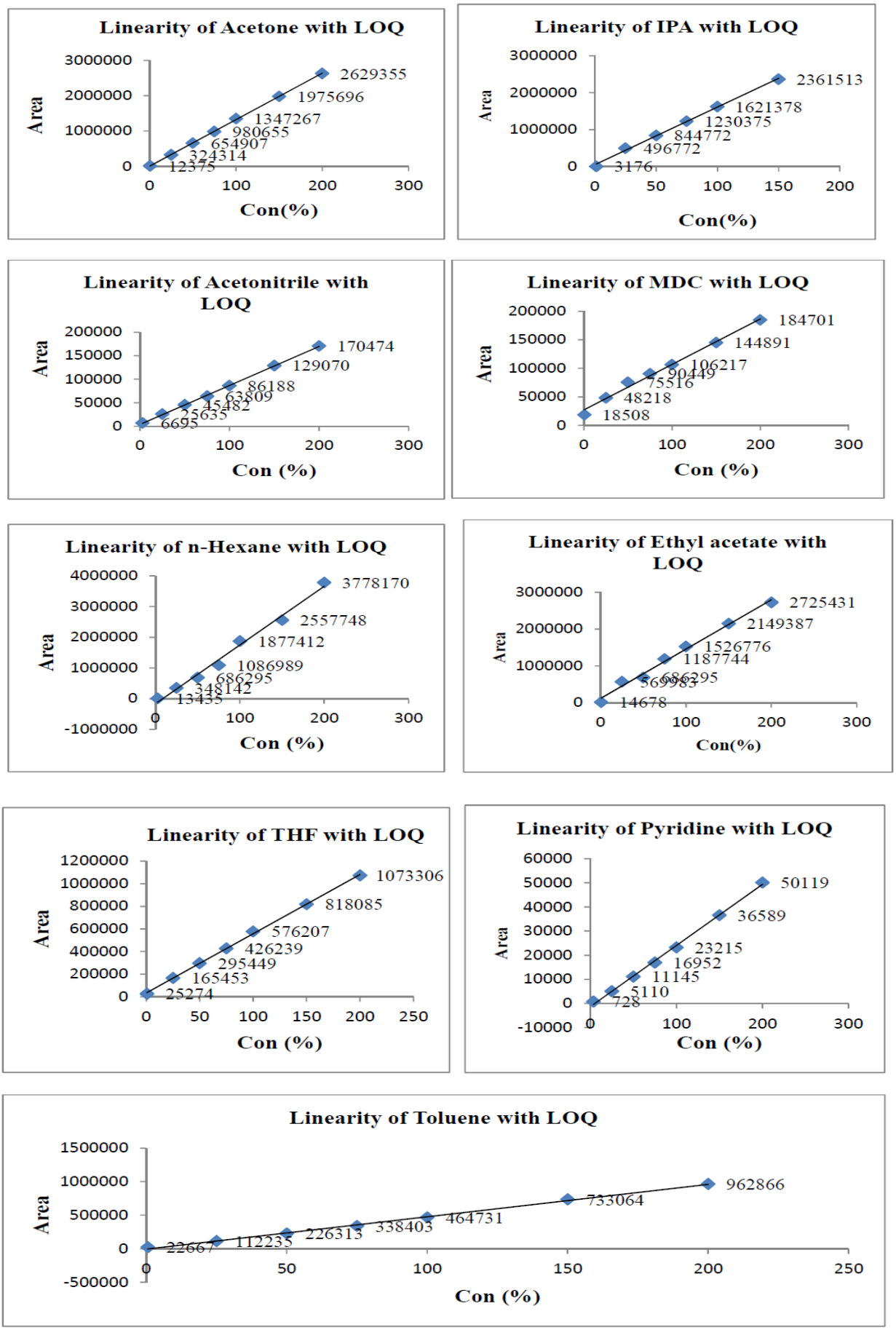


Fig. 6: Linearity with LOQ Graphs of Nine OVI'S.

LOQ-Precision: Results indicate an acceptable level of precision at LOQ level for the analytical system. The % relative standard deviation (%RSD) of area obtained from six standard vials was calculated to show the precision at LOQ. An acceptance criterion for precision was taken to be not more than NMT 15% for six vials. Result and graph as shown in Table 6 and Fig 7.

Table. 6: LOQ-Precision data.

	Acetone	IPA	Acetonitrile	MDC	n-Hexane	EA	THF	Pyridine	Toluene
Run-1	16795	8912	8119	8138	33976	14637	24868	47445	21868
Run-2	16824	8541	7526	8131	33984	14282	24328	44570	21515
Run-3	16830	10015	8085	8206	34166	14329	24406	53921	21999
Run-4	16918	8886	7876	8194	34094	14419	24624	47249	22003
Run-5	16770	10427	7946	8070	33653	14425	24195	57584	21982
Run-6	17059	8950	7981	8212	33657	14762	24831	47567	22355
ACVG	**16866**	**9289**	**7922**	**8159**	**33922**	**14476**	**24542**	**49723**	**21954**
STDV	**107**	**748**	**214**	**55**	**218**	**186**	**276**	**4938**	**271**
%RSD	**0.63**	**8.06**	**2.70**	**0.68**	**0.64**	**1.28**	**1.13**	**9.93**	**1.23**

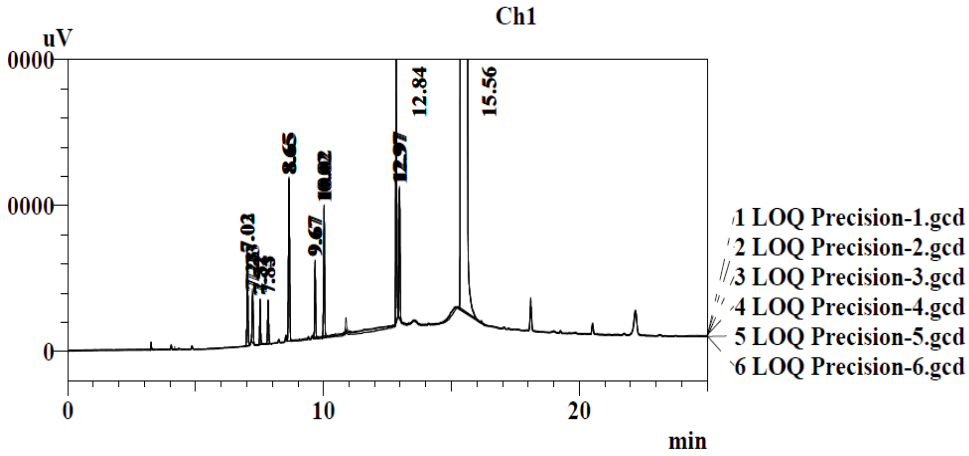


Fig. 7: LOQ-Precision %RSD Graphs of Nine OVI'S.

Accuracy: The accuracy was evaluated by the % recoveries of residual solvents spiked with the sample. An acceptance criterion for accuracy was that the recovery should be in the range of 85-115%.Results indicating that the method has an acceptable level of accuracy [Table 7].

Table. 7: %Recovery data for Nine OVI'S.

OVI	Avg.Sample Area	Avg. STD Area	Avg. 50% area	Avg.100% Area	Avg.150% Area	50% Recovery	100% Recovery	150% Recovery
Acetone	36165	1306320	665396	1427988	2159111	99.34	106.6	108.34
IPA	92269	1306435	681268	1455519	2178474	90.17	104.4	106.46
Acetonitrile	5531	85366	43989	87932	133767	90.10	96.53	100.15
MDC	14754	61187	43651	74331	114123	94.45	97.37	108.27
n-Hexane	50215	1755239	892361	1907144	2472410	95.36	105.8	92.00
EA	6233	1296139	679655	1340487	1989573	103.91	102.9	102.01
THF	8804	653425	356678	685409	1056909	106.48	103.6	106.93
Pyridine	-----	24630	11733	25655	34618	95.27	104.2	93.70
Toluene	2891	486221	233749	507210	770913	94.96	103.7	105.30

LOQ-Accuracy: Accuracy of the methods was assured by applying the standard addition technique. The % recovery was calculated. The mean % recovery of each solvent at LOQ level should be within 100+15%. Results obtained were within the limits indicating the method as accurate and are shown in Table 8.

Table. 8: LOQ %Recovery data for Nine OVI'S.

	Acetone	IPA	Acetonitrile	MDC	n-Hexane	EA	THF	Pyridine	Toluene
Run-1	24954	175629	14177	23009	87981	24005	34487	54137	26397
Run-2	24853	174215	14023	24685	86341	23756	33074	54232	25507
Run-3	24879	173257	14325	23314	82402	20263	30168	53852	23144
Average	**24895**	**174367**	**14175**	**23669**	**85575**	**22675**	**32576**	**54074**	**25016**
STD Area	**16866**	**9289**	**7922**	**8159**	**33922**	**14476**	**24542**	**49723**	**21954**
In Sample	**6334**	**165441**	**5531**	**14754**	**50215**	**6233**	**8804**	**0**	**2891**
% Recovery	**110.05**	**96.09**	**109.11**	**109.27**	**104.24**	**113.58**	**96.86**	**108.75**	**100.78**

Ruggedness: Ruggedness of the method was evaluated by performing the sample analysis in six replicates using different analyst on different days and the results are summarized as shown in Table 9. The %RSD values of less than 15.0% for Acetone, IPA, Acetonitrile, and Dichloromethane, n-Hexane, Ethyl acetate, Tetra Hydro Furan, Pyridine and Toluene content indicate that the method adopted is rugged.

Table. 9: Ruggedness data for Nine OVI'S.

Name of Impurity	Day-1			Day-2			Analyst-1	Analyst-2
	%RSD An-1	%RSD An-2	%RSD An-1&2	%RSD An-1	%RSD An-2	%RSD An-1&2	%RSD Day-1&2	%RSDDay-1&2
Acetone	1.06	0.9	0.98	1.04	1.09	1.28	1.33	2.19
IPA	11.37	9.37	9.93	3.96	2.24	3.07	8.8	7.42
Acetonitrile	4.31	4.29	4.12	0.98	1.42	1.17	3.06	3.07
MDC	2.46	0.91	3.59	0.89	2.38	5.75	3.49	11.71
n-Hexane	1.91	2.01	2.21	7.65	2.22	5.41	8.2	4.87
EA	1.06	1.16	1.6	0.86	1.3	2.26	1.35	4.34
THF	0.92	0.72	0.97	1.54	0.53	7.38	1.64	5.66
Pyridine	13.09	11.44	11.73	4.17	2.62	3.41	10.94	9.94
Toluene	2.1	2.53	2.27	1.33	1.62	1.43	1.68	2.04

Robustness: This study was performed by making small and deliberate variations in the method parameters. The variation in the column flow 2.3 ml/min and 2.7ml/min, column oven temperature 75°C and 85°C was done and the results obtained were within the acceptance criteria indicating the method is robust within the specified range. % RSD values were less than 15% as shown in Table 10.

Table. 10: Robustness data for Nine OVI'S.

Name of Impurity	Flow rate(ml/min)			Column Temperature(°C)		
	2.3ml/min	2.5ml/min	2.7ml/min	75°C	80°C	85°C
Acetone	0.87	1.34	0.9	1.47	1.34	2.2
IPA	6.39	10.10	7.00	6.18	10.10	7.24
Acetonitrile	2.21	4.27	2.44	2.5	4.27	3.91
MDC	2.58	4.47	1.53	2.27	4.47	2.4
n-Hexane	1.19	5.96	1.66	5.81	5.96	5.12
EA	1.45	2.44	1.02	1.64	2.44	2.5
THF	0.92	1.63	0.9	1.59	1.63	2.22
Pyridine	8.08	10.58	7.82	6.09	10.58	8.15
Toluene	1.36	2.73	1.12	1.64	2.73	2.97

CONCLUSION

As per ICH guidelines, the quantification of residual solvents is mandatory for all the pharmaceutical products and excipients before their release into the market because of its potential toxicity and may be the interference with the quality. Based on this point, we developed a method with an objective of development and validation of simple analytical method for simultaneous quantification of residual solvents present in Topiramate by HS-GC with flame ionization detector. The residual solvents Acetone, IPA, Acetonitrile, Dichloromethane, n-Hexane, Ethyl acetate, Tetra Hydro Furan, Pyridine and Toluene were well separated from each other and quantified by the proposed method. This method was also applied for the quantification of residual solvents in the marketed Topiramate, which were present in ppm specification limits as per ICH guidelines. The proposed method was validated as per the ICH guidelines and the results revealed that the method was scientifically. Finally we conclude that the proposed method can be effectively applied for the quantification of residual solvents present in Topiramate.

This investigation may be helpful to the manufacturers for controlling and minimization of the residual solvents. And this method was found to be applicable for the routine analysis of the Topiramate in pharmaceutical industry.

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