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Original Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION BY HEADSPAVE GAS CHROMATOGRAPHY FOR RESIDUAL SOLVENTS IN IGURATIMOD

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Abstract: Testing of residual solvent is a primary requirement for any drug substance. These studies provide information about the residual solvents content in Iguratimod with simple, accurate, precise by headspace gas chromatography. Method by head space gas chromatography with flame ionization detector has been developed and validated to detect and quantitate the specified solvents. Baseline separation in-between the peaks were been achieved by suing capillary column with a flame ionization detector. Percentage recovery obtained in the range of 80-120% and the method is linear for all the specified solvents as per synthesis route of synthesis of Iguratimod. Range for these method is listed for each solvent - Ethanol (50ppm - 7500ppm), Acetone (50ppm - 7500ppm), Isopropyl alcohol (50ppm - 7500ppm), Acetonitrile (20ppm - 615ppm), Dichloromethane (30ppm - 900ppm), Ethyl acetate (50ppm - 7500ppm), Methanol (30ppm - 4500ppm), Pyridine (20ppm - 300ppm), Dimethyl formamide (88ppm - 1320ppm), Benzene (0.2ppm - 3ppm), Nitrobenzene (3ppm - 45ppm). All five methods are having the coefficient of variation (r) not less than 0.99. This proposed methodology was found precise, linear and accurate for the specified range for respective solvents.

Keywords: Iguratimod, validation, development.

Introduction: Residual solvents in drug substance or drug products are a potential toxic risk factor and are major concerns for manufacturer. These residual solvents can affect the quality and stability of active pharmaceutical ingredient and pharmaceutical dosage form. Thus, acceptable levels of these residual solvents are incorporated as per ICH guidelines. Residual solvents can be classified into four different classes due to their toxicity level and potential environmental hazard.

Class 1 solvents are to be avoided because these are known carcinogens and can harmful to humans as well as environment, but can be used with rationale.

Class 2 solvents are to be limited use due to their inherent toxicity.

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Class 3 solvents can be used where these can be removed by synthetic process because these solvents are low toxic potential to humans.
Class 4 these solvents don't have adequate toxicological data.

All these solvents can be analyzed by chromatographic techniques such as static headspace gas chromatography (HS-GC).

Experimentation:

All five methods were analyzed by gas chromatographic instrument using flame ionization detector (FID).

CHROMATOGRAPHIC CONDITIONS					
Method	Method-I	Method-II	Method-III	Method-IV	Method-V
Instrument	GCHS with autosampler	GCHS with autosampler	GCHS with autosampler	GCHS with autosampler	GC with autosampler
Column (Capillary column)	RTX-624 (30 m X 0.53 mm X 3.0µm).	RTX-624 (30 m X 0.53 mm X 3.0µm).	RTX-624 (30 m X 0.53 mm X 3.0µm).	RTX-624 (30 m X 0.53 mm X 3.0µm).	DB-1 (30m X 0.32 mm X 1.0 µm).
GC Parameters :					
Initial oven temp.	40°C	40°C	80°C	45°C	45°C
Initial hold time	10 minutes	10 minutes	5 minutes	6 minutes	2 minutes
Ramp	8°C/minute	8°C/minute	10°C/minute	10°C/minute	15°C/minute
Oven temp.II	200°C	200°C	220°C	220°C	320°C
Hold time II	5 minutes	5 minutes	2 minutes	2 minutes	2 minutes
Carrier gas	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Nitrogen
Flow	1.5 ml/min.	1.5 ml/min.	2 ml/min.	3 psi	2 ml/min.
Split ratio	20:1	20:1	10:1	10:1	10:1
Injector temp.	200°C	200°C	180°C	180°C	240°C
Detector temp.	240°C	240°C	240°C	240°C	320°C
Range:	1	1	1	1	-
Attenuation:	-6	-6	-6	-6	-
Head space parameters:					
Vial temp.	90°c	90°c	95°c	100°c	-
Needle temp.	100°c	100°c	100°c	110°c	-
Transfer line temp.	110°c	110°c	110°c	120°c	-
Headspace carrier pressure	15 psi	15 psi	15 psi	15 psi	-
GC cycle time	45 min.	45 min.	25 min.	35 min.	-
Time for Vial equilibration	20 min.	20 min.	20 min.	20 min.	-
Pressurization time	3 min	3 min	3 min	3 min	-
Injection volume	0.2ml	0.2ml	0.24ml	0.4ml	5 µl
Needle withdraw time	0.2 minute	0.2 minute	0.2 minute	0.2 minute	-
Thermostat time	20 min.	20 min.	20 min.	20 min.	-

System suitability criteria (RSD for area of replicate standard injections)	Not more than : 15.0%	Not more than : 15.0%	Not more than : 15.0%	Not more than : 15.0%	Not more than : 15.0%

Method -I (For Ethanol, ethyl acetate, 2-propanol, acetone, Acetonitrile, dichloromethane)
Method-II (For Methanol)
Method-III (For Pyridine and Dimethyl formamide)
Method-IV (Benzene)
Method-V (Nitrobenzene)

Procedure: Set the gas chromatograph and condition as mentioned above. In blank monitoring there should not be the baseline drift as well as no interference of any peak at retention time of the analyte peak. Inject blank, standard and sample as per approved protocol.

Calculate the relative standard deviation for area response of six replicate standard injections as system suitability criteria.

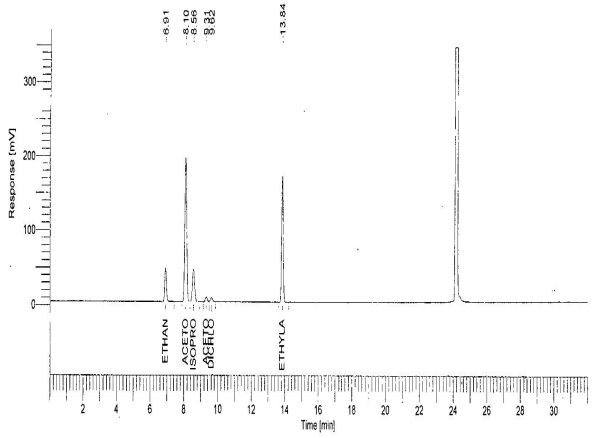
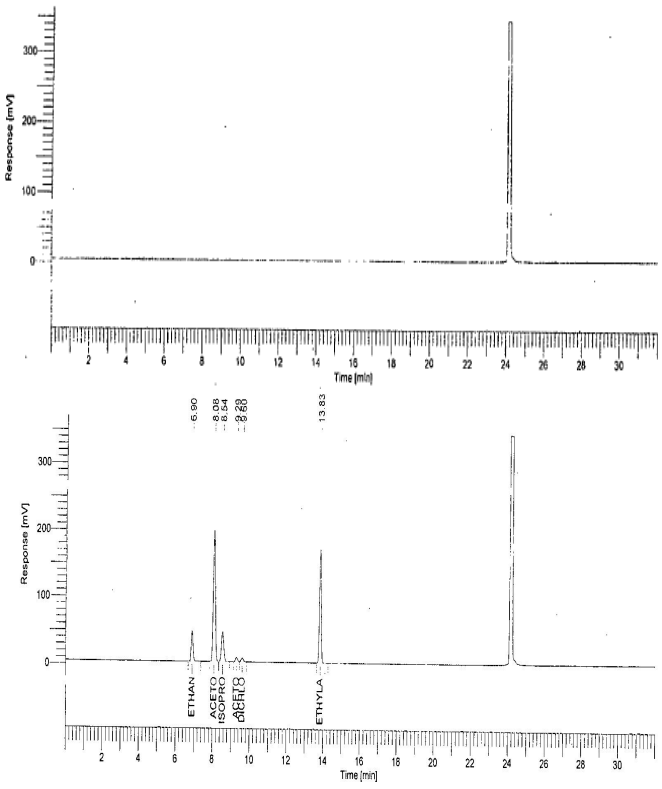
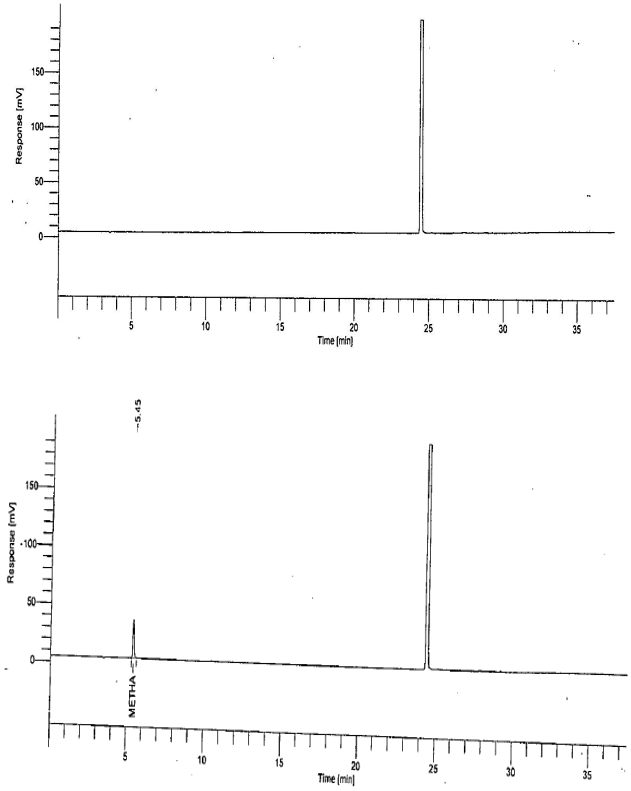


Fig. 1: Method-I: Representative chromatograms of blank, standard and spike test.





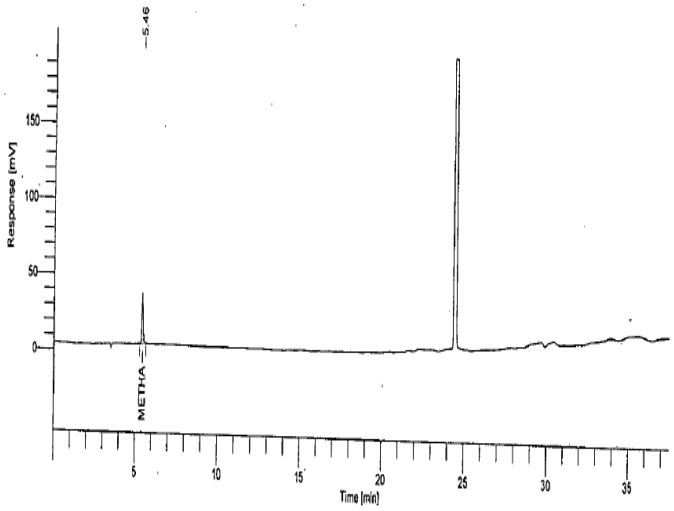


Fig. 2: Method-II: Representative chromatograms of blank, standard and spike test.

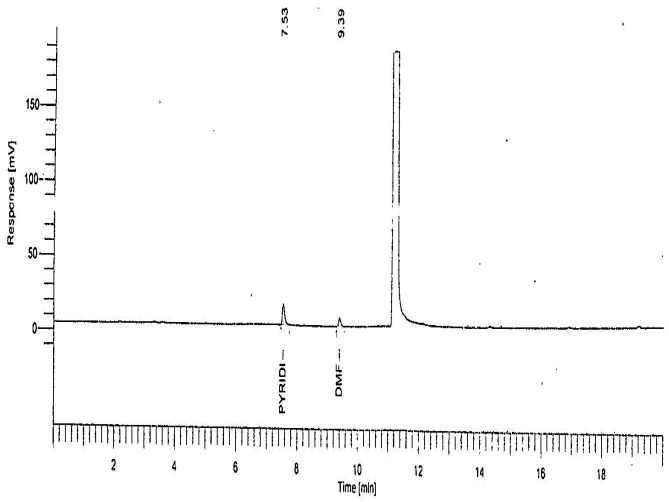
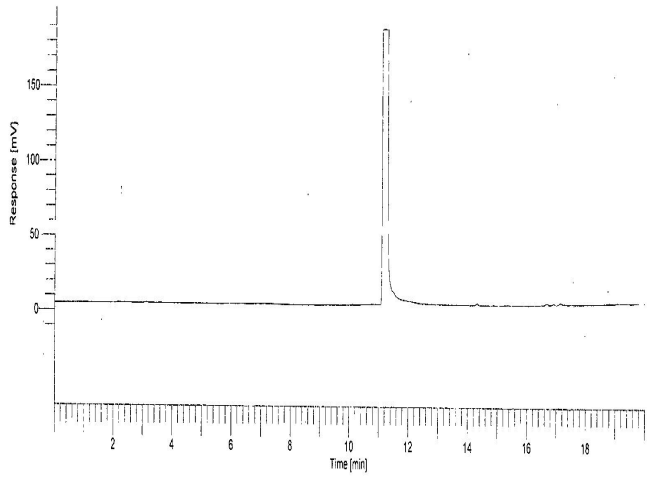
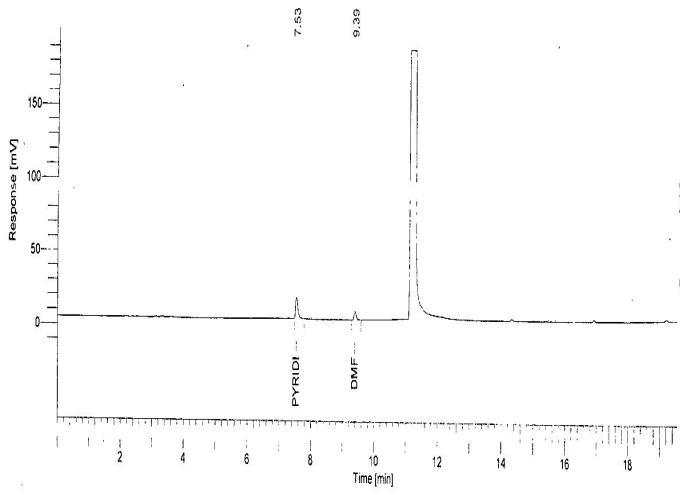
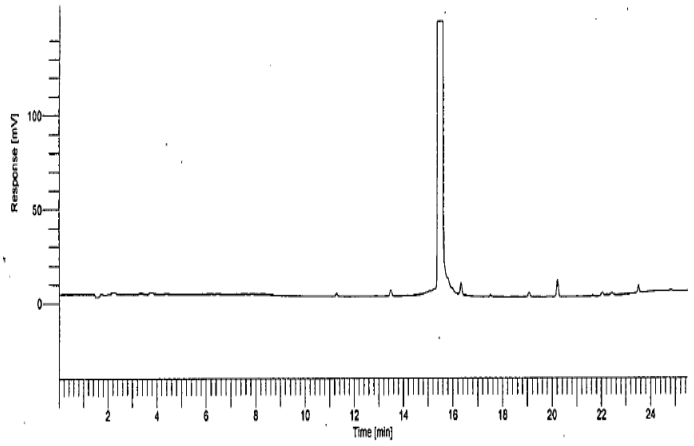
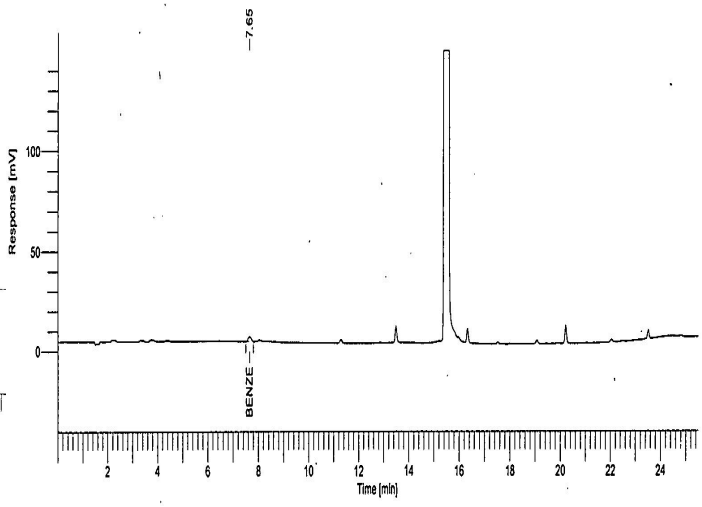


Fig. 3: Method-III: Representative chromatograms of blank, standard and spike test.









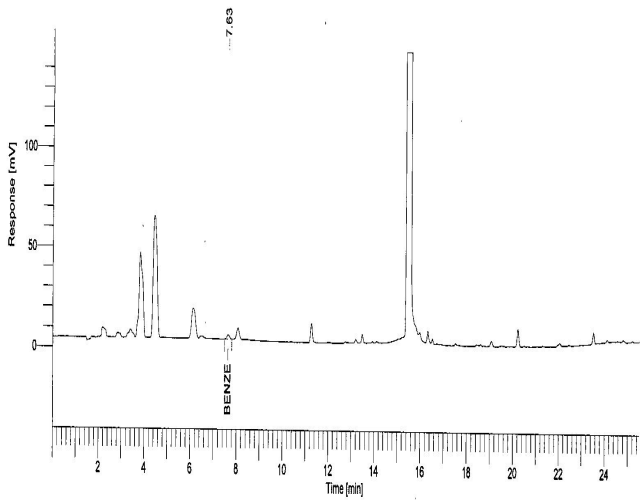
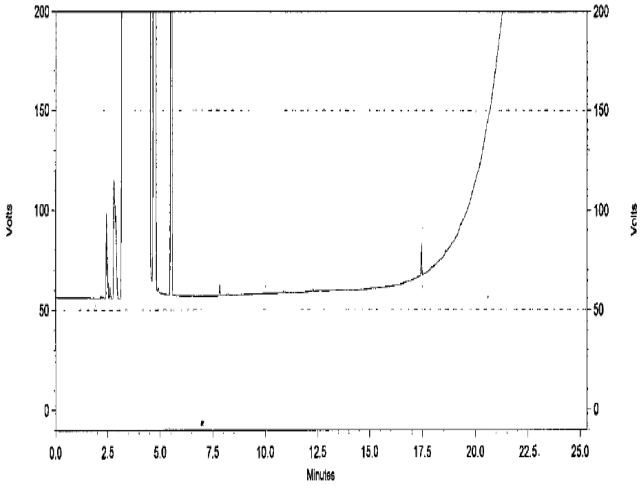
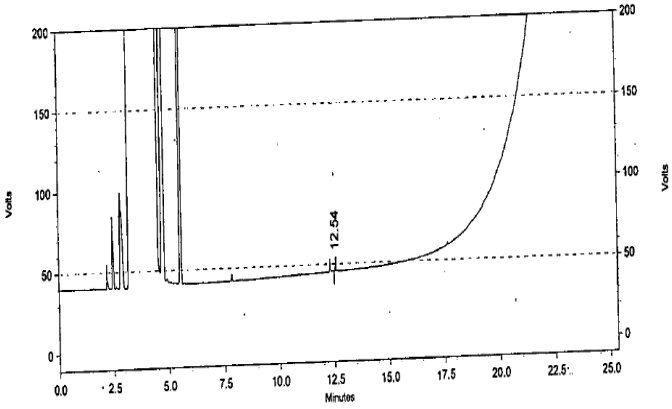


Fig. 4: Method-IV: Representative chromatograms of blank, standard and spike test.





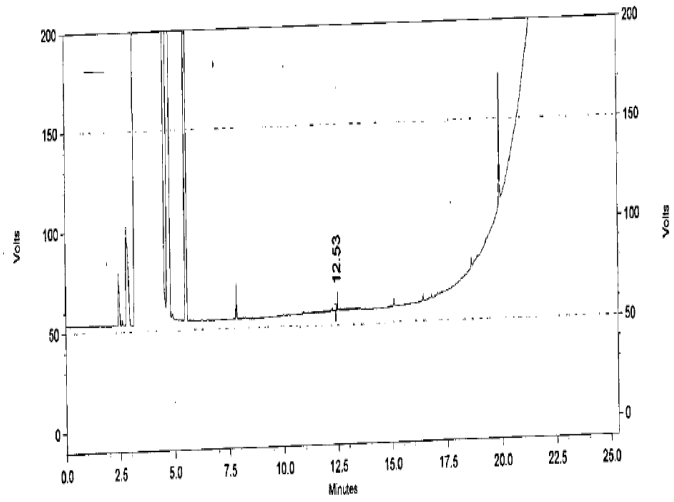


Fig. 5: Method-V: Representative chromatograms of blank, standard and spike test.

Results and Discussion: In this validation activity, analytical method by using head space gas chromatography instrument was developed and validated for quantification of solvents Ethanol, Ethyl acetate, 2-Propanol, Acetone, Acetonitrile, Dichloromethane, Pyridine, Dimethyl formamide, Methanol, Benzene and Nitrobenzene in Iguratimod. The method was validated as per ICH guideline for the parameters like selectivity, limits of detection and quantitation, linearity, precision and recovery as well as robustness (deliberate change in chromatographic conditions). Analytical results obtained by using all the five methods are well within the acceptance criteria. The test methods were validated and had good reproducibility, linearity and recovery for the respective solvents as per synthetic route of synthesis.

Selectivity: Capillary column selection was done due to standard stationary phase, which has very good baseline separations of analyte and diluents. All the five methods show good peak shapes for all the analyte peaks with excellent column efficiency. No any blank chromatogram shows any interference wrt to analyte peaks.

Specificity: Specificity was performed to demonstrate non-interference of other peaks with analyte peak during sample analysis.

Specificity has been performed by injecting blank, individual solvent, standard, test and spiked test. Representative chromatograms are shown for all method in Fig.1, Fig.2, Fig.3, Fig.4 and Fig.5.

System Precision: System precision demonstrates that the chromatographic system gives precise measurements for analytical method with replicate measurements at target concentration.

Method precision: Method precision demonstrates that the analytical method provides the precise results for replicate measurements of homogenous sample.

Linearity: Linearity proves the direct correlation between test results and concentration of analyte in sample. The linearity study was carried for solvents (Ethanol, Ethyl acetate, 2-Propanol, Acetone, Acetonitrile, Dichloromethane, Pyridine, Dimethyl formamide, Methanol, Benzene and Nitrobenzene) from LOQ concentration to 150% of specification level.

Conc.(ppm)	Average area
502.88	104591
2514.38	529195
3771.57	786988
5028.77	1041291
6285.96	1311814
7543.15	1573437
Slope =	208.2317
Correlation coefficient=	1.0000
Squared correlation coefficient=	0.9999

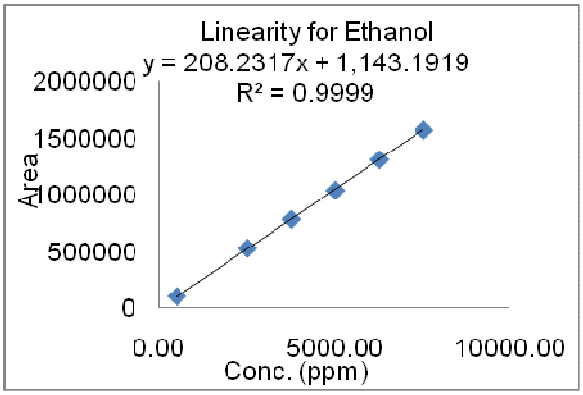


Fig. 6: Linearity plot for Ethanol

Conc.(ppm)	Average area
501.68	339772
2508.39	1648586
3762.58	2572829
5016.78	3397072
6270.97	4221316
7525.17	5045559
Slope =	672.9087
Correlation coefficient=	0.9999
Squared correlation coefficient=	0.9997

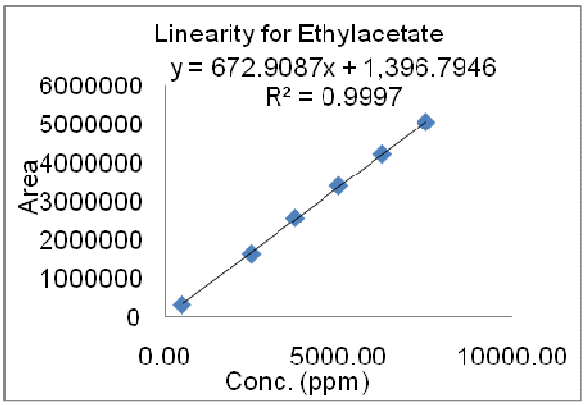


Fig. 7: Linearity plot for Ethyl acetate

Conc.(ppm)	Average area
501.28	121875
2506.39	635175
3759.59	958663
5012.78	1258175
6265.98	1597693
7519.17	1922212
Slope =	255.9086
Correlation coefficient=	0.9999
Squared correlation coefficient=	0.9998

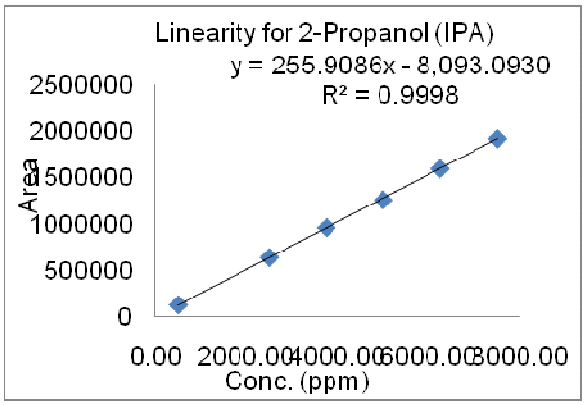


Fig. 8: Linearity plot for 2-Propanol

Conc.(ppm)	Average area
500.38	453762
2501.90	2288711
3752.84	3481217
5003.79	4574623
6254.74	5768029
7505.69	6861435
Slope =	916.8195
Correlation coefficient=	0.9999
Squared correlation coefficient=	0.9999

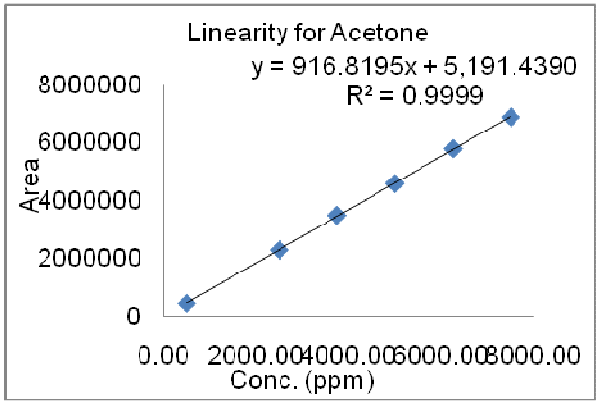


Fig. 9: Linearity plot for Acetone

Conc.(ppm)	Average area
41.28	15375
206.39	77686
309.59	115068
412.79	153357
515.98	196464
619.18	230603
Slope =	375.0366
Correlation coefficient=	0.9998
Squared correlation coefficient=	0.9995

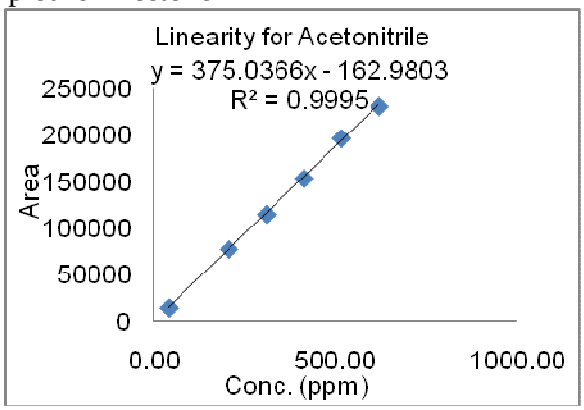


Fig. 10: Linearity plot for Acetonitrile

Conc.(ppm)	Average area
61.90	16828
309.49	83804
464.24	121522
618.98	165568
773.73	205985
928.47	251302
Slope =	269.0169
Correlation coefficient=	0.9998
Squared correlation coefficient=	0.9995

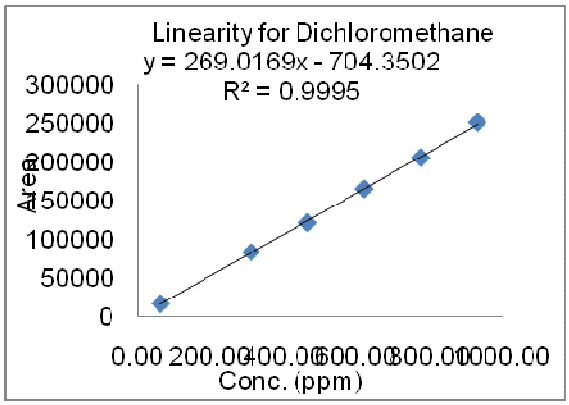


Fig. 11: Linearity plot for Dichloromethane

Conc.(ppm)	Average area
302.48	11514
1512.40	55527
2268.60	83875
3024.80	113441
3781.00	138139
4537.20	164771
Slope =	36.3445
Correlation coefficient=	0.9997
Squared correlation coefficient=	0.9995

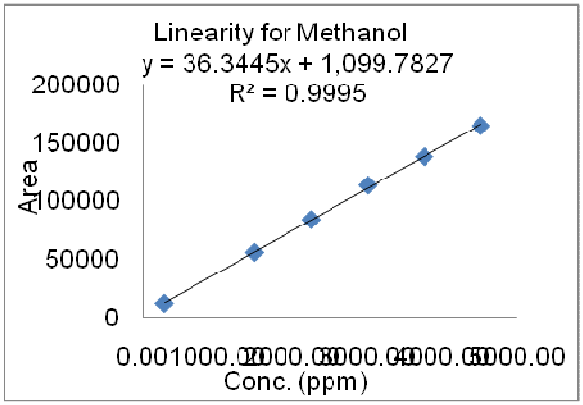


Fig. 12: Linearity plot for Methanol

Conc.(ppm)	Average area
20.94	7848
104.70	37404
157.04	56516
209.39	74088
261.74	93060
314.09	112023
Slope =	354.5921
Correlation coefficient=	1.0000
Squared correlation coefficient=	0.9999

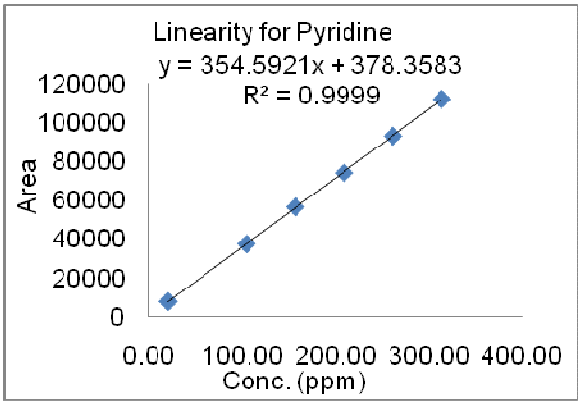


Fig. 13: Linearity plot for Pyridine

Conc.(ppm)	Average area
89.17	2460
445.85	13819
668.78	19977
891.71	26639
1114.63	32599
1337.56	39495
Slope =	29.3541
Correlation coefficient=	0.9997
Squared correlation coefficient=	0.9993

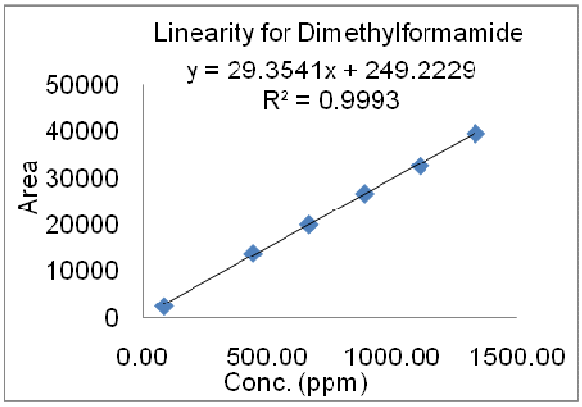


Fig. 14: Linearity plot for Dimethyl formamide

Conc.(ppm)	Average area
0.20	1962
1.01	9360
1.52	14546
2.03	19925
2.53	24047
3.04	28989
Slope =	9575.2004
Correlation coefficient=	0.9996
Squared correlation coefficient=	0.9992

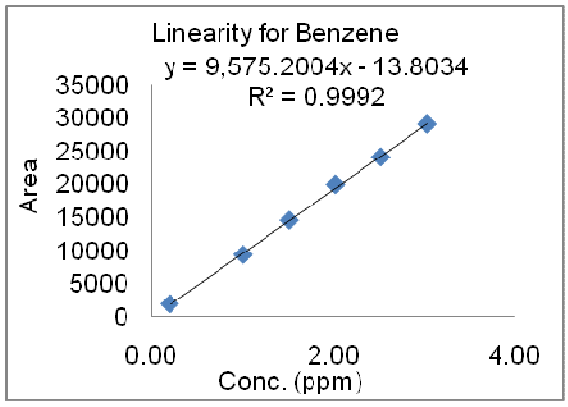


Fig. 15: Linearity plot for Benzene

Conc.(ppm)	Average area
3.11	3532
15.57	17567
23.35	26715
31.13	35533
38.92	45091
46.70	53230
Slope =	1148.7674
Correlation coefficient=	0.9999
Squared correlation coefficient=	0.9998

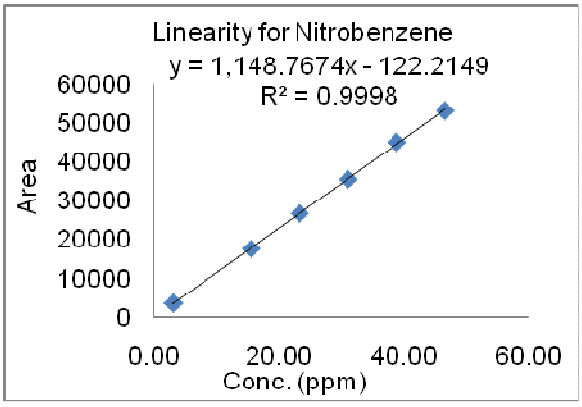


Fig. 16: Linearity plot for Nitrobenzene

LOD (limit of detection) and LOQ (limit of quantitation) determination:

LOD: Detection of lowest amount of analyte peak in sample to be analyzed.

LOQ: Quantification of lowest amount of analyte peak in sample to be analyzed.

LOD and LOQ can be determined by different method like signal to noise ratio, residual standard deviation, visual basis, etc.

Accuracy: Accuracy is the closeness of obtained results with the true value.

Robustness: Robustness can be demonstrated by deliberate change in chromatographic condition obtained results are well within acceptable criteria wrt to standard chromatographic conditions.

VALIDATION SUMMARY REPORT						
Method	Method-I	Method-II	Method-III	Method-IV	Method-V	Acceptance criteria
Specificity	No interference observed	No interference observed	No interference observed	No interference observed	No interference observed	No interference should observed at retention time of analyte
System Precision	RSD below 15.0%	RSD below 15.0%	RSD below 15.0%	RSD below 15.0%	RSD below 15.0%	RSD for area of replicate standard injections should be NMT 15.0%
Method Precision	RSD below 10.0%	RSD below 10.0%	RSD below 10.0%	RSD below 10.0%	RSD below 10.0%	RSD for test results should be NMT 10.0%
Linearity	Correlation coefficient more than 0.999	Correlation coefficient more than 0.999	Correlation coefficient more than 0.999	Correlation coefficient more than 0.999	Correlation coefficient more than 0.999	Correlation coefficient should be NLT 0.98
Accuracy	RSD for recovery of analyte is between 80.0% to 120.0%	RSD for recovery of analyte is between 80.0% to 120.0%	RSD for recovery of analyte is between 80.0% to 120.0%	RSD for recovery of analyte is between 80.0% to 120.0%	RSD for recovery of analyte is between 80.0% to 120.0%	RSD for recovery of analyte should be between 80.0% to 120.0%
Robustness Flow(±0.1ml /min),Temp. (±2.0°C)	RSD below 10.0%	RSD below 10.0%	RSD below 10.0%	RSD below 10.0%	RSD below 10.0%	RSD for test results should be NMT 10.0%

Method -I: For Ethanol, ethyl acetate, 2-propanol, acetone, Acetonitrile, dichloromethane

Method-II: For Methanol

Method-III: For Pyridine and Dimethyl formamide

Method-IV: For Benzene

Method-V: For Nitrobenzene

Table 1: Validation summary report

Conclusions: The analytical method proposed for the quality control of Iguratimod to analyze the residual Ethanol, Ethyl acetate, 2-Propanol, Acetone, Acetonitrile, Dichloromethane, Pyridine, Dimethyl formamide, Methanol, Benzene and Nitrobenzene contents, met the validation requirements. Results were obtained are well with globally accepted validation criteria. The method was sensitive, linear, accurate and precise. The drug substance was

analyzed under validated method conditions and the concentrations of residual Ethanol, Ethyl acetate, 2-Propanol, Acetone, Acetonitrile, Dichloromethane, Pyridine, Dimethyl formamide, Methanol, Benzene and Nitrobenzene was much lower than their maximum ICH limits.

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