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METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CLASS II SLOVENTS IN LULICONAZOLE BY HSGC-FID

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1. ABSTRACT

Method Development and validating the HS-Gas chromatography was carried on DB-624, (30mx0.53mm) 3.0micron column, using quantified solvents and by modifying the temp. of oven, flow of carrier gas with Flame ionization detector. The process was linear at 25-150 µg /ml for solvents Methanol, Cyclohexane, Methyl isobutyl ketone and Toluene (r^2>0.999). So, developed method shows good agreement with increased conc. Levels of Luliconazole. Recovery studies determined at 50%, 100% and 150% levels, it was inferred to be in acceptance limits 80% to 120%. Precision performed with 6 replicates using the solvents Methyl isobutyl ketone, Tolune, Cyclohexane, methanol using DMSO as diluent was inferred that the RSD% in limits. Recovery experiments indicated the absence of interference from commonly encountered diluent and API. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 10 for Methanol, Cyclohexane, Methyl isobutyl ketone and Toluen. The method found to be within limits for detection and quantification limit.

KEYWORDS: Luliconazole, HS-GC, FID Detectors, Residual solvents.

2. INTRODUCTION

Quality investigation plays a very important role in quality specification establishment of chemical drugs. The number of drugs introduced into the market every year .very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs. Solvents used in the manufacture of active pharmaceutical ingredients (APIs) or drug substances and excipients or in the formulation of drug products are often necessary.

Literature survey reveals that no analytical method was reported earlier for estimation of residual solvents in Luliconazole by HS-GC. The main aim is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for estimation of class II solvents in Luliconazole.

Table 1: Literature review.

Author	Title
N. Jahnavi and VS. Saravanan*	Analytical method for residual solvents determination in glibenclamide by gas chromatography (GC/FID) with head space.
N. Jahnavi and VS. Saravanan*	Analytical method for residual solvents determination in omeprazole by HSGC/FID.
Clecio S. Ramos	Residual solvents estimation as per validation guidelines as per ICH in 5 drug substances using head space and flame ionization detector.

Solubility determination of Luliconazole by various Residual solvents. Determine the Physical properties like Boiling point, Solubility, Polarity etc Optimize the Gas chromatography conditions for proper resolution and retention times. Validate the developed method as per ICH guidelines.

Luliconazole, sold under the brand name Lulimac, is used to treat athlete's foot that is between the toes (Interdigital tinea pedis). To treat jock itch (Tinea cruris), and ringworm (Tinea corporis).

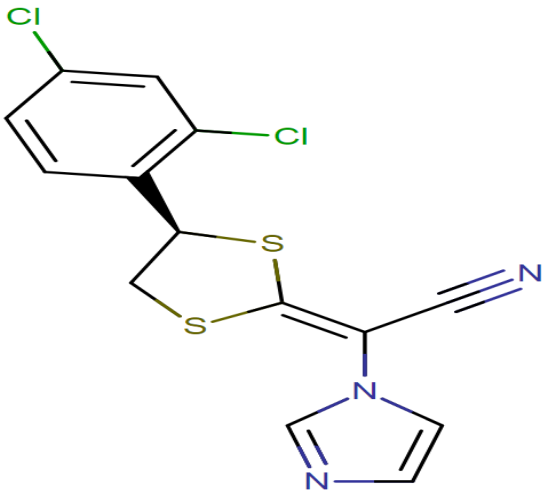

Fig 1: Chemical structure of Luliconazole.

IUPAC Name: 2-[(2E,4R)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-2-(1H-imidazol-1-yl)acetonitrile.
Molecular Formula: $C_{14}H_9Cl_2N_3S_2$

Molecular Weight: 247.247.

Category: Antifungal Agent

Mechanism of Action
Luliconazole acts by inhibition of lanosterioldemethylase enzyme, as this enzyme is the main component of cell membrane of fungi.

Indication
Indicated for the treatment of tinea corporis caused by the organisms Trichophyton rubrum and Epidermophyton floccosum. Indicated for the treatment of tinea cruris caused by the organisms Trichophyton rubrum and Epidermophyton floccosum.

Indicated for the treatment of interdigital tinea pedis caused by the organisms Trichophyton rubrum and Epidermophyton floccosum.

3. MATERIALS AND METHODS

Drug used: Luliconazole API.

Table 3.1: Instruments used.

Software for Data Acquisition	Open labs EZchrome
Electronic balance	Metler Toledo
Gas Chromatography Make	Agilent Infinity
Modelof GC	7697A
Column used in GC	DB-624 column,(30mx0.53mm) 3.0µm

Table 3.2: Reagents used.

Toluene	GC Grade(Make: Sigma Aldrech)
Cyclohexane	
Methylisobutyl ketone	
Dimethyl sulfoxide	GC Grade(Make: Qualigens)
Methanol	

4. SELECTION AND DESCRIPTION OF PARTICIPANTS

4.1 Solubility Studies for active entity

The solubility of Luliconazole (active entity) is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF) and low solubility in water. In this two solvents DMF and DMSO, DMSO has high solubility so DMSO has diluent.

These studies are carried out at 25 ^{0} C.

Solvents quantified
1.0 Methanol
2.0 Cyclohexane
3.0 Toluene
4.0 Methyl iso butyl ketone

4.2 Determination of Boiling Points

Table 4.1: Boiling Points.

S.No	Solvents Name	Temperature(°C)
01	Methanol	64.7
02	Cyclohexane	81.0
03	Toluene	110.6
04	Methyliso butyl ketone	117-118

Diluent: Use Dimethyl sulfoxide (DMSO).

Preparation of Blank

Transfer 5.0 ml of diluent in headspace vial containing 200mg of sodium chloride and seal the vial immediately.

Standard Sock-I Preparation

Weigh accurately about 500 mg of Methanol, 500 mg of Cyclohexane, 500 mg of Toluene 500mg of mg Methyliso butyl ketone in 100ml flask containingabout20 ml of diluent, make up to volume with diluent and shake well.

Standard Sock-II Preparation

Take2 ml of above solution in 100 mlflask containing about 20 ml diluent, make up to volume with diluent. Withdraw 5 ml of above solution intoGC vial with 0.2gm NaCl andseal the vial.

Test Sample Preparation

Weigh accurately about 500 mg of test sample (Lulucinazole API) and transfer in to 25mL volumetric flask add 15mL of Diluent, vortex it for 5min. Then made the capacity with Dimethyl sulfoxide mix well.

4.3 METHOD DEVELOPMENT OF RESIDUAL SOLVENTS

GC Parameter and Condition

Split Ratio : 5:1 Column
Detector Temp: 2500C
Oven Temp: 100^0C
Column:DB-624 column, (60mx0.50mm) 3.0µm
Carrier Gas: Nitrogen
Flow: 1.2 ml/min.

Head Space Parameters

Vial Temp. : 80°C
Loop Temp. : 95°C
Transfer line Temp. : 105°C
GC cycle Time : 40 min.

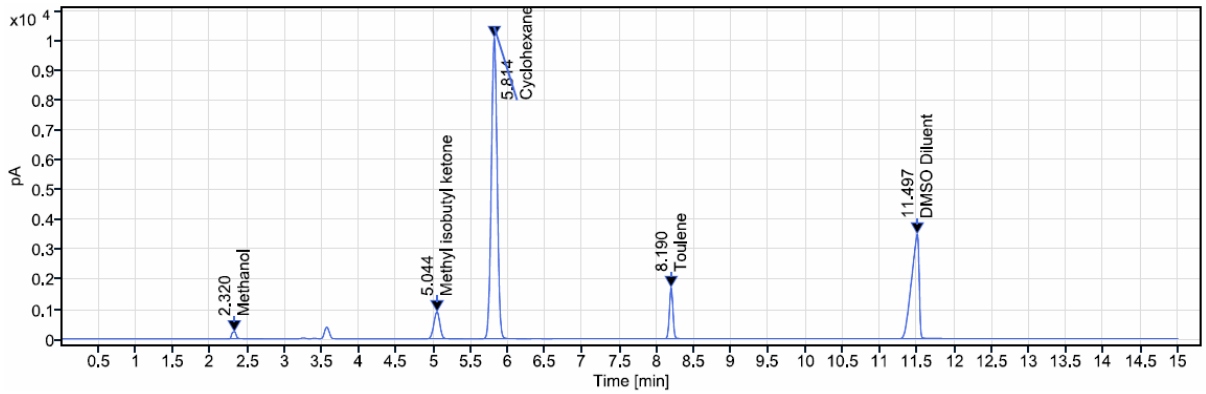

Fig 1: Chromatogram of Optimized Trial.

Observation

This Trial taken as a Optimised Trial, since all peaks observed with high resolution, theoret9ical plate count and asymmetry factor were in acceptance limits.

5. TECHNICAL INFORMATION

5.1 System Suitability and System Precision

Sock-I Preparation

Weigh accurately about 500 mg of Methanol, 500 mg of Cyclohexane, 500 mg of Toluene 500mg of mg Methyliso butyl ketone in 100ml Volumetric flask containing about20 ml of diluent, make up to volume with diluent and shake well.

Standard Sock-II Preparation

Withdrawn 2 ml of into 100 ml flask containing 20 ml DMSO, and final capacity with DMSO.

Standard prepared in to six head space vials and injected in to HS-GC and calculated %RSD for responses.

5.2 Specificity by Direct comparison method

There is no interference of Diluent with the solvent peak and no interference of the API peak at the retention time of the solvent peaks.

Preparation of Diluent: Use Dimethyl sulfoxide (DMSO).

Preparation of Blank
Transfer 5.0 ml of diluent in headspace vial containing 200mg of sodium chloride and seal the vial immediately.

Stock-I Preparation
Weigh accurately about 500 mg of Methanol, 500 mg of Cyclohexane, 500 mg of Toluene and Methyliso butyl ketone in 100ml flask with 20 ml DMSO and final mark with DMSO.

Stock-II Preparation
Withdrawn 2 ml of above solution in 100 ml flask with 20 ml diluent, make with diluent.

Withdrawn 5 ml and transferred into GC system with 0.2gm sodium chloride andseal the vial.

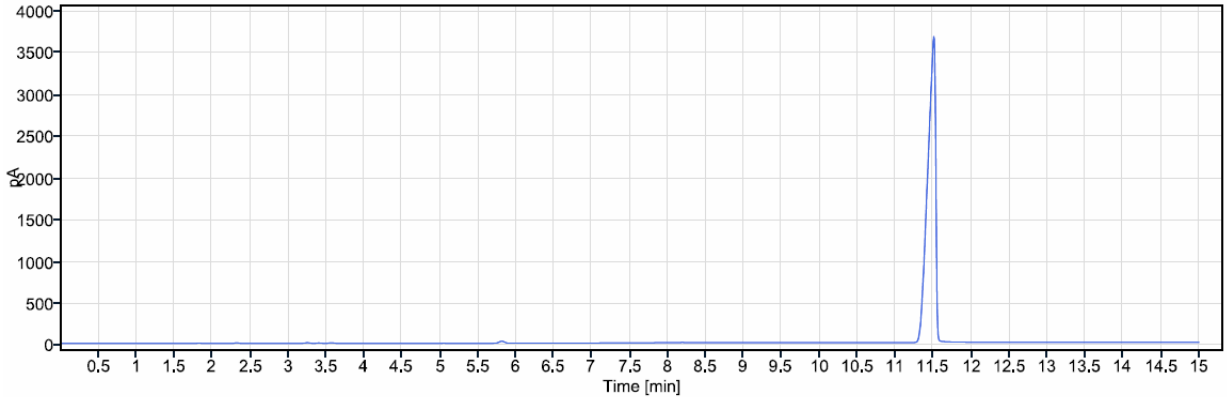

Fig 5.2.1: Blank chromatogram for specificity.

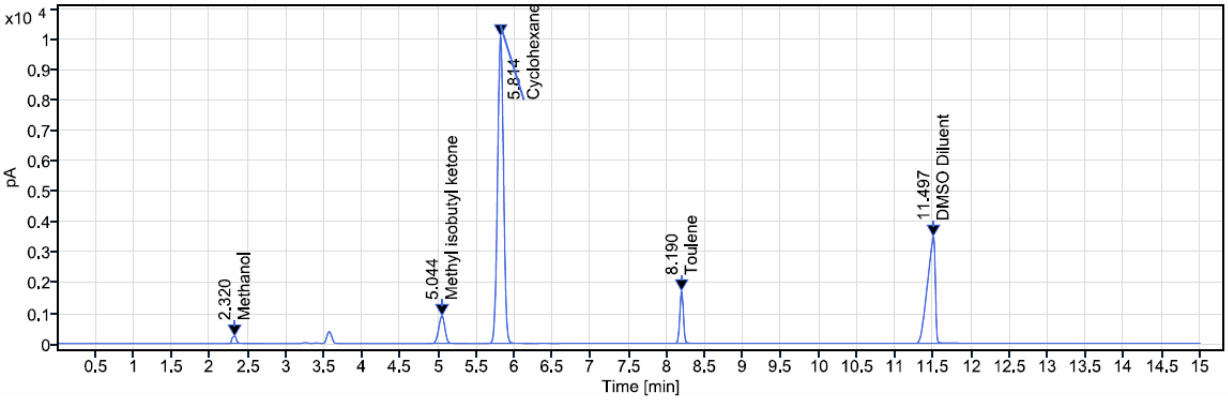

Fig 5.2.2: Specificity of standard.

Observation
Diluent or API peaks are not interfering with the Solventpeaks i.e., Methanol, Methyl Isobutyl ketone, cyclohexane and Toluene.

5.3 Linearity and range
Standard Sock-I Preparation
Linearity Stock-I Preparation
Weighed 500 mg Methanol, 500 mg Cyclohexane, 500 mg Toluene, 500mg Methyliso butyl ketone in 100ml flask mixed well and final capacity done using diluent.

Linearity Stock-II Preparation
Transferred 2ml into 100 ml flask having 20 ml diluent, made upto capacity with diluent.

Table 5.1: Linearity.

Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml) Methanol, Methyl Isobutyl ketone, cyclohexane and Toluene
0.5	100	25
1.0	100	50
1.5	100	75
2.0	100	100
3	100	150

5.4 Accuracy

Accuracy of the method was determined by Recovery studies. To the API (pre analyzed sample), the SOLVENTS were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% & 150%.

Standard Preparation

About 250mg Methanol, 250mg Cyclohexane, 250mg Toluene, 250mg Methyliso butyl ketone in 50ml flask with 20 ml of DMSO, make up to volume with diluent and shake well.

50% Accuracy

Transfer 2 ml of above solution in 200 ml volumetric flask containing about 20 ml diluent, make up to volume with diluent.

100% Accuracy

Transfer 6 ml of above solution in 200 ml volumetric flask containing about 20 ml diluent, make up to volume with diluent.

150% Accuracy

Transfer 6 ml of above solution in 200 ml volumetric flask containing about 20 ml diluent, make up to volume with diluent.

5.5 Precision

Method precision

Standard Sock-I Preparation

About 250mg Methanol, 250mg Cyclohexane, 250mg Toluene, 250mgMethyliso butyl ketone in 50ml flask with 20 ml of DMSO, make up to volume with diluent and shake well.

Standard Sock-II Preparation

Pipette out 10 ml of above solution in 200 ml volumetric flask containing about 20 ml diluent, make up to volume with diluents.

5.6 Limit of Detection

$$LOD = \frac{3.3\sigma}{S}$$

Where, σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

5.7 Limit of Quantification

$$LOQ = \frac{10\sigma}{S}$$

Where, σ = the standard deviation of the response
S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

6. RESULTS

Table 6.1: System suitability of solvents.

Sovent Name	Methanol		Methyl isobutyl ketone		Cyclohexane		Toulene	
S.No	Rt	Area	Rt	Area	Rt	Area	Rt	Area
1	2.32	1016.93	5.044	5143.22	5.814	54941.480	8.190	5580.940
2	2.318	910.05	5.040	4561.43	5.811	49365.290	8.189	4918.150
3	2.317	961.89	5.040	4721.07	5.811	48614.080	8.188	5163.260
4	2.316	977.64	5.038	4817.60	5.810	50130.360	8.188	5210.900
5	2.316	938.12	5.039	4590.56	5.809	48183.130	8.187	4944.670
6	2.316	1017.17	5.039	4975.44	5.809	50257.040	8.187	5448.210
avg	2.3172	970.30	5.040	4801.55	5.811	50248.563	8.1882	5211.022
stdev	0.0016	42.81	0.002	226.22	0.002	2439.477	0.0012	265.366
%RSD	**0.07**	**4.41**	**0.04**	**4.71**	**0.032**	**4.85**	**0.01**	**5.09**

Acceptance Criteria: %RSD of responses of each solvents should be NMT 10%
Observation: %RSD less than 10%.

Table 6.2: Linearity of Methanol.

S.No.	Conc.(µg/ml)	Area
1	25	283.75
2	50	545.42
3	75	772.21
4	100	986.52
5	150	1472.87

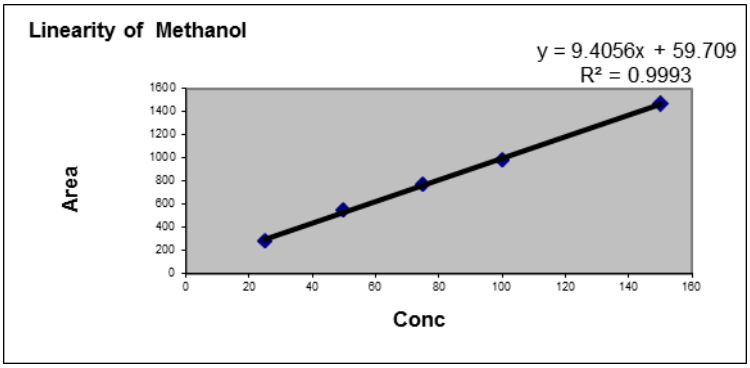

Fig 6.1: Methanol Linearity graph.

Table 6.3: Methyl isobutyl ketone linearity.

S.No.	Conc.(µg/ml)	Area
1	25	1351.010
2	50	2665.460
3	75	3832.060
4	100	4953.210
5	150	7335.000

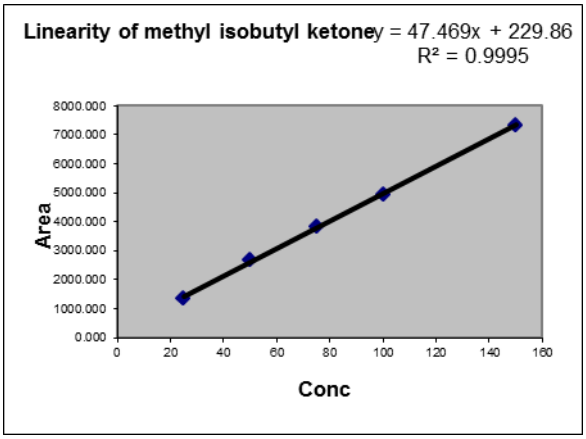

Fig 6.2: Methyl isobutyl ketone-Linearity graph.

Table 6.4: Cyclohexane-linearity.

S.No.	Conc.(µg/ml)	Area
1	25	10722.25
2	50	23715.3
3	75	35431.25
4	100	47375.78
5	150	73116.38

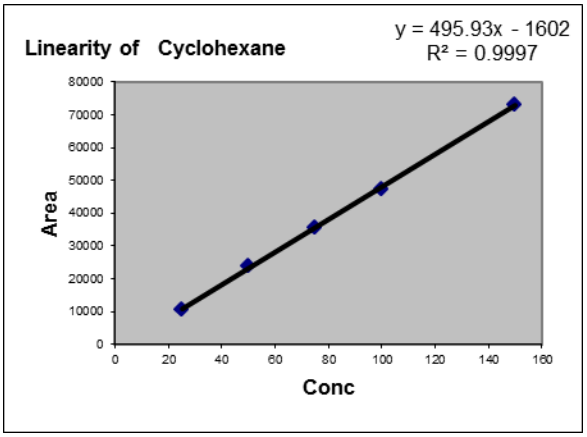

Fig 6.3: Linearity graph of cyclohexane.

Table 6.5: Linearity of Toluene.

S.No.	Conc.(µg/ml)	Area
1	25	1490.10
2	50	2995.35
3	75	4235.52
4	100	5162.93
5	150	7913.38

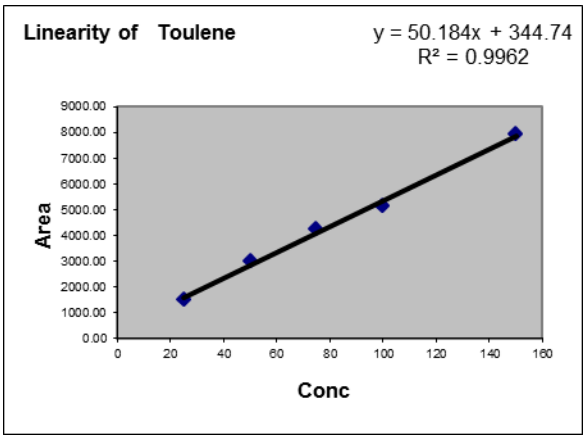

Fig 6.4: Linearity graph of toluene.

Limits Methanol, Methyl Isobutyl ketone, cyclohexane and Toluene responses of area and conc. should not be less than 0.99.

Observation

Methanol, Methyl Isobutyl ketone, cyclohexane and Toluene is >0.999 the correlation coefficient for linear curve obtained between concentration vs. Area is well within limits.

Table 6.7: **Recovery results for Solvents.**

S.No	Name of the Parameter	Methanol	methyl isobutyl ketone	Cyclohexane	Toluene
01	50% Recovery	113.5	116.9	115.6	116.2
02	100% Recovery	100.7	99.9	91.4	99.5
03	150% Recovery	107.6	115.8	100.6	105.3
Average		107.3	110.9	102.5	107.0
%RSD		6.0	8.6	11.9	7.9

Observation

The percentage mean recovery of all solvents were obtained between 80% to 120%.

Table 6.8: Results for Method precision of solvents.

S.No	Name of the Parameter	Methanol in ppm	methyl isobutyl ketone in ppm	Cyclohexane in ppm	Toulene in ppm
01	Method Precision-I	100.2	98.6	100.7	102.3
02	Method Precision-II	104.5	99.5	96.3	97.5
03	Method Precision-III	103.6	99.6	102.5	102.3
04	Method Precision-IV	105.2	99.8	104.5	104.5
05	Method Precision-V	107.8	101.5	102.3	103.6
06	Method Precision-VI	104.2	102.3	100.7	104.2
Average		104.3	100.3	101.2	102.4
STD DEV		2.46556	1.39056	2.76743	2.57371
%RSD		2.4	1.4	2.7	2.5

Observation
Test results for Above solvents wereshowing that the %RSD of obtained ppm results are within limits.

Table 6.9: LOD and LOQ of solvents.

S.No	Name of the Parameter	Methanol in ppm	methyl isobutyl ketone in ppm	Cyclohexane in ppm	Toluene in ppm
01	Limit of Detection	12.22	12.98	13.15	13.18
02	Limit of Quantification	37.05	39.35	39.84	39.93

Table 6.10: LOD and LOQ.

S.No	Name of the Parameter	Methanol in ppm	methyl isobutyl ketone in ppm	Cyclohexane in ppm	Toluene in ppm
01	LOQ-1	98.5	101.4	104.5	96.5
02	LOQ-2	97.4	103.6	102.3	101.4
03	LOQ-3	92.3	99.7	97.8	102.6
04	LOQ-4	93.6	98.6	99.5	101.3
05	LOQ-5	102.2	99.4	98.6	97.5
06	LOQ-6	103.2	99.7	97.2	99.4
Average		97.9	100.4	100.0	99.8
STD DEV		4.4	1.8	2.8	2.4
%RSD		4.5	1.8	2.8	2.4

Observation
All LOQ solutions % Recovery obtained between 70 to 130%.

7. DISCUSSION

SNO.	PARAMETER	OBSERVATION	ACCEPTANCE CRITERIA
1.	System suitability %RSD	Less than 10%	Not more than 10%
2.	Specificity	No interference of diluent or API peaks with the solvents.	No interference of diluents with the solvent peak and no interference of API peak at the retention time of the solvent peaks.
3.	Linearity Slope – P1 P2 P3 P4 Correlation coefficient	50-150 µg/ml 0.999 0.999 0.999 0.996 Less than and equal to 0.999	 Should not be lesser than 0.999
4.	Accuracy Mean % recovery	Between 80% to 120%	between 80% to 120%
5.	Precision % RSD	7.12%	NMT15.0%

8. CONCLUSION

Method for the estimation of Residual solvents of Methanol, Cyclohexane, Methyl isobutyl ketone and Toluene in Lulucinazole API was found to beaccurate and high resolution and shorter retention time makes this method more acceptable and cost effective.

9. ACKNOWLEDGEMENTS

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13. Indian Pharmacopoeia, Vol. I, II & III, Government of India, Ministry of Health and family Welfare, Controller of Publications, Delhi, 2018, http://www.ijpsi.org/Papers/Vol2(3)/Version-1/H233641.pdf_ Development and validation of a headspace gas chromatographic method for determination of residual solvents in five drug substances.
14. The intension of this paper was to review and discuss some of the current analytical procedures including gas chromatographic (GC) and other alternative techniques which are used for residual solvents determination. Katarzyna Grodowska* and Andrzej Parczewski[1] et al., *ptfarm.pl/pub/File/act2010/1_2010/013-026.pdf.*