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Development of Validated Method for Determination of Residual Solvents in Guaifenesin and Imidazole Alcohol by Gas Chromatography (GC/FID) with Head Space

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ABSTRACT

Residual solvents (RS) are not desirable substances in the final pharmaceutical product so their acceptable limits have been published in pharmacopoeias and ICH guidelines. In the present work, a simple and sensitive gas chromatographic method has been developed for the determination of residual solvents in Guaifenesin and imidazole alcohol. Analysis was performed by headspace GC/FID method on Shimadzu 2014 system with auto sampler HT 200H. Carrier gas nitrogen was used with constant flow rate of 4.2 mL/min and the separation of residual solvents were achieved on DB-624 column. The thermostat temperature was 100 °C for 30 minute for each vial and after the equilibration the vials were pressurized and injected on GC column. FID detector was used for detection. The parameter for which the method was validated included specificity, limit of detection and quantification, linearity, precision, accuracy and robustness. The method was successfully used to quantify the levels of specified limit for residual solvents in Guaifenesin and imidazole alcohol bulk drug.

Keywords: Guaifenesin, Emidazole Alcohol, Triconazole, Validation, GC/FID, Residual Solvents.

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INTRODUCTION

Organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products are not completely removed by practical manufacturing techniques, all this solvents should be removed to the extent possible to meet product specifications, good manufacturing practices, or other quality based requirements. In an attempt to harmonize with the ICH Guidelines, the USP has proposed a more comprehensive method in the current USP 30/NF 25 for compendia method for identifying and quantifying residual solvents. Many pharmaceutical products must be analyzed for residual solvents at different stages of their development (raw material, intermediate products, and final product). Organic solvents such as methanol, acetone, dichcloromethane, isopropyl alcohol and toluene are frequently used in pharmaceutical industry. The manufacturing of new active pharmaceutical ingredients (APIs) under GMP condition commands to control adequently the quality of the different ingredient happening in the synthesis. Organic residual solvent have therefore to be controlled and their purity has to be determined before any GMP synthesis.

Headspace gas chromatography method has been used for determination of residual solvents in pharmaceutical compounds. Direct injection of analytes evaporated through equilibration between liquid (or solid) phase and gas phase the GC system minimized the contamination of GC system and the deterioration of GC column. Volatile residual solvents are accumulated prior to analysis.

Triconazole (Imidazole Alcohol) is a triazole antifungal agent available for intravaginal use. It is structurally related to imidazole-derivative antifungal agents, although terconazole and other triazoles have 3 nitrogens in the azole ring. By inhibiting the 14-alpha-demethylase (lanosterol 14-alpha-demethylase), Terconazole inhibits ergosterol synthesis. Depletion of ergosterol in fungal membrane disrupts the structure and many functions of fungal membrane leading to inhibition of fungal growth.

Guaifenesin is an expectorant. It helps loosen congestion in chest and throat, making it easier to cough out through the mouth. Guaifenesin is used to reduce chest congestion caused by the common cold, infections, or allergies [1-7].

The aim of this study is to develop HS-GC method for analysis of residual solvent in Guaifenesin and imidazole alcohol *pharma. The residual solvents compared to standard solvents and the ICH standard residual solvents limit.*

EXPERIMENT

The analysis was performed on Shimadzu Gas Chromatograph GC-2014 with Headspace Auto sampler HT 200H with flame ionization detector. The injection temperature was 190 ^{o}C and detector temperature was 290 ^{o}C. Column was DB-624 with serial no. (30 m long, 0.53 mm

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internal Diameter coated with 3.0 um film of 6 % Cyanopropylphenyl 94 % Dimethyl polysiloxane). Split ratio of injection 1: 4, Oven temperature was maintain at 40ºC for 5 min ,and then raised at rate of 10ºC/min to 170 ºC ,maintain for 7 min. Total run time was 25 min. Nitrogen was used as a carrier gas at a constant flow rate of 4.2 mL/ min. The instrument and headspace condition used for the analysis are outlined in Table 1 and 2 respectively [8-13].

Table-1: Instrument conditions

GC Run Time	: 25 min
Column Oven Temperature:	**:** 40ºC-5min-@10ºC/min-170ºC-7 min
Injection Temperature	: 190^{o}
Detector Temperature	: 290^{o} C
Inlet Pressure	: 21.1 kpa (about 4.2 mL/min)
Linear velocity	: 32 cm/sec
Injection Volume (Head space)	: 1 mL
Split Ratio	1:4
Carrier Gas	Nitrogen
Detector	Flame Ionization Detector

Table-2: Head space conditions

Equilibration Temperature	100^{o} C
Equilibration Time	: 30 min.
Transfer line Temperature	: 115^{o} C
Vial Volume	: 20 mL
Syringe Rinsing	: Thrice
Injection Volume	1 mL by Head space
Syringe Filling Speed	25 mL/min
Injection Speed	15 mL/min
GC Cycle Time	: 35 min

Reagents:

Methanol, Acetone, Methylene dichloride (MDC), Isopropyl alcohol(IPA), Toluene, dimethyl formamide and dimethyl sulphoxide used were Analytical grade reagents. Guaifenesin and imidazole alcohol bulk drug sample was ob-tained from Shree danvantary pharmaceutical analysis and research center, Surat.

Preparation of standard solution:

Mixture of requisite concentration for solvents was obtained by mixing appropriate aliquots of stock in dissolving solvent with respected to sample concentration. For Guaifenesin, the working concentration of solvents in the solution is as 5000 µg/mL for IPA and 890 µg/mL

fortoluene prepared in dimethyl formamide diluents. For Imidazole alcohol, the working concentration of solvents in the solution is as 3000 µg/mL for methanol, 5000 µg/mL for acetone, 600 µg/mL for MDC and 890 µg/mL for toluene prepared in dimethyl sulphoxide diluent.

Preparation of Blank and sample solution:

Weighed accurately 0.1 gm of Guaifenesin in head space (HS) vial. Add 5.0 mL of DMF into a HS vial and seal the vial immediately with PTFE silicon septa closure and secured the closure with an aluminium cap. For imidazole alcohol, weighed accurately 1.0 gm of sample in head space (HS) vial. Add 5.0 mL of DMSO into a HS vial and seal the vial immediately with PTFE silicon septa closure and secured the closure with an aluminium cap. For the Blank solution, pipette out 5 mL respective diluents into a HS vial and the vial were closed with PTFE silicon septa closure and secured the closure with an aluminium cap.

VALIDATION:

The validation was done by evaluating specificity, limit of detection and quantitation, linearity, accuracy, repeatability, and precision of residual solvents as was indicated in the International Conference on harmonization (ICH) guidelines Q2B "validation of analytical procedures: methodology.

Specificity:

Specificity denoted to resolving power of system. Resolution of the analyte peak from the nearest peak is not less than 1.5. The specificity of the analytical method was determined by injecting blank solution and the individual and Mix solution of residual solvents under the same experimental conditions and find out parameters like resolution, theoretical plates, tailing factor.

Detection Limit (LOD) and Quantification Limit (LOQ :

A series of solutions were prepared by quantitative dilutions of the stock solution of solvents. Each solution was injected into the chromatograph in triplicate and the mean peak area was calculated. A graph of mean peak area against concentration in µg/mL was plotted and the equation of regression line and the residual standard deviation was determined. LOD and LOQ determined by statistical formula.

LOD= 3.3 SD/Slope LOQ= 10 SD/Slope
Where, SD is standard deviation

RESULT AND DISCUSSION

The result for the residual solvents in the sample (in µg/mL) using the following formula.

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$$\frac{(A-B)}{(C-D)} \times \frac{W_S}{W_t} \times E \times 10^6$$

Where, A is peak area response of solvent in test preparation, B is peak area response of solvent interference from blank preparation, C is peak area response of solvent in standard preparation, D is peak area response of solvent interference from blank preparation, Ws is weight of component in standard in gm, Wt is weight of sample taken in gram, E is dilution factor.

Specificity:

There was no interference of dissolving solvent at the retention time of Toluene and ethyl acetate and all peaks were well re-solved from each other. Hence the method was found specific. Specificity parameters showed in table-3.

Table-3: Specificity Parameters

Sample Drug	Solvents	RT	Resolution	Theoretical Plates	Tailing Factor
Guaifenesin	**IPA**	4.146	--	9448.308	1.053
	Toluene	10.928	46.121	123815.475	1.025
	DMF	13.032	9.716	28276.030	0.583
Imidazole Alcohol (Triconazole)	**Methanol**	2.556	--	8543.981	1.119
	Acetone	4.073	10.754	10453.409	1.073
	MDC	4.778	5.327	35287.66	1.106
	Toluene	11.167	37.982	112345.241	1.039
	DMSO	14.923	52.269	324313.145	0.864
Acceptance Criteria		--	NLT 1.5	NLT 5000	NMT 1.5

Precision: Six replicate injections of standard solution for system precision were analyzed as per the proposed method and the chromatograms obtained. The standard deviation and percentage relative standard deviation (% RSD) was calculated. For the precision of method and system the % RSD for six solvents complies with acceptance criteria of less than 2%, hence the method and system is said to be précised.

Linearity: A linear relationship evaluated across the range of concentration of analyte solvents (25% to 150% Concentration) and calculate the correlation coefficient, y-intercept and slope of the regression line. The acceptance criteria of correlation coefficient should be more then 0.99. Linearity of solvents showed in table-4 and figures 1-6

ISSN: 0975-8585

Tabel-4: Linearity of residual solvents of Imidazole and Guaifenesin

Linearity for Imidazole alcohol								Linearity for Guaifenesin			
Methanol		Acetone		MDC		Toluene		IPA		Toluene	
Level µg/mL	RSD	Level µg/mL	RSD	Level µg/mL	RSD	Level µg/mL	RSD	Level µg/mL	RSD	Level µg/mL	RSD
750	9.17%	1250	4.98%	150	5.15%	222.5	4.44%	1250	1.47%	222.5	1.47%
1500	1.27%	2500	0.60%	300	0.99%	445	0.61%	2500	6.18%	445	4.19%
2250	1.41%	3750	0.97%	450	1.21%	667.5	1.23%	3750	1.26%	667.5	1.27%
3000	0.82%	5000	0.55%	600	1.85%	890	1.69%	5000	3.95%	890	1.61%
3750	1.39%	6250	0.48%	750	1.23%	1112.5	0.49%	6250	1.53%	1112.5	1.72%
4500	0.09%	7500	0.24%	900	1.68%	1335	0.21%	7500	1.52%	1335	0.58%

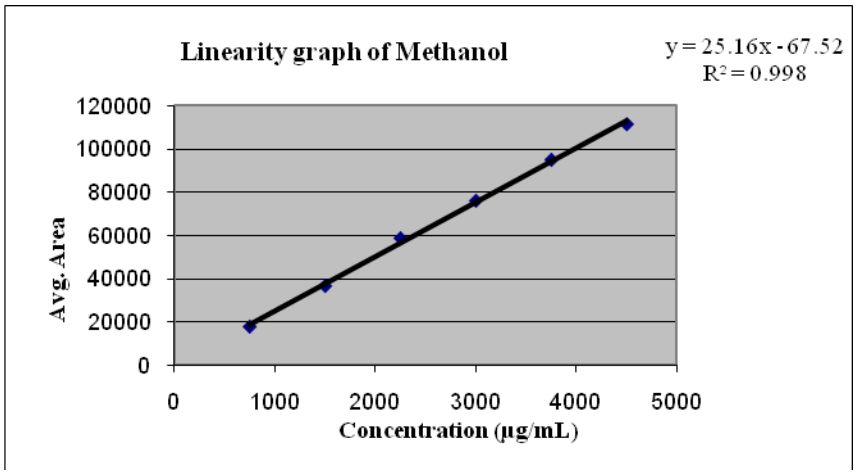

Figure-1: Linearity graph of Methanol

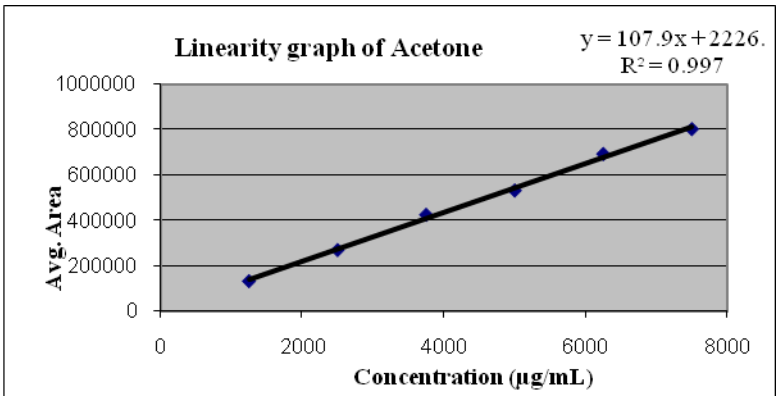

Figure-2: Linearity graph of Acetone

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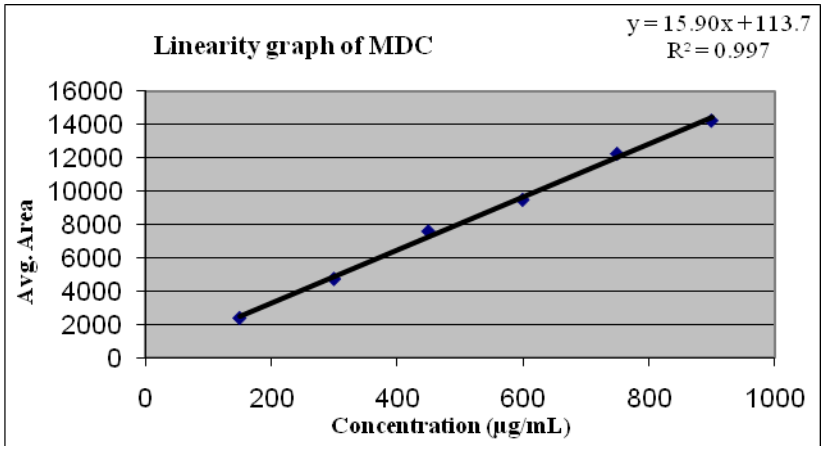

Figure-3: Linearity graph of MDC

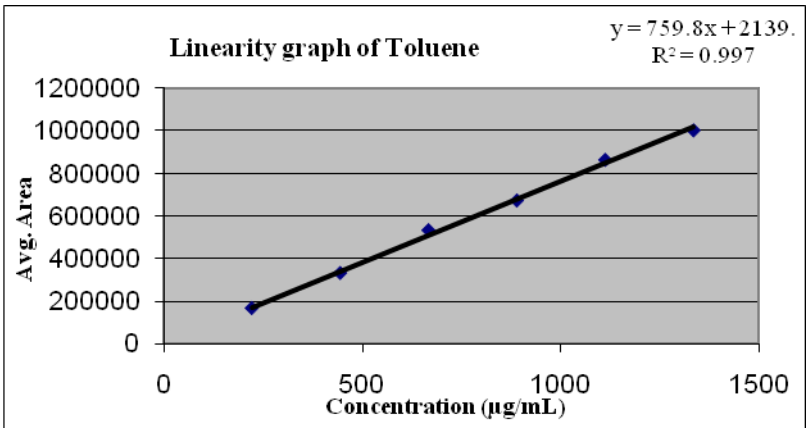

Figure-4: Linearity graph of Toluene

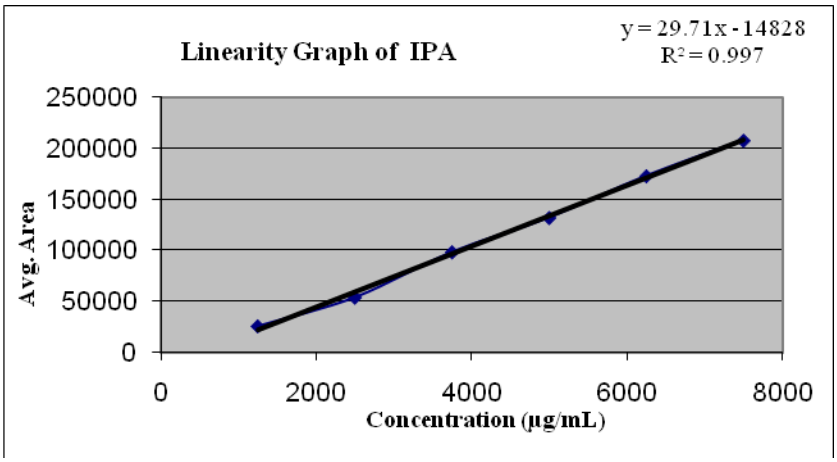

Figure-5: Linearity graph of IPA

ISSN: 0975-8585

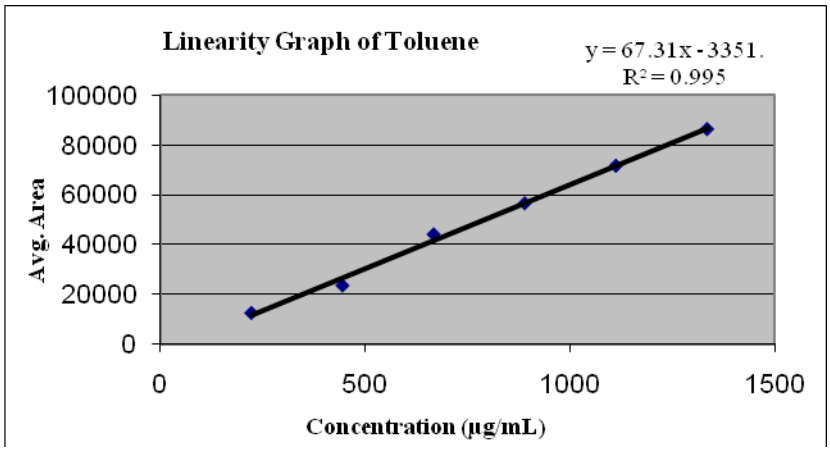

Figure-6: Linearity graph of Toluene (Guaifenesin)

LOD & LOQ (Limit of Detection & Limit of Quantification)

The LOD and LOQ were calculated by instrumental method LOD is determined as the lowest amount to detect and LOQ is the lowest amount to quantify by the detector. The value for the limit of detection and limit of quantification showed in table No-5.

Tabel-5: Limit of detection and Limit of quantification

Bulk Drug	Solvents	Linearity			LOD	LOQ
		RSD Range	r^2	Slope	(µg/mL)	
Imidazole Alcohol	Methanol	0.09-9.17%	0.998	25.16	5.97	18.10
	Acetone	0.24-6.23%	0.997	107.9	3.24	9.83
	MDC	0.99-6.21%	0.997	15.90	3.54	10.73
	Toluene	0.21-6.83%	0.997	759.8	2.76	8.36
Guaifenesin	IPA	1.26-6.18%	0.995	28.64	63.11	191.25
	Toluene	0.58-9.74%	0.996	65.95	29.93	90.71

Accuracy / % Recovery (By Standard Addition Method)

Accuracy of the method was ascertained by standard addition method at 3 levels. Standard solution quantity equivalent to 50 %, 100% and 150 % were added in Sample. The amount recovered by the method was compared to the amount added. Percent deviation was calculated at each levels and a grand average across all the levels was also calculated. The acceptances criteria of recovery at each level is 90.0 – 110.0%.

% Recovery = (Area of solvent in spiked sample-Area of solvent in Sample)*100/Area of solvent in standard

% Recovery calculated showed in table-6

ISSN: 0975-8585

Tabel-6: Accuracy / % Recovery

Solvents	Range (%, µg/mL)	Recovery1	Recovery 2	Recovery 3	% RSD
Methanol	2500 µg/mL (50 %)	102.56	102.76	100.46	1.25
	5000 µg/mL (100%)	97.33	101.63	102.04	2.60
	7500 µg/mL (150%)	101.58	100.32	100.97	0.62
Acetone	445 µg/mL (50%)	98.60	97.40	96.76	0.96
	890 µg/mL (100%)	95.56	100.86	99.11	2.74
	1335 µg/mL (150%)	99.91	97.06	99.96	1.68
MDC	2500 µg/mL (50 %)	100.43	97.08	98.84	1.70
	5000 µg/mL (100%)	96.78	99.55	98.08	1.41
	7500 µg/mL (150%)	100.38	97.14	99.15	1.65
Toluene	2500 µg/mL (50 %)	97.62	94.74	94.24	1.91
	5000 µg/mL (100%)	96.78	102.38	102.47	3.24
	7500µg/mL (150%)	92.09	94.49	92.44	1.39
IPA	2500 µg/mL (50 %)	95.32	97.75	106.76	6.03
	5000 µg/mL (100%)	95.26	98.97	94.80	2.37
	7500 µg/mL (150%)	96.96	95.00	96.34	1.04
Toluene	2500 µg/mL (50 %)	95.32	93.08	95.87	1.56
	5000 µg/mL (100%)	95.26	93.80	96.32	1.33
	7500 µg/mL (150%)	99.76	101.34	101.34	0.90

Robustness:

There was no significant difference in the results for Methanol, Acetone, MDC, IPA and Toluene obtained by the normal method and those obtained by carrying out deliberate changes in the method. Hence the method was found robust with respect to change in the flow rate for the carrier gas and incubation temperature in head space. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

Ruggedness:

The ruggedness was established by determining residual solvents using the same chromatographic system and the same column by two analysts on a different day. The assay result indicated that the method was capable with high precision. Additionally, good separations were achieved, which suggested that the method was selective for all components under the test.

CONCLUSION

The method developed for the analysis of residual solvents in *Guaifenesin and Imidazole Alcohol*, is rapid, sensitive, accurate and rugged. The method is quite faster with a run time of 25 minutes and achieves to address the residual solvents at the prescribed range of limits. The method exhibits a good range of quantization**.**

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