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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RESIDUAL SOLVENTS IN METHOCARBAMOL BY GAS CHROMATOGRAPHY (GC/FID) WITH HEAD SPACE

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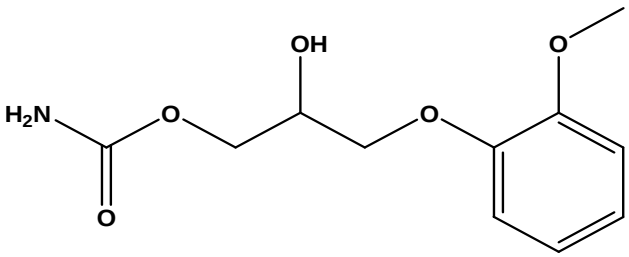
ABSTRACT

An analytical method for the quantification of residual solvents in Methocarbamol was established using a static headspace gas chromatography (HSGC) coupled with a flame ionization detector (FID). Methanol, IPA, Toulene and Benzene as residual solvents determined in Methocarbamol. Analysis was performed by headspace GC/FID method on Agilent 7890A system. Nitrogen was used as carrier gas with constant flow rate of 4.5 mL/min and the separation of residual solvents were achieved on DB-624 column. The thermostat temperature was 50°C-hold for 8 min-raise @ 10°C/min to 230^{0}C for 10 min. The %RSD for six injections obtained are with in acceptance criteria. The percentage recovery ranges obtained from 94% and 108%.The correlation coefficients obtained are greater than 0.99. The method parameters were validated included specificity, limit of detection and quantification, accuracy, linearity, precision, and robustness. A new, simple, specific, accurate and precise method was validated according to the International Conference on Harmonization (ICH) guidelines.

KEYWORDS: Methocarbamol, Validation, GC/FID, Residual Solvents, ICH.

INTRODUCTION

Methocarbamol



Methocarbamol is a carbamate which belongs to a class of medications called muscle relaxants and they reduce muscle spasm[1]. Though the exact mechanism of action of methocarbamol was not established, it's postulated to be via a mechanism similar of carbamate, inhibition of acetylcholinesterase at synapses in the autonomic nervous system, neuromuscular junction, and central nervous system. Methocarbamol has no direct effect on the contractile mechanism of striated muscle, the motor end plate or the nerve fiber. Methocarbamol is a commonly used, centrally acting muscle relaxant and has not been linked to instances of liver injury.

MATERIALS AND METHODS

The working standard of Methocarbamol was obtained as gift sample from Granules INDIA PVT Ltd, Hyderabad., India.

Table 1: Chemicals and Reagents.

Chemicals and Reagents	Make
Methanol	Merck
Benzene	Merck
Toluene	Merck
Dimethyl Sulfoxide	Merck

Table 2: Instruments Used.

Instruments	Make and Model
Analytical Balance	Mettler XS205
Gas Chromatography	Agilent 7890A
Gas Chromatography	Agilent 6850

PREPARATION OF SOLUTIONS

Preparation of Methanol Standard stock solution[2-5]

Dissolve 75 mg of methanol in 50 mL volumetric flask, then diluted to the mark with DMSO.

Preparation of IPA Standard stock solution

Dissolve 125 mg of IPA in 50 mL volumetric flask, then diluted to the mark with DMSO.

Preparation of Toluene Standard stock solution

Dissolve 22 mg of toluene in 50 mL volumetric flask, then diluted to the mark with DMSO.

Preparation of Benzene Standard stock solution

Dissolve 20 mg of benzene in 50 mL volumetric flask, then diluted to the mark with DMSO. Further dilute 5 mL to 10 mL with DMSO.

Preparation of Standard solution

Then dilute 20 mL each of the above Methanol, IPA, and Toluene Standard stock solutions, 1 mL of Benzene Standard stock solution to 100 mL DMSO. This solution contains 3000 ppm of Methanol, 5000 ppm of IPA, 890 ppm of Toluene and 2 ppm of Benzene with respect to the sample. Add 5.0 mL of this solution to 20 mL headspace vial then cap and seal the vial immediately.

Preparation of Sample solution

Weigh approximately 500.0 mg of sample and transfer to a 20 mL headspace vial add 5 mL of DMSO, then cap and seal the vial immediately. Vortex the sample until it is fully dissolved.

Preparation of Spiked sample

Weigh approximately 500.0 mg of sample and transfer to a 20 mL headspace vial add 5 mL of Standard Solution, then cap and seal the vial immediately. Vortex the sample until it is fully dissolved.

METHOD DEVELOPMENT

Selection of the solvent

As Methocarbamol is a polar drug it can be easily dissolved in Dimethyl Sulfoxide (DMSO)[7-8] DMSO is an organo sulphur compound. It is a polar aprotic solvent which dissolves both nonpolar and polar compounds and is miscible in wide range of organic solvents as well as water. Its boiling point was 189^0C, as it has high boiling point it is mostly used to detect the presence of residual solvents.

Optimised Chromatographic conditions

Column	: DB-624
Dimension	: 30 meters x 0.53 mm ID (3µm)
Detector	: FID
Detector temperature	: 250^0C
Injector temperature	: 180^0C
Injector volume	: 1.0 mL
Conditions	: 50°C-hold for 8 min-raise @ 10°C/min to 230^0C for 10 min
Run time	: 40 min
Split ratio	: 1:5
Carrier Gas	: 4.5 mL/min (Nitrogen)

Optimised Head space conditions

Bath temperature	: 125°C
Loop temperature	: 135°C
Transfer line temperature	: 145°C
Vial equilibration temperature	: 30 min
Pressurize temperature	: 0.5 min
Loop fill time	: 0.2 min
Loop equilibration time	: 0.2 min
Injection time	: 1.0 min
GC cycle time	: 45 min

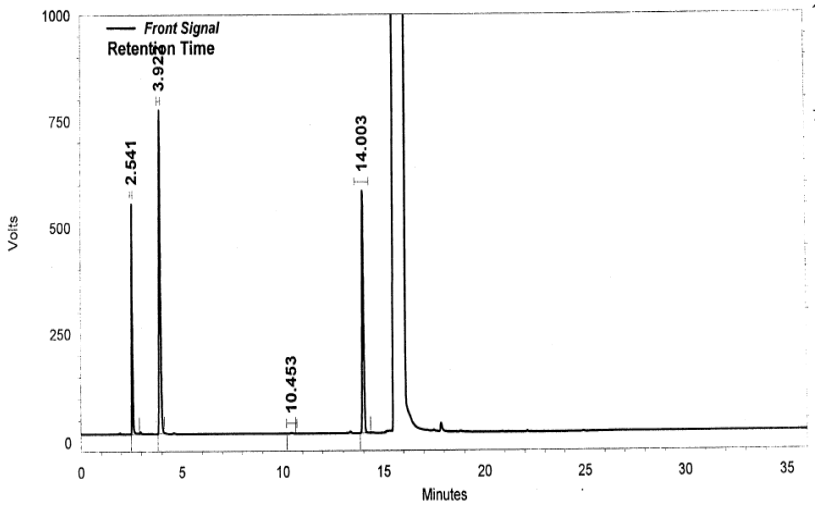


Figure: 1 Optimised Chromatogram.

METHOD VALIDATION

System Suitability

System suitability tests are an integral part of any chromatographic analysis method which is used to verify reproducibility of the chromatographic system. Six standard injections were injected and average is taken, SD and %RSD were calculated.[9-14]

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the form of diluents, solvents and working standards.

Individually blank i.e. the diluent, methanol standard stock solution, IPA standard stock solution, benzene standard stock solution, toluene standard stock solution were injected and retention time was noted. Then the prepared standard solution was injected 6 times and %RSD was calculated.

PRECISION

System precision

The precision of an analytical procedure "expresses the closeness of agreement between a series of measurement obtained from multiple sampling from the same homogenous sample under the prescribed conditions". Precision of an analytical procedure is usually expressed the variance standard deviation of coefficient of variation of a series of measurement.

Method Precision

Demonstrate the method precision by preparing six different preparations from single batch as per the test method, in which Residual solvents are spiked at the respective specification level. Determine the Residual solvents of these samples and evaluate the precision of the method by computing the percentage-relative standard deviation.

Intermediate precision

Carry out the precision study by preparing the sample six times as per the protocol representing a single batch, by two different analysts, on two different days, on two different columns and on two different instruments.

LOD and LOQ

The limit of detection is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, by using a specific method under the required

experimental conditions.LOD and LOQ of residual solvents are determined based on the residual standard deviation and slope of the linearity data.LOD and LOQ linearity solutions are prepared, injected and area of the solvent peaks are recorded.

Linearity

The linearity of an analytical method is the ability to elicit test results that are directly proportional to the analyte concentration. Linearity of residual solvents are determined over the range of LOQ to 150 %.Prepared the Linearity solutions as per the protocol**,** injected the solutions into the GC system and record the areas. Plotted a graph of concentration in %(in X-axis) V/S Average area (in Y-axis).Evaluated the correlation co-efficient between concentration and average area.

Accuracy

Accuracy of the test method is demonstrated by preparing recovery samples (i.e. spiking sample with known quantity of solvents) at the level of LOQ, 50 %, 100 % and 150 % of specification limit. Prepare the recovery samples in triplicate in each level.

50 % Accuracy solution

Dilute 0.5 mL of the Standard stock solution-1 and 10 mL of Standard Stock preparation-2 to 100 mL DMSO.

100 % Accuracy solution

Dilute 1.0 mL of the Standard stock solution-1 and 10 mL of Standard Stock preparation-2 to 100 mL DMSO.

150 % Accuracy solution

Dilute 1.5 mL of the Standard stock solution-1 and Standard stock preparation-2 to 100 mL DMSO.

50 % Accuracy Spiked Sample

Weigh approximately 500 mg of sample and transfer to a 20 mL headspace vial. Add 5.0 mL of 50 % Accuracy solution then cap and seal the vial immediately, vertex the sample until it is fully dissolved. Prepare in triplicates.

100 % Accuracy Spiked Sample

Weigh approximately 500 mg of sample and transfer to a 20 mL headspace vial. Add 5.0 mL of Standard solution then cap and seal the vial immediately, vertex the sample until it is fully dissolved. Prepare in triplicates.

150 % Accuracy Spiked Sample

Weigh approximately 500 mg of sample and transfer to a 20 mL headspace vial. Add 5.0 mL of 150% Accuracy solution then cap and seal the vial immediately, vertex the sample until it is fully dissolved. Prepare in triplicates.

Robustnesss

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. Flow rate is changed to ±0.35mL/min from actual flow rate i.e. 3.5mL/min.

Bench Top Stability of solutions

Stability of the analytial solutions was determined after 24 hrs when stored at room temperature.

RESULTS AND DISCUSSION

A simple HS-GC method was adopted for the determination of residual solvents in the given sample of Methocarbamol. To optimise the proposed method, all of the experimental conditions were investigated. HS-GC method was preferred as the sample is injected in the form of vapour. The best resolution and retention time is obtained by using nitrogen as a carrier gas and FID as a detector.[15-18]

The system suitability for the method was found to be with in the acceptance criteria i.e. NMT 15% for individual solvents for atleast 6 injections. Retention time for residual solvents were clearly specified individually and also in spiked standard solution. The method was also found to be precise for the conditions given.

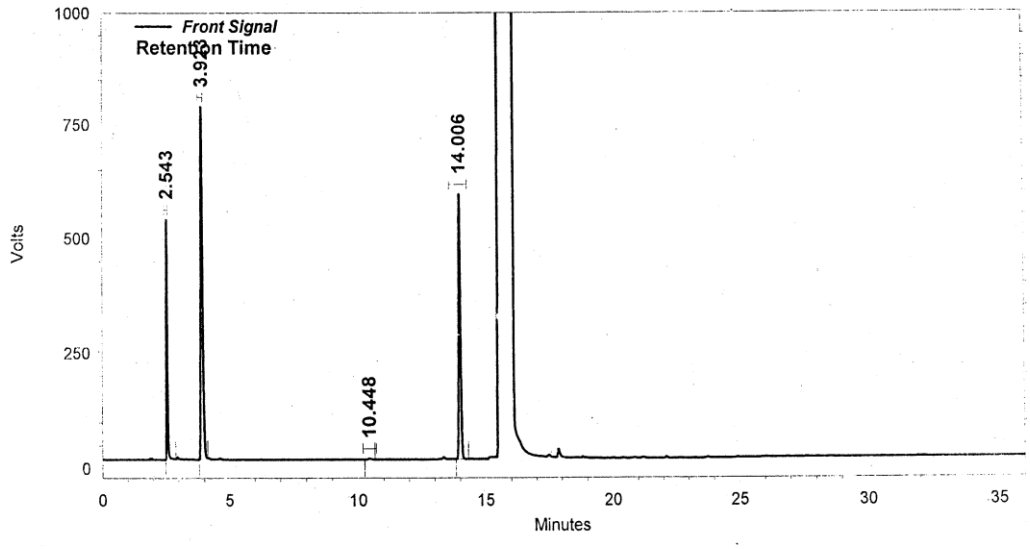


Figure: 2 Chromatogram for System suitability.

Specificity

Table 3: Results for Individual solvents and spiked standard.

Solvent Name	Retention Time(min)		Resolution
	Individual	Spiked	
Methanol	2.535	2.541	-
Iso Propyl alcohol	3.923	3.922	12.81
Benzene	10.432	10.453	37.80
Toluene	14.003	14.003	20.79

Table 4: Results for spiked sample.

S.No	Name of the Solvent			
	Methanol	IPA	Benzene	Toluene
1.	11415111	31048779	102770	22646919
2.	11586030	31418105	100059	22987069
3.	11467377	31012452	99729	22723222
4.	11439472	30908433	100701	22624229
5.	11523517	31051253	99851	22794752
6.	11565050	31258755	100018	22999466
Avg	11499426	31116296	100521	22804193
Std.Dev	69451.82	186634.9	1151.78	156347.6
%RSD	0.60	0.60	1.15	0.69

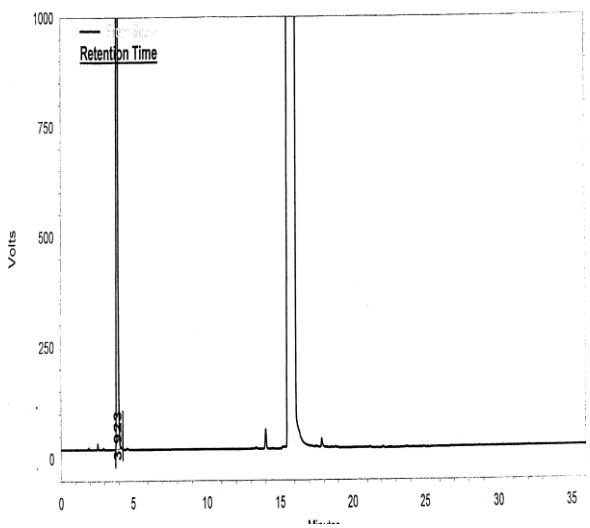


Figure-3 Methanol

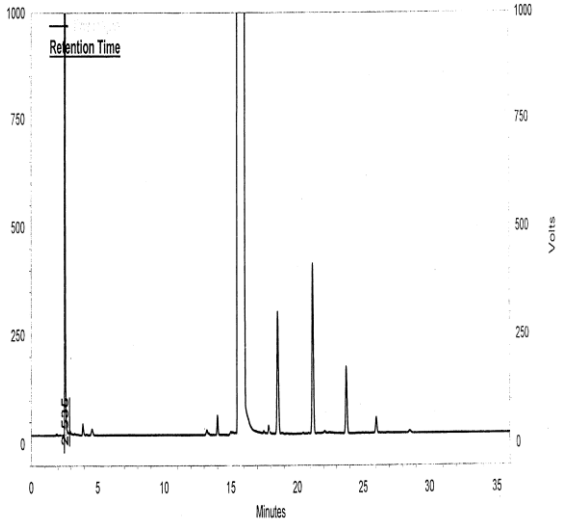


Figure-4 IPA

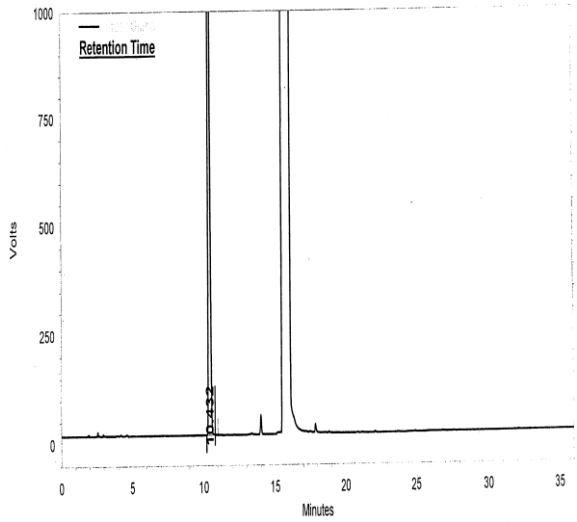


Figure 5: Benzene

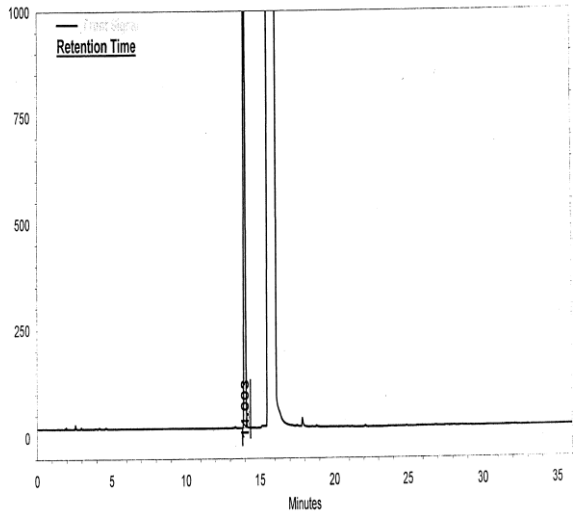


Figure 6: Toluene

PRECISION

Table 5: Results for System Precision.

S.No	Name of the Solvent			
	Methanol	IPA	Benzene	Toluene
1.	11173863	31645577	102416	23333900
2.	11141913	30914523	99388	22658559
3.	11160836	30751470	99536	22543787
4.	11619644	32102769	100866	23474963
5.	11278236	30827644	99973	22506054
6.	11325428	31051504	100010	22846095
Avg	11283320	31215581	100365	22893893
Std.Dev	179939.9	539527.6	1129.52	415188.8
%RSD	1.59	1.73	1.13	1.81

Table 6: Results for Method Precision.

S.No	Name of the solvent			
	Methanol	IPA	Benene	Toluene
1.	11296277	32060864	142415	22983508
2.	11424606	33170366	146660	22874332
3.	11300143	31898389	143610	22897082
4.	11555850	33307267	145861	23043962
5.	11370371	32101279	145863	23059605
6.	11480365	32098661	144698	23027142
Avg	11404602	32439471	144851	22980939

Intermediate precision

Carry out the precision study by preparing the sample six times as per the protocol representing a single batch, by two different analysts, on two different days, on two different columns and on two different instruments.

Table 7: Results for Intermediate precision.

(1st day, 1st analyst, 1st column, 1st system)

S.No	Name of the solvent			
	Methanol	IPA	Benzene	Toluene
1.	11296277	32060864	142415	22983508
2.	11424606	33170366	146660	22874332
3.	11300143	31898389	143610	22897082
4.	11555850	33307267	145861	23043962
5.	11370371	32101279	145863	23059605
6.	11480365	32098661	144698	23027142
Avg	11404602	32439471	144851	22980939
Std.Dev	102794.8	625126.5	1600.89	78356.37
%RSD	0.90	1.93	1.11	0.34

Table-8 Results for Intermediate precision.

(2nd day, 2nd analyst, 2nd column, 2nd system)

S.No	Name of the solvent			
	Methanol	IPA	Benzene	Toluene
1.	1966.417	5473.723	15.8187	3310.993
2.	2015.743	5764.442	16.926	3476.541
3.	1967.851	5345.589	15.3002	3208.741
4.	2015.548	5624.185	16.2155	3375.038
5.	1751.802	4294.701	11.6908	2511.872
6.	1812.049	4538.773	12.4742	2669.001
Avg	1921.568	5173.569	14.7376	3092.031
Std.Dev	111.955	607.8229	2.13846	401.2592
%RSD	5.83	11.75	14.51	12.98

Linearity

Linearity was determined by constructing the calibration curve from concentration LOQ to 150% and graph was found to be linear over the given concentrations. The correlation coefficients were 0.9995 for methanol, 0.9998 for IPA, 0.9991 for benzene, and 0.9998 for toluene respectively.

Table 9: Results for Linearity.

S.No	Methanol		IPA		Benzene		Toluene	
	Actual Conc.	Avg. area	Actual Conc.	Avg. area	Actual Conc.	Avg. area	Actual Conc.	Avg. area
LOQ	36.43	151987	2.52	24170	0.67	25225	0.35	69839
25%	759.05	2488194	1262.05	6963886	0.92	32564	232.2	4143926
50%	1518.1	5035506	2524.1	14070312	1.12	42712	464.4	8271667
75%	2277.15	7372583	3786.15	20854337	1.67	59828	696.6	12295770
100%	3036.2	10142484	5048.2	28483357	2.23	78042	928.8	16634578
125%	3795.25	12491416	6310.25	35122623	2.79	95150	1161.0	20570855
150%	4554.3	15342322	7572.3	42835902	3.4	116589	1393.2	25090959
Slope	3343.20		5637.33		32877.2		17888.8	
Correlation coefficient	0.9995		0.9998		0.9991		0.9998	

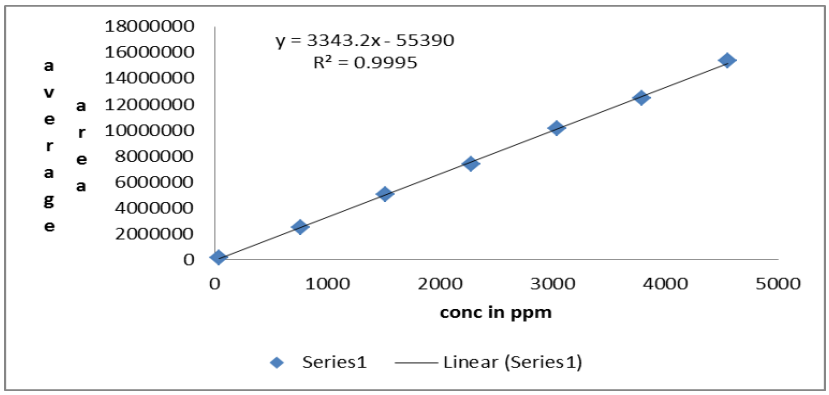


Fig 7: Linearity Graph for Methanol.

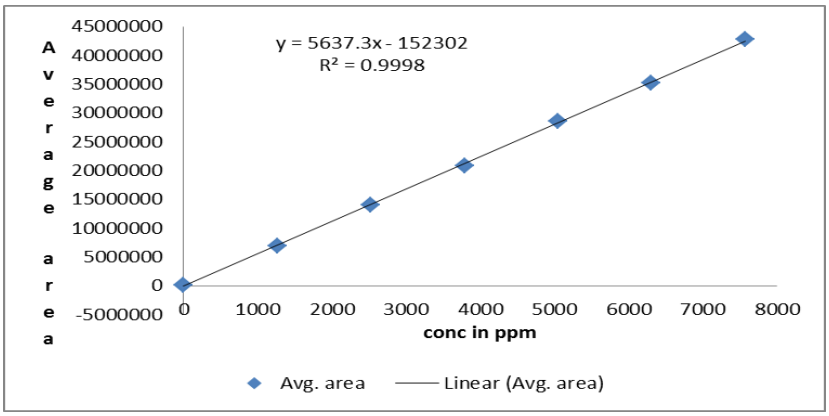


Fig-8 Linearity Graph for IPA.

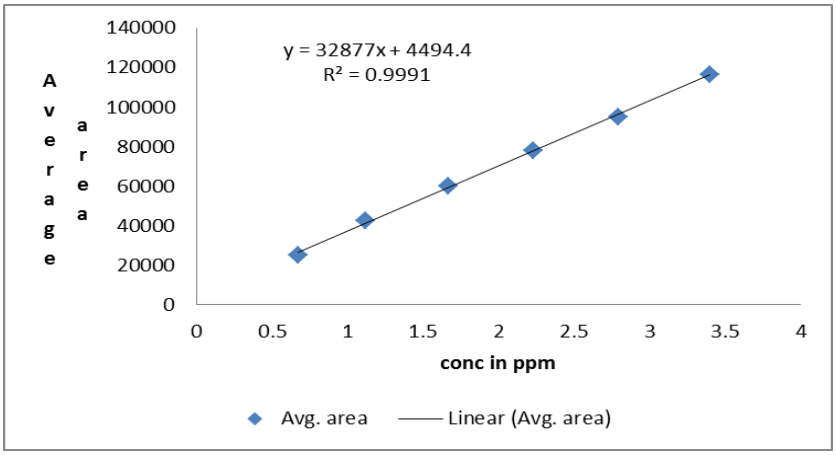


Fig-9 Linearity Graph for Benzene.

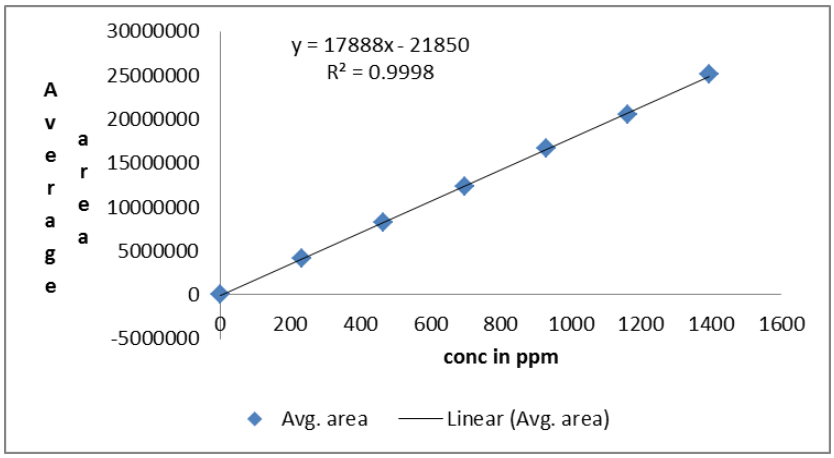


Fig-10 Linearity Graph for Toluene.

LOD and LOQ

Dissolve 100 mg of Benzene, 5000 mg of Methanol and 5000 mg of IPA and 445.0 mg of Toluene in to a 100 mL volumetric flask containing about 20 mL of DMSO. Dilute and bring the volume with DMSO, mix thoroughly. Further dilute 2 mL to 100 mL with DMSO. Prepare the linearity solutions for residual solvents at lower concentrations as follows.

Table-10 Results for LOD and LOQ Stock Solution.

S.No	LOD and LOQ stock sol. taken in mL	Diluted to vol. with diluent(mL)	Conc in ppm			
			Methanol	IPA	Benzene	Toluene
1.	0.2	100.0	20	20	1.8	0.4
2.	0.3	100.0	30	30	2.7	0.6
3.	0.4	100.0	40	40	3.6	0.8
4.	0.5	100.0	50	50	4.5	1
5.	1.0	100.0	100	100	8.9	2

Table 11: Results for LOD and LOQ.

Solvent	Methanol	IPA	Benzene	Toluene
LOD	11.40ppm	0.82ppm	0.16ppm	0.09ppm
LOQ	34.55ppm	2.50ppm	0.49ppm	0.26ppm

Accuracy

Accuracy is performed to know the %drug recovery. The recovery was found about the concentrations of LOQ, 50%, 100%, 150%

Table 12: Results for %Recovery drug.

Concentration in %	Average %Recovery			
	Methanol	IPA	Benzene	Toluene
LOQ	94	98	97	102
50%	105	106	95	108
100%	101	103	102	106
150%	103	105	101	102

Acceptance criteria is 85-115%.

Robustness

The robustness study indicated that the selected factors remain unaffected by small variations in the flow rate, which were 3.15 mL/min and3.85 mL/min. Hence the method was robust by changing the flow rate.

Table 13: Results for Robustness (3.15 mL/min flow).

S.No	Methanol	IPA	Benzene	Toluene
1.	10689660	30353093	82939	18142727
2.	10488336	29558036	81071	17464707
3.	10664995	30000987	84983	17578788
4.	10548990	29732641	83604	17570518
5.	10452759	29346334	81501	17326447
6.	10525113	29569266	81474	17518384
Avg	10561642	29760060	82595	17600262
Std.Dev	95701.8	363007.17	1524.14	281306.93
%RSD	0.91	1.22	1.85	1.6

Table 14: Results for Robustness (3.85 mL/min flow).

S.No	Methanol	IPA	Benzene	Toluene
1.	11209997	31305585	85570	18483291
2.	11284160	31778207	85728	184838810
3.	11054466	30838045	83907	18188503
4.	11235666	31328987	85962	18420054
5.	11078362	30893242	84468	18261342
6.	11424162	31960018	87743	18861829
Avg	11214469	31300681	85563	18449805
Std.Dev	136691.9	411607.7	1332.68	235254.6
%RSD	1.22	1.32	1.56	1.28

Bench Top Stability

Different standard stock solutions are prepared for validating the developed method. These solutions should be stable on storage. so they are tested for stability after storing for 24hrs at room temperature. After storage also the values were found to be within the acceptance criteria i.e. %RSD is found to be NMT 15%.

Table 15: Results for Solution Stability.

Interval	S.No	Methanol area	IPA area	Benzene area	Toluene area
Initial hrs	Injection-1	10760850	30586058	116296	17502113
	Injection-2	10898410	30706454	117411	17574006
	Injection-3	10973035	30746675	114661	17476336
	Injection-4	10905313	30721207	116506	17591477
	Injection-5	10803789	30443393	117506	17414874
	Injection-6	10811701	30167983	116133	17297460
After 24 hrs	Injection-1	10741819	29939570	103519	17188297
	Injection-2	10850109	30087135	105231	17185977
	Injection-3	10697009	29948487	106550	17348440
	Injection-4	10714840	29669341	102726	17032155
	Injection-5	10403526	28915879	100393	1659672
	Injection-6	10434776	29152408	101975	16757202
	Average	10749598	30090383	109909	17246751
	Std.Dev	174838.04	607824.4	6998.21	317041.4
	%RSD	1.63	2.02	6.37	1.84

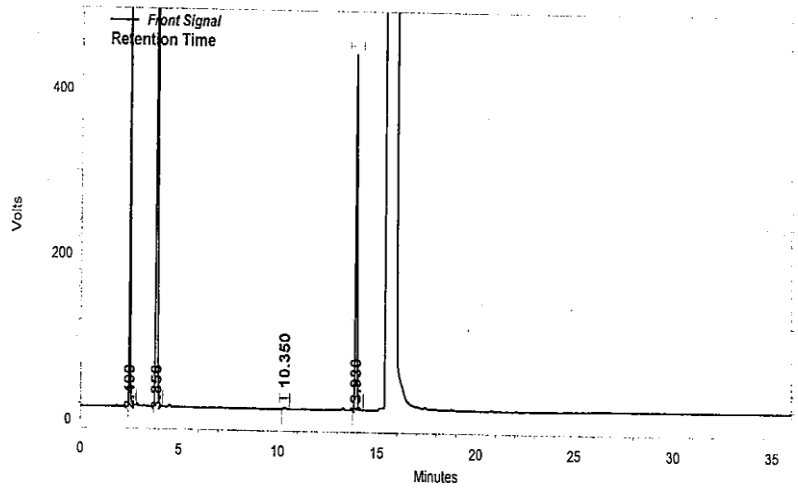


Figure: 11 Stability of Analytical solutions at initial hours.

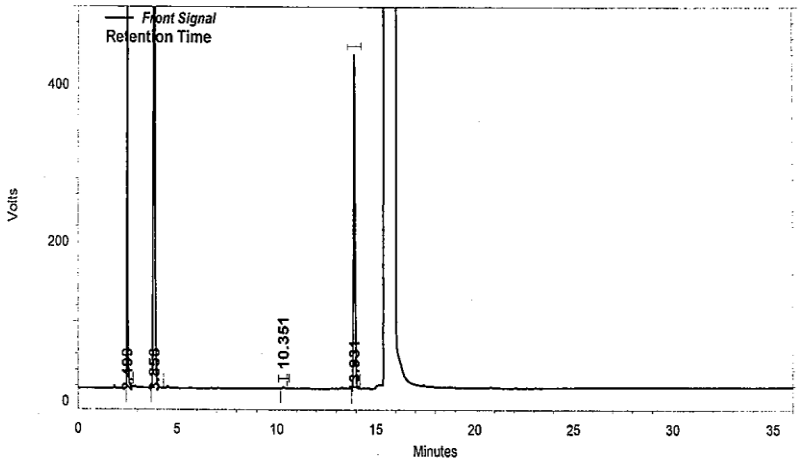


Figure: 12 Stability of Analytical solutions after 24hrs.

SUMMARY AND CONCLUSION

In the present study, a simple and reproducible method for the determination of residual solvents in Methocarbamol by HS-GC was developed. The method developed has the advantages like greater sensitivity, good precision and accuracy, required run time, strong separation power. The proposed method being precise and sensitive can be used for the determination of residual solvents in the pure drug of Methocarbamol.

It can be concluded that the proposed methods are fully validated and were found to be sensitive, accurate, robust and inexpensive. This method can also be used for the multiple components.

From the results obtained it is evident that all the values of the validated parameters were found to be within the acceptance criteria mentioned.

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