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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF RESIDUAL SOLVENTS IN METHOCARBAMOL PURE DRUG BY HS-GC

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ABSTRACT

A simple HS-GC method for the determination of residual solvents in methocarbamol using nitrogen as the carrier gas at 3.5mL/min with DB-624 (30 meters X 0.53 mm ID) as column using FID as detector was developed. The developed method was validated and parameters were to be found within the limits of USP. The retention time for residual solvents individually and in spiked standard solution was determined. The %RSD for six injections should be NMT15%. The percentage recovery ranges from 85-115%. The correlation coefficient $R^2 \geq 0.999$. The limit of detection and limit of quantification was found to be specific. Precision, method precision and intermediate precision was found to be within the acceptance limit. Finally the sample was tested for the presence of residual solvents mainly benzene as it is a class1 solvent and should be avoided.

Keywords: Methocarbamol, DB-624, FID, %RSD.

INTRODUCTION

Residual solvents are the organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. These solvents are not completely removed by practical manufacturing techniques[2]. Since there is no therapeutic benefit from residual solvents, these solvents should be removed. As benzene is a class1 solvent it should not be present in our sample i.e. methocarbamol. Methocarbamol is a central muscle relaxant. Chemically it is 2-hydroxy-3-(2-methoxy phenoxy) propylcarbamate. Its mechanism of action may be due to central nervous system depression and has no direct action on the contractile mechanism of straited muscle, the motor end plate or the nerve fibre.

Literature survey has reported that several analytical methods were found for the quantitation, and simultaneous determination of methocarbamol by HPLC [8-12], RP-LC [13], isocratic SFC method [5] and chemometric method [3], pharmacokinetic properties [14], GC [1]. The aim of the present study was to prove the absence of residual solvents mainly benzene in the pure drug of methocarbamol.

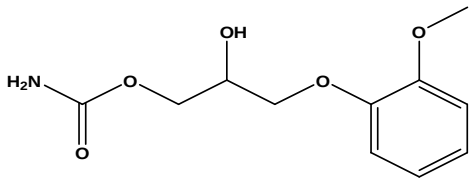

Fig. 1: Chemical structure of methocarbamol

EXPERIMENTAL

Head space Gas chromatography

The analysis was performed on Agilent gas chromatography model no 7890A and 6850 using DB-624 as the column and FID detector with nitrogen as the carrier gas [4].

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 vapor
Conditions	50°C-hold for 8min-Raise @10°C/min to 230°C hold for 10min
Runtime	40 minutes.
Split Ratio	1:5
Carrier Gas	3.5 mL/min. (Nitrogen)
Makeup Gas	25 mL/min. (Nitrogen)

Head space conditions

Bath temperature	125°C
Loop temperature	135°C
Transfer line temperature	145°C
Vial equilibration time	30 min.
Pressurize time	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

Sample and Standards

Reagents: Methanol, IPA, benzene, toluene, di methyl sulfoxide (DMSO) were obtained from Merck -Mumbai.

Standard stock preparation-1

Dissolve 40mg of benzene in 100mL volumetric flask, then diluted to the mark with DMSO. Further dilute 5mL to 100mL with DMSO.

Standard stock solution-2

Accurately transfer 150mg of methanol and 250mg of IPA and 44.5mg of toluene into a 100mL volumetric flask, containing about 20mL of DMSO. Dilute and bring the volume with DMSO, mix thoroughly.

Standard preparation

Dilute 1mL of the standard stock solution-1 and 20mL of standard stock solution-2 into a 100mL volumetric flask and bring to volume with DMSO. Mix thoroughly. Add 5mL of this solution to 20mL headspace vial then cap and seal the vial immediately.

Sample preparation: Weigh approximately 500mg of sample and transfer to a 20mL headspace vial add 5mL of DMSO, then cap and seal the vial immediately. Vortex the sample until it is fully dissolved.

Procedure: Prepared solutions are taken into 2mL headspace vial, sealed with aluminium closure. These standards are run under the specified conditions and retention times are noted to calculate %RSD.

Method Validation

The parameters like specificity, linearity, precision, accuracy, robustness, system suitability were performed that are mentioned in the International conference on harmonisation (ICH) guidelines [6].

Specificity is performed to know the retention time for the residual solvents individually and in spiked sample solution.

Linearity was done to know the test results which are directly proportional to the concentration of analyte in the sample. It was performed from LOQ to 150% and results were found to be within the limits.

Precision was validated to know the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample. %RSD for precision was also found to be NMT 15%.

Accuracy is the amount of drug recovered from the spiked sample. It is assessed by 9 determinations over a minimum of 3 concentration levels covering the specified range.

Robustness is tested by introducing small variations in method parameters. From the results it was observed that the method remain unaffected.

System suitability is performed to ensure that the complete testing system is suitable for intended application.

Finally the sample is checked for the presence of residual solvents especially benzene.

RESULTS

All the validated parameters were found to be within the limits. Linearity is performed from 50-150% and graph obtained was linear showing correlation coefficient $R^2 \geq 0.999\%$. Drug recovery should be 85-105%. System suitability for 6 injections %RSD was found to be NMT 15%.

CONCLUSION

From the results obtained we can conclude that all the results are within the acceptance criteria i.e. %RSD for atleast of 6 injections is NMT 15% as per the USP [12].

Table 1: Specificity

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

Linearity

Table 2: Linearity Table for Methanol

S. No	Methanol	
	Actual Conc.	Avg. Area
LOQ	36.43	151987
25%	759.05	2488194
50%	1518.1	5035506
75%	2277.15	7372583
100%	3036.2	10142484
125%	3795.25	12491416
150%	4554.3	15342322
Slope	3343.20	
Correlation coefficient	0.9995	

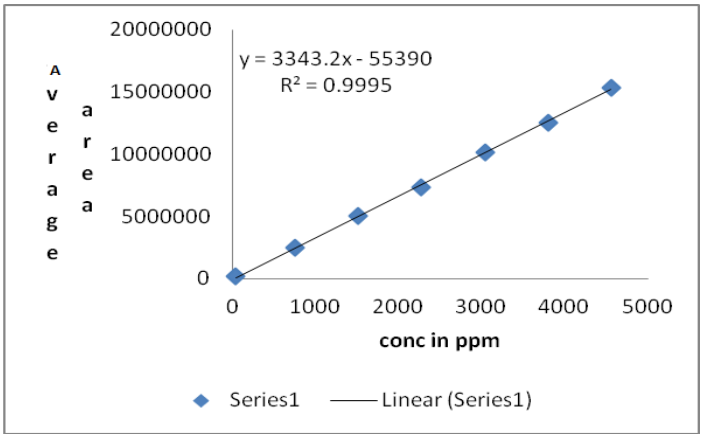

Fig. 2: Linearity Graph for Methanol

Table 3: Linearity Table for IPA

S. No	IPA	
	Actual Conc.	Avg. area
LOQ	2.52	24170
25%	1262.05	6963886
50%	2524.1	14070312
75%	3786.15	20854337
100%	5048.2	28483357
125%	6310.25	35122623
150%	7572.3	42835902
Slope	5637.33	
Correlation coefficient	0.9998	

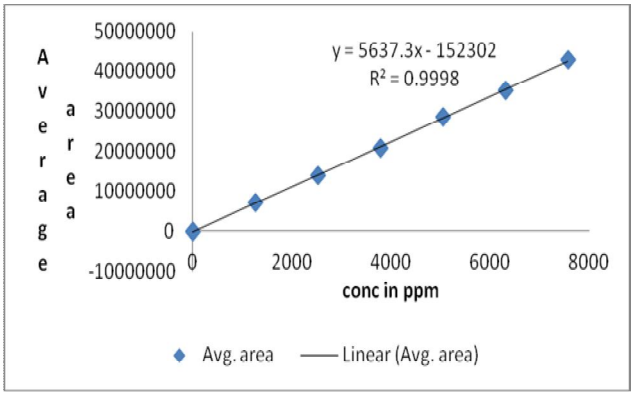

Fig 3: Linearity Graph for IPA

Table 4: Linearity Table for Benzene

S. No	Benzene	
	Actual Conc.	Avg. area
LOQ	0.67	25225
25%		
50%	1.12	42712
75%	1.67	59828
100%	2.23	78042
125%	2.79	95150
150%	3.4	116589
Slope	32877.2	
Correlation coefficient	0.9991	

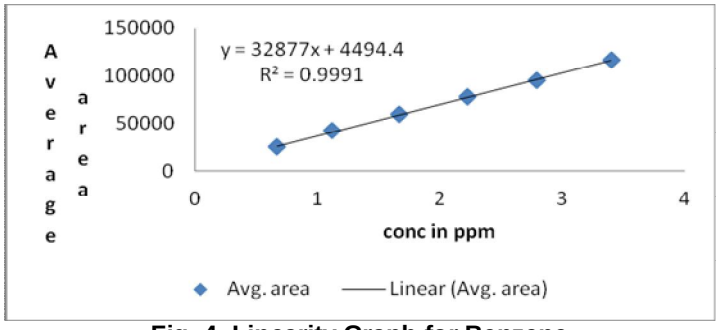

Fig. 4: Linearity Graph for Benzene

Table 5: Linearity Graph for Toluene

S. No	Toluene	
	Actual Conc.	Avg. area
LOQ	0.35	69839
25%	232.2	4143926
50%	464.4	8271667
75%	696.6	12295770
100%	928.8	16634578
125%	1161.0	20570855
150%	1393.2	25090959
Slope	17888.8	
Correlation coefficient	0.9998	

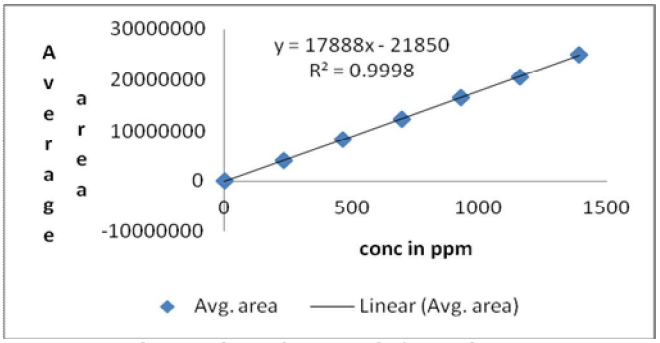

Fig. 5: Linearity Graph for Toluene

Table 6: Accuracy

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

Table 7: 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

Robustness

The flow rate was changed ±0.35mL/min from that of the original one i.e. 3.5mL/min. The obtained results show that it has not affected by change in flow rate.

Batch Analysis

Finally the prepared methocarbamol pure drug was tested for the presence of residual solvents mainly benzene. Prepare the test solution in duplicate consecutively for 10 batches, inject the prepared two test solutions in to the gas chromatograph and record the peak responses. Subtract the area counts at solvent retention time in blank injection from the area counts obtained due to test preparation. Calculate the content in ppm of residual solvents by using average area from Test solution against to the solvent peak areas obtained from six standard injections. Consecutive 10 batches shall be injected for the estimation of solvent profile. Inject another five batches spiked with LOD level and five more batches shall be spiked with LOQ level, inject these samples for better monitoring of residual solvents in Methocarbamol.

Calculate the residual solvent content by using the

Following formulae:

Calculation

$$\frac{\text{Area of solvent in test solution X conc. in mg/mL of Solvent in standard solution X } 10^6}{\text{Ave. Area of solvent in Standard solution X Conc. in mg/mL of Sample solution}}$$

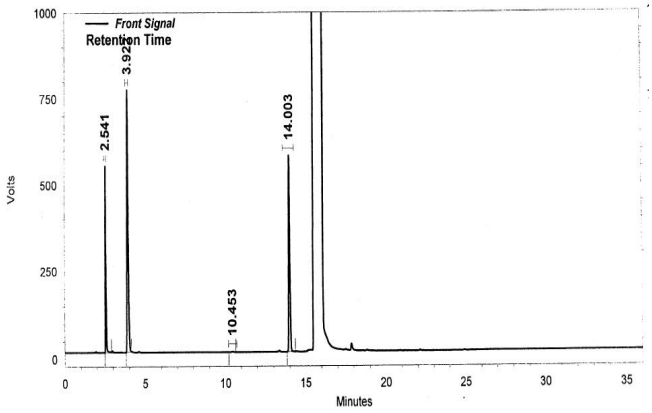

Fig 6: Optimised chromatogram

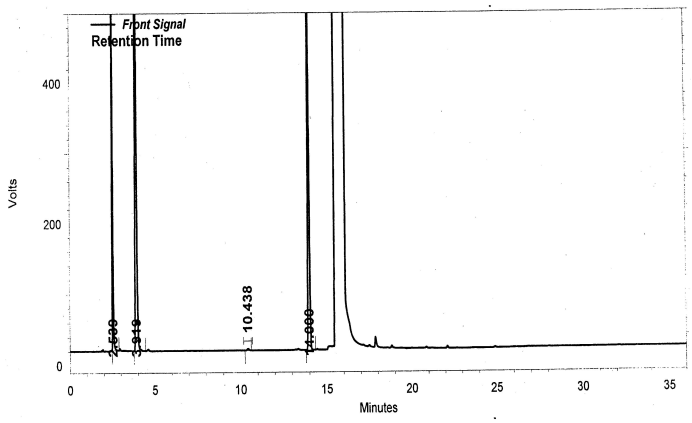

Fig 7: Standard Stock Solution

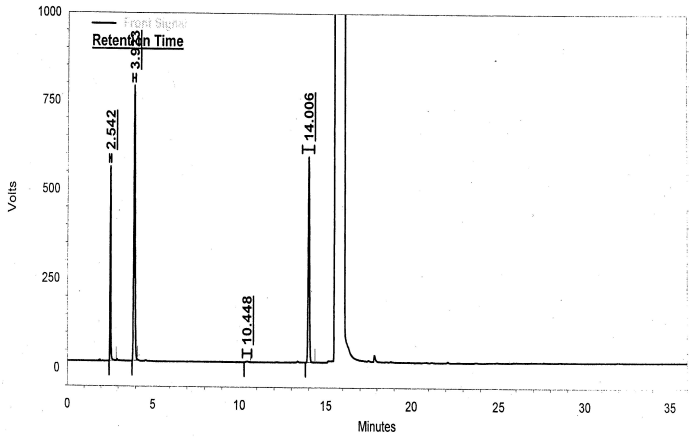

Fig 8: Accuracy

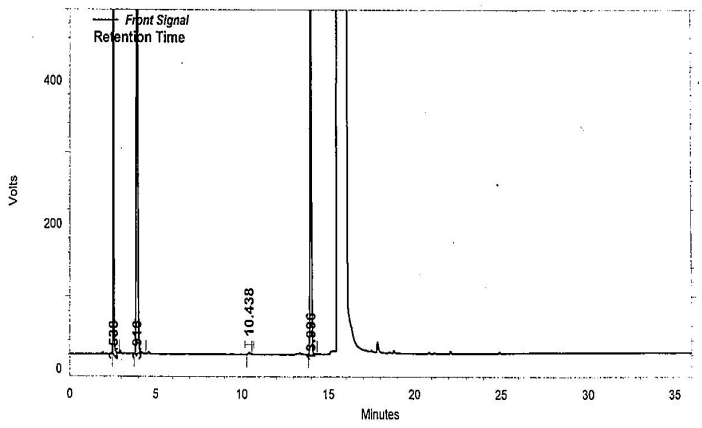

Fig 9: Spiked sample

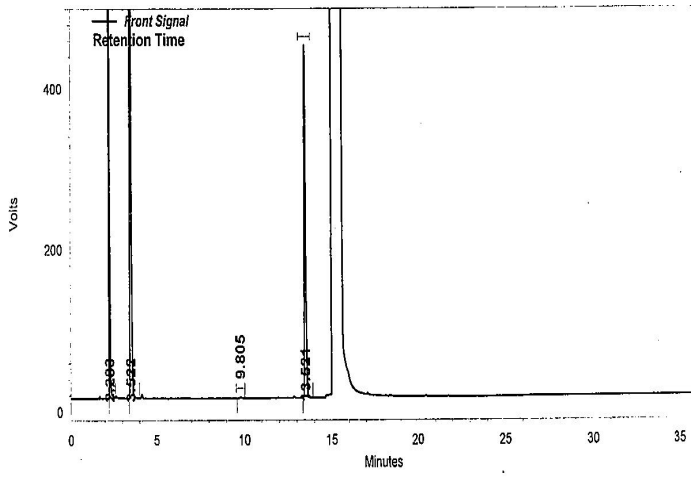

Fig 10: Robustness

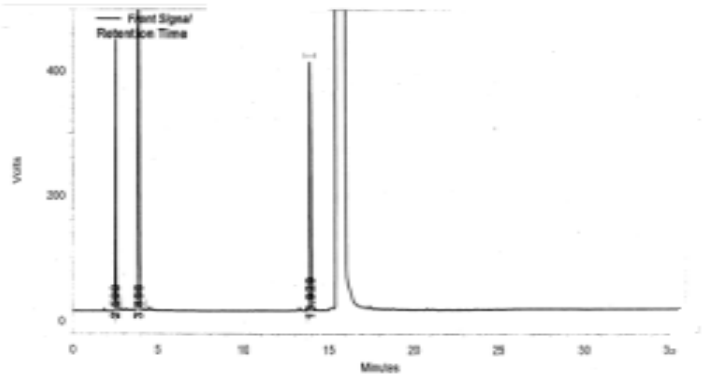

Fig 11: Standard-1

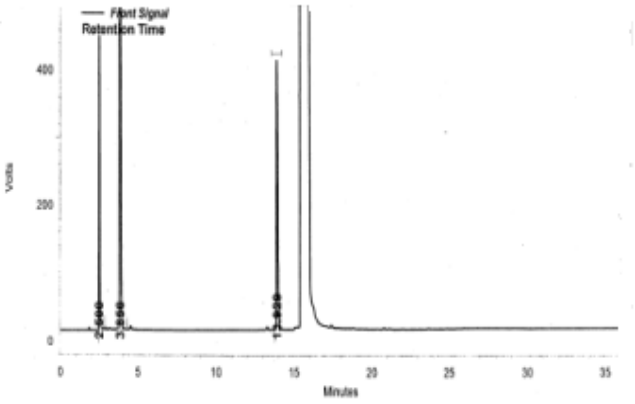

Fig 12: Standard-2

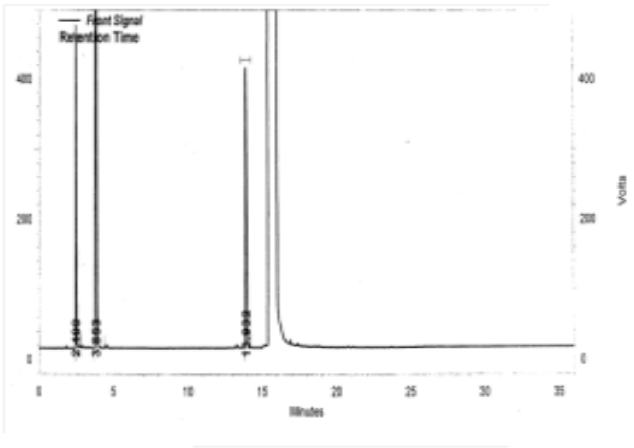

Fig 13: Standard-3

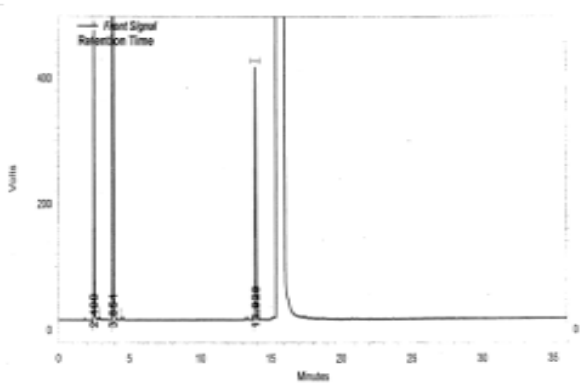

Fig 14: Standard-4

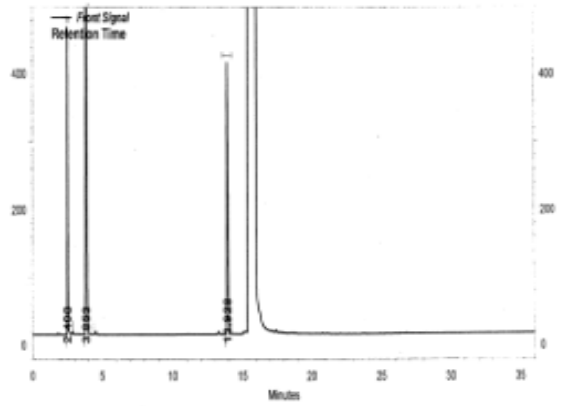

Fig 15: Standard-5

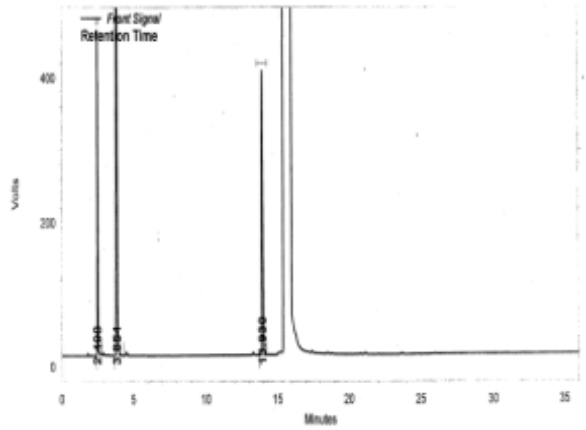

Fig 16: Standard-6

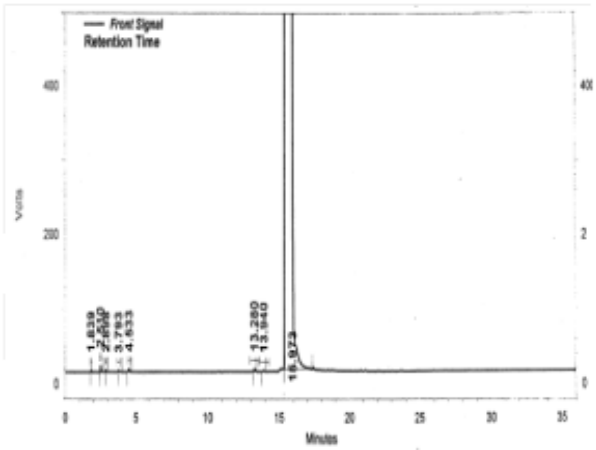

Fig 17: Blank

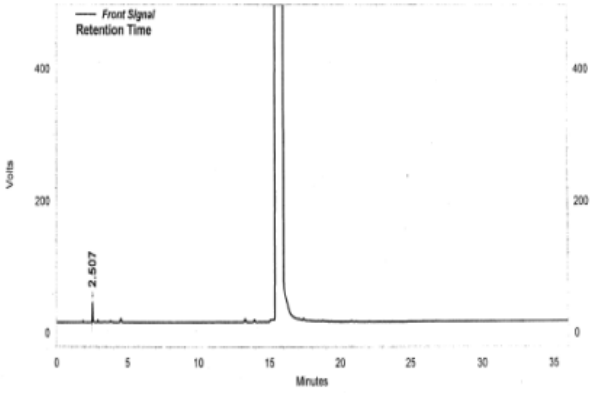

Fig 18: Methocarbamol Sample-1

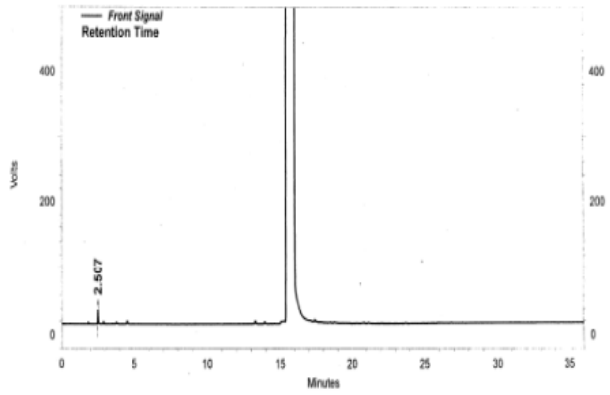

Fig 19: Methocarbamol Sample-1

From these chromatograms obtained from the sample we can observe that no peak was found at the retention time of the benzene. By this it was concluded that our sample is pure and free from residual solvents.

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