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Research Article

VALIDATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF COMMON COUGH AND COLD INGREDIENTS IN MULTICOMPONENT ORAL DRUG PRODUCTS

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ABSTRACT

This research was done to develop a potential, reliable fast and efficient analytical method for various dosage forms (eg. Syrup, tablet, suspension etc) which could estimate all the major components of a cough and cold multicomponent formulation and also this method was validated. All common used components like pheniramine maleate, phenylephrine hydrochloride, acetaminohen, dextromethorphan hydrobromide, guafenesin, chlorpheniramine maleate, diphenhydramine in cough and cold category of products also covering more than one dosage forms (eg. Syrups, suspensions, tablet etc) was used thus making the methodology by and large universal for the entire range of cough and cold products. This would help in using almost a common method of analysis for separation of most of the components instead of using a lot of separate methods for the same product or using a common method of analysis for a range of different dosage form of cough and cold category for a common set of components. This would save time and cost and ensure optimum usage of resources. Mobile phase of 0.01M 1-octane sulphonic acid sodium salt monohydrate (pH adjusted to 2.80 with 0rthophosphoric acid): Acetonitrile in a gradient ratio was used with a flow rate of 1.0 ml/min on a C18, 25 X 4.6 mm id, 5µ column at a wavelength of 264nm. This method was validated for parameters as accuracy, repeatability, reproducibility, robustness, linearity, limit of detection and limit of quantification. This HPLC method was found to be specific, linear, precise ,accurate, reproducible and robust and can be easily used for determination of common cough and cold analytes in a formulation as the results were found to be well within the acceptance range.

Keywords: High Performance Liquid Chromatography (HPLC), Over the Counter (OTC).

1.0 INTRODUCTION

Cough and cold category is one of the major illness in the medical field. Almost everyone suffers from cough and cold some time or the other thus making these products very valuable. The selection of analytical methods is determined by several factors such as speed, convenience, specificity, accuracy, precision, sensitivity, selectivity, cost, availability of instruments, technical expertise and the number of samples to be analyzed etc. This shows the need of improved methods for analysis.

There are many methods reported which are developed for components used in cough and cold products.

But these methods are developed either for individual components or only for that particular dosage form or for that particular product or to determine some components from a particular dosage form.
HPLC, with Ultraviolet, Fluorimetry or Mass Spectroscopy(MS) detectors are most widely used. Other techniques include Ultraviolet-Visible Spectroscopy, Thin layer Chromatography (TLC), Gas Chromatography(GC), GC/MS, Capillary Electrophoresis and multivariate spectrophotometric method have been used to determine few of these compounds. A number of conventional methods have been applied to present series. Pseudoephedrine and Acetaminophen have been determined Spectrophotometrically and Gas Liquid Chromatography (GLC) is also used. Guafenesin has been determined by GLC.Spectrophotometric, GLC or methods requiring TLC separation when applied to samples such as cough mixtures can be lengthy and or subject to interferences by the matrix of the sample, thus making them unsuitable for simultaneous assay.
Simultaneous capillary gas chromatographic determination of guafenesin, dextromethorphan, and diphenhydramine in cough –cold syrup was performed earlier.[1]
Similarly HPLC Method for the Simultaneous Determination of acetaminophen, phenylephrine, Dextromethorphan and Chlorpheniramine in pharmaceutical Formulations was used.[2]
Simultaneous determination of phenylpropanolamine hydrochloride, dextromethorphan hydrobromide and chlorpheniramine maleate in formulations by reversed-phase liquid chromatography was done.[3]
Simultaneous determination of Phenylephrine Hydrochloride, Guafenesin, and Chlorpheniramine Maleate in cough Syrup by Gradient Liquid Chromatography literature is available.[4]
Simultaneous capillary gas chromatographic determination of guafenesin, dextromethorphan, and diphenhydramine in cough –cold syrup.[5]
Some of the methods are very time consuming and expensive and a lot of problems are associated with the method itself ie. Sensitivity to the method, reproducibility , ruggedness thus giving us a need to develop a coherent method which will overcome all these issues.
As of now there are methods in which a single component is developed or multicomponent are developed from a particular dosage forms.
There is no analytical method for simultaneous determination of most of the compounds mentioned earlier for cough and cold category of products.
This research was done to develop a potential, reliable fast and efficient analytical method for various dosage forms (eg. Syrup, tablet, suspension etc) which could estimate all the major components of a cough and cold multicomponent formulation .
The validation for parameters as accuracy, repeatability, reproducibility, robustness, linearity, limit of detection (LOD) and limit of quantification (LOQ) was undertaken as per ICH Q4.

1.1 Chemical structures of all analytes[6]

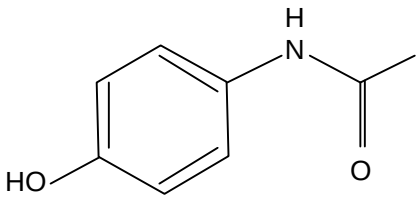

Fig. 1 : Paracetamol

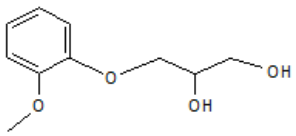

Fig. 2: Guaifenesin

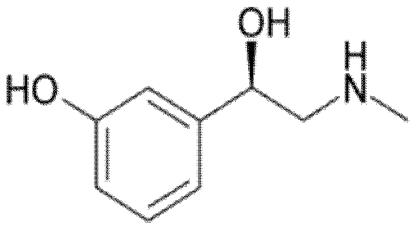

Fig. 3: Phenylephrine Hydrochloride

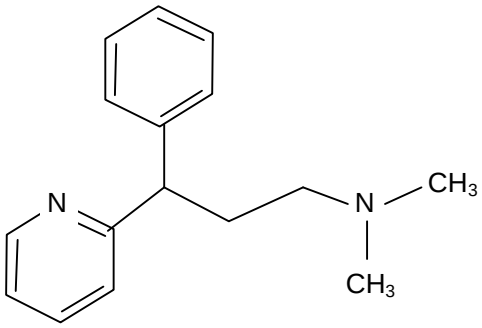

Fig. 4: Pheniramine Maleate

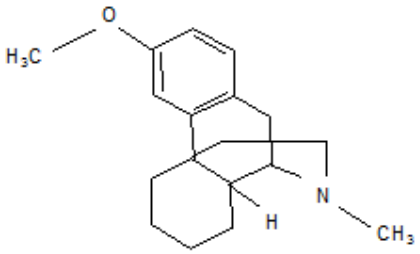

Fig. 5: Dextromethorphan

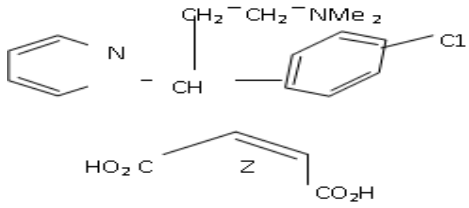

Fig. 6: Chlorpheniramine Maleate

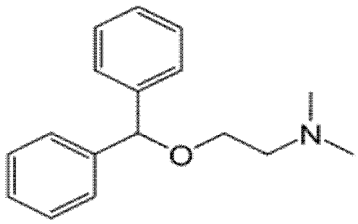

Fig. 7: Diphenhydramine

2.0 MATERIAL AND METHODS

Individual standards of all seven analytes – acetaminophen (USP), guafenesin (USP), pheniramine maleate (IP), phenylephrine hydrochloride (USP), diphenhydramine (IP), chlorpheniramine maleate (IP), dextromethorphan (USP) were used. Ammonium phosphate dibasic AR, ammonium phosphate monobasic AR, 1- octane suphonic acid sodium salt monohydrate AR , acetonitrile (HPLC grade) and milli Q water were also used in all the work. Phosphoric acid AR for pH adjustment and triethylamine HPLC grade was used.[7]

2.1 Equipment / Material needed - Table 1.0

For testing (Chemicals)	Testing equipment
1. 1-Octane Sulfonic Acid Sodium salt monohydrate	1. HPLC system
2. Ammonium Phosphate Dibasic AR	2. Ultrasonic bath
3. Ammonium Phosphate monobasic AR	3. Analytical balance
4. Phosphoric acid AR	4. Glass apparatus
5. Water (Milli-Q)	5. Column- Phenomenex luna, 100A, 5 um, 4.6 x 250mm
6. Acetonitrile (HPLC Grade)	6. Filtration Assembly
7. Triethylamine AR	7. pH meter

2.2 Selection of the most suitable wavelength for UV

S.No	Components	λmax1	λmax2	λmax3	λmax4
1.	Pheniramine maleate	262.0	223.6		
2.	Guafenesin	275.0	226.0	210.2	
3.	Diphenhydramine	264.6	258.8	253.0	215.6
4.	Dextromethorphan	287.6	280.4	227.2	207.8
5.	Chlorpheniramine maleate	269.8	262.6	220.0	207.8
6.	Acetaminophen	259.2	211.6		
7.	Phenylephrine hydrochloride	276.0	218.2		

Hence a wavelength of 264 nm was selected as all the components were detected at this wavelength.

2.3 Mobile Phase Preparation

In the present research work, initially the mobile phase used was methanol: water, it was observed that analytes moved from the origin with methanol and water but not convincingly. Hence methanol was replaced by acetonitrile, which gave better separation and resolution. Along with that phosphoric acid was also incorporated in the mobile phase as the modifier to reduce tailing and achieve sharper spots. Hence the mobile phase was then altered to buffer (Sodium octane salt of sulphonic acid monohydrate: Acetonitrile: Phosphoric acid in the gradient manner.With this mobile phase, good resolution between all the three components was seen with suitable Rf values. [8]
Thus optimized mobile phase used for separation was
Solvent A: Acetonotrile
Solvent B: Buffer.

2.3.1 Buffer

It was prepared by dissolving about 2.34 g of 1-Octane sulfonic acid sodium salt monohydrate AR in 1000 ml of water in a borosilcate glass container to obtain a concentration of about 0.01M . Adjusted the pH to 2.8 ± 0.1 with phosphoric acid, and filtered through a 0.45µ nylon membrane filter. All solutions as well as the mobile phases were filtered through 0.45µ nylon membrane filter. Mobile phases were freshly prepared weekly and degassed prior the use.

2.3.2 Mobile phase A

Acetonitrile was used as mobile phase A

2.3.3 Mobile phase B:

Buffer prepared above was used as mobile phase B

Gradient: Table 2.0

Sr. No.	Time	Flow	%A	%B	%C	%D	Curve
1.	----------	1.00	20.0	80.0	0.0	0.0	----------
2.	3.50	1.00	20.0	80.0	0.0	0.0	6
3.	40.00	1.00	40.0	60.0	0.0	0.0	6
4.	47.00	1.00	40.0	60.0	0.0	0.0	6
5.	48.00	1.00	20.0	80.0	0.0	0.0	6
6.	60.00	1.00	20.0	80.0	0.0	0.0	6

2.3.4 Diluent A

Dissolved 0.094 g of ammonium phosphate monobasic and 0.108 g of ammonium phosphate dibasic in 250 mL of water. Added 750 ml of acetonitrile and 3.0 mL of triethylamine. Mixed and adjusted to pH 6.8 ± 0.05 with phosphoric acid.

2.3.5 Diluent B

Prepared sufficient quantity by mixing buffer pH 2.8 and Acetonitrile 40:60 in a suitable container.

2.4 Preparation of the solutions

Standard solutions of acetaminophen, guaifenesin, pheniramine maleate, phenylephrine hydrochloride, diphenhydramine ,chlorpheniramine maleate, dextromethorphan were prepared as mentioned below.

Stock Standard Solution A: Accurately weighed and transferred about 50 mg of acetaminophen, guaifenesin, pheniramine maleate, phenylephrine hydrochloride, diphenhydramine ,chlorpheniramine maleate, dextromethorphan standard into a 50 mL volumetric flask individually and added 60 mL of diluent A. Sonicated to dissolve and dilute to volume with diluent A and mixed well. (1000 ppm)

Stock Standard Solution B: Transfer 5.0 ml of Stock standard solution A individually into a separate 20 ml volumetric flasks, diluted to volume with diluent A and mixed well. (250 ppm).

2.5 Combined Standard Preparation

1. Transfered 5.0 mL of dextromethorphan hydrobromide and diphenhydramine hydrochloride stock standard solution A into a 50 mL volumetric flask, diluted to volume with diluent B and mixed well. (100 ppm)
2. Transfered 2.0 mL of paracetamol, guaiphenesin, pheniramine maleate, phenylephrine hydrochloride and chlorpheniramine maleate stock standard solution B (250 ppm) into same 50 mL volumetric flask, diluted to volume with diluent B and mixed well. (10 ppm).

2.6 Preparation of work standard solution

Prepared the sample stock solution into diluent A. Further diluted the solution to get final concentration of acetaminophen (10 ppm), guaiphenesin (10 ppm), pheniramine maleate (10 ppm), phenylephrine hydrochloride (10 ppm), diphenhydramine (100 ppm), chlorpheniramine maleate (10 ppm), dextromethorphan (100 ppm) with diluent B.

2.7 Preparation of Placebo

Placebo is prepared using all the excipients except the analytes namely acetaminophen, guaiphenesin , pheniramine maleate, phenylephrine hydrochloride, diphenhydramine, chlorpheniramine maleate,dextromethorphan. The criteria to select the placebo was to have all the common excipients used in cough and cold products as a part of the placebo without the main components. This was done to check for any kind of interferences from the placebo.

2.8 Column Selection

Various columns were used during method development to ensure all the components were eluted efficiently. Thus column used for separation was Phenomenex luna, 5 µ, 4.6 X 250 mm Flow rate selection and injection volume selection A lot of flow rates were tried out to get the best chromatogram eluted. Finally flow rate of 1ml/min was selected Different volumes from 10µl - 100 µl was tried out. Finally injection volume of 25 µl was used Thus optimized flow rate of 1 ml/min and injection volume of 25µl was selected Visualization, detection and quantification

There are many methods for the detection and visualisation of the separated components on HPLC.[9] Paracetamol,Guaiphenesin, Pheniramine Maleate, Phenylephrine Hydrochloride, Dextromethorphan hydrobromide, Diphenhydramine hydrochloride and Chlorpheniramine maleate exhibit absorbance in the UV region of the electromagnetic spectrum. Hence detection and quantification was performed in

absorbance mode using Ultra violet spectrophotometer. During the method development, the wavelength chosen for further quantification was 264 nm.
Hence it was observed that Paracetamol, Guafenesin, Phenylephrine Hydrochloride, Dextromethorphan, Diphenhydramine Hydrochloride, Pheniramine Maleate, Chlorpheniramine maleate could be determined by HPLC. Thus, the proposed HPLC method developed can be successfully applied for the routine quality control analysis of Paracetamol, Guafenesin, Phenylephrine Hydrochloride, Dextromethorphan, Diphenhydramine Hydrochloride, Pheniramine Maleate, Chlorpheniramine maleate from their fixed dosage form.

2.9 Optimized chromatographic conditions Table 3.0

SR No.	PARAMETER		DESCRIPTION
1.	1	Instrument	HPLC
	2	Pump	Gradient pump
	3	Injector	Autosampler
	4	Column	Phenomenex Luna C18, 25cm x 4.6 mm i.d., 5µm.
	5	Detector	UV- Visible detector
	6	Wavelength	264nm
	7	Recorder	Empower -2 chromatography software
2.	Mobile Phase		0.01M 1-octane sulphonic acid sodium salt monohydrate (pH adjusted to 2.80 with 0rthophosphoric acid): Acetonitrile in a gradient ratio
3.	Flow Rate		1.0 ml/ min
4.	Volume		25µl

3.0 Method validation

3.1 Specificity

The placebo was spiked with paracetamol, guaiphenesin, phenylephrine hydrochloride, pheniramine maleate, chlorpheniramine maleate, dextromethorphan and diphenhydramine hydrochloride and were chromatographed to check the interference of the placebo.[10]

3.2 Linearity

Linearity was studied by preparing seven standard solutions of paracetamol, guaiphenesin, phenylephrine hydrochloride, pheniramine maleate, chlorpheniramine maleate, dextromethorphan and diphenhydramine hydrochloride at the concentration range of 70% to 120 % of working concentration from a stock solution and each concentration was injected in triplicate and chromatographed .[11]

3.3 Detection and quantification limits

The parameters of detection limit (DL) and quantitation limit (QL) were determined on the basis of the ICH Guidelines for the Validation of Analytical Procedures. Both parameters were evaluated with the ‘‘signal-to-noise’’ approach with a ration 3:1 for DL and 10:1 for QL.

3.4 Precision and repeatability

The precision of the employed HPLC method was determined by repeatability (intra-day) and intermediate precision (inter-day) with a standard solution . Both parameters were measured by different analyst.[12]

3.5 Robustness

The evaluation of robustness was carried out with regard to flow rate of solution. Three different flowrates (0.9, 1.0 and 1.1 ml/min) were tested in triplicate measurements.

3.6 Accuracy

Accuracy is performed by injecting in triplicate concentrations at 80%,100% and 120% of working concentration. The recovery is calculated against a standard preparation.

3.7 Stability of solution

Analysis was done after 8 and 16 hours versus a freshly prepared standard solution on each occasion. The content of all the analytes was calculated against a standard and compared.

4.0 RESULTS AND DISCUSSION

The proposed HPLC method developed was successfully applied for the routine quality control analysis of paracetamol, guafenesin, phenylephrine hydrochloride, dextromethorphan, diphenhydramine hydrochloride, pheniramine maleate, chlorpheniramine maleate from their fixed dosage form.

4.1 Validation of the method developed

4.1.1 Specificity

The chromatogram of standard showed peaks at retention time appoximately 4.1 min, 9.7 min, 11.3 min, 29.0 min, 35.9 min, 40.3 min, 42.9 min for paracetamol, guaiphenesin, phenylephrine hydrochloride, pheniramine maleate, chlorpheniramine maleate, dextromethorphan and diphenhydramine approximately. Readily identifiable is that the components are clearly separated from the additives. The specificity of the method has been obtained by comparison of the single compound chromatograms with the chromatogram of the standard solution and blank .The purity of each compound was certificated by the manufacturer.[13]

This indicates that the presented HPLC method is selective and suitable for the detection of the seven common cough and cold active components in a formulation used to treat cough and cold.

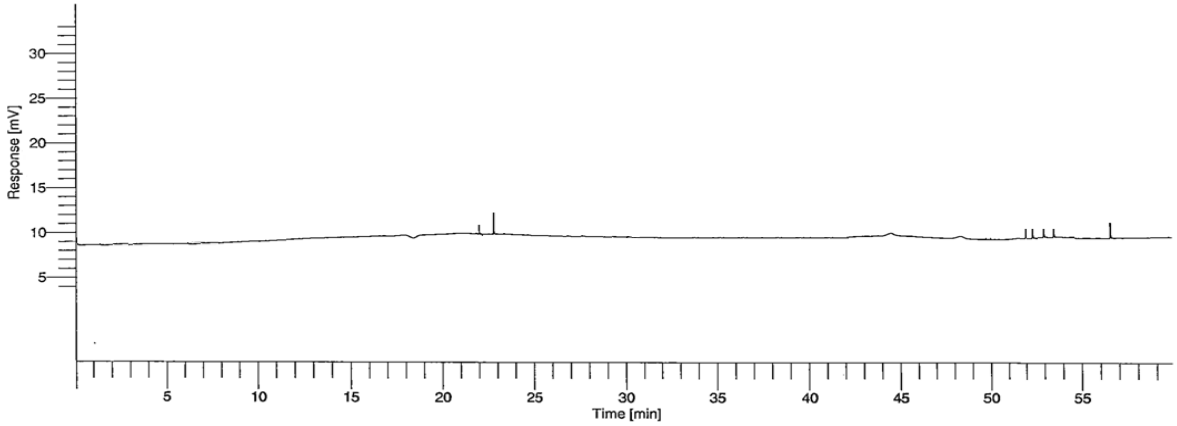

Fig. 8.0: Specificity for Paracetamol Guaiphenesin, Pheniramine Maleate, Phenylephrine Hydrochloride, Dextromethorphan hydrobromide, Diphenhydraminehydrochloride and Chlorpheniramine maleate (BLANK)

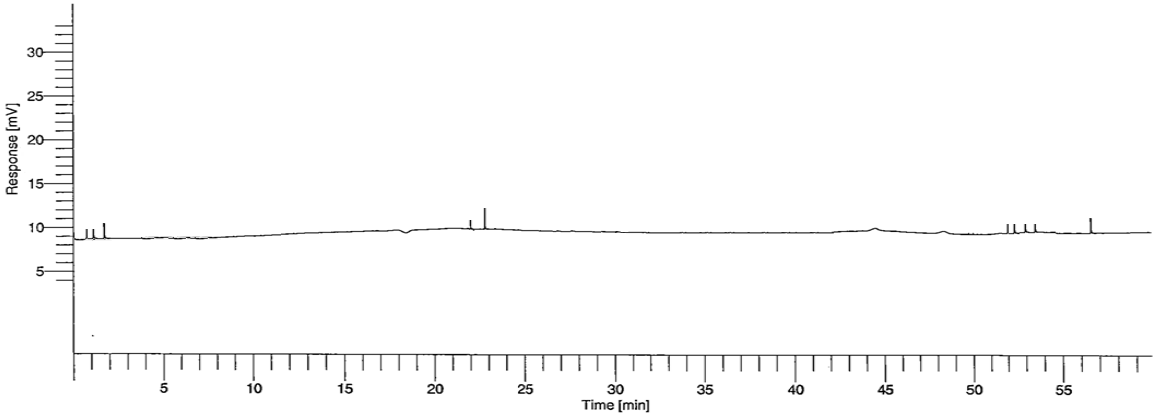

Fig. 9.0: Specificity for Paracetamol, Guaiphenesin, Pheniramine Maleate, Phenylephrine Hydrochloride, Dextromethorphan hydrobromide, Diphenhydramine hydrochloride and Chlorpheniramine maleate (PLACEBO)

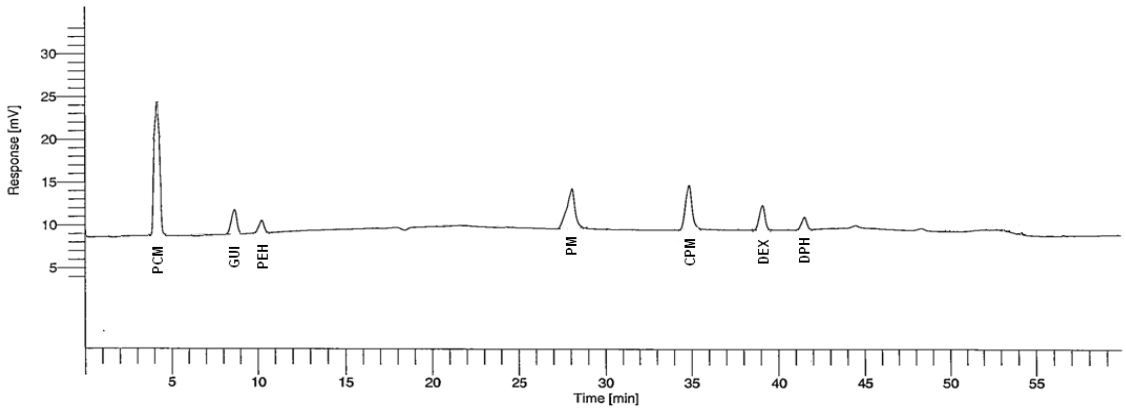

Fig. 10.0: Specificity for Paracetamol, Guaiphenesin, Pheniramine Maleate, Phenylephrine Hydrochloride, Dextromethorphan hydrobromide, Diphenhydramine hydrochloride and Chlorpheniramine maleate (STANDARD)

4.1.2 Linearity, DL, QL

Calibration curves for every single component were carried out in aqueous solution as well as in the drug matrix and were found to be linear with a correlation coefficient of 0.999 in most of the cases. DL and QL were determined in relation to the lowest concentrated drug. Measures were performed in triplicate.[14]

Limit of detection

The least concentration that can be effectively detected is found.

$$\textbf{LOD} = \frac{3.3 \times \text{SD of Intercept}}{\textbf{Mean slope}}$$

Limit of quantification

The least concentration that can be effectively quantified is the limit of quantification.

$$\textbf{LOQ} = \frac{10 \times \text{SD of Intercept}}{\textbf{Mean slope}}$$

Linearity was studied by preparing six standard solutions of Paracetamol, Guaiphenesin, Phenylephrine Hydrochloride, Pheniramine Maleate, Chlorpheniramine maleate, Dextromethorphan hydrobromide and Diphenhydramine hydrochloride at the concentration range of 10% to 120 % of working concentration from a stock solution and each concentration was injected in triplicate and chromatographed as per the procedure.

Linearity 10%: 3.5 ml of stock standard solution A (IV) and (V) + 1.4 ml of PCM, GUI, PM, PEH and CPM Stock Standard Solution B -→ 50 ml with diluent B and mix well.

Linearity 40%: 3.5 ml of stock standard solution A (IV) and (V) + 1.4 ml of PCM, GUI, PM, PEH and CPM Stock Standard Solution B -→ 50 ml with diluent B and mix well.

Linearity 80%: 4.0 ml of stock standard solution A (IV) and (V) + 1.6 ml PM, PEH and CPM Stock Standard Solution B -→ 50 ml with diluent B and mix well.

Linearity 100%: 5.0 ml of stock standard solution A (IV) and (V) + 2.0 ml PM, PEH and CPM Stock Standard Solution B -→ 50 ml with diluent B and mix well.

Linearity 120%: 6.0 ml of stock standard solution A (IV) and (V) + 2.4 ml PM, PEH and CPM Stock Standard Solution B -→ 50 ml with diluent B and mix well.

Linearity Graph: Table 4.0:

Concentration in %	PCM	GUI	PM	PEH	DEX	DPH	CPM
10	45768	12272	21895	1478	8943	4155	20679
40	188616	50615	99195	5853	36378	16958	90237
80	386028	100279	194351	11780	71092	34776	175916
100	471988	125773	246406	14873	89585	42974	221971
120	564994	150954	290730	17846	107999	52122	271614
Average	331479	87978	170516	10366	62800	30197	156083
y-Intercept	159.5	-124.64	-832.89	-64.15	57.22	-297.16	-1864.17
Correlation	0.9998	1	0.9998	1	0.9999	0.9999	0.9997
Slope	4733.13	1258.62	2447.83	149.01	896.32	435.63	2256.39
LOD	**0.2087**	**1.4759**	**0.2861**	**1.1444**	**1.3**	**0.1665**	**0.4171**
LOQ	**0.6323**	**4.4723**	**0.8668**	**3.4678**	**3.9384**	**0.5044**	**1.2638**

4.1.3 Precision and repeatability

Precision and repeatability data was done in the form of intra- and inter-day variations .The precision of the method has an RSD of 0.05 % for repeatability and 0.05 % for precision, which comply with the defined requirements. All measurements were produced in triplicate.[15]

i) Repeatability: Six replicate injections of a standard solutions were chromatographed and the relative standard deviation of the peak areas was calculated.

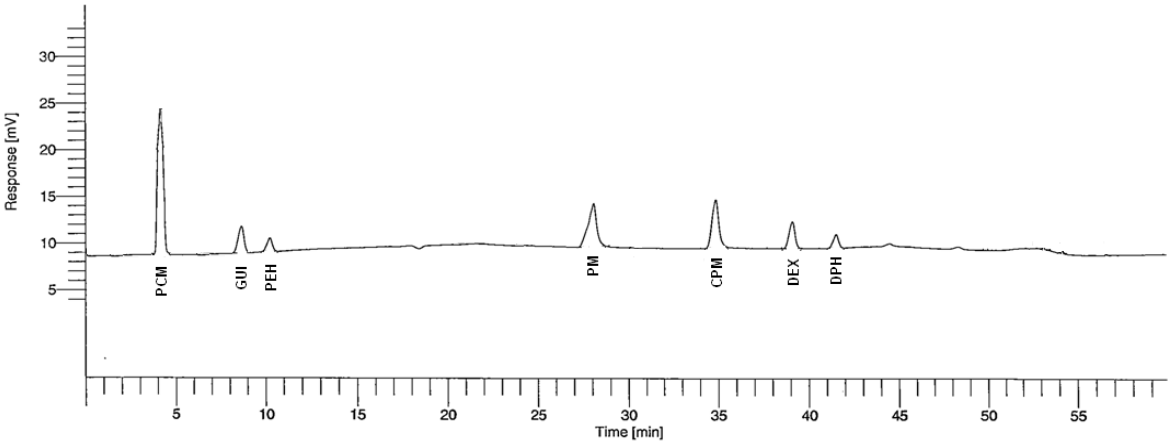

Fig. 11.0: STANDARD

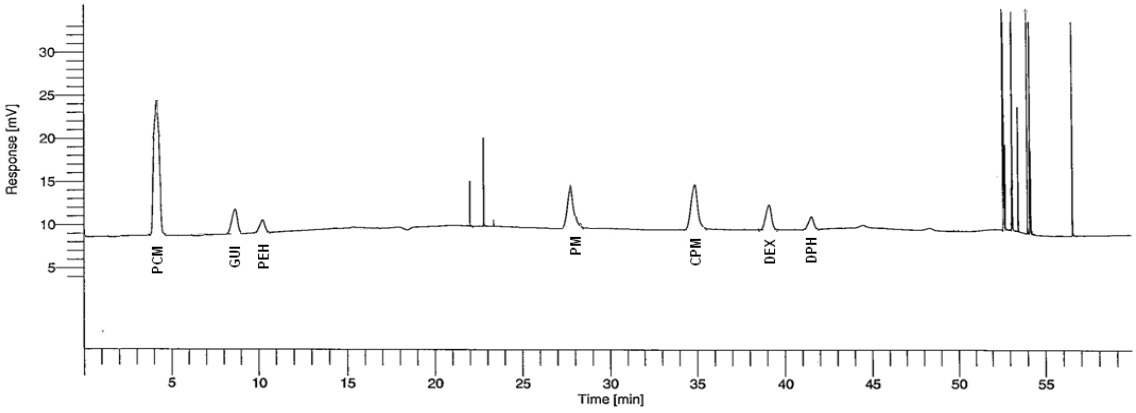

Fig. 12.0: SAMPLE

Where, Paracetamol = PCM ,Guaiphenesin = GUI, Pheniramine Maleate= PM, Phenylephrine Hydrochloride= PEH, Dextromethorphan hydrobromide= DEX, Diphenhydramine hydrochloride= DPH, Chlorpheniramine maleate= CPM.

ii) Intermediate precision: Ruggedness

Analysis was carried out on same batch on different day by different Chemist as per methodology.

Day 1, Chemist 1,Column 1, Instrument: Agilent Technologies 1200 series 1, Column: Phenomenex Luna C18, 25cm x 4.6 mm i.d., 5µm.
Day 2, Chemist 2, Column 2, Instrument: Agilent Technologies 1200 series 1, Column: Phenomenex Luna C18, 25cm x 4.6 mm i.d., 5µm.

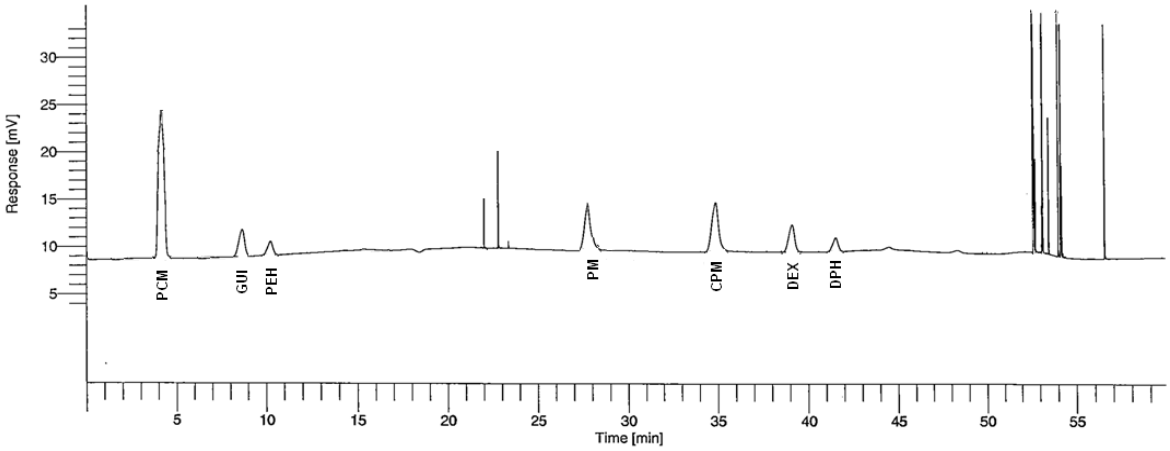

Fig. **13.0:** Intermediate precision: Ruggedness **(SAMPLE)**

4.1.4 Robustness

Flow rate was examined to evaluate the role in the developed method. Three different flowrates (0.9, 1.0 and 1.1 ml/min) were tested.
In this parameter the effect of the stability of the solution, changing the flow rate on the content of paracetamol, guaiphenesin, phenylephrine hydrochloride, pheniramine maleate, chlorpheniramine maleate, dextromethorphan hydrobromide and diphenhydramine hydrochloride was studied.[16]

i. Stability of solution

Analysis was done after 8 and 16 hours versus a freshly prepared standard solution on each occasion.

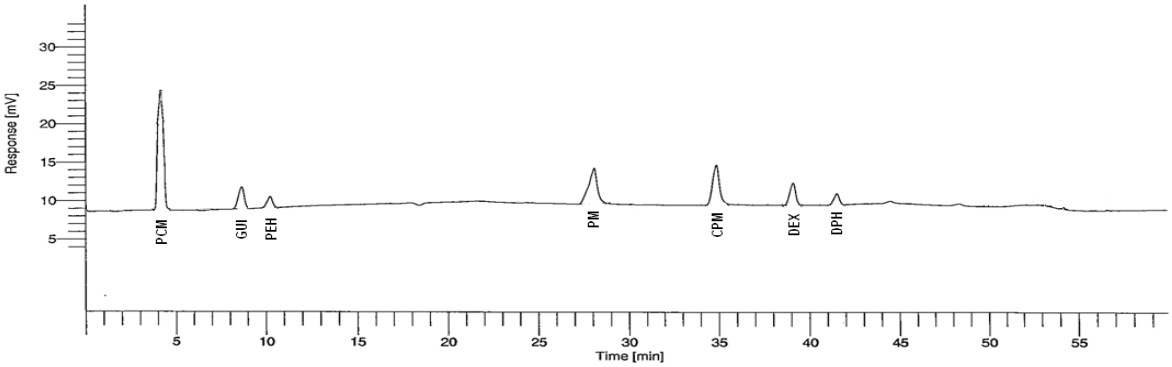

Fig. **14.0**: Stability of solution **(STANDARD)**

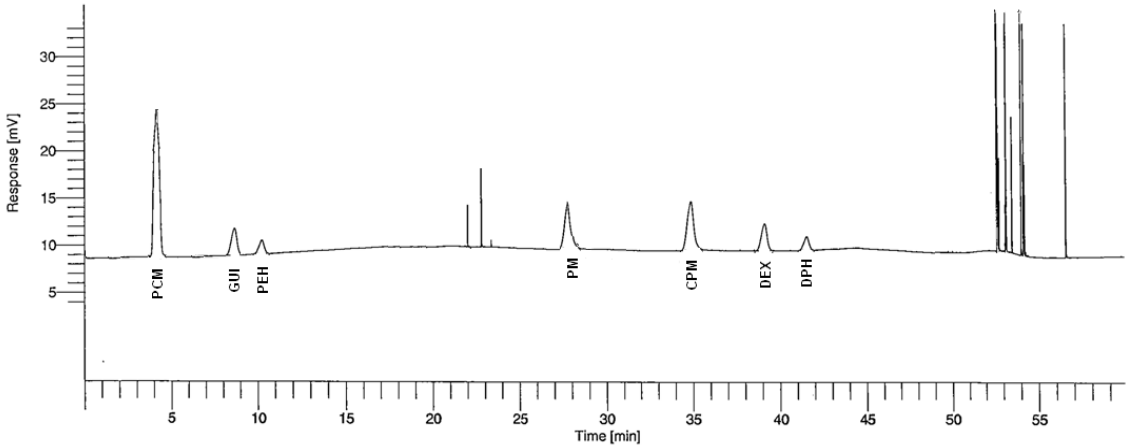

Fig. **15.0**: Stability of solution **(SAMPLE)**

Stability of solution after 8 hours

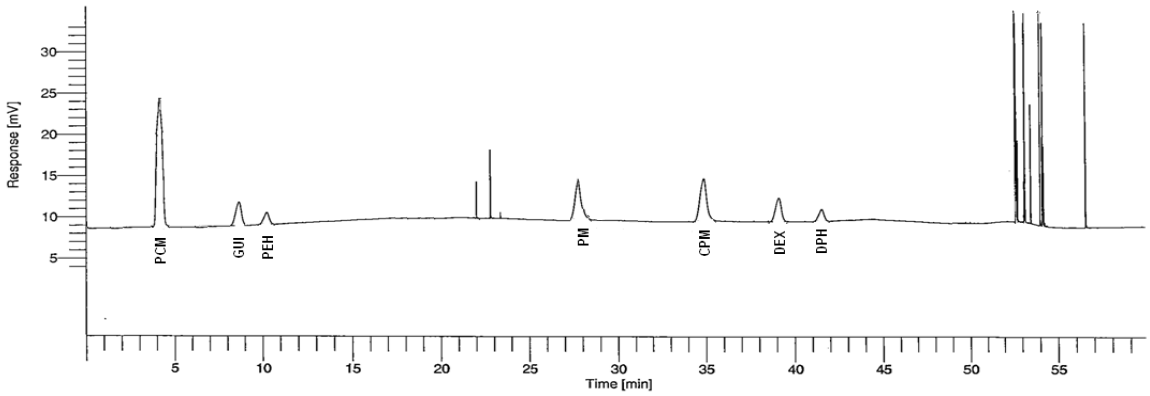

Fig. 16.0: Stability of solution after 8 hours (SAMPLE)

Stability of solution after 16 hours

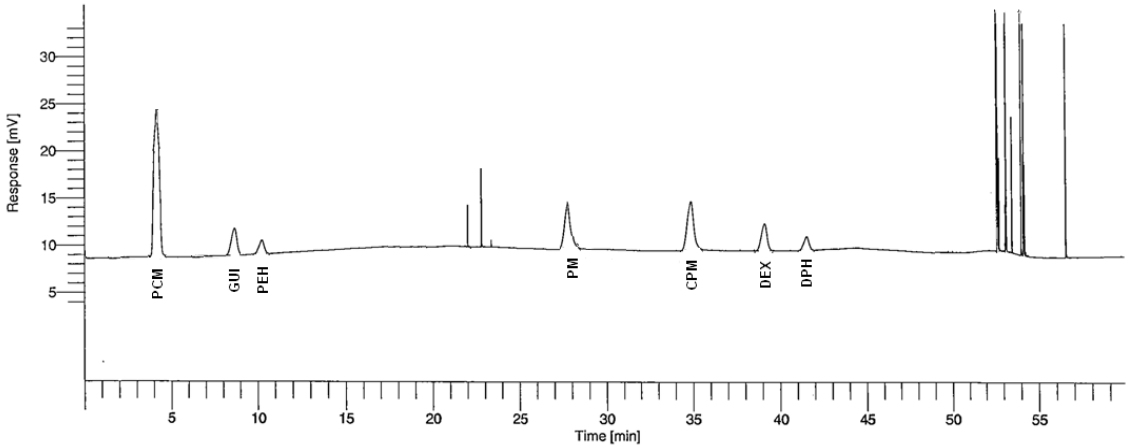

Fig. 17.0: Stability of solution after 16 hours (SAMPLE)

ii. Change of flow rate:

Flow rate will be changed within ± 0.1 ml versus a standard solution.

a. Initial Flow rate 1.0ml/min:

b. Flow rate changed to 0.9ml/min:

c. Flow rate changed to 1.1ml/min:

The content of Paracetamol, Guaiphenesin, Phenylephrine Hydrochloride, Pheniramine Maleate, Chlorpheniramine maleate, Dextromethorphan hydrobromide and Diphenhydramine hydrochloride with the 3 different flow rate was found to be identical. No appreciable change was observed.

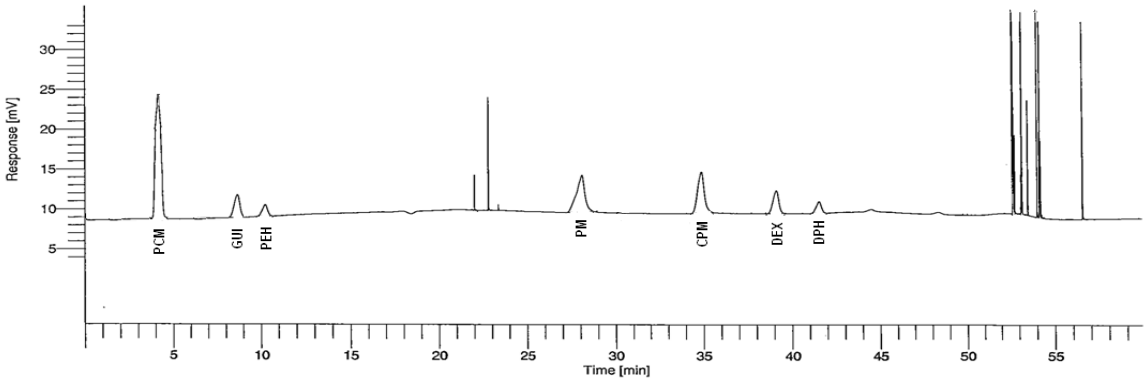

Fig. 18.0: GRAPH FOR FLOW RATE 1.0 ml/min (SAMPLE)

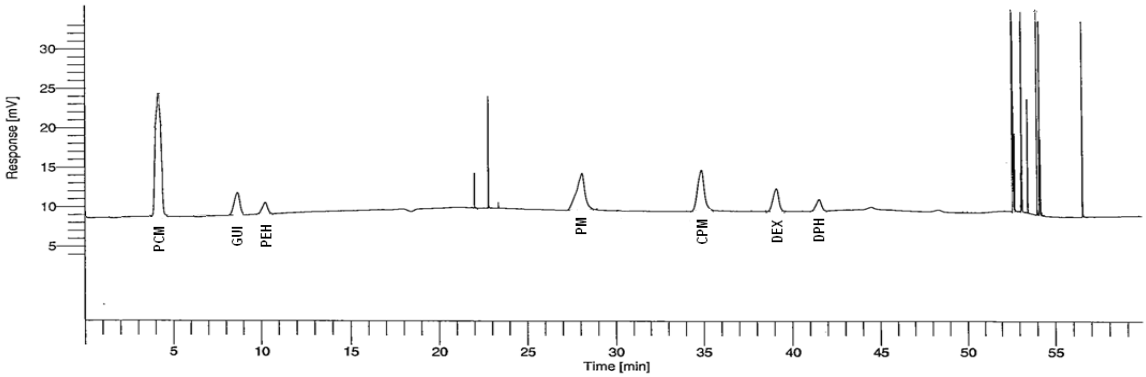

Fig. 19.0: GRAPH FOR FLOW RATE 0.9 ml/min (SAMPLE)

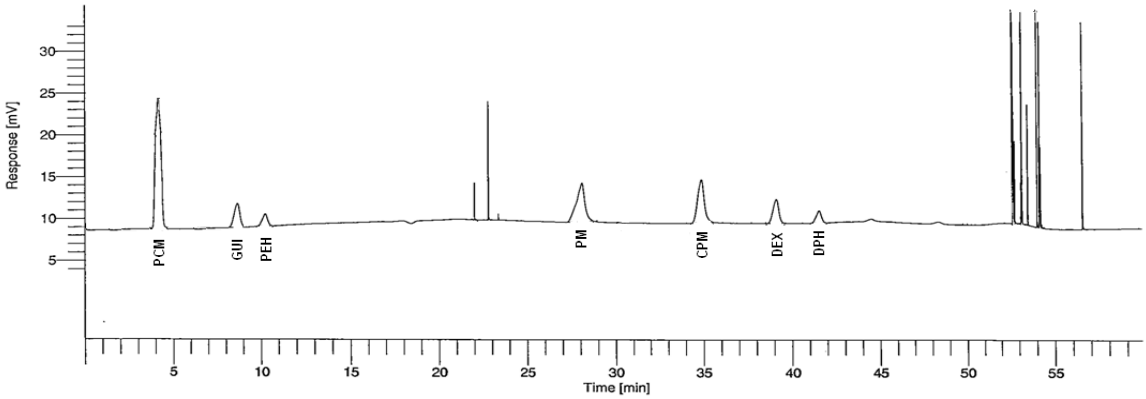

Fig. 20.0: GRAPH FOR FLOW RATE 1.1 ml/min (SAMPLE)

4.1.5 Accuracy

Accuracy is performed, by injecting in triplicate, concentrations at 80%, 100% and 120% of working concentration. The recovery is calculated against a standard preparation. (17)

Perform the same on samples by injecting in triplicate, concentrations at 80%, 100% and 120% of working concentration. The recovery is calculated against a standard preparation by using placebo.

Accuracy 80%: Transfer 4.0 ml of stock standard solution A (IV) and (V) + Transfer 1.6 ml of PCM, GUI, PM, PEH and CPM Stock Standard Solution B into 50 ml volumetric flask and dilute to mark with diluent B and mix well.

Accuracy 100%: Transfer 5.0 ml of stock standard solution A (IV) and (V) + Transfer 2.0 ml of PCM, GUI, PM, PEH and CPM Stock Standard Solution B into 50 ml volumetric flask and dilute to mark with diluent B and mix well.

Accuracy 120%: Transfer 6.0 ml of stock standard solution A (IV) and (V) + Transfer 2.4 ml of PCM, GUI, PM, PEH and CPM Stock Standard Solution B into 50 ml volumetric flask and dilute to mark with diluent B and mix well.

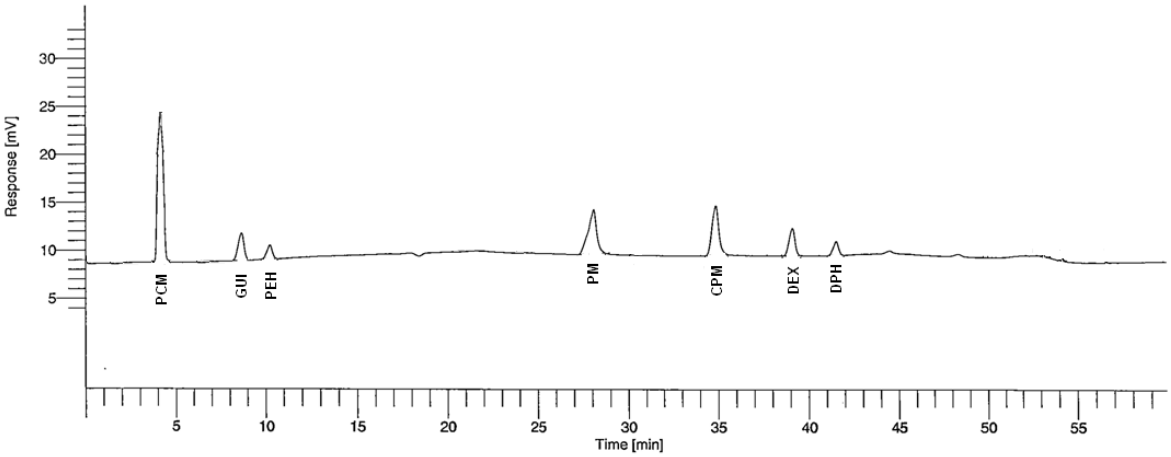

Fig. **21.0:** Accuracy for Paracetamol Guaiphenesin, Pheniramine Maleate, Phenylephrine Hydrochloride, Dextromethorphan hydrobromide, Diphenhydraminehydrochloride and Chlorpheniramine maleate **(STANDARD)**

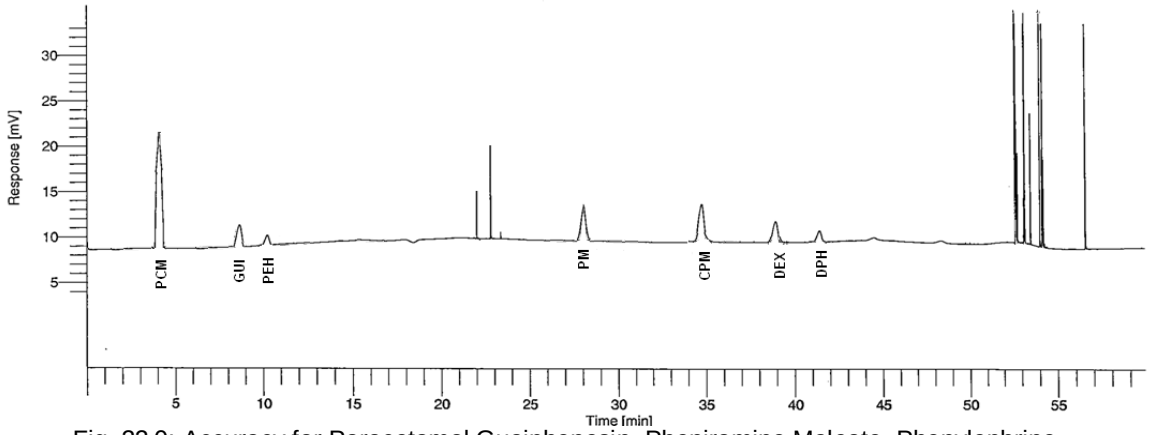

Fig. 22.0: Accuracy for Paracetamol Guaiphenesin, Pheniramine Maleate, Phenylephrine Hydrochloride, Dextromethorphan hydrobromide, Diphenhydraminehydrochloride and Chlorpheniramine maleate (SAMPLE- 80 %)

SAMPLE- 100 %

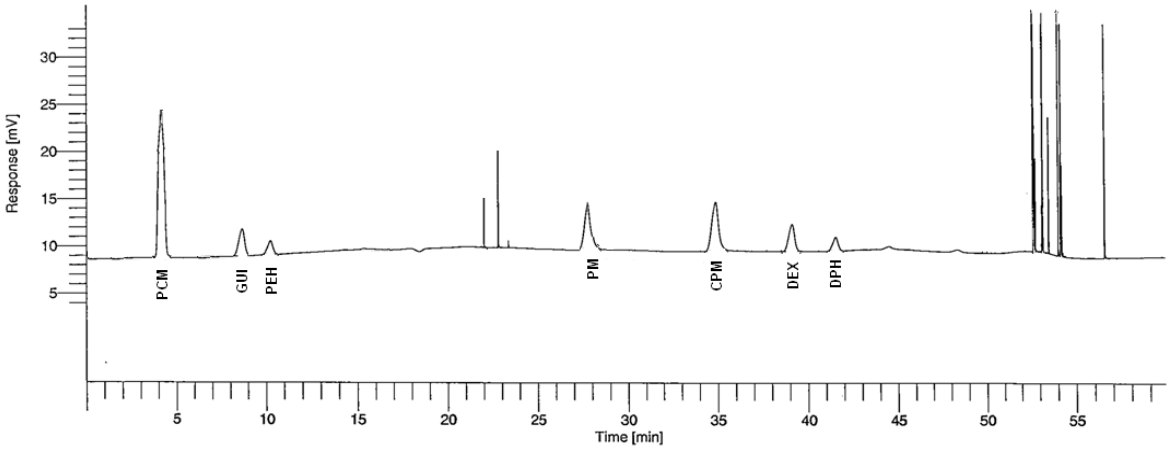

Fig. **23.0**: Accuracy for Paracetamol Guaiphenesin, Pheniramine Maleate, Phenylephrine Hydrochloride, Dextromethorphan hydrobromide, Diphenhydraminehydrochloride and Chlorpheniramine maleate (SAMPLE- 100 %)

SAMPLE- 120 %

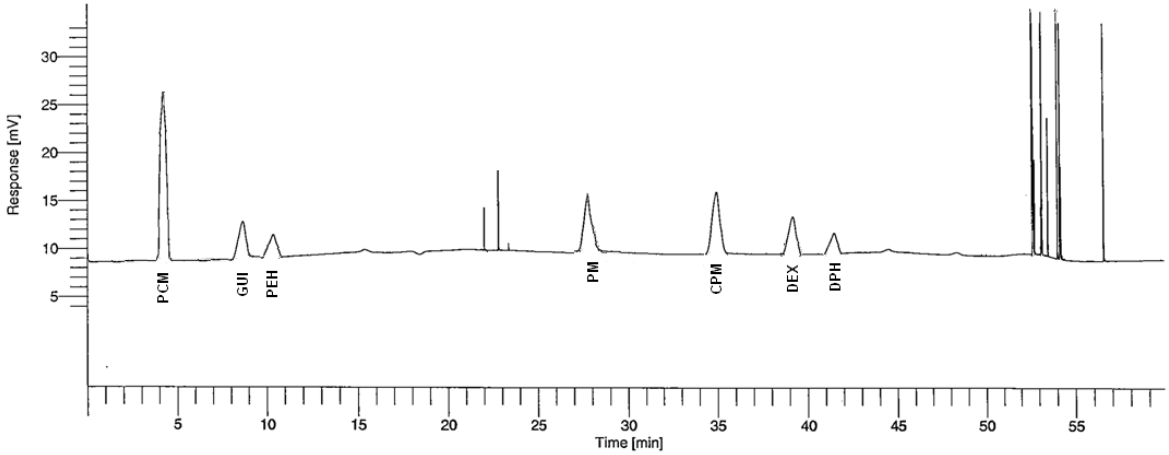

Fig. 24.0: Accuracy for Paracetamol Guaiphenesin, Pheniramine Maleate, Phenylephrine Hydrochloride, Dextromethorphan hydrobromide, Diphenhydraminehydrochloride and Chlorpheniramine maleate (SAMPLE- 120 %)

5.0 CONCLUSION

The aim of this work was to validate the seven active ingredients Acetaminophen,Guafenesin, Phenylephrine maleate, Phenylephrine Hydrochloride, Pseudoephrine Hydrochloride, Chlorpheiramine Maleate, Dextromethorphan Maleate which are the common ingredients in most of the cough and cold formulations via HPLC in one single run.

The optimal analysis conditions were found to be with buffer pH of 2.80 and only minimal changes are allowed as resulting from robustness tests.

The method passed a series of validation tests including precision, linearity and repeatability and is therefore well suited for the determination of common ingredients in cough and cold formulations.

Chromatographic methods are specific, sensitive, accurate, precise and reproducible. Thus these methods are preferred over other nonspecific techniques such as titrimetric and spectrophotometric methods. Hence these methods are used in the analysis of bulk drugs, drug intermediates and finished products.

The proposed high performance thin layer chromatographic method and high performance liquid chromatographic method find applications in routine quality control and in standardization. The proposed methods are simple and do not require elaborate sample preparation. HPLC has become a powerful tool for analysis of pharmaceutical products. Mixtures used for the treatment of cough and colds may be complexes containing several active ingredients including a decongestant, antihistamine, analgesic, preservatives, dyes and flavors. The active materials cover a range of structures with widely varying polarities and include both acidic and basic compounds.

A number of conventional methods have been applied to present components. Cough and cold pharmaceutical preparation are one of the most extended formulations in the world and have got many pharmaceutical forms : syrup, suspension, sachets, capsules and tablets. Pheniramine maleate, pseudoephrine hydrochloride are widely used in combination with other drugs for the clinical treatment of common cold, sinusitis, bronchitis and respiratory allergies. dextromethorphan hydrobromide, guafenesin are used as cough suppressants, antitussives for the relief of non productive cough and cold preparations. The most common formulation can be either liquid or suspension that requires the addition of preservative. Due to the characteristic and diverse properties inherent to their formulation ,these preparations offer an analytical problem.

Some of these methods take lot of time and are costly thus not making the entire product analysis cost effective. Also there are a lot of problems associated with the method itself ie. Sensitivity to the method, reproducibility , ruggedness thus giving us a need to develop a coherent method which will overcome all these issues.

As of now there are methods in which a single component is developed or multicomponent are developed from a particular dosage forms.

Setting the optimized chromatographic parameters, high performance liquid chromatographic method is the simplest. HPLC method is an efficient separation technique. Use of high efficiency columns with large theoretical plates coupled with sensitive detection helps in separation and detection of various compounds in the sample. Quantitative TLC is the technique, which can be viewed complimentary and not competitive with HPLC.

Simplicity, specificity, accuracy and precision of the proposed HPLC methods make them suitable choice for routine quality control analysis. The degree of sensitivity, availability of instruments, cost and speed required will decide the choice of the method to be employed.

This methodology can be used as a base for determining the common ingredients in cough and cold formulations thus saving lot of time and expenses thus making the medicine available to the patient in a timely and effective manner. This platform can thus be used for any other type of common ailments where the common ingredients in the formulation can be determined in a single method.

6.0 ACKNOWLEDGEMENT

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I express my sincere thanks to Dr. Kunal Saxena (MD, Rusan Pharma) for his continuous support and inspiration and belief that I can excel.

7.0 AUTHOR CONTRIBUTION

I have been able to complete my research under the guidance of Dr. Nitin Borkar where my work started from collecting data for literature survey to completing the research.

Dr. Nitin Borkar the co-author to my paper has been instrumental in guiding me throughout the research work and also helping me to write this paper methodologically with sufficient data. He has been the one to analyse all the data and finally structuring this paper.

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