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Research Article

METHOD DEVELOPMENT OF AN SIMULTANEOUS DETERMINATION OF COMMON COUGH AND COLD INGREDIENTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HLPC) IN MULTI COMPONENT COUGH AND COLD ORAL DRUG PRODUCTS

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ABSTRACT

A common simultaneous HPLC method has been developed for the quantification of common analytes used in cough and cold products. The seven analytes acetaminophen, guafenesin, pheniramine maleate, phenylephrine hydrochloride, diphenhydramine, chlorpheniramine maleate, dextromethorphan are separated out. HPLC separation was achieved on a C18 column with a gradient system optimized for all seven analytes with different chemical structures.

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

1.0 INTRODUCTION

Cough and cold segment in the OTC is the major area of self medication. Aspirin drug combinations do not require a doctor's prescription as these analytes are previously approved prescription drugs now deemed safe enough for use without a medical practitioner's supervision like ibuprofen.

HPLC with ultraviolet, fluorimetry or mass spectroscopy (MS) is most widely used. [1] Other techniques include ultraviolet-visible spectroscopy, thin layer chromatography (TLC), gas chromatography (GC), GC/MS, capillary electrophoresis and multivariate spectrophotometric methods.[2] There is no analytical method for simultaneous determination of most of the common ingredients for major analytes in the cough and cold category.

Dosage forms used for the treatment of cough and colds are complex mixtures containing several active analytes as decongestants, antihistamines, analgesics, antitussive, expectorants, preservatives, dyes and flavors available as syrup, suspension, powders, capsules and tablets. The active materials cover widely ranging polarities and include both acidic and basic compounds. Analytical methodologies are reported for analgesic (eg acetaminophen), an antitussive (eg dextromethorphan), and antihistamine (eg chlorphenaramine Maleate) and a nasal decongestant (eg phenylephrine Hydrochloride). Guaifenesin was estimated simultaneously in the presence of acetaminophen, pseudoephedrine, folcodine.[3] However this method was not stability indicating method. Acetaminophen has been analysed with salts of chlorphenaramine , dextromethorphan, phenylephrine in tablets using HPLC.[4]

Several methods use ion pairing reagents. They require use of hydrophobic additives either cationic such as triethylamine, hexalamine or anionic such as alkyl sulfonate. They tend to absorb very strongly on the stationary phase leading to difficulty in recovering initial column properties. Also use of ion pairing agents in mobile phase enhances the retention time of most components thereby increasing analysis time hence may not be preferred method of choice[5].

Pharmacopoeial methods reported for each drug are generally not suitable for simultaneous determination due to interferences from other

analyte peaks. Using multiple methods of each analyte with different column, mobile phase, flow rates and sample preparations is uneconomical and time consuming in this low cost self medication market. Gradient method has been developed.

The goal of this article is to develop a potential, reliable fast and efficient chromatographic conditions for various dosage forms which can estimate all the major components of a cough and cold multi component formulation.

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). Ammonium phosphate dibasic AR , Ammonium phosphate monobasic AR , 1- octane suphonic acid sodium salt monohydrate AR , Acetonitrile (HPLC grade) and Milli Q water was used in all the work. Phosphoric acid AR for pH adjustment and triethylamine HPLC grade was used.

2.1 Buffer

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 0.45-µ nylon membrane filter. All solutions as well as the mobile phases were filtered through a 45-mm nylon membrane filter. Mobile phases were freshly prepared weekly and degassed prior the use.

2.2 Mobile phase A

Acetonitrile was used as mobile phase A

2.3 Mobile phase B

Buffer prepared above was used as mobile phase B

2.4 Diluent A

Dissolve 0.094 g of ammonium phosphate monobasic, and 0.108 g of ammonium phosphate dibasic, in 250 ml of water. Add 750 ml of acetonitrile and 3.0 ml of triethylamine. Mix and adjust to pH 6.8 ± 0.05 with phosphoric acid.

2.5 Diluent B

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

2.6 Preparation of the solutions

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

2.7 Stock Standard Solution A

Accurately weigh and transfer about 50 mg of acetaminophen, guaifenesin, pheniramine maleate, phenylephrine hydrochloride, diphenhydramine, chlorpheniramine maleate, dextromethorphan standard into a 50 ml volumetric flask individually and add 60 ml of Diluent A, sonicate to dissolve and dilute to volume with Diluent A and mix well. (1000 ppm)

2.8 Stock Standard Solution B

Transfer 5.0 ml of Stock Standard Solution A individually into a separate 20 ml volumetric flasks, dilute to volume with Diluent A and mix well. (250 ppm)

2.9 Combined Standard Preparation

1) Transfer 5.0 ml of Dextromethorphan hydrobromide and Diphenhydramine hydrochloride Stock Standard Solution A into a 50 ml volumetric flask, dilute to volume with Diluent B and mix well. (100 ppm).
2) Transfer 2.0 ml of paracetamol, guaiphenesin, pheniramine maleate, phenylephrine hydrochloride & chlorpheniramine maleate stock standard solution B (250 ppm) into same 50 ml volumetric flask, dilute to volume with Diluent B & mix well.(10 ppm)

2.10 Preparation of sample preparation

Prepare the sample stock solution into Diluent A. Further dilute the solution and get final concentration of acetaminophen (10 ppm), guaiphenesin (10 ppm), pheniramine maleate(10 ppm), phenylephrine hydrochloride(10 ppm), diphenhydramine (10ppm), chlorpheniramine maleate(10 ppm), dextromethorphan (100 ppm) with Diluent B.

3.0 METHOD DEVELOPMENT

Method development is based on several considerations. It is preferable to have maximum sample information to make development fast and desired for intended analytical method application, Physical and chemical properties are most preferable as primary information. Moreover, separation goal needs to be defined at beginning so that

appropriate method can be developed for the purpose. Method development can be based on sample and goal as well as available resources for chromatography but few basic steps for method development are discussed as below[6].

3.1 Steps in method development [7]

- Sample information
- Define separation goals
- Sample pretreatment, need of special HPLC procedure
- Selection of detector and detector settings
- Selection of LC method; preliminary run; estimate best separation conditions
- Optimize separation conditions
- Check for problems or requirement for special procedure

3.2 Sample information

1. Number of compounds present
2. Chemical structure of compounds
3. Chemical nature
4. Molecular weight of compounds
5. Ka Value(s) of compounds
6. Sample solubility
7. Sample stability and storage
8. Concentration range of compounds in sample
9. UV spectra of compounds or properties for detection of compounds.

3.3 Below mentioned are different analytes which were used for the separation by HPLC method

S.No	Analyte	pKa	Acidic/Basic	Category
1.	Acetaminophen	9.0	Acidic	Analgesic
2.	Dextromethorphan	9.85	Basic	Antitussive
3.	Diphenhydramine	8.98	Basic	Antihistaminic
4.	Phenylephrine hydrochloride	8.97	Acidic	Decongestant
5.	Chlorpheniramine maleate	9.13	Acidic	Anihistaminic
6.	Pheniramine maleate	9.48	Basic	Anithistaminic
7.	Guaifenesin	13.42	Acidic	Expectorant

3.4 Selection of various chromatographic conditions

3.4.1 Mode of Separation

In case of HPLC the method of separation used is reverse phase mode of separation. In this case water will be one of the components of the mobile phase. The unmodified stationary phases include silica, cellulose, starch, polyamides and chitin. Modified stationary phases include silicas containing cyanodiol, amino, thiol groups. For HPLC the most frequently used stationary phase is silica[8].

Hence reverse phase mode of separation was chosen for HPLC determination.

3.4.2 Selection of stationary phase

The introduction of a technique using alkyl chains covalently bonded to the solid support created a hydrophobic stationary phase, which has a stronger affinity for hydrophobic compounds. The use of a hydrophobic stationary phase can be considered the opposite, or "reverse", of normal phase chromatography - hence the term "reversed-phase chromatography".[9] Reversed-phase chromatography employs a polar (aqueous) mobile phase. As a result, hydrophobic molecules in the polar mobile phase tend to adsorb to the hydrophobic stationary phase, and hydrophilic molecules in the mobile phase will pass through the column and are eluted first. Hydrophobic molecules can be eluted from the column by decreasing the polarity of the mobile phase using an organic (non-polar) solvent, which reduces hydrophobic interactions. The more hydrophobic the molecule, the more strongly it will bind to the stationary phase, and the higher the concentration of organic solvent that will be required to elute the molecule.[10]

Any inert non-polar substance that achieves sufficient packing can be used for reversed-phase chromatography[11]. The most popular column is an octadecyl carbon chain (C18)-bonded silica (USP classification L1) with 297 columns are commercially available. This is followed by C8-bonded silica (L7 - 166 columns), pure silica (L3 - 88 columns), cyano-bonded silica (L10 - 73 columns) and phenyl-bonded silica (L11 - 72 columns)[12]. Note that C18, C8 and phenyl are dedicated reversed-phase resins, while cyano columns can be used in a reversed-phase mode depending on analyte and mobile phase conditions[13]. It should be noted at this point that not all C18 columns have identical retention properties. Surface functionalization of silica can be performed in a monomeric or a polymeric reaction with different short-chain organosilanes used in a second step to cover remaining silanol groups (end-capping). While the overall retention mechanism remains the same, subtle differences in the surface chemistries of different stationary

phases will lead to changes in selectivity.[14]
The sorbents have a pore size of 40 to 100 A°. Thus silica gel having pore diameter of 60 A° was chosen as a stationary phase material for the present research work.
Hence, Reverse phase HPLC was used for the research work.

3.4.3 Mobile phase optimization
Mixtures of water or aqueous buffers and organic solvents are used to elute analytes from a reversed-phase column. The solvents must be miscible with water, and the most common organic solvents used are acetonitrile, methanol, and tetrahydrofuran. Other solvents can be used such as ethanol or 2-propanol . Elution can be performed isocratically (the water-solvent composition does not change during the separation process) or by using a solution gradient (the water-solvent composition changes during the separation process, usually by decreasing the polarity). The pH of the mobile phase can have an important role on the retention of an analyte and can change the selectivity of certain analytes.[15]
Since most APIs have acidic and /or basic functionalities , their ionization state is controlled by both solution pH and acidic dissociation constants (ie K_a values). These different chemical species (cationic, neutral or anionic) often have vastly different properties with respect to water solubility, volatility, UV absorption and reactivity with chemical oxidants. The ionized form is usually more water soluble while the neutral form is more lipophilic and has higher membrane permeability. From dissociation constants the major species of pharmaceuticals present in the environment can be estimated.
Consequently it is very important to know dissociation constants for environmentally relevant API's in order to estimate their occurrence , fate and effects.
Knowledge of pka values as a function of solvent composition is also useful in applying liquid chromatography for the separation of ionizable compounds. The chromatographic retention of ionizable compounds strongly depends on the pKa of the compound and mobile phase pH.
It is advisable to have the pH of mobile phase within approximately ± 2 units of pK_a. Whenever acidic or basic samples are to be separated it is advisable to control mobile phase pH by adding a buffer solution. Reversed phase HPLC separations are generally carried out with C8 or C18 bonded phase silica based columns that are stable in pH ranges 2.0 and 8.0. Thus, buffers able to control pH between 2.0 to 8.0 are desirable. For reverse phase chromatography, a buffer concentration of 10-50 mM is usually adequate.
The general strategy for optimizing the mobile phase in HPLC is to adjust the solvent strength by replacing a pure solvent by another, or by varying the proportions of the weak solvent and the strong solvent in a mixture so that the R_f values are in the range of 0.2 – 0.7. While keeping the optimized solvent strength constant, the solvent composition is then modified to obtain selectivity necessary to achieve the desired separation. But with complex multicomponent samples, it may not be possible to achieve the required separation with binary solvent system. In such cases ternary or quaternary mixtures provide more opportunities to fine-tune the selectivity while maintaining the solvent strength constant.[16]

The solvents should fulfil the following requirements

- The solvent should be of adequate purity at reasonable cost.
- The solvent should be unreactive with the sample and the stationary phase.
- The solvent should be miscible with the other solvents of the mobile phase.
- The solvent should be stable in air and should have low boiling point to facilitate easy removal after chromatographic development.
- The solvent should be of suitable viscosity and surface tension.
- The solvent should have linear partition isotherm.
- The solvent should have vapour pressure that is neither very low nor very high.
- The properties like flammability and toxicity are important due to their impact on safety and disposal.

The selection of the mobile phase is of prime importance in the development of a chromatographic technique for proper elution, resolution, spot definition, symmetrical peak shapes and reproducibility of the analytes.[17]
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 R_f values.

Table 1: Mobile phase optimization

S.No	Mobile phase	Result
1.	Potassium phosphate buffer, pH 2.5 : Acetonitrile (85 : 15)	All peaks were not seen namely dexmethorphan, diphenhydramine HCL
2.	Potassium phosphate buffer, pH 2.5 : Acetonitrile (90 : 10)	All peaks were not seen
3.	Potassium phosphate buffer, pH 2.5 : Methanol (90 : 10)	All peaks were not seen
4.	Potassium phosphate buffer, pH 6.0: Acetonitrile (90 : 10)	All peaks were not seen
5.	Potassium phosphate buffer, pH 6.0 : Methanol (90 : 10)	All peaks were not seen
4.	Potassium phosphate buffer, pH 3.5: Acetonitrile (90 : 10)	All peaks were not seen
5.	Potassium phosphate buffer, pH 3.5 : Methanol (90 : 10)	All peaks were not seen
6.	Potassium phosphate buffer, pH 2.5 :TFA added 1% :Acetonitrile (85 : 15)	All peaks were not seen
7.	1-Octane Sulfonic Acid Sodium salt, Monohydrate buffer ,pH2.8 with Phosphoric Acid : Acetonitrile	All peaks were seen

Thus optimized mobile phase used for separation was Solvent A: Acetonotrile Solvent B: Buffer Buffer: Dissolve about 2.34 g of 1-Octane Sulfonic Acid Sodium salt, Monohydrate AR grade in 1000 ml of Milli Q water in a suitable glass container to obtain a concentration of about 0.01M 1-Octane Sulfonic Acid Sodium salt solution. Adjust the pH to 2.8 ± 0.1 with Phosphoric Acid, and filter through a 0.45-µ nylon membrane filter. Degas the solution.

Gradient

Programmed Flow

Accelerate to 10.0 mL/min in:

Pump Mode: Gradient | 2.00 | min (5.00 mL/min/min)

	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

3.4.5 Column Selection

Table 2: Various columns were used during method development to ensure all the components were eluted efficiently

S.No	Column type	Result
1.	Zorbax SB_CN, 5 µ, 4.6 X 250 mm	All peaks were not seen namely dexmethorphan, diphenhydramine HCL
2.	Hypersil Gold, 5 µ, 4.6 X 250 mm	All peaks were not seen
3.	Thermoscientific, Hypersilgold , 4.6 X 250 mm	All peaks were not seen
4.	Zorbax C18, 5 µ, 4.6 X 250 mm	All peaks were not seen
5.	Zorbax C18, 5 µ, 4.6 X 150 mm	All peaks were not seen
6.	Thermoscientific, Hypersilgold , 4.6 X 150 mm	All peaks were not seen
7.	Zorbax NH2, 5 µ, 4.6 X 250 mm	All peaks were not seen
8.	Inertsil ODS 3V, 5 µ, 4.6 X 250 mm	All peaks were not seen
9.	Inertsil ODS 3V, 5 µ, 4.6 X 150 mm	All peaks were not seen
10.	Phenomenexluna, 5 µ, 4.6 X 150 mm	All peaks were not seen namely dexmethorphan, diphenhydramine HCL, split peaks were seen
11.	Phenomenexsynergi max RP, 4 µ, 4.6 X 250 mm	Phenylephrine peak was not seen
12.	Phenomenexluna, 5 µ, 4.6 X 250 mm	This column showed all the peaks

Thus column used for separation was Phenomenexluna, 5 µ, 4.6 X 250 mm

3.4.6 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.

3.4.7 Selection of the most suitable detector

Before the first sample is injected during the HPLC method development one must be sure that the detector selected will sense all sample components of interest. There are lot of detectors used like UV, photo diode array, mass spectroscopy, fluorescence, refractive index detector. Normally variable wavelength UV detector is the first choice of the chromatographers, because of their convenience and applicability for most organic samples. UV spectra can be obtained by PDA detector. When the UV response of the sample is inadequate, other detector or derivative HPLC method can be used.[18]

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.

In the present research work the most suitable wavelength was decided when a lambda max was found out for all the components. The wavelength chosen for further quantification was 264 nm.

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.

3.5 Placebo interference

The specificity of the HPLC method was determined by injecting individual component, placebo preparation, where in no interference was observed for any of the components and no interference of any of the excipients is observed from the placebo preparation at the retention of Paracetamol, Guafenesin, Phenylephrine Hydrochloride, Dextromethorphan, Diphenhydramine Hydrochloride, Pheniramine Maleate, Chlorpheniramine maleate.

3.6 Information of sample

The important information concerning sample composition and properties like number of compounds present in the sample, concentration range of compounds in the sample preparation, the other properties like the chemical structure, molecular weight, pKa value, UV spectra, solution stability and solubility should be there.

Based on the composition of the sample, choice of the initial conditions for an HPLC was decided.

Sample comes in various forms such as solutions ready for injections, solutions that require dilution, addition of internal standard or other volumetric manipulation, solids must be dissolved or extracted from formulation matrix and sample that requires pretreatment to remove interferences and to protect the column from damage.

Sample preparation is an essential part of HPLC analysis to provide a reproducible and homogenous solution that is suitable for injection onto the column. Best results are obtained when the composition of the sample solvent is close to that of the mobile phase, since this minimizes baseline disturbance and does not affect the sample retention and resolution.

Samples used were of syrups, tablets, powders in sachets. The sample preparation was designed in order to have all the ingredients in a common mixture so that it can analyzed in a single run.

Prepare the sample stock solution into Diluent A. Further dilute the solution equal concentration of standard preparation into a Diluent B.

Take sample equivalent to get final concentration of Dextromethorphan hydrobromide and Diphenhydramine hydrochloride 100ppm and Paracetamol,

Guaiphenesin, Pheniramine Maleate, Chlorpheniramine maleate 10ppm. Phenylephrine Hydrochloride and

Samples Areas

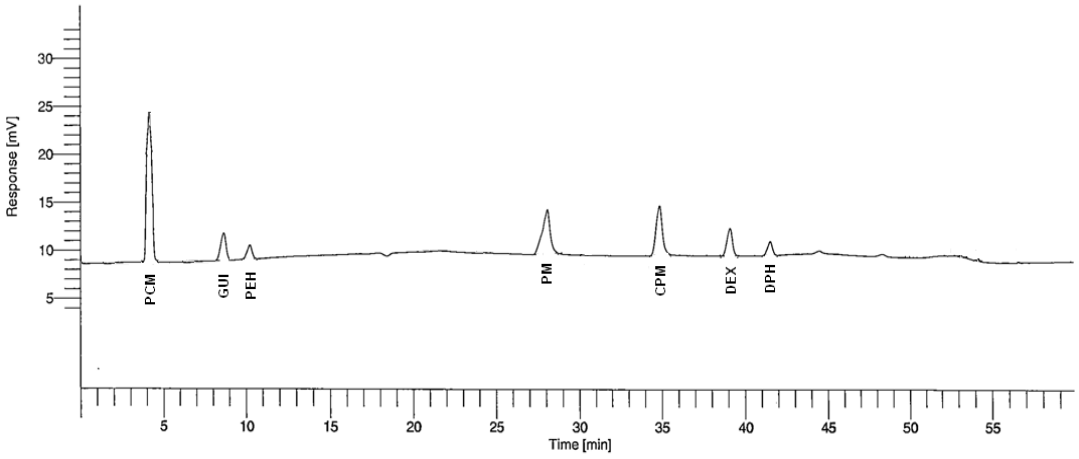
Injection No:	PCM	GUI	PM	PEH	DEX	DPH	CPM
1	470645	125673	246135	14830	89831	42722	222879
2	470648	125677	246132	14828	89833	42721	222880
3	470623	125680	246130	14827	89835	42728	222885
Average	470639	125677	246132	14828	89833	42724	222881
%	99.99	100.00	100.00	99.96	99.99	100.00	100.00

The order of elution of peak is as follows: Paracetamol, Guaiphenesin, Phenylephrine Hydrochloride, Pheniramine Maleate, Chlorpheniramine maleate, Dextromethorphan hydrobromide and Diphenhydramine hydrochloride.

Standard Areas

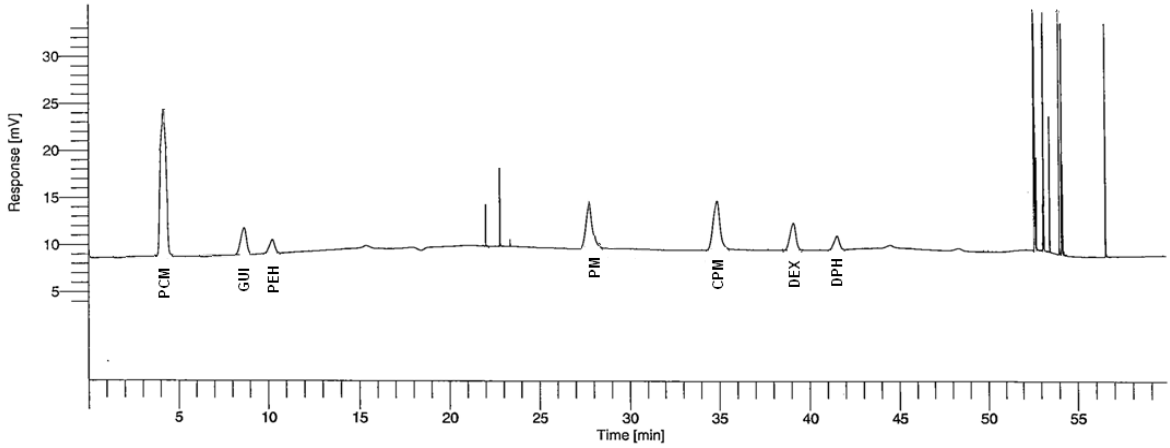
Assay for Paracetamol Guaiphenesin, Pheniramine Maleate, Phenylephrine Hydrochloride, Dextromethorphan hydrobromide, Diphenhydraminehydrochloride and Chlorpheniramine maleate (Standard and Sample Areas)

Injection No:	PCM	GUI	PM	PEH	DEX	DPH	CPM
1	470679	125685	246139	14834	89831	42726	222884
2	470675	125683	246136	14832	89833	42724	222885
3	470676	125682	246138	14836	89835	42724	222883
4	470679	125683	246140	14835	89839	42727	222883
5	470678	125681	2461437	14832	89837	42727	222886
6	470677	125682	246135	14836	89839	42725	222883
Average	470677	125683	246138	14834	89838	42726	222884
SD	1.63	1.37	1.87	1.83	1.17	1.38	1.26
RSD	0.0003	0.0011	0.0008	0.0124	0.0013	0.0032	0.0006



STANDARD

Fig. 1: Assay for paracetamol, guafenesin, pheniramine maleate, phenylephrine hydrochloride, dextrometorphan hydrobromide, diphenhydramine hydrochloride and chlorphenimarine maleate (Standard)



SAMPLE

Fig. 2: Assay for paracetamol, guafenesin, pheniramine maleate, phenylephrine hydrochloride, dextrometorphan hydrobromide, diphenhydramine hydrochloride and chlorphenimarine maleate (Sample)

4.0 CONCLUSION

Hence it was observed that paracetamol, guafenesin, phenylephrine hydrochloride, dextromethorphan, diphenhydramine hydrochloride, pheniramine maleate, chlorpheniramine maleate could be determined by HPLC. Thus, the research proposes HPLC method development which 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.

Developed chromatographic conditions are as follows

SR No.	PARAMETER	DESCRIPTION
1.	Instrument	
	Pump	Gradient pump
	Injector	Autosampler
	Column	Phenomenex Luna C18, 25 cm x 4.6 mm i.d., 5µm.
	Detector	UV- Visible detector
	Wavelength	264nm
	Recorder	Empower -2 chromatography software
2.	Mobile Phase	0.01M 1-octana 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.	Injection Volume	25µl

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6.0 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.

REFERENCES

1. Alaa El-Gindy, Samy Emara, Mostafa K Mesbah and Ghada M Hadad: Liquid Chromatography and Chemometric-Assisted Spectrophotometric Methods for the Analysis of Two Multicomponent Mixtures Containing Cough Suppressant Drugs. Journal of AOAC International. 2005;88(4):1069-1080.
2. Ghanekar AG and Das Gupta V. Quantitative determinations of two decongestants and an antihistamine in combination using paired ion high pressure liquid chromatography. Journal of Pharmaceutical Sciences. 1978;67:873-874.
3. Shenoy KR, Krishnamurthy KS. Iyengar V and Harsha J. Simultaneous determination of phenylpropanolamine hydrochloride, dextromethorphan hydrobromide and chlorpheniramine maleate in formulations by reversed-phase liquid chromatography Indian Journal of Pharmaceutical Sciences. 2002;64(4):398-400.
4. Laura Carnevale. Simultaneous determination of Acetaminophen, Guafenesin, Pseudoephedrine, Pholcodine, and Paraben Preservatives in Cough Mixture by High- Performance Liquid Chromatography. Journal of Pharmaceutical Sciences. 1983;72(2): 196-198.
5. Caroll DI, Dzidic, Stillwell RN, Haegele KD and Horning EC. Analytical Chemistry. 1975;47:2369-2373.
6. Settle FA. Handbook of instrumental techniques for analytical chemistry, ed: Pearson Education, Singapore. 2004.
7. Khopkar SM. Basic Concept of Analytical Chemistry. Published by Wiley Eastern Limited, New Delhi, 1984.
8. Skoog DA, West DM, Holler FJ and Crouch SR. Analytical Chemistry – Introduction ,7th edition; Published by Saunders College Publishing; USA. 1999.
9. Tswett MS and Ber Disc Botb Ges. M-S Twestt & Chromatography. 1906;24: 316.
10. Tswett MS and Ber Disc Bot Ges. M-S Twestt & Chromatography. 1906;24:384.
11. Robards K, Haddad PR and Jackson PE. Priciples and Practice of Modern Chromatographic Methods, Published by Academic Press Limited. London. 1994;203.
12. Synder L, Kirkland J and Glajch J. Practical HPLC Method Development. In: Difficult Separations: The use of other separation variables and procedures. New York, Chichester, Brisbane, Toronto, Singapore. 1988; 125-137.
13. Synder L, Kirkland J and Glajch J. Practical HPLC Method Development. In: Difficult Separations: The use of other separation variables and procedures. New York, Chichester, Brisbane, Toronto, Singapore. 1988;61-64.
14. Synder L, Kirkland J and Glajch J. Practical HPLC Method Development. In: Difficult Separations: The use of other separation variables and procedures. New York, Chichester, Brisbane, Toronto, Singapore. 1988; 231-239.
15. Snyder L, Kirkland J and Glajch J. Practical HPLC Method Development. In: Getting started .Ed. Wiley - Interscience, New York, Chichester, Brisbane, Toronto, Singapore. 1988;5-6.
16. Snyder L, Kirkland J and Glajch J. Practical HPLC Method Development. In: Getting started .Ed. Wiley - Interscience, New York, Chichester, Brisbane, Toronto, Singapore. 1988;1-4.
17. Snyder L, Kirkland J and Glajch J. Practical HPLC Method Development, In: Systematic Method Development. Ed. Wiley- Interscience New York, Chichester, Brisbane, Toronto, Singapore. 1988;85-89.
18. Snyder L, Kirkland J and Glajch J. Practical HPLC Method Development, In: Systematic Method Development. Ed. Wiley- Interscience New York, Chichester, Brisbane, Toronto, Singapore. 1988;54-71.