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A Novel RP-HPLC Method for Simultaneous Estimation of Guaifenesin, Chlorpheniramine Maleate and Dextromethorphan in Pharmaceutical Formulation

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ABSTRACT
A simple and efficient liquid chromatographic method has been developed and validated for the simultaneous determination of Guaifenesin, Chlorpheniramine Maleate and Dextromethorphan in cough and cold syrup. The separation of the analytes was achieved within 15 min, employing a mixture of 45:25:30 (v/v/v) ratio, adjusted to pH 5.5 using orthophosphoric acid as isocratic mobile phase, pumped at 0.7ml/min through a C_{18} column (particle size 5µm, 250x4.6mm). The analytes were detected at 265nm. Statistical experimental design and graphic representations were used for optimizing the mobile phase composition. The linearity of the calibration in the relevant ranges 50µg/ml - 500µg/ml, method accuracy (RSD <2.0%), repeatability (RSD<2.0%) and intermediate precision, were verified. The validated method was successfully employed for the routine analysis of a syrup pharmaceutical preparation against cough and cold, showing satisfactory analytes recoveries and precision.

KEYWORDS: Guaifenesin, Chlorpheniramine Maleate, Dextromethorphan, Liquid Chromatography

I. INTRODUCTION

Cough is caused when mechanical and/or chemical stimuli trigger sensory receptors in the airways. Drugs with mucolytic properties that have an inhibitory effect on the cough reflex itself are used as part of cough-suppressant therapy. This kind of treatment aims to lessen coughing both frequently and severely. The best combinations involve an expectorant combined with an antitussive and anti-histaminic agent. One such combination frequently prescribed in of Guaifenesin, Dextromethorphan and Chlorpheniramine.

Guaifenesin (GUA) is a widely used expectorant, useful for the symptomatic relief of respiratory conditions.It is a mucoactive drug, acts by loosening mucus in the airways and making coughs more productive. It is used for relief of wet cough and chest congestion due to the common cold. Its empirical formula is $C_{10}H_{14}O_4$ and IUPAC name is (+)-3-(2-methoxyphenoxy)-propane-1,2-diol, which corresponds to a molecular weight of 198.21. It is a white or slightly grey crystalline substance with a slightly bitter aromatic taste.Guaifenesin has a central carbon atom that is attached to two hydroxyl (-OH) groups and a methoxy (-OCH3) group. The carbon atom is also connected to a benzene ring that has another methoxy group attached to it.The molecule has a chiral centre at the second carbon atom, which means that it can exist in two enantiomeric forms (mirror-image isomers) that are non-superimposable. However, the commercial form of guaifenesin is a racemic mixture of the two enantiomers.Fig.: 1

Dextromethorphan (DEX) is the dextrorotatory enantiomer of the methyl ether of levorphanol and stereoisomer of levomethorphan. Dextromethorphan is an antitussive (cough suppressant) drug and used for pain relief and psychological applications. Its empirical formula is C8H25NO, and its IUPAC name is (1S,9S,10S)-4-methoxy-17-methyl-17-azatrtracyclo[7.5.3.01,10.02,7]he[ptadeca-2(79),3,5-trienewhich corresponds to a molecular weight of 271.4. It is a white powder. Fig.:2

Chlorphenamine is a tertiary amino compound that is propyl amine which is substituted at position 3 by a pyridine-2-yl group and a p-chlorophenyl group and in which the hydrogens attached to the nitrogen are replaced by methyl groups.3-(4-chlorophenyl)-N,N-dimethyl-3-pyridin-2-ylpropan-1-amine is the IUPAC name of the Chlorpheniramine Maleate (CPM). The empirical formula for Chlorpheniramine Maleate is C20H23ClN2O4. Chlorpheniramine Maleate is an H-1 receptor blocker. Chlorpheniramine Maleate is

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an antihistamine used to relief symptoms of allergy, hay fever, and the common cold. These symptoms include rash, watery eyes, itchy eyes/nose/throat/skin, cough, runny nose, and sneezing. Fig.:3

Several analytical methods have been developed for the separation and quantitation of GUA, CPM and DEX individually from different matrices.

Wilcoxet al. [4]illustrated the simultaneous determination of guaifenesin in commercially available capsule dosage form and guaifenesin-codeine in a commercial cough syrup dosage form by HPLC method.Qi et al. [5] developed simple HPLC method in whichsimultaneous determination of acetaminophen, caffeine and chlorpheniramine maleate in a new tablet formulation. Harsono et al. [6]has developedgas chromatographic (GC) method for the simultaneous determination of combinations of acetaminophen, phenylpropanolamine hydrochloride, guaifenesin, pseudoephedrine hydrochloride, caffeine, chlorpheniramine maleate, and dextromethorphan hydrobromide in cough and cold tablets and syrups. A tablet dosage form by Liquid Chromatography (LC) method in which Chlorpheniramine Maleate and Dexamethasone were present were determined by Moyano et al [7].By Koranyet al. [8] three pharmaceutical combinations were analysed by HPLC method. Some RP-HPLC and TLC spectrodensitometric method also described for analysis of quaternary mixture of oxomemazine , sodiumbenzoate , guaifenesin , and paracetamol in syrup and suppositories by NF Farid et al. [9].Buyuktuncelet al [10] proposed liquid chromatographic [LC] method for pseudoephedrine HCI, pheniramine maleate, acetaminophen, guaifenesin, pyrilamine maleate, chlorpheniramine maleate, triprolidine HCI, dextromethorphan HBr, diphenhydramine HCI in cough and cold pharmaceutical dosage form such as syrups and tablets andReidet al [11] also developed LC method for of pseudoephedrine, dextromethorphan, and triprolidine in cough and cold syrup. Salomi et al. [12] RP-HPLC method for Simultaneous estimation of bulk and pharmaceutical formulations such as guaifenesin and dextromethorphan and Yuliana et al.[13]developed analytical HPLC method for Dextromethorphan Hydrobromide, Chlorpheniramine Maleate, and Potassium Sorbate in cough syrup.

Thus, this literature review revels that no as such RP-LC method is available for simultaneous estimation of GUA, DEX and CPM. That is why this research to establish and validate a simple, economical, accurate and reproducible procedurefor the simultaneous quantitative analysis of the pharmaceutical formulation was performed.

To our knowledge, no analytical method has been reported for the simultaneous determination of this combination of GUA, DEX and CPM cough and cold syrup formulation. So, we investigated the different buffer concentrations, pH values, and flow rate on various chromatographic parameters such as plates, symmetry factor, resolution, retention time, peak areas. The development of a new LC analytical method for the simultaneous quantitative determination of GUA, DEX and CPM is done successfully.

II. MATERIALS AND METHODS

Chemicals and Reagents

Guaifenesin, Chlorpheniramine Maleate and Dextromethorphan were received from the formulation research and development laboratory of Dr. Reddy's Laboratories Ltd. HPLC grade methanol (gradient grade) by Thermo Fisher Scientific India Pvt. Ltd., HPLC Grade Acetonitrile Special by Fisher Scientific and Orthophosphoric acidabout 85% by Central Drug House (P) LTD.7/28 Vardaan House, Daryaganj, New Delhi – 110002 (INDIA).Potassium dihydrogen Orthophosphate anhydrous purified by central drug house (p) Ltd.Ultipor N66 NYLON 6, 6membrane 0.2µm (47mm) filer paper by life sciences. All the other chemicals used were of AR grade.

Equipment

The chromatographic system (Shimadzu Kyoto, Japan) consisted of a Shimadzu LC-20 AT Prominence solvent delivery module, a manual Rheodyneinjector with a 25µl fixed loop, and an SPD -20A Prominence UV-VIS detector. Hamilton syringe was used for injecting the samples.The separation was performed on a Phenomenex C18 column (particle size 5µm, 250x4.6mm)at an ambient temperature chromatographic data was recorded and processed using a LC solution (software).

Chromatographic Conditions:

To estimate the concentrations of CPM, DEX, and GUA simultaneously, we utilized reverse phase chromatography with a Phenomenex RP C18 Column. Based on the solubility of the drugs, reverse phase chromatography was deemed the

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most effective method. The mobile phase consisted of a mixture of methanol, acetonitrile, and 0.025M phosphate buffer in a 45:25:30 (v/v/v) ratio, adjusted to pH 5.5 using orthophosphoric acid. Prior to use, both the mobile phase and working solution were filtered through a 0.2µm nylon filter and degassed using a sonicator. To determine the appropriate wavelength for simultaneous determination of CPM, DEX, and GUA, solutions of these compounds were scanned on a UV-Vis spectrophotometer in the range of 200-400 nm. Based on overlaid UV spectra, a suitable wavelength of 265nm was chosen for monitoring these drugs.

Preparation of Mobile Phase solution:

An amount of 1.0206 gm of Potassium dihydrogen phosphate was dissolved in 300ml distilled water in 1000ml volumetric flask. Then 450ml HPLC Grade methanol and 250ml of HPLC Grade acetonitrile was added and then mixed well. The pH was adjusted to 5.5 with the help of orthophosphoric acid. The solution was sonicated for 10 min for proper mixing after sonication filtration is done by 0.2µm Nylon 6, 6 membrane (47mm) filter paper with the help of vacuum pump. The degassing of mobile phase was done by sonicated for 20 min.

Preparation of standard solution:

Weighed 10mg CPM, 10 mg DEX and 25mg GUA precisely in separate volumetric flask and dissolved in methanol to obtain stock concentration of 1mg/ml. Then prepared 5 volumetric flask with different concentration and make up volume by methanol.

Method Validation

The proposed method was validated as per ICH guidelines Q2B.

Calibration Curve

In this experiment, we analysed five different concentrations of GUA (Guaifenesin), DEX (Dextromethorphan), and CPM (Chlorpheniramine Maleate). To construct the calibration curve, we tested these concentrations within a specified range of 50-500 µg/ml. To generate the calibration plots, we performed replicate analysis at each concentration level. We then used the least square method within Microsoft Excel Program to evaluate the linear relationship between the concentrations and the measured values.

Repeatability, Precision

To determine the injection repeatability, we conducted six consecutive injections using the same sample. Similarly, to examine the analysis repeatability, we performed six different injections using samples prepared through the same procedure. In both cases, the prepared standard mixture was used. To assess instrument precision, we performed six replicates of the standard solution mixture at three concentration levels within a specific time interval.On the other hand, the inter-day assay precision was conducted over a specific time period.

Limit of Detection and Limit of Quantification

LOD and LOQ were determined by kSD/s where k is a constant (3 for LOD and 10 for LOQ), SD is the standard deviation of the analytical signal, and s is the slope of the concentration versus response graph.

Accuracy

To ensure accuracy in our analysis, we employed the standard addition method to determine the recoveries of GUA, DEX and CPM. This involved adding (80%, 100%, and 120 %) of known amounts of the standards to the pre-analysed sample solution and obtaining chromatograms. By measuring the peaks areas and fitting these values to the straight-line equation of the calibration curve, we were able to estimate the amounts of standards present in the solutions. This approach allowed us to accurately determine the recoveries of GUA, DEX and CPM in the sample solution and ensure the reliability of our analysis.

Specificity

Specificity is the ability of the analytical method to measure analyte response in the presence of interferences present in the sample matrix. It was checked by detecting the analytes of interest in synthetic laboratory formulation. The resolution of the intented peaks were determined. Furthermore, the proposed method was also applied to marketed formulation.

System Suitability

System suitability test was performed to evaluate the chromatographic parameters such as retention factor, column efficiency, separation factor, HETP asymmetry of peaks, no. of theoretical plates and resolution between two consecutive peaks) before the validation of run. So,

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there are three replicate injections of the standard solution and prepared solution were prepared for the specificity procedure respectively.

Robustness

It evaluates by keeping chromatographic conditions unaffected by small variation of these parameters: (1) Detection wavelength: changed from 265 to 270 nm for GUA, DEX and CPM. (2) Column: using another column. (3) Solvent Brand: Acetonitrile and methanol.

Standard solutions were injected three times for each change. System suitability parameters like resolution, peak asymmetry, theoretical plates, retention factor, and RSD were calculated for each peak. Recoveries and % RSD were calculated for each component during each change.

III. RESULT AND DISCUSSION

Chromatographic Conditions

For the simultaneous determination of GUA, DEX, and CPM in tertiary mixtures or formulations, an easy HPLC technique was developed. All of the experimental settings were examined with the objective to optimise the proposed HPLC procedure.Due to the disadvantages of the normal phase, such as peak tailing caused by the hydration of silica with water, reversed phase separation was adopted.

Using a mobile phase consisting of methanol, acetonitrile, and 0.025M phosphate buffer in a 45:25:30 (v/v/v) ratio, adjusted to pH 5.5 using orthophosphoric acid, an adequate resolution was attained.

Validation

Linearity

The calibration curves (n=3) created for the standards GUA, CPM, and DEX were linear over the concentration range of 50-500 µg/ml. Peak regions of the standards were plotted vs concentration, and the resulting curve underwent linear regression analysis.Following a linear regression analysis, the coefficients of determination for GUA, DEX, and CPM, with % RSD values ranging from 0.5 to 2% across the concentration range under study, were found to be 0.9973, 0.9961, and 0.9994, respectively.

Precision

The precision result of the solution is shown in Table 2, where it was determined that the RSD values of retention time were both less than 2% for intra-day and inter-day precision.

Limit of Detection and Limit of Quantification

The LOD and LOQ were found to be 0.68, 1.31, 2.04 and 1.266, 4.36, 6.8 for GUA, DEX and CPM respectively.

Accuracy

Table 4 shows the results of the recovery investigations. The % RSD demonstrated that the devised analytical method demonstrated dependability and accuracy for the measurement of GUA, DEX and CPM in syrup formulation.

Specificity

Results were satisfactory, demonstrating that the suggested method has high specificity for determining the standards in tertiary mixtures and formulations.

System suitability

Peak resolution was satisfactory, and no interferences were seen. As a result, it can be said that the process works with the chosen column and solvent brand. The capacity factor, separation factor, number of theoretical plates, HETP, asymmetry of the peaks, and resolution between consecutive peaks were all evaluated as part of a system suitability test (Table 1).

Robustness

It evaluates by the selected factors remained unaffected by small variation of these parameters and studied each factor of mean obtained. The recovery obtained individually and mean were between 98 and 102% for GUA, DEX and CPM. It can therefore be concluded that the method is consistent for the detection of wavelength, selected column and solvent brand. The robustness studies indicated that the chosen factors were unaffected by minor modifications in those variables. For GUA, DEX, and CPM, recovery rates were obtained individually and the mean were between 98% and 102%.

Assay of Marketed Formulation

Table 5 displays the assay findings for GUA, DEX, and CPM in commercial formulations, which ranged from 96% to 101%.

IV. CONCLUSION

Simultaneous estimation of drug substances in combination formulations has always

been a challenge for analysts. The estimation of drugs in syrup formulations further aggravates the problem of easy estimation of compounds. The present manuscript reports a simple, sensitive and rapid chromatographic method for estimation of a commonly used cough syrup.

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Table No. 1 Intra-day and Inter-day precision of the developed method (n= 3)

PARAMETERS	GUA	CPM	DEX
Retention time (min)	10.15	11.36	12.59
Detection Wavelength (nm)	265	265	265
LOD (μgml^{-1})	0.38	1.31	2.04
LOQ (μgml^{-1})	1.266	4.36	6.8
Linearity range (μgml^{-1})	50-500	50-500	50-500
Correlation Coefficient (height)	0.9939	0.9886	0.9967
Correlation Coefficient (area)	0.9973	0.9961	0.9994
Regression equation (height)	y = 3871.1x - 124413	y = 477.83x - 12432	y = 1364.7x - 44378
Regression equation (area)	y = 92981x - 2000000	y = 16251x - 326580	y = 32430x - 789724
Capacity factor (K)	0.6551381	0.85222575	1.05001595
Theoretical Plates	2676.25395	2542.052597	2725.192656
HETP(mm)	0.093414154	0.098346	0.091737
Resolution (Rs)	---	0.840906	0.68459
Separation Factor (α)	---	1.31038086	1.23947097
Asymmetry (As)	0.93	1.02	0.92

Table No.2 Intra-day and Inter-day precision of the developed method (n= 3)

Components ($\mu g\ ml^{-1}$)	Intra-day				Inter-day			
	Retention time		Peak Area		Retention time		Peak Area	
	Mean	%RSD	Mean	SD	Mean	%RSD	Mean	SD
GUA 1	10.544	0.720	2948734	949403.3	10.251	1.94	2390394	159791.3
2	10.594	0.726	3929129	274278.9	10.287	2.08	3940651	290573.5
3	10.708	0.396	6428486	908881.1	10.373	3.40	6460414	954034.1
DEX 1	12.471	0.040	529295.5	75306.17	11.257	1.14	511520.5	50168.52
2	12.454	0.44	707048.5	137131.3	11.235	1.81	638715.5	40493.88
3	12.590	0.32	941545	333963.7	11.296	0.89	761280	79030.5
CPM 1	11.22	0.240	2973045.5	81303.84	12.572	1.24	2772236	202683.7
2	11.160	0.310	2919014.5	131782.8	12.523	2.04	2918584	132391.6
3	11.255	0.133	1025302.5	289999.3	12.588	0.88	3089257	260235.8

Table No. 3 Robustness of the method (n = 3)

Chromatographic change factor	Recovery, % components			
	Level	GUA	DEX	CPM
Solvent Brand				
CDH	1	98.23 %	99.1%	97.62%
Qualigens (Thermo Fischer)	2	98.68%	99.62%	96.45%
Wavelength (nm)				
263 nm	-2	98.55%	100.1%	99.26%
265 nm	0	99.13%	98.53%	97.65%
267 nm	2	98.52 %	99.36 %	100.2%

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Column Brand				
Phenomenex	1	98.76 %	99.63%	97.63%
Hypersil	2	98.35%	99.63%	100.4%

Table No. 4 Recovery test (n = 3)

Components	Quantity added %	Total quantity present	Amount quantity found	Recovery	% RSD
GUA	0	50	49.77	99.54	0.74
	80	90	90.61	100.67	0.94
	100	100	99.87	99.87	0.76
	120	110	109.48	99.52	0.74
DEX	0	5	4.89	102.4	1.88
	80	9	8.99	99.88	0.70
	100	10	10.05	100.5	1.87
	120	11	11.02	100.182	1.36
CPM	0	2.5	2.45	98	1.82
	80	4.5	4.48	99.55	0.64
	100	5	4.99	99.8	0.66
	120	5.5	5.45	99.09	0.72

Table No. 5 Results of Assay of Formulation

Formulation	Concentration in mg in Syrup (5ml)	GUA (50)	CPM(2.5)	DEX(50)
GUA	50	97.35%	99.28%	98.33%
CPM	2.5	96.54%	97.34%	96.32%
DEX	50	100.34%	102.1%	100.96%

Fig.: 1 Chemical Structure of Guaifenesin

Fig.:2 Chemical Structure of Dextromethorphan

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Fig.:3 Chemical Structure of Chlorphenamine

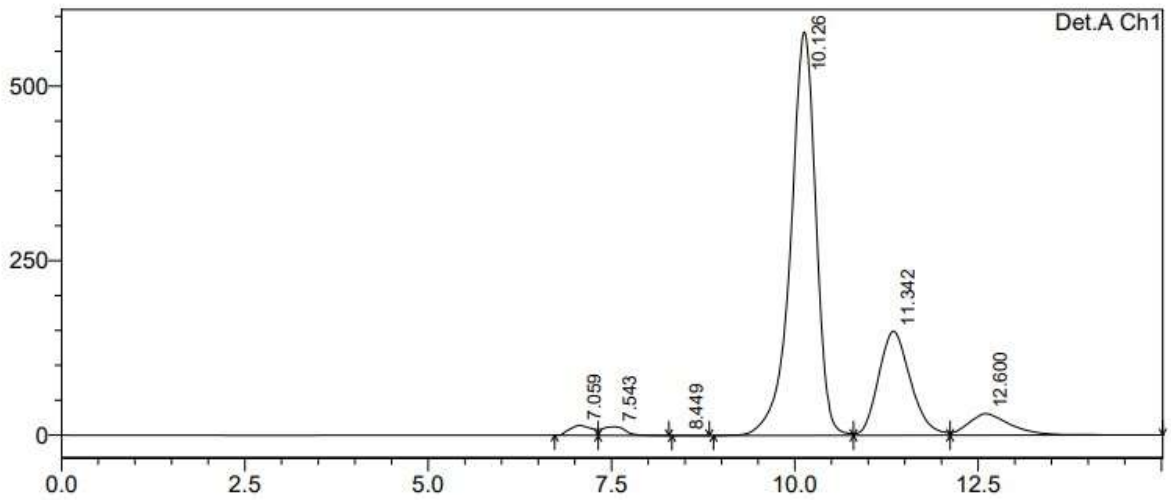

Fig. 4 Chromatogram of CPM, GUA and DEX standards in combination

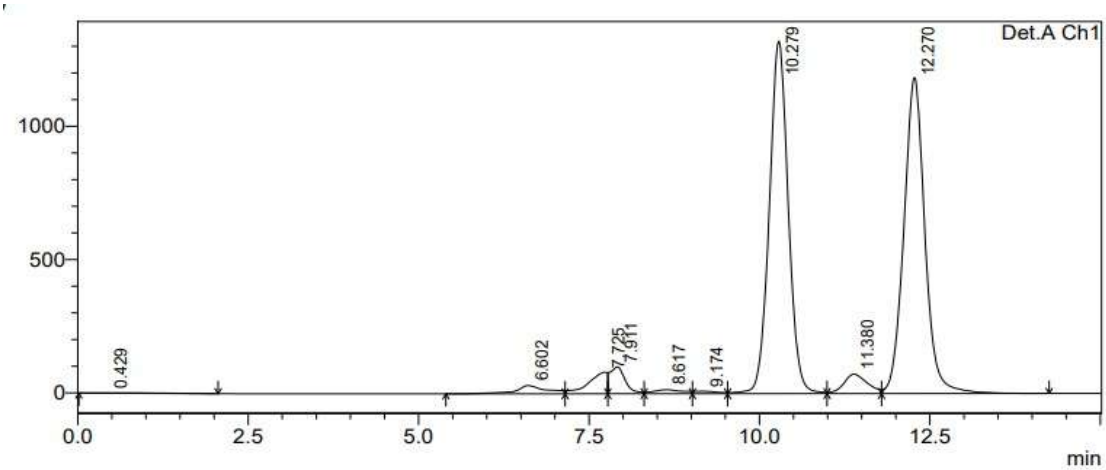

Fig. 4 Chromatogram of estimation of CPM, GUA and DEX in Syrup formulation