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A NOVEL VALIDATED ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF GUAIPHENESIN, CHLORPHENIRAMINE MALEATE AND BROMHEXINE HYDROCHLORIDE IN BULK AND COMBINED DOSAGE FORM BY RP-HPLC

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ABSTRACT

The current study focuses on developing a simple, precise and accurate RP-HPLC method for simultaneous analysis of Bromhexine hydrochloride, Guaiphenesin and Chlorpheniramine maleate in bulk and combined dosage forms. The chromatographic conditions were standardized using Hypersil BDS column (150×4.6mm, particle size 5µ) with UV detection at 225nm and mobile phase consist of 0.01M potassium dihydrogen phosphate buffer (pH adjusted to 3 with 1% ortho phosphoric acid): acetonitrile: methanol (45:5:50 v/v/v) with flow rate 0.8mL/min. The injection volume was 10µL and run time was about 10 minutes. The retention times of Guaiphenesin, Chlorpheniramine maleate and Bromhexine hydrochloride have been found to be 2.861 min, 4.050 min and 7.273 min respectively. The calibration curves were linear with a correlation coefficient of 0.9994, 0.9992 and 0.9992 over a

concentration range of 5.0µg/mL-25.0µg/mL for Bromhexine hydrochloride, 10.0µg/mL-50.0µg/mL for Guaiphenesin and 5.0µg/mL-25.0µg/mL for Chlorpheniramine maleate respectively. Method was accurate with % recovery of 99.88%, 100.17% and 99.59% and for Bromhexine hydrochloride, Guaiphenesin and Chlorpheniramine maleate. The method was evaluated for validation parameters such as specificity, linearity, precision, accuracy, limit of detection and quantification and robustness according to International Conference on Harmonization (ICH) Q2R1 guidelines.

Keywords: Guaiphenesin, Chlorpheniramine maleate, Bromhexine hydrochloride, ICH, RP-HPLC

INTRODUCTION

Guaiphenesin (GUA) is 3-(2-methoxy phenoxy) propane-1,2-diol **(Figure 1)** with molecular formula of $C_{10}H_{14}O_4$ and molecular weight 198.22 gm/mol. GUA is used as an expectorant to cure cough caused by minor upper respiratory infections and illnesses like sinusitis, pharyngitis and bronchitis that are worsened by viscous mucus and congestion **[1]**.

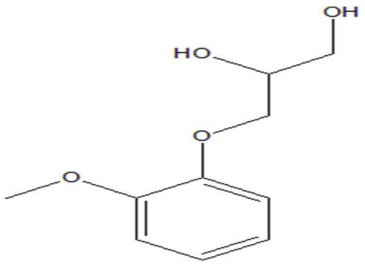

Figure 1: Structure of GUA

Chlorpheniramine maleate (CPM) is [3-(4-chlorophenyl)-3-(pyridin-2-yl) propyl] dimethylamine **(Figure 2)** with molecular formula of $C_{20}H_{23}ClN_2O_4$ and molecular weight of 390.9 gm/mol. It is a histamine H1 antagonist that is used to treat the symptoms of hay fever, asthma, urticaria and rhinitis. It acts as antipruritic, histamine antagonist, serotonin uptake inhibitor, antidepressant, and anti-allergic substance. It is a tertiary amino molecule that related to the monochlorobenzenes and pyridine group **[2]**.

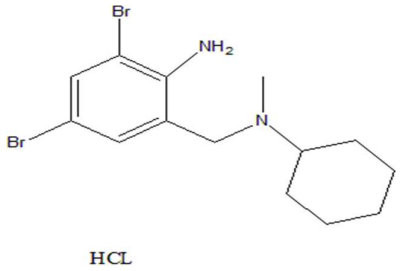

Figure 2: Structure of CPM

Bromhexine hydrochloride (BRH) is 2,4-dibromo-6-[[cyclohexyl(methyl)amino] methyl] aniline; hydrochloride (Fig.3) is a secretolytic that also has mucolytic action with molecular formula of $C_{14}H_{20}Br_2N_2.HCl$ and molecular weight 412.59 gm/mol. This causes the respiratory tract to produce more serous mucus, which thins the phlegm and reduces the viscosity of the mucus. This improves the cilia's ability to carry phlegm out of the lungs and contributes to its secretomotoric action **[3]**. This helps to remove mucus from the respiratory tract and may help with respiratory diseases like abnormal viscid

mucus, excessive mucus output, and poor mucus transport.

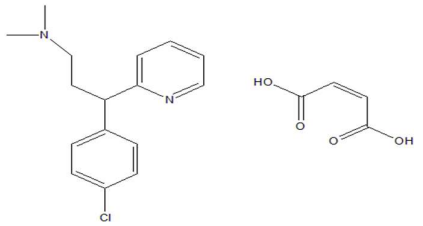

Figure 3: Structure of BRH

An extensive literature search confirmed that there was only one reported analytical method for the simultaneous estimation of Guaiphenesin, Chlorpheniramine maleate and Bromhexine hydrochloride. As a result, we presented an accurate, cost-effective analytical method for the simultaneous estimation of Guaiphenesin, Chlorpheniramine maleate and Bromhexine hydrochloride in bulk and combined dose form

MATERIALS AND METHODS

Materials:

Guaiphenesin, Chlorpheniramine maleate, Bromhexine HCl, methanol, acetonitrile HPLC grade water and OPA of analytical grade were obtained from national scientific products. The tablet formulation containing 50mg of GUA, 4mg of CPM and 4mg of BRH were purchased.

Chromatographic Conditions:

HPLC 2030 C 3D Plus Shimadzu instrument equipped with PDA (Photo Diode Array) detector is utilised and data was analyzed by using LAB SOLUTIONS software. The chromatographic conditions were standardized using Hypersil BDS column, (150×4.6mm, particle size 5µ) with UV detection at 225nm and mobile phase consisting of 0.01 M KH_2PO_4 buffer (pH adjusted to 3 with 1% OPA): acetonitrile: methanol (45:5:50 v/v/v) with flow rate 0.8mL/min. The injection volume was 10µL and run time was about 10 minutes.

Preparation of Standard Stock Solution:

About 10mg of each GUA, CPM and BRH were accurately weighed and transferred into a separate 10mL clean dry volumetric flask and dissolved in 3/4th volume with methanol and made up to 10 mL with methanol to obtain 1000 µg/mL.

Preparation of Working Stock Solutions for Chlorpheniramine Maleate and Bromhexine hydrochloride:

From the stock solutions 1mL each solution of CPM and BRH was transferred into another 10mL volumetric flask and each volumetric flask made up to 10mL with methanol to obtain 100 µg/mL.

Preparation of Sample Solution:

About twenty tablets (each tablet contains 4mg of BRH, 50mg of GUA and 4mg of CPM were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Weight equivalent to one tablet powder of GUA, CPM and BRH was dissolved in 3/4th volume with methanol. The above solution was sonicated for 5 min and filtered using 0.45µm membrane filter

under vacuum filtration. Dilute the filtered solution to 100ml with methanol.

Preparation of Buffer (pH 3) (0.01 M KH_2PO_4)

An accurately weighed 0.6804 gm of KH_2PO_4 (0.01M) was transferred into 500 mL volumetric flask, followed by addition of 300 mL of HPLC grade water. Resulting solution was shaken for 5 mins and sonicated for 15 min. Then resulting solution was filtered using 0.45µm membrane filter under vacuum filtration and transferred to another 500 mL volumetric flask and volume was make up to mark with HPLC grade water. Then pH adjusted to 3 with 1% ortho phosphoric acid.

Optimized Chromatographic Conditions:

For separation and resolution various chromatographic conditions were explored. The Hypersil BDS (1504.6mm, 5m) column was found to be adequate. Peak purity of Guaiphenesin, Chlorpheniramine maleate and Bromhexine hydrochloride was determined using a photo diode array detector and 225 nm was found to be acceptable for detecting three drugs with adequate sensitivity. A mobile phase of 0.01M KH_2PO_4: Acetonitrile: Methanol (45:5:50 v/v/v) provided adequate resolution between the peaks at a flow rate of 0.8mL/min. The retention times of Guaiphenesin, Chlorpheniramine maleate and Bromhexine hydrochloride was found to be 2.861 min, 4.050 min, and 7.273 min, respectively.

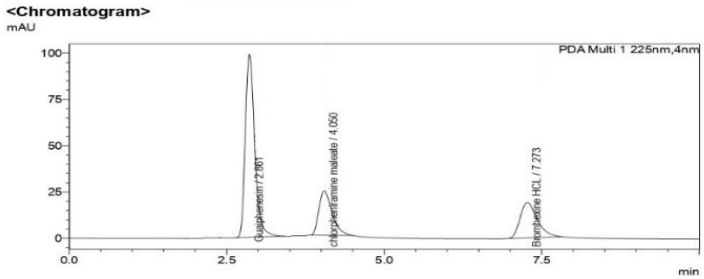

Figure 4: Optimized Chromatogram

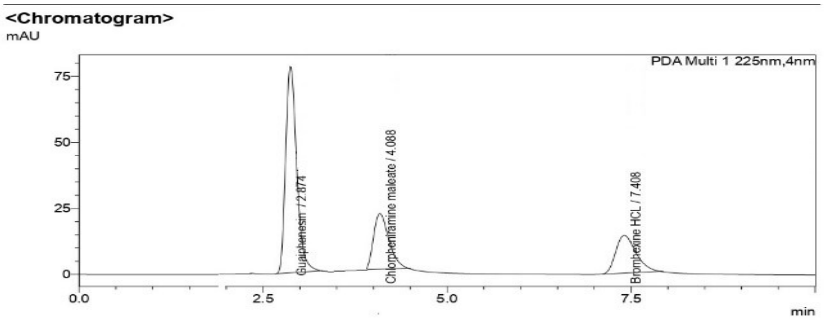

Figure 5: Sample Chromatogram

Method Validation:

The optimized chromatographic method was validated according to the ICH Q2R1 guidelines for the validation of parameters like linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness. The following parameters were evaluated**.**

RESULTS AND DISCUSSION

System Suitability:

The method's system suitability was demonstrated by injecting the optimal chromatogram concentration for six times to measure parameters such as retention time, % RSD, resolution, number of theoretical plates and tailing factor the results were calculated and compared with standard specification of system and data was represented in **Table 1**.

Acceptance Criteria: The %RSD should not be NMT 2.0

Specificity:

The chromatograms of the blank, standard and sample were compared to determine the method's specificity. There was no interaction of the blank with the analyte present in the standard and sample when they were injected into the system and the chromatograms were recorded.

Linearity:

The method's linearity was investigated by establishing calibration curves with various concentrations of standard solutions **(Figure 6, 7, 8)**. GUA linearity was demonstrated at 10-50 µg/mL, while CPM and BRH linearity was established at 5-25 µg/mL. Parameters like slope, intercept and regression equation were calculated from the resulting data and represented in **Table 2**.

Acceptance Criteria: The regression(R^2) should not be NLT 0.999

Precision:

The method's precision was determined by injecting the reference solution (40 µg/ml of GUA, 20 µg/ml of CPM and BRH) in six replicates and the data was displayed in **Table 3**.

Acceptance Criteria: The % RSD should be NMT 2.0

Accuracy:

The method's accuracy was determined by comparing the % recovery of the analyte added to weighted amounts at levels of 50%, 100%, and 150 % to the declared amounts using tablet formulation (Cofex^{TM}), and the results were shown in **Table 4**.

Acceptance Criteria: The mean % recovery should be NLT 98% and NMT 102%.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD for GUA, CPM and BRH were found to be 1 µg/mL, 0.5 µg/mL and 0.5 µg/mL, respectively, and the LOQ for GUA, CPM and BRH were found to be 3 µg/mL, 1.5 µg/mL and 1.5 µg/mL. This

indicates the method's sensitivity.

Robustness:

Robustness was tested at flow rates of 0.6 mL/min and 1.0 mL/min, as well as a shift in the MP ratio from 0.01M KH_2PO_4 buffer: Acetonitrile: Methanol (45:5:50 v/v/v) to 40:5:55 v/v/v and 50:5:45 v/v/v, with data for robustness listed in **Table 5**.

Acceptance Criteria: The % RSD should be NMT 2.0.

Assay:

The % purity of GUA, CPM and BRH was found to be 99.03%, 99.51% and 99.38 % respectively. **Table 6**.

Data

Acceptance criteria: The % assay should be 98%-102%.

Table 1: Data of System Suitability

Injection No.	Guaiphenesin	Chlorpheniramine maleate	Bromhexine HCl
	Peak Area	Peak Area	Peak Area
1	1041289	362091	367012
2	1049763	373795	371860
3	1057139	365408	361198
4	1065528	376376	369655
5	1056377	371577	368473
6	1068902	370893	366328
AVG	1056500	370023.3	367421
SDV	10124.22	5328.701	3630.147
%RSD	0.96	1.44	0.99

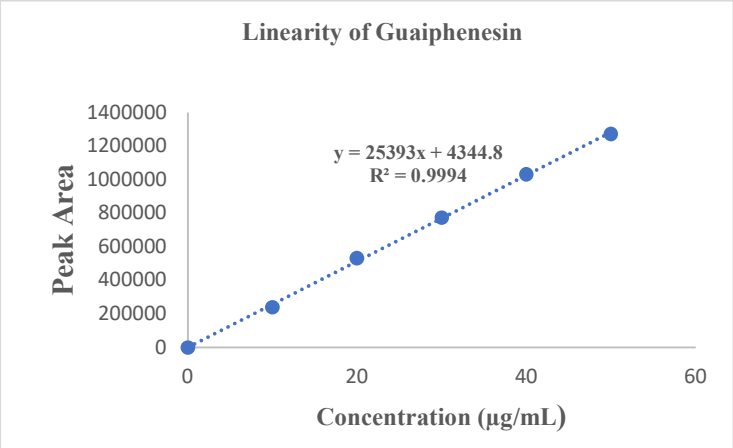

Figure 6: Linearity of GUA

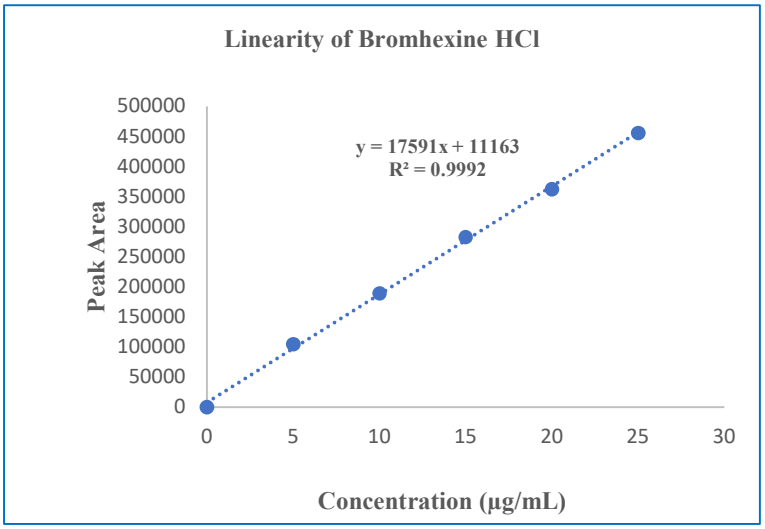

Figure 7: Linearity of CPM

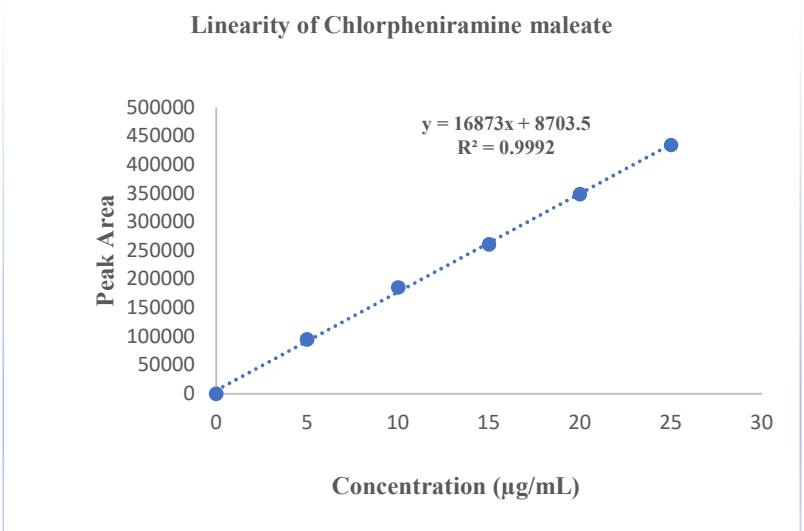

Figure 8: Linearity of BRH

Table 2: Data of Linearity

GUA		CPM		BRH	
Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area
10	239756	5	95268	5	104611
20	532155	10	185957	10	189039
30	773726	15	261069	15	282701
40	1031775	20	348866	20	362452
50	1273564	25	434353	25	455572
R^2= 0.9994		R^2= 0.9992		R^2= 0.9992	

Table 3: Data of System Precision and Method Precision

Injection No	System Precision			Method Precision		
	GUA	CPM	BRH	GUA	CPM	BRH
	Peak area	Peak area	Peak area	Peak area	Peak area	Peak area
1	1045762	373152	371252	1043692	367294	361285
2	1052913	361256	365479	1053627	362572	368512
3	1059782	365472	367054	1059428	375874	372584
4	1056562	361218	368415	1064184	372528	361099
5	1065895	372159	362398	1058452	364275	366395
6	1071126	362719	361576	1069641	361277	364387
AVG	1058673	365996	366029	1058171	367303.3	365710.3
SDV	9086.168	5394.462	3669.856	8940.531	5807.35	4430.905
%RSD	0.86	1.47	1.00	0.84	1.58	1.21

Table 4: Data of Accuracy

Drug	% Level	Standard Peak area	Sample peak area	% Recovery	% Average Recovery	% Overall Mean Recovery
Guaiphenesin	50%	1056500	534881	100.62	101.62	100.17%
		1056500	538881	101.29		
		1056500	538568	101.12		
	100%	1056500	1050023	99.35	99.22	
		1056500	1049023	99.15		
		1056500	1048203	99.16		
	150%	1056500	1588520	100.04	100.29	
		1056500	1594211	100.47		
		1056500	1595123	100.38		
Chlorpheniramine maleate	50%	370023	184524	99.11	99.10	99.59%
		370023	184852	99.20		
		370023	184658	99.00		
	100%	370023	370129	99.99	100.26	
		370023	371256	100.19		
		370023	372456	100.61		
	150%	370023	552513	99.35	99.41	
		370023	552456	99.41		
		370023	553625	99.47		
Bromhexine hydrochloride	50%	367421	184456	99.77	99.94	99.88%
		367421	185126	100.05		
		367421	185258	100.02		
	100%	367421	367245	99.92	99.82	
		367421	366968	99.74		
		367421	366865	99.80		
	150%	367421	551256	99.82	99.89	
		367421	551348	99.91		
		367421	552365	99.95		

Table 5: Data of Robustness

S. No	Parameters	GUA			CPM			BRH		
		RT (min)	Peak area	% RSD	RT (min)	Peak area	% RSD	RT (min)	Peak area	% RSD
1	Change in flow rate- 0.6mL/min	3.776	1367873	0.79	5.272	458875	0.51	9.360	469437	0.40
		3.774	1383413		5.270	462210		9.369	472129	
2	Change in flow rate- 1mL/min	2.294	843857	0.80	3.207	290932	0.06	5.714	349563	0.57
		2.297	853464		3.216	290670		5.739	352401	
3	Change in MP composition- 40:5:55% v/v/v	2.189	842637	0.21	2.791	317895	0.13	4.377	310289	0.75
		2.125	845242		2.714	317308		4.299	313604	
4	Change in MP composition- 50:5:45% v/v/v	2.545	889205	0.64	4.026	298035	0.90	8.383	316729	0.75
		2.559	881143		4.046	301866		8.395	320130	

Table 6: Data of Percentage Assay

Formulation	Label claim (mg)	% Assay
Guaiphenesin	50 mg	99.03%
Chlorpheniramine Maleate	4 mg	99.51%
Bromhexine HCl	4 mg	99.38%

DISCUSSION

The retention time for GUA, CPM and BRH was 2.861 min, 4.050 min and 7.273 min respectively. Calibration curves were linear with correlation coefficient 0.9994, 0.9992 and 0.9992 over a concentration range of 10.0µg/mL-50.0µg/mL for GUA, 5.0µg/mL-25.0µg/mL for CPM and 5.0µg/mL-25.0µg/mL for BRH respectively. Accuracy was achieved for the three drugs with % recovery of 100.17%, 99.59% and 99.88%. The precision results were within limits and the approach was proven to be robust, with a % RSD limit of NMT 2.0. The acceptance limits were satisfied for all parameters, including theoretical plates, resolution, % RSD and tailing factor.

CONCLUSION

A simple and efficient RP-HPLC method was developed and validated for simultaneous estimation of GUA, CPM and BRH in bulk and combined dosage form. The acceptance limits were satisfied for all parameters, including theoretical plates, resolution, tailing factor and % RSD. As a result, the proposed method can be used in routine analysis of the combined dosage form. The method was evaluated for validation parameters such as specificity, linearity, precision, accuracy, LOD and LOQ and robustness according to International Conference on Harmonization (ICH) Q2R1 guidelines.

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