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SIMULTANEOUS ESTIMATION OF PARACETAMOL, GUAIPHENSIN, PHENYLEPHRINE HCl, CHLORPHENIRAMINE MALEATE AND BROMOHEXINE HCl IN COMBINED TABLET DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATO-GRAPHY

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Keywords:

Paracetamol, Guaiphenesin, Phenylephrine HCl, Chlorpheniramine maleate, Bromhexine HCl, RP-HPLC, Validation, ICH Guidelines

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ABSTRACT: A reverse phase high performance liquid chromatographic method was developed for the estimation of paracetamol, guaiphenesin, phenylephrine HCl, chlorpheniramine maleate and bromhexine HCL in a single tablet dosage form. This method was proved to be simple, accurate, precise and robust. The chromatographic separation was achieved on a symmetry C-8 column with dimensions of 150 X 4.6mm, 3.5µm. All the five components were separated by the gradient elution of the mobile phase A consisting of buffer 10mM KH_2P0_4 and 3.7mM of an ion pair reagent, octane-1-sulphonic acid sodium salt. The pH of the mobile phase A was adjusted to 4.0 with ortho phosphoric acid and the mobile phase B consisted of a mixture of methanol and acetonitrile in the ratio of 3:2. The wavelength of absorption was 220nm and the flow rate was fixed at 1.0 ml/min. The retention times of paracetamol, guaiphenesin, phenylephrine HCL, chlorpheniramine maleate and bromhexine HCl were found to be 5.73, 17.46, 18.29, 21.78, and 23.55 respectively. The method was validated according to the ICH guidelines for specificity, LOD, LOQ, precision, accuracy, linearity, ruggedness and robustness. The linearity range for all the drugs were performed from 10% to 150% with respect to the test concentration strength. The co-relation coefficients obtained were ≥ 0.999. The mean %recoveries for all the drugs were found to be in the range of 98 to 102%. The above validated method can be used for the analysis of combined dosage form of all these five drugs.

INTRODUCTION: Paracetamol (N-(4-hydroxyphenyl) acetamide, **Fig. 1**) is classified as a mild analgesic. It is commonly used for reducing the body temperature (antipyretic). Recent studies suggested that it is highly selective for COX-2 and it does not significantly inhibit the production of the pro-clotting thromboxanes.

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Guaiphenesin ((*RS*)-3-(2-methoxyphenoxy) propane-1, 2-diol, **Fig. 2**) is a cough remedy sold over the counter and taken orally to assist the expectoration of phlegm from the airways in acute respiratory tract infections by increasing the volume and reducing the viscosity of secretions in the trachea and bronchi. It also stimulates the flow of respiratory tract secretions, allowing ciliary movement to carry the loosened secretions upward toward the pharynx.

Phenylephrine ((*R*)-3-[-1-hydroxy-2-(methylamino) ethyl] phenol, **Fig. 3**) is a selective α_1-adrenergic receptor agonist used primarily as a decongestant.

Phenylephrine is marketed as a substitute for the decongestant pseudoephedrine, though clinical studies differ regarding phenylephrine's effectiveness in this role.

Chlorpheniramine maleate (3-(4-chlorophenyl)-*N*, *N*-dimethyl-3-pyridin-2-yl-propan-1-amine, Fig: 4) is a first generation alkylamine antihistamine used in the prevention of symptoms of allergic conditions such as rhinitis and urticaria.

Bromhexine (2, 4-dibromo-6-{[cyclohexyl (methyl) amino] methyl} aniline, Fig: 5) a synthetic derivative of vasicine is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus. Its mechanism is by increasing the production of serous mucus in the respiratory tract and makes the phlegm thinner and less viscous.

This contributes to a secretomotoric effect by helping the cilia transport the phlegm out of the lungs. For this reason it is often added to cough syrups.

Most of the cold and cough OTC remedies contain combinations of analgesic, antipyretic, antihistaminic, expectorants, nasal decongestant and mucolytics. Usually a combination of HPLC and UV methods are used for the estimation of the individual components of such formulations. The literature survey (1-21) revealed that so far no single method has been reported for simultaneous determination of paracetamol, guaiphenesin, phenylephrine HCl, chlorpheniramine maleate and bromhexine HCl combination by HPLC. We report here development and validation of a single HPLC method for all the above components.

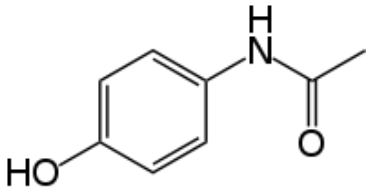


FIG. 1: STRUCTURE OF PARACETAMOL

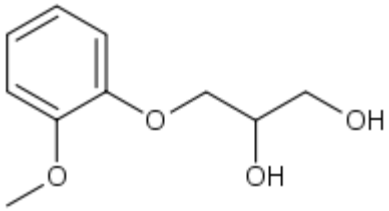


FIG. 2: STRUCTURE OF GUAIPHENSIN

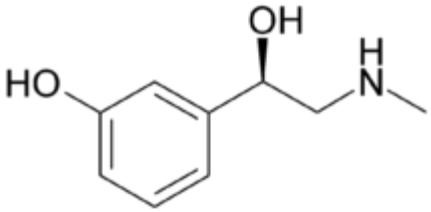


FIG. 3: STRUCTURE OF PHENYLEPHRINE

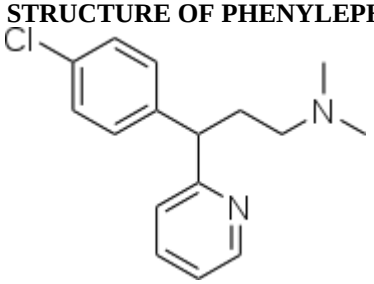


FIG. 4: STRUCTURE OF CHLORPHENIRAMINE

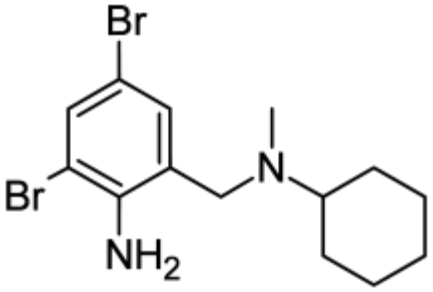


FIG. 5: STRUCTURE OF BROMOHEXINE

MATERIALS AND METHODS:

Chemicals: Paracetamol, guaiphenesin, phenylephrine HCl, chlorpheniramine maleate and bromhexine HCl were obtained from Natco Pharma Ltd., India. Methanol (HPLC Grade), acetonitrile (HPLC Grade), potassium dihydrogen phosphate and octane-1-sulphonic acid sodium salt were purchased from MERCK, Mumbai.

Ortho phosphoric acid (AR Grade) was purchased from Qualligens fine chemicals, Mumbai. Milli Q water was used throughout the experiment. Tablet dosage form containing paracetamol 325mg, guaiphenesin 100mg, phenylephrine HCl 5mg, chlorpheniramine maleate 2mg and bromhexine HCl 8mg was procured from the local pharmacy store.

Equipment: Chromatographic system of Shimadzu Prominence LC-20AT equipped with SIL-20AC Autosampler, CTO-10AS-vp Column Oven and SPD-20A UV-Visible detector was used for performing analysis. Chromatographic separation was achieved on Symmetry C8 (150 X 4.6mm, 3.5µ) column. LC Solutions software was used for acquisition of the data.

Mixed Standard Stock Solution: Standard stock solution of paracetamol (3.25mg/ml), guaiphenesin (1mg/ml), phenylephrine HCl (0.5mg/ml), chlorpheniramine maleate (0.2mg/ml) and bromhexine HCl (0.8mg/ml) was prepared by transferring 325mg of paracetamol, 100mg of guaiphenesin, 50mg of phenylephrine HCl, 20mg of chlorpheniramine maleate and 80mg of bromhexine HCl into a 100ml volumetric flask and diluting the sample with the diluent containing water and methanol in 1:1 ratio. It was then sonicated for 10 min and made upto the volume with the diluent. The working standard solution was prepared by further diluting 10ml of the above stock solution to 100ml.

Test Sample Preparation: Twenty tablets were weighed, their mean weight was determined and they were crushed and finely powdered. An equivalent weight of the tablet content was transferred into a 100 ml volumetric flask containing 80 ml of diluent, ultrasonicated for 30 mins and made up to the volume. The solution was filtered through a 0.45µ syringe filter and injected for the estimation of phenylephrine HCl, chlorpheniramine maleate and bromhexine HCl. As the responses of Paracetamol and guaiphenesin were very high these two drugs were estimated by further diluting 5 ml of the above solution to 10ml.

The standard and test solutions of the paracetamol, guaiphenesin, phenylephrine HCl, chlorpheniramine maleate and bromhexine HCl were 0.325mg/ml, 0.1mg/ml, 0.05mg/ml, 0.02mg/ml and 0.08mg/ml respectively.

RESULTS AND DISCUSSIONS:

Optimization of chromatographic conditions: For optimization of the method two organic solvents methanol and acetonitrile were used individually and in combination. Different buffers like phosphate, acetate and citrates were used but phosphates with ion pair reagent octane 1-sulphonic acid at pH 4.0 gave good peak shapes. Organic solvent in combination of methanol and acetonitrile gave good resolution between the peaks. Analysis was performed on a Shimadzu prominence LC-20AT liquid pump using a gradient elution (time (min) / % B: 0/10, 8/10, 13/45, 20/75, 25/75, 30/10, 35/10) with a C8 (150 X 4.6mm, 3.5µm) column and mobile phase A consisting of buffer 10mM KH_2P0_4 with ion pair reagent 3.7mM octane-1-sulphonic acid sodium salt, pH adjusted to 4.0 with ortho phosphoric acid and mobile phase B of methanol and acetonitrile in the ratio of 3:2 The wavelength of absorption was 220nm and a flow rate of 1.0ml/min. The retention times of paracetamol, guaiphenesin, phenylephrine HCl, chlorpheniramine maleate and bromhexine HCl were 5.73, 17.46, 18.29, 21.78, and 23.55 min respectively. The typical chromatogram is given in **Fig. 6**.

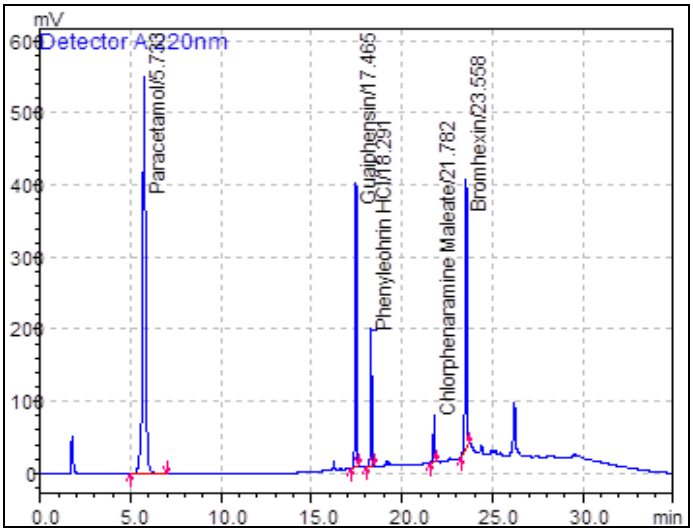


FIG. 6: TYPICAL CHROMATOGRAM

Method Validation: The optimized chromatographic method was validated according to the ICH guidelines Q2 (R1) for evaluating the specificity, accuracy, precision, LOD, LOQ, linearity and robustness.

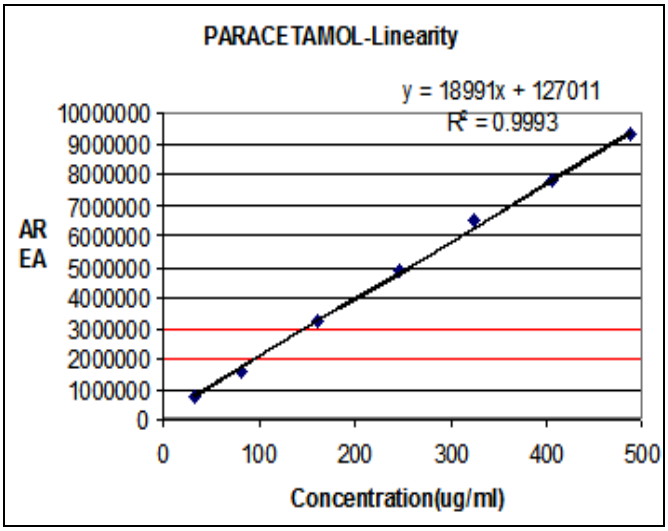
Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection represents the concentration of the analyte that would yield signal-to-noise ratio of 3 and the limit of quantification represents concentration of the analyte that would yield signal-to-noise ratios of 10. LOD of 0.5µg/ml and LOQ of 1.5µg/ml were established.

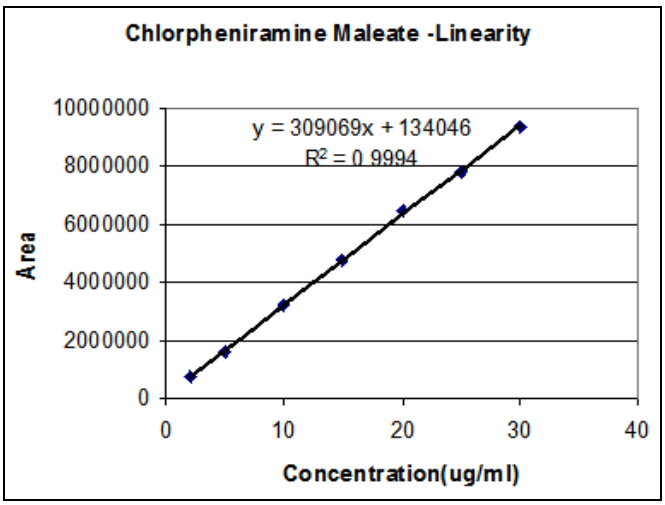
Linearity: Linearity for the above drug combination was determined in the range of 10% to 150% of the working standard concentration. The co-relation coefficients (r^2) obtained were ≥ 0.999. The concentration range for paracetamol was 32 to 488µg/ml, guaiphenesin was 10 to 150µg/ml, phenylephrine HCl was 5 to 75µg/ml, chlorpheniramine maleate was 2 to 30µg/ml and bromhexine was 8 to 120µg/ml.

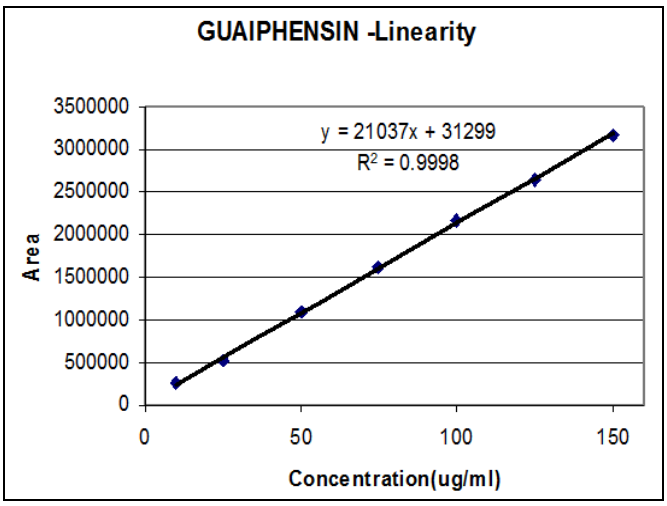
The linearity curves are represented in **Fig. 7** and the linearity overlay chromatogram is represented in **Fig. 8**.

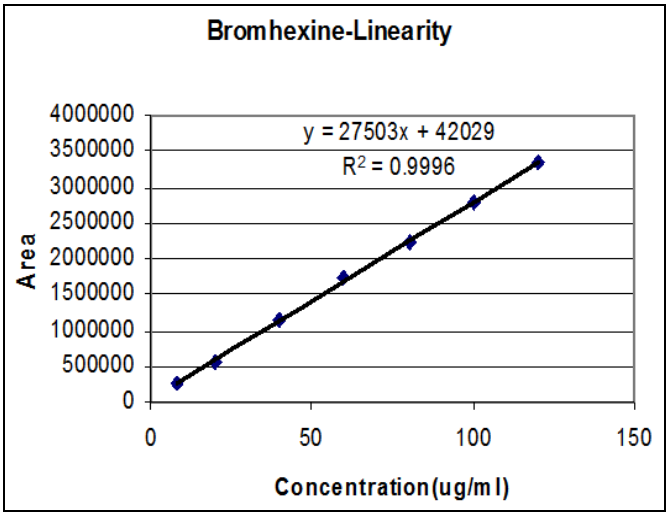
Accuracy: Accuracy was determined by external standardization method. A known concentration of standards in the range of 80%, 100% and 120% were added to the preanalyzed test samples. At each level triplicate samples were analyzed and the mean percentages recoveries were calculated. The mean percentage recoveries were in the range of 100.12 to 102.2 for paracetamol, 98.5 to 100.95 for guaiphenesin, 100.2 to 102.2 for phenylephrine HCl, 98.67 to 101.6 for chlorpheniramine maleate and 99.79 to 100.6 for bromhexine.

Precision: The precision expressed as %RSD was determined by intra-day and inter-day precision. The %RSD for six replicates was found to be less than 1.0% indicating good repeatability. The values obtained for repeatability are given in the **Table 1.**









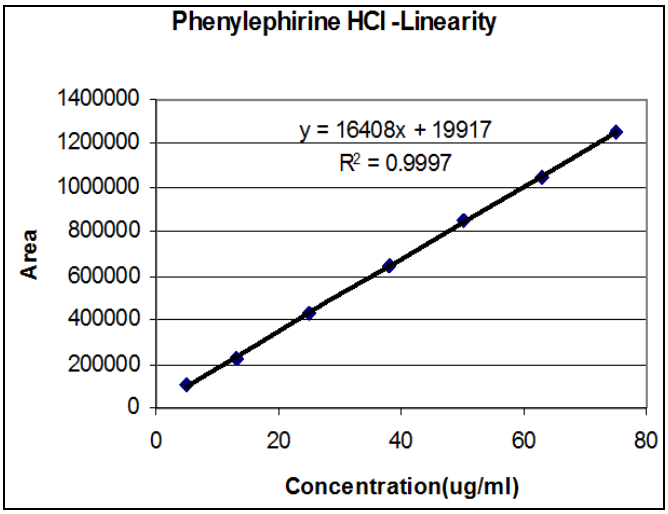


FIG. 7: LINEARITY CURVES

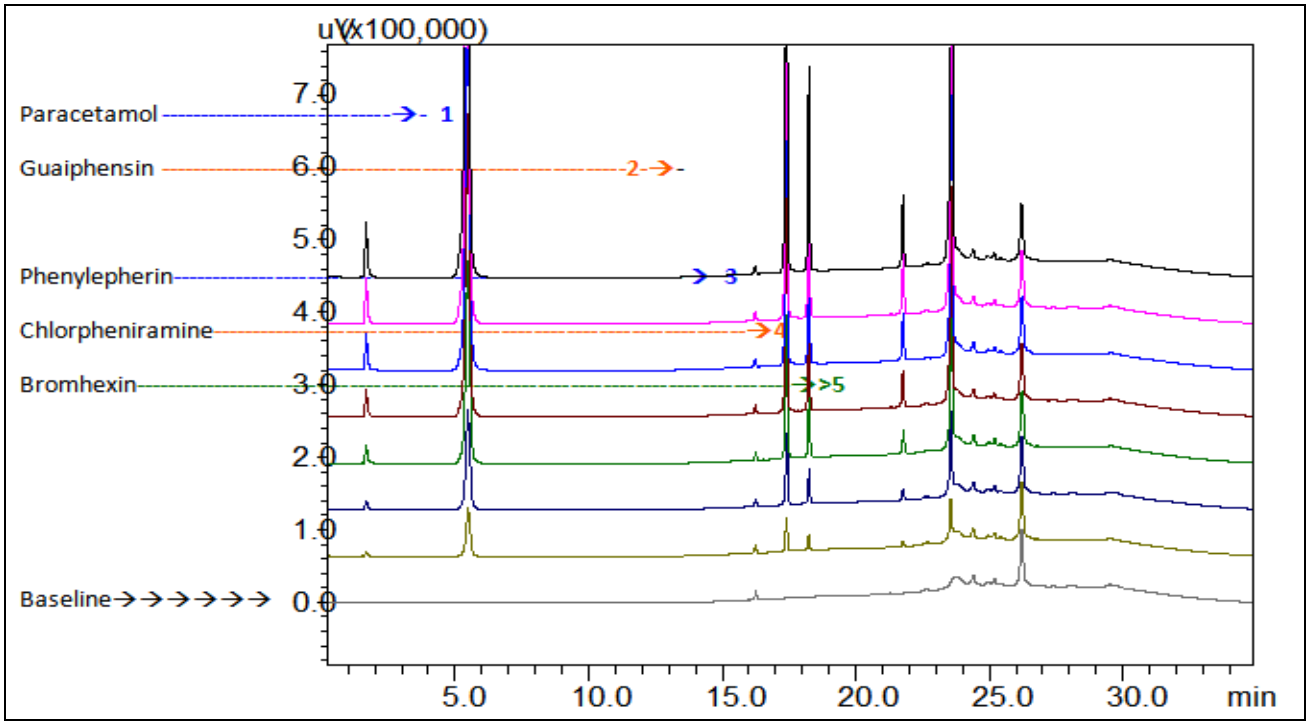


FIG: 8 OVER-LAY CHROMATOGRAM OF LINEARITY

TABLE 1: PRECISION OF THE METHOD

S. No.	Name of the Drug	%RSD of peak area	
		DAY-1	DAY-2
1	Paracetamol	0.038	0.148
2	Guaiphenesin	0.090	0.7
3	Phenylephrine HCl	0.106	0.82
4	Chlorpheniramine maleate	0.438	0.588
5	Bromhexine HCl	0.428	0.450

System suitability: System suitability tests are an integral part of chromatographic methods and are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. The system suitability parameters obtained were summarized in the **Table 2**.

Specificity: The present method demonstrates good resolution between the peaks and was found to be free of interferences from the excipients and therefore the method was proved to be specific.

TABLE 2: SYSTEM SUITABILITY PARAMETERS

S. No.	Drug	Retention Time	Tailing Factor	Resolution	Theoretical Plates
1	Paracetamol	5.733	0.947	-	5981
2	Guaiphenesin	17.465	0.945	52.508	215997
3	Phenylephrine HCl	18.291	1.024	5.954	331278
4	Chlorpheniramine Maleate	21.782	0.968	25.676	362211
5	Bromhexine HCl	23.558	0.831	11.146	293237

Robustness: The chromatographic method was deliberately changed for flow rate, pH variation and percent organic variation in the gradient elution to demonstrate the robustness of the method. The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2.0%. The results of the robustness are given in **Table 3**.

Assay of commercial combined tablet formulation of paracetamol, guaiphenesin, phenylephrine HCl, chlorpheniramine maleate and bromhexine HCl: This validated method was applied to evaluate the assay of commercial tablet containing paracetamol, guaiphenesin, phenyl-ephrine HCl, chlorpheniramine maleate and bromhexine HCl. The experimental results are in good agreement with the label claim.

The average result of the two different lots analyzed using the proposed method is shown in **table 4**.

TABLE 3: ROBUSTNESS OF THE METHOD

S. No.	Name of the Drug	%RSD of peak area						
		Flow variation (ml/min)			pH Variation		Altered %B	
		Actual	0.9	1.1	3.8	4.2	8%	12%
1	Paracetamol	0.148	0.318	0.394	0.148	0.155	0.287	0.199
2	Guaiphenesin	0.70	0.346	0.392	0.63	0.25	0.387	0.21
3	Phenylephrine HCl	0.82	0.297	0.313	0.371	0.328	0.290	0.27
4	Chlorpheniramine maleate	0.58	0.97	0.864	0.357	0.346	0.38	0.468
5	Bromhexine HCl	0.45	0.94	0.724	0.123	0.537	0.46	0.098

TABLE 4: ASSAY OF TABLET FORMULATION

Drug	Labelled amount (mg)	Amount found (mg)	% Amount found
Paracetamol	325	327.9	100.9
Guaiphenesin	100	94.4	94.4
Phenylephrine HCl	5	4.925	98.5
Chlorpheniramine maleate	2	1.986	99.3
Bromhexine HCl	8	8.008	100.1

CONCLUSION: It can be concluded that a simple, accurate, precise, and robust method was developed for the simultaneous estimation of paracetamol, guaiphenesin, phenylephrine HCl, chlorpheniramine maleate and bromhexine HCl in a tablet dosage form. The validation parameters were well within the acceptable limits and thus the proposed method can be used for the routine quality control tests for the above drug combination in the tablet dosage form.

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