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Original Article

STABILITY INDICATING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS DETERMINATION OF PHENYLEPHRINE HYDROCHLORIDE, CHLORPHENIRAMINE MALEATE, PARACETAMOL, GUAIPHENESIN AND BROMHEXINE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

Objective: The objective of the present study is to develop simple, rapid, sensitive, accurate and economic stability-indicating ultra-performance liquid chromatographic (UPLC) method for the simultaneous quantification of phenylephrine hydrochloride, chlorpheniramine maleate, paracetamol, guaiphenesin and bromhexine hydrochloride in bulk and tablet dosage form.

Methods: The separation of drugs in the chromatographic column was accomplished on Hibar C18 (100 mm x 2.1 mm, 1.6 µm) column at a detection wavelength of 220 nm. The mobile phase was a combination of sodium phosphate monobasic monohydrate buffer (p^H was adjusted to 3.5 with orthophosphoric acid) and acetonitrile in the ratio of 70:30 % v/v which was pumped at a flow rate of 0.3 ml/min. The column temperature was maintained at 30 °C and the injection volume was 0.3 µl. Forced degradation studies of drugs were carried out using acid, base, peroxide, light and heat.

Results: All the five drugs have been eluted within 3 min. The retention times were found to be 0.834 min, 1.199 min, 1.600 min, 1.979 min and 2.525 min for phenylephrine, chlorpheniramine maleate, paracetamol, guaiphenesin and bromhexine respectively. The correlation coefficient (r^2) was found to be 0.999 for all the drugs. The recovery levels were found to be in the range of 98.06 % to 100.28 %. RSD values of drugs were found to be below 2 %. The results of limit of detection and quantitation specified the sensitivity of the developed method. Significant degradation of drugs as a result of stress studies was found in acid, base and peroxide, but they were slightly degraded in photolytic and thermal conditions. The method has effectively resolved the degraded products. All the validation parameters were found to be within the limits according to International Conference on Harmonization (ICH) guidelines.

Conclusion: A simple and rapid UPLC method was established for the determination of five drugs. Hence, the proposed method can be employed for the quality control of specified drugs in bulk and pharmaceutical formulation even in the presence of degradation products.

Keywords: Phenylephrine hydrochloride, Chlorpheniramine maleate, Paracetamol, Guaiphenesin, Bromhexine hydrochloride, Stability indicating UPLC method

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INTRODUCTION

Phenylephrine hydrochloride [fig. 1] is chemically 3-[(1R)-1-hydroxy-2-(methylamino) ethyl] phenol hydrochloride is a sympathomimetic amine that acts predominantly on α-adrenergic receptors and it is mainly used to treat nasal congestion.

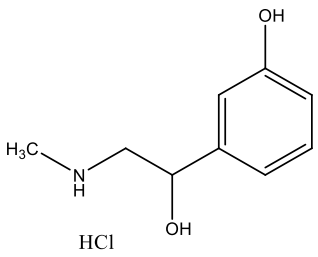

Fig. 1: Structure of phenylephrine hydrochloride

Chlorpheniramine maleate [fig. 2] is chemically but-2-enedioic acid; 3-(4-chlorophenyl)-N, N-dimethyl-3-pyridine-2-ylpropan-1-amine is a histamine H1-receptor antagonist used in allergic reactions, hay fever, rhinitis, urticaria and asthma.

Paracetamol [fig. 3] is chemically N-(4-hydroxyphenyl) acetamide is a p-aminophenol derivative is commonly used for its analgesic and antipyretic effects.

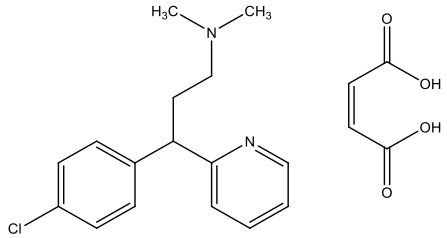

Fig. 2: Structure of chlorpheniramine maleate

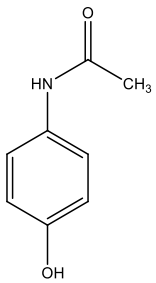

Fig. 3: Structure of paracetamol

Guaiphenesin [fig. 4] is chemically 3-(2-methoxyphenyl) propane-1,2-diol is an expectorant and also has some muscle relaxing action.

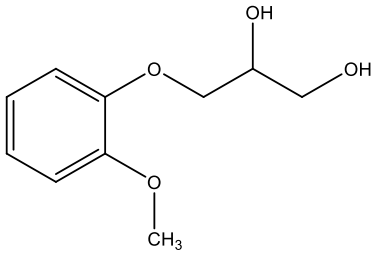

Fig. 4: Structure of guaiphenesin

Bromhexine hydrochloride [fig. 5] is chemically 2,4-dibromo-6-[[cyclohexyl(methyl) amino]methyl] aniline; hydrochloride act as an expectorant or mucolytic agent.

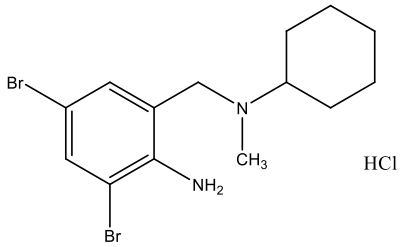

Fig. 5: Structure of bromhexine hydrochloride

The combination of phenylephrine hydrochloride, chlorpheniramine maleate, paracetamol, guaiphenesin and bromhexine hydrochloride is available as tablets in the market till date which is used to treat cough, fever, cephalalgia, cold, catarrh, flu, hay fever and allergies due to food. Extensive literature survey revealed various analytical techniques for the estimation of mentioned drugs individually [1] and with other combinations [2-14]. An RP-HPLC method has been reported for the simultaneous estimation of phenylephrine hydrochloride, chlorpheniramine maleate, paracetamol, guaiphenesin and bromhexine hydrochloride in bulk and tablet dosage form [15]. In this method the retention time of analytes was high with a run time of 35 min thereby increasing the solvent consumption and leading to longer analysis time. The mode of separation was gradient and has used two mobile phases one of which was a combination of potassium dihydrogen phosphate buffer with octane-1-sulphonic acid reagent. Usage of buffers reduces the life of the column and preparation of mobile phases consumes more time, thereby increasing the analysis time. Hence, a rapid method is needed for the estimation of these drugs which can be attained by using UPLC technique with high sensitivity and less time of analysis. In addition to this, no UPLC method has been reported for the assay of specified drugs either individually or simultaneously in bulk and pharmaceutical formulation. Therefore, it was thought appropriate to develop stability-indicating ultra-performance liquid chromatographic procedure that serves as a rapid, simple, accurate, and sensitive method for the simultaneous estimation of phenylephrine hydrochloride, chlorpheniramine maleate, paracetamol, guaiphenesin and bromhexine hydrochloride in bulk and tablet dosage form. Further, it was aimed to validate the developed method as per validation of analytical procedures i.e. ICH guidelines: Q2[R1] Validation of Analytical Procedures: Text and Methodology.

MATERIALS AND METHODS

Chemicals and reagents

The commercially available formulation (Kuff Q NF tablets) containing phenylephrine hydrochloride 10 mg, chlorpheniramine maleate 2 mg, paracetamol 325 mg, guaiphenesin 100 mg and bromhexine hydrochloride 8 mg, manufactured by Intas Pharmaceuticals Limited were purchased from local pharmacy. Standard drugs were obtained from Spectrum Pharma Research Solutions, Hyderabad. Orthophosphoric acid, acetonitrile of analytical reagent grade and HPLC grade water was purchased from Rankem. Sodium phosphate monobasic monohydrate of AR grade was purchased from Sisco research laboratories.

Instrument and equipment

Acquity UPLC H-Class system (Waters) consists of a binary solvent manager, autosampler with UV detector. The output signal was monitored and processed using empower 2 software. Sonicator (LMUC-2, Labman scientific), p^H meter (AD 1020, ADWA) and analytical balance (ER-200A, AFCOSET) were used.

Chromatographic conditions

The chromatographic column used was Hibar C18 (100 mm x 4.6 mm x 1.8 µm). The separation was achieved on isocratic mode. The mobile phase consists of sodium phosphate monobasic monohydrate buffer whose p^H was adjusted to 3.5 with orthophosphoric acid and acetonitrile in the ratio of 70:30 %v/v was pumped at a flow of 0.3 ml/min. The column temperature was maintained at 30 °C with an injection volume of 0.3 µl and the detector was monitored at 220 nm with a total run time of 5 min.

Preparation of buffer solution

The buffer solution of 50 mmol sodium phosphate monobasic monohydrate was prepared by weighing 6.89 g of sodium phosphate monobasic monohydrate and transferred into 1000 ml volumetric flask. About 950 ml of milli-Q water was added, degassed and sonicated for 20 min and finally, the volume was made up with water then the p^H was adjusted to 3.5 with orthophosphoric acid.

Preparation of diluent

The diluent was prepared by taking water and acetonitrile in the ratio 50:50.

Preparation of standard solution

The standard stock solution was prepared by weighing 2.5 mg of phenylephrine hydrochloride, 0.5 mg of chlorpheniramine maleate, 81.25 mg paracetamol, 25 mg of guaiphenesin and 2 mg of bromhexine hydrochloride and transferred to 25 ml volumetric flask. To this 10 ml of diluent was added, sonicated for 25 min and the volume was made up with diluent. From the above standard stock solution 1 ml was transferred into 10 ml volumetric flask and made up to the volume with diluent so as to get final concentration of 10 µg/ml of phenylephrine hydrochloride, 2 µg/ml of chlorpheniramine maleate, 325 µg/ml of paracetamol, 100 µg/ml of guaiphenesin and 8 µg/ml of bromhexine hydrochloride.

Preparation of sample solution

From the blend of 20 tablets, the weight of powder equivalent to one tablet was transferred into a 100 ml volumetric flask, to this 50 ml of diluent was added and sonicated for 25 min; further the volume was made up with diluent and filtered. From the filtered solution 1 ml was transferred into 10 ml volumetric flask and made up to the volume with diluent.

Preparation of samples for forced degradation

During stress studies, degradation in acidic medium was carried out by taking 1 ml of standard stock solution, to this 1 ml of 1 N HCl was added and refluxed for 30 min at 60 °C. The resultant solution was neutralized by adding 1 ml of 1 N NaOH solution and diluted to 10 ml with diluent. Forced degradation in the basic medium was performed by taking 1 ml of standard stock solution, to this 1 ml of 1 N NaOH was added and refluxed for 30 min at 60 °C. The resultant solution was neutralized using 1 N HCl solution and diluted to 10 ml with diluent. For oxidative degradation, 1 ml of 10 % hydrogen peroxide was added to 1 ml of standard stock solution. The solution was kept for 30 min at 60 °C. The resultant solution was diluted to 10 ml with diluent. For thermal degradation, the standard stock solution was taken in a beaker and placed in oven at 60 °C for 6 h and 1 ml from the resultant solution was diluted to 10 ml with diluent. For photostability studies, a volume of 5 ml from stock

solution was taken in a beaker and kept under UV light in UV chamber for 3 d. From the resultant solution 1 ml was taken and diluted up to 10 ml with diluent.

Method validation

The developed method was validated for specificity, accuracy, precision, linearity, limit of detection (LOD), the limit of quantitation (LOQ) and robustness according to ICH guidelines [16].

Specificity

Specificity of the method was performed to analyse the interference of solvent and impurities with the analyte peaks. To determine this, blank and generated impurities by forced degradation were injected into the chromatographic system and observed for any interfering peaks at the retention time of analytes peak.

Accuracy

Accuracy was performed to ensure the reliability and accuracy of the method by recovery studies which was carried out by standard addition method. The known quantities of pure drugs were added to the pre-analyzed sample at three concentration levels 50 %, 100 %, 150 % in three replicates and contents were reanalyzed by the proposed method and the percent recovery were calculated.

Precision

Precision was performed to check the repeatability of the developed method. The standard solution was prepared at working concentration and injected six times into the chromatographic system. The percentage relative standard deviation was calculated.

Linearity

The linearity of the method was determined by preparing the solutions at five different concentration levels with standard drugs, which was from 25 % to 150 % of target assay concentration. The calibration curves were plotted between peak area and concentration. The least square linear regression were carried and correlation coefficient (r^2) values were observed to check the linearity.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD is the minimum concentration at which analyte can be detected and calculated from the linearity curve by applying the formula:

$$LOD = 3.3 \times SD/S$$

LOQ is the lowest concentration of the analyte that can be estimated quantitatively by applying the formula:

$$LOQ = 10 \times SD/S$$

Where SD=Standard deviation of y-intercepts; S=Slope of the calibration curve.

Robustness

Robustness of the developed method was established by deliberately changing the method parameters such as flow rate (±0.1 ml/min), column temperature (±5˚C) and mobile phase ratio (±5 %) to evaluate the impact of these conditions on the method and the system suitability parameters were evaluated for the studies.

Forced degradation studies

Forced degradation or stress testing was undertaken to demonstrate specificity during the development of stability-indicating assay method. It is the specific one, which evaluates the drug in the presence of its degradation products. The percentage of drug degraded under stressed conditions was calculated and also observed for the interference of degraded products peak at the retention times of analyte peaks.

RESULTS AND DISCUSSION

Method development

After many trials made by altering the chromatographic conditions like mobile phase, p^H of the selected mobile phases, detection wavelengths and injection volume to resolve all the five drugs, the obtained peaks were sharp with good resolution using a mobile phase of sodium phosphate monobasic monohydrate buffer, p^H adjusted to 3.5 with orthophosphoric acid and acetonitrile in the ratio 70:30 %v/v which was pumped at a flow rate of 0.3 ml/min and at a detection wavelength 220 nm. The corresponding optimized chromatogram obtained for a standard solution of analytes was shown [fig. 6]. The system suitability parameters such as theoretical plates, resolution, tailing factor were determined and presented in the table 1 and the parameters were found to be within limits. All the drugs were eluted within 3 min with isocratic mode which specify an absolute rapidity of the method when compared to the reported RP-HPLC method where the elution of drugs took up to 25 min on gradient mode [15].

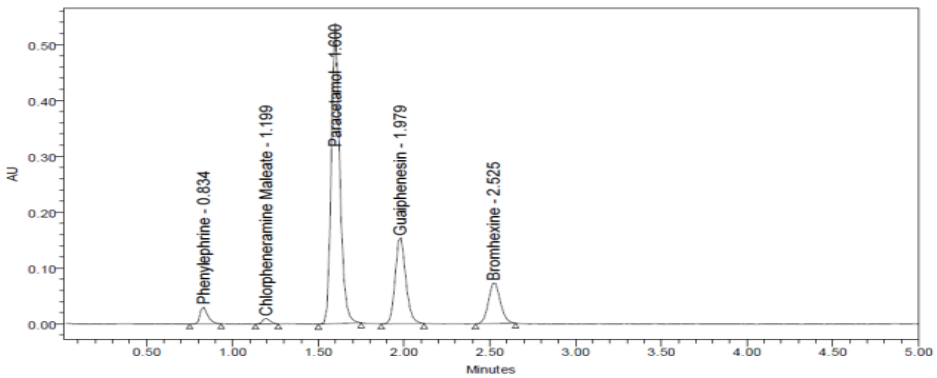

Fig. 6: Typical chromatogram of standards

Table 1: Results of system suitability studies

Drug name	Retention time* (min)	Resolution*	Plate count*	Tailing factor*	Peak area*
Phenylephrine	0.834±0.03	-	6576.1±173.7	1.3±0.01	96250±544.5
Chlorpheniramine maleate	1.199±0.10	4.2±0.2	3164.8±451.6	1.1±0.14	31280±220.7
Paracetamol	1.600±0.10	4.4±0.1	4795.6±81.1	1.2±0.03	1938223±15007.9
Guaiphenesin	1.979±0.01	3.6±0.1	5048.3±175.2	1.1±0.01	683832±1809.6
Bromhexine	2.525±0.02	4.5±0.1	6379.3±96.7	1.1±0.01	387727±2718.4

*Each value is represented as a mean±SD of 6 observations

Method validation

Specificity

After the injection of blank and degradation samples into the chromatographic system, no interfering peaks have been observed at the retention times of analyte peaks. Hence, the method was found to be specific for the estimation of drugs.

Accuracy

The results of accuracy were presented in table 2. Recovery studies were carried out at three concentration levels for three times each. From the accuracy data, it was found that the recoveries were within the specified range. The results of accuracy decisively specify that the recovery values were within the acceptance range of 98-102 %. Hence, the developed method was accurate for the determination of the above mentioned drugs.

Precision

From the results of precision studies which were tabulated in table 3, the RSD values for peak areas were found to be less than 2 %. This surely assures that the developed method was precise and repeatable.

Table 2: Results of accuracy studies

Name of the analyte	Accuracy level (%)	*Concentration spiked (µg/ml)	* Concentration found (µg/ml)	*Mean % recovery
Phenylephrine	50	5	4.94	98.92
	100	10	9.95	99.55
	150	15	14.97	100.28
Chlorpheniramine maleate	50	1	0.99	99.16
	100	2	2.01	100.03
	150	3	2.96	98.72
Paracetamol	50	162.5	161.35	99.29
	100	325	322.49	99.22
	150	487.5	487.18	99.93
Guaiphenesin	50	50	49.62	99.25
	100	100	99.76	99.76
	150	150	150.17	100.11
Bromhexine	50	4	3.97	99.44
	100	8	7.94	99.34
	150	12	12.01	100.07

*Average of triplicate determinations, Acceptence criteria: % recovery must be 98%-102%

Table 3: Results of precision studies

Name of the analyte	RSD* (%)
Phenylephrine	0.2
Chlorpheniramine maleate	0.4
Paracetamol	0.6
Guaiphenesin	0.4
Bromhexine	0.5

*RSD values for six determinations, RSD: Relative Standard Deviation, Acceptance criteria: RSD =<2 %

Linearity and range

The results of linearity were given in table 4 and the representative calibration plots were shown [fig. 7-11]. The data was treated by least-square linear regression analysis and the correlation coefficients for all the drugs was found to be 0.999 over the specified range of 25-150 % of target assay concentrations. The results indicate linear and directly proportional relationship between the concentration of specified range and response which meet the method validation acceptance criteria (r^2 must be 0.990-1) and hence the method was said to be linear for the specified concentration range.

LOD and LOQ

By applying the formula method, the LOD values were found to be 0.06 µg/ml for phenylephrine, 0.03 µg/ml for chlorpheniramine maleate, 1.06 µg/ml for paracetamol, 0.89 µg/ml for guaiphenesin and 0.09 µg/ml for bromhexine. The LOQ values were found to be 0.17 µg/ml for phenylephrine, 0.08 µg/ml for chlorpheniramine maleate, 3.22 µg/ml for paracetamol, 2.71 µg/ml for guaiphenesin and 0.28 µg/ml for bromhexine. The results of LOD and LOQ perfectly indicate the sensitivity of the developed UPLC method.

Robustness

The degraded sample solutions were prepared as per the test method and injected at different variable conditions, system suitability parameters were assessed. The results of robustness was presented in table 5 and 6, it was found that the parameters such as tailing factor, plate count were within the limits even after small but deliberate alterations. From the results, it was concluded that even small changes that have made in the chromatographic conditions did not affect significantly on system suitability parameters and found to be within limits. Hence, the developed method was robust.

Table 4: Results of linearity

Name of the analyte	Linearity range (µg/ml)	Correlation coefficient (r^2)
Phenylephrine	2.5-15	0.999
Chlorpheniramine maleate	0.5-3	0.999
Paracetamol	81.25-487.5	0.999
Guaiphenesin	25-150	0.999
Bromhexine	2-12	0.999

Acceptance criteria: Correlation coefficient (r^2) must be from 0.999-1

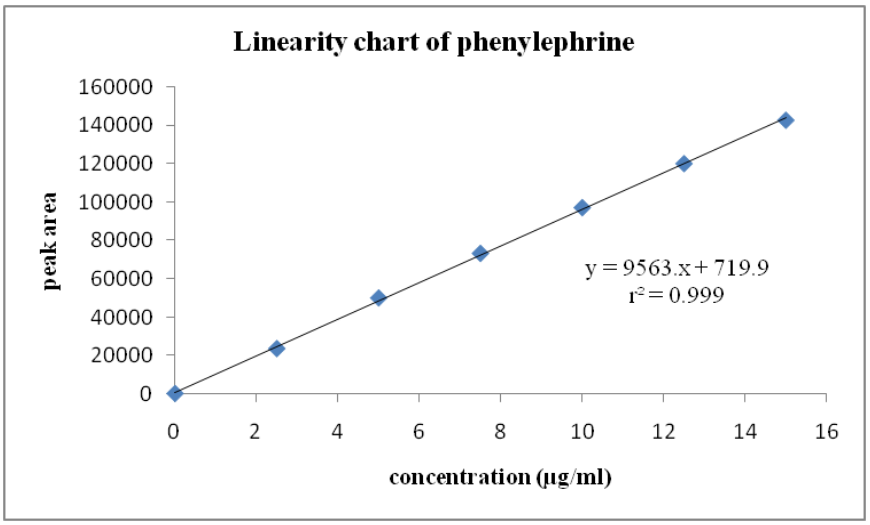

Fig. 7: Calibration plot of phenylephrine

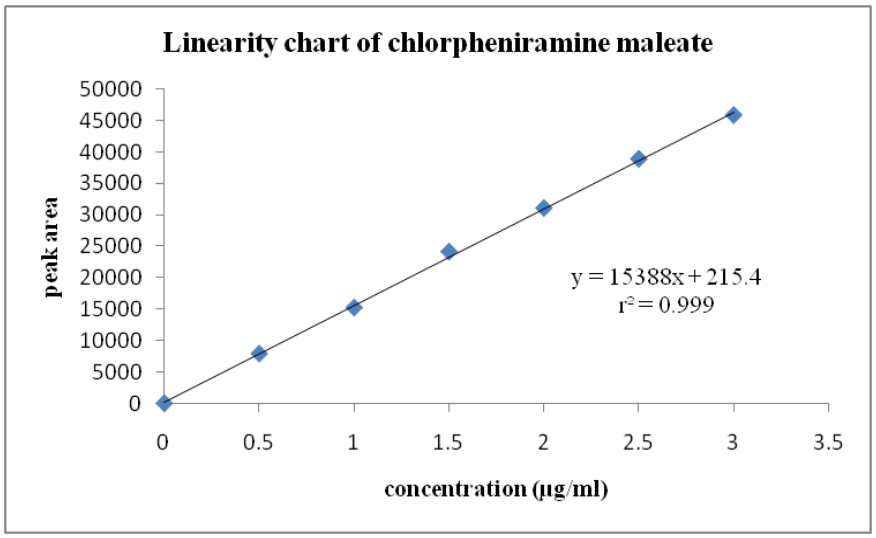

Fig. 8: Calibration plot of chlorpheniramine maleate

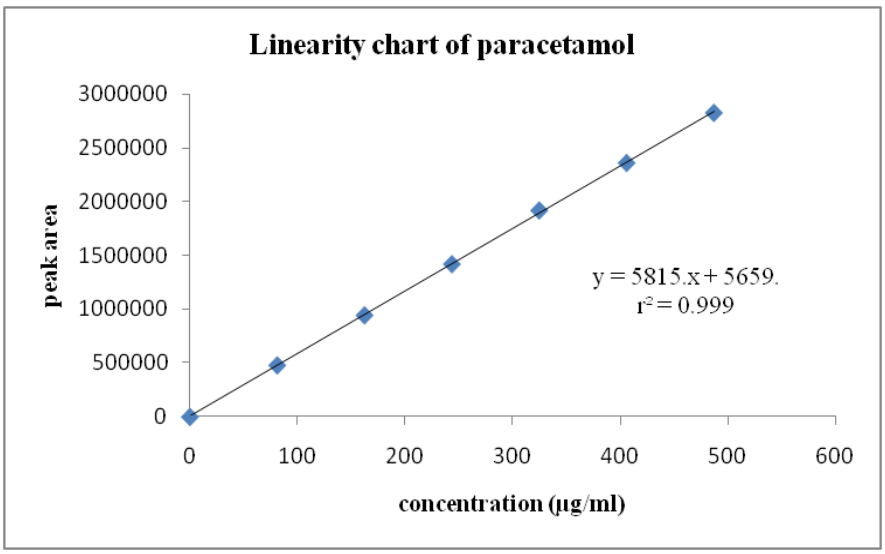

Fig. 9: Calibration plot of paracetamol

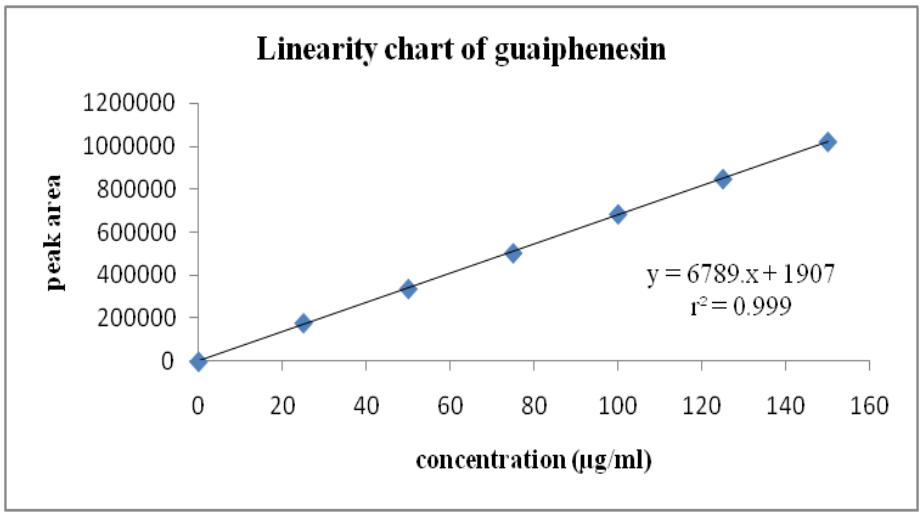

Fig. 10: Calibration plot of guaiphenesin

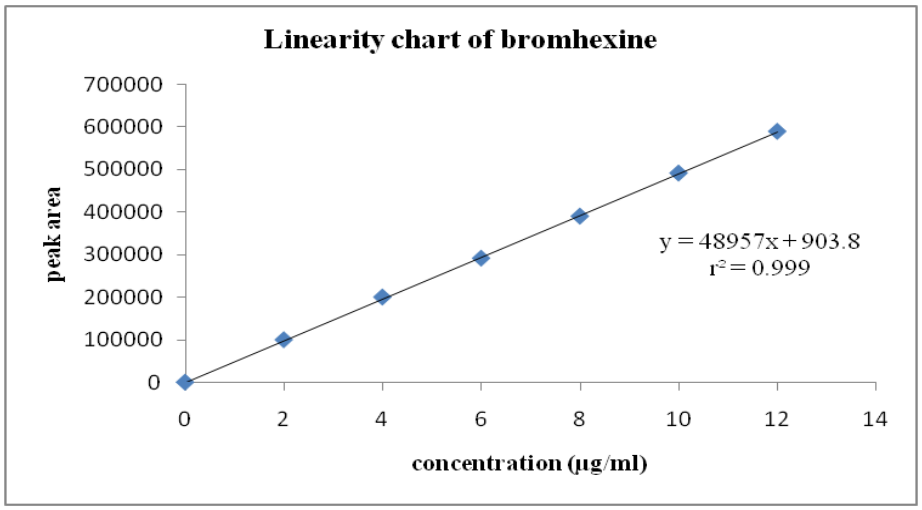

Fig. 11: Calibration plot of bromhexine

Table 5: Results of robustness for phenylephrine and chlorpheniramine maleate

Parameter	Phenylephrine		Chlorpheniramine maleate	
	Tailing*	Plate count#	Tailing*	Plate count#
Less flow rate (0.2 ml/min)	1.42	6589	1.08	3195
More flow rate (0.4 ml/min)	1.38	6482	1.11	2451
Less column temperature (25 ° C)	1.41	6465	1.22	3077
More column temperature (35 ° C)	1.40	6781	1.05	2607
Less organic phase (75:25)	1.32	6467	1.53	1681
More organic phase (65:35)	1.17	6752	1.12	2187

Acceptance criteria: *Tailing: <2, #Plate count: >2000

Table 6: Results of robustness for paracetamol, guaphenesin and bromhexine

Parameter	Paracetamol		Guaiphenesin		Bromhexine	
	Tailing*	Plate count#	Tailing*	Plate count#	Tailing*	Plate count#
Less flow rate (0.2 ml/min	1.21	5195	1.16	5535	1.12	6676
More flow rate (0.4 ml/min)	1.19	4695	1.14	5360	1.14	6182
Less column temperature (25 ° C)	1.21	4880	1.14	5285	1.10	6340
More column temperature (35 ° C)	1.19	4632	1.09	5213	1.07	6765
Less organic phase (75:25)	1.12	5032	1.10	5446	1.07	7070
More organic phase (65:35)	1.22	5129	1.07	5346	1.12	6554

Acceptance criteria: *Tailing: <2, #Plate count: >2000

Forced degradation studies

Standard and degraded samples were injected into the system with a run time of 10 min and the results were given in table 7. The degradation assay chromatograms were shown [fig. 12-16]. The percentage of drug degraded in solution was calculated. The degradation products produced as a result of stress studies did not interfere with the detection of analytes. Significant degradation was found in the presence of acid, base and peroxide. It was found that the drugs were slightly degraded in photolytic and thermal conditions. Therefore, the assay was considered as stability-indicating.

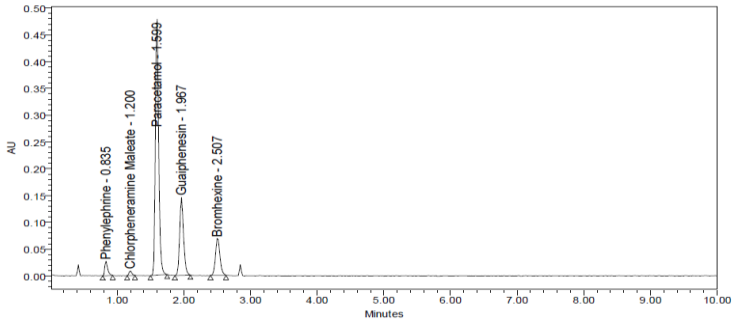

Fig. 12: Chromatogram of acid degradation

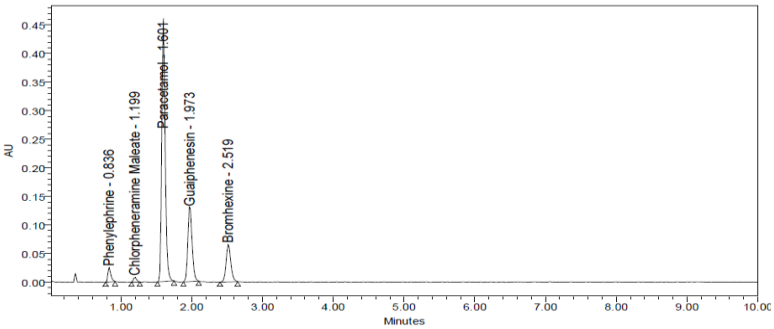

Fig. 13: Chromatogram of base degradation

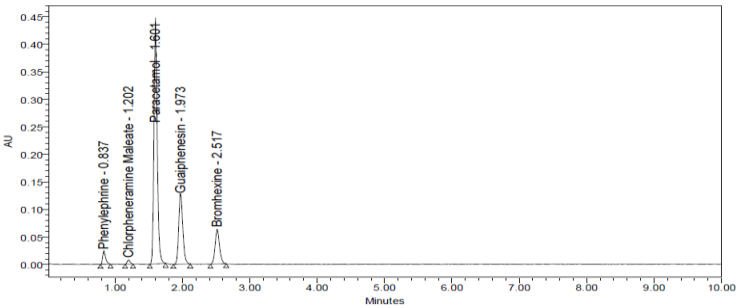

Fig. 14: Chromatogram of peroxide degradation

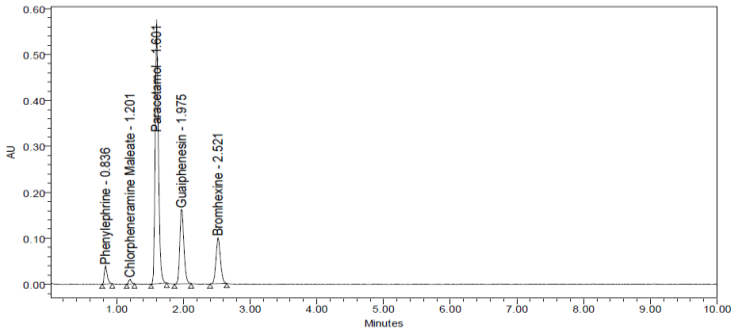

Fig. 15: Chromatogram of thermal degradation

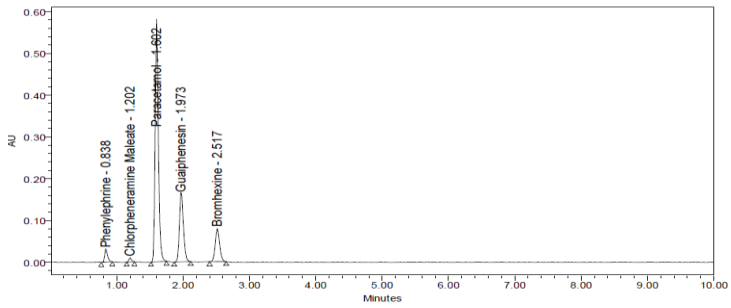

Fig. 16: Chromatogram of photodegradation

Table 7: Results of forced degradation studies

Stress conditions	Phenylephrine	Chlorpheniramine maleate	Paracetamol	Guaiphenesin	Bromhexine
	% Degraded				
Acid	4.60	5.50	4.56	5.09	5.85
Base	3.91	4.72	3.73	4.27	4.99
Peroxide	3.64	3.82	3.06	3.78	3.44
Thermal	2.82	2.36	2.23	2.36	2.66
Photolysis	1.81	1.91	1.80	1.91	1.33

Assay of marketed formulation

The applicability of the developed method was verified by assaying the marketed formulation. Kuff Q NF tablets were analyzed by standard comparison method. The prepared standard and sample solutions were injected into the chromatographic system and the amount of drug present in the formulation was calculated and the results were tabulated in table 8. The results complied with the label claim and specify that the developed method was successfully applied for quality control of formulation.

Table 8: Results of assay of formulation

S. No.	Phenylephrine	Chlorpheniramine maleate	Paracetamol	Guaiphenesin	Bromhexine
% Assay					
1	99.86	99.83	99.63	99.93	99.22
2	99.90	99.66	99.32	99.25	100.59
3	99.95	99.45	99.05	99.87	99.61
4	99.85	99.67	99.86	99.10	99.49
5	99.86	100.53	100.87	99.06	99.91
6	100.48	99.84	99.58	99.38	100.35
Mean	99.98	99.83	99.72	99.43	99.86

Acceptance criteria: % Assay must be in the range of 98-102 %

CONCLUSION

The developed isocratic, reverse phase UPLC method was found to be rapid, simple, accurate, precise and stability-indicating for the simultaneous estimation of phenylephrine hydrochloride, chlorpheniramine maleate, paracetamol, guaiphenesin and bromhexine hydrochloride in bulk and pharmaceutical formulation. The method can be used to estimate the drugs either individually or simultaneously in bulk and pharmaceutical formulation. This method has effectively resolved all the drugs and their degradation products. Thus, it was found to be more specific and selective for the estimation of above-specified drugs. The flow of mobile phase was set at very low rate, and has very less elution time and hence the method has advantages of less solvent consumption and faster analysis. Therefore, the developed method can be considered as economic for the estimation of five drugs simultaneously. Finally, it was concluded that the developed analytical method can be employed for the routine quality control of drugs in bulk and pharmaceutical formulation even in the presence of degraded impurities.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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