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RESEARCH ARTICLE

RP-HPLC METHOD FOR DETERMINING THE LEVELS OF GUAIPHENESIN AND PHENYLEPHRINE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, precise and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of guaiphenesin and phenylephrine in their tablet dosage form. The chromatographic conditions were standardized using an Inertsil column,C18 (150x4.6 ID) 5µm with UV detection at 277nm , and the mobile phase consisted of sodium dihydrogen phosphate buffer: Acetonitrile (30:70).The retention times of guaiphenesin and phenylephrine were About 3.497 min for Guaifenesin and 2.353min for Phenylephrine, respectively. The calibration curves were linear with correlation coefficients of 0.9987, 0.9988, 0.9981 and 0.9981 over a concentration range of 4.0–24.0 µg/ml for guaiphenesin, 5.0–30.0 µg/ml for phenylephrine, respectively. The proposed method has been validated according to the ICH guidelines and was successfully applied to estimate the levels of two drugs in a combined formulation with good accuracy and precision.

Keywords: RP-HPLC; Guaifenesin; Phenylephrine; ICH guidelines.

INTRODUCTION

Combinations of decongestant and antihistamine preparations are widely used for a cough and cold treatments. Guaifenesin, chemically named 3-(2-methoxyphenoxy)propane-1,2-diol (Fig. 1), is an expectorant agent used in the treatment of respiratory disorders associated with viscid or excessive mucus[1,2].Guaifenesin is thought to act as an expectorant by increasing the volume and reducing the viscosity of secretions in the trachea and bronchi. It has been said to aid in the flow of respiratory tract secretions, allowing ciliary movement to carry the loosened secretions upward toward the pharynx. Thus, it may increase the efficiency of the cough reflex and facilitate removal of the secretions. Guaifenesin has muscle relaxant and anticonvulsant Properties and may be acting as an NMDA receptor antagonist.

The literature survey reveals several HPLCmethods that were reported for their simultaneous determination along with several other active ingredients,which exist as various combinations in a cough–cold mixtures [3]. These methods include liquid chromatography [4], liquid gas chromatography [5], and gas chromatography with mass detection [6], combined formulations using HPLC [7–9] and UV spectrophotometry [10–13].

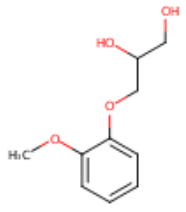


Figure: 1

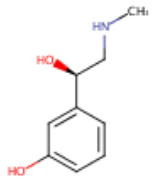


Figure: 2

Phenylephrine 3-[(1R)-1-hydroxy-2-(methyl amino) ethyl] phenol is a selective α_1-adrenergic receptor agonist of the phenethylamine class used primarily as a decongestant, as an agent to dilate the pupil, and to increase blood pressure (Fig. 2) is a powerful and is widely used for symptomatic relief of the common cold and allergic rhinitis [14]. A literature survey shows that several HPLC methods have been reported for Phenylephrine alone and in combination in pharmaceuticals, such as liquid chromatographic [15–17], HPTLC [18], spectrophotometry [19] and micellar electrokinetic chromatography [20]. The chemical structures of DEX are shown in Fig. 1, 2. The combination of these drugs is used as an antitussive and mucolytic in bronchitis and chronic pulmonary conditions. Several analytical techniques have been reported in the literature, most commonly liquid chromatography [23, 24], first and second derivative UV spectrophotometric techniques.

There is no method reported for the simultaneous estimation of guaiphenesin and phenylephrine in a combined dosage form. Therefore, we communicate here a rapid and cost-effective quality-control tool and a reliable method for the simultaneous assay of mixtures of these two drugs. The method should have sufficient accuracy and precision and permit a simple and time-saving assay for a mixture of guaiphenesin and phenylephrine.

MATERIALS AND METHODS

Apparatus

To develop a suitable LC method for the analysis of guaiphenesin and phenylephrine in their combined dosage form, different mobile phases were tried. The chromatographic system consists of a pump (Shimadzu LC 10AT VP) with a universal loop injector (Rheodyne 7725i) with an injection capacity of 20µL. The detector consists of a photodiode array detector (PDA), SPD-10 AVP UV-Visible detector and an Inertsil C18(150x4.6 ID) 5µm column. The equipment was controlled by a PC workstation equipped with CLASS M 10-VP software (Shimadzu, Kyoto, Japan). A UV/Visible double beam spectrophotometer (Shimadzu Model 1700) was employed with a spectral bandwidth of 1 nm and a wavelength accuracy of 0.3 nm (with automatic wavelength correction using a pair of 1 cm matched quartz cells).

Reagents and materials

Pure drug samples of guaiphenesin and phenylephrine were generously obtained as a gift from TABLIKE and SCHON Pharmaceutical (Indore, India). The tablet dose form, Gilphex TR (Label claim: 388 mg, 10.0 mg phenylephrine), was procured from the local market (manufactured by Gil Pharmaceuticals Pvt. Ltd., Roorkee, India). HPLC grade methanol and acetonitrile were obtained from Merck (Mumbai, India).

Chromatography conditions

The solubility of the two drugs indicated that the reverse phase chromatographic method would be the best option for the simultaneous estimation of guaiphenesin and phenylephrine. The mobile phase consists of a sodium dihydrogen phosphate buffer: Acetonitrile (30:70) adjusted to pH 5.5. The mobile phase and working solutions were filtered through a 0.2 µm nylon filter and degassed using a sonicator before use. To determine the

appropriate wavelength for the simultaneous determination of guaiphenesin and phenylephrine solutions of these compounds were scanned on a UV–vis spectrophotometer in the range 200–400 nm. The suitable wavelength to monitor these drugs was chosen from the overlaid UV spectra (277 nm).

Preparation of standard stock solutions

Standard stock solutions of guaiphenesin and phenylephrine were prepared separately by accurately weighing 10.0 mg of each of guaiphenesin and phenylephrine (reference standard), transferring it to a 100 ml volumetric flask and dissolving it in 20.0 ml of HPLC grade Acetonitrile. The solutions were sonicated in a bath sonicator for 10 min to ensure complete solubilization. After sonication, the volume was brought to 100 ml with same HPLC grade Acetonitrile at a final concentration of 0.1 mg/ml (100µg/ml) of each reference standard. A combined standard solution containing guaiphenesin and phenylephrine was prepared by adding 160 mg,40 mg, of each reference standard, respectively, transferring it to a 1000 ml volumetric flask, and adding 200 ml of HPLC grade methanol. The solution was sonicated in a bath sonicator for 10 min to ensure complete solubilization. After sonication, the volume was brought to 1000 ml with same diluent, to result in final concentrations of 160µg/ml , 40µg/ml of guaiphenesin and phenylephrine respectively.

Estimation from pharmaceutical dosage form

Twenty tablets of Gilphex TR were weighed to calculate the average weight of one tablet. They were homogenized to a fine powder, transferred to a 1000.0 ml volumetric flask, dissolved in 200.0 ml of diluent (HPLC grade Acetonitrile) and sonicated in a bath sonicator for 20.0 min to ensure complete solubilization. After sonication, the supernatant was transferred to a 1000.0 ml volumetric flask by filtering through Whatman #41 filter paper. The residue was washed three times with 10.0 ml of Acetonitrile and the combined filtrate was brought to1000.0 ml with the same diluent to achieve final concentrations of 160.0µg/ml, 40.0µg/ml of guaiphenesin and phenylephrine, respectively.

A constant volume of the sample solution was injected six times under the conditions described above.The chromatogram showed that the retention times of guaiphenesin and phenylephrine were 3.497 min for Guaifenesin and 2.353min for Phenylephrine respectively, with a resolution of 3.15 between guaiphenesin and phenylephrine. The capacity factor, tailing factor, theoretical plate number results are reported in Table 1. The total run time was 20 min. The peak areas were measured at 277 nm for guaiphenesin and phenylephrine respectively, and their concentrations in the samples were determined using a multi-level calibration curve and the linear regression equation using the same conditions on the same HPLC system.

Table.1 : Data for the evaluation of the system suitability.

Property	Guaiphenesin	Phenylephrine
Rt	3.497	2.353
Tf	1.09	1.04
K	1.562	1.217
N	6359	1276
Rs	3.98	2.72

***Rt*, retention time; *Tf*, tailing factor; *K*_, capacity factor; *N*, number of theoretical plates; *Rs*, resolution.**

Preparation of solutions to determine linearity

From the standard stock solution 1, 100.0µg/ml of each guaiphenesin and phenylephrine, of the different working standards were prepared at the following concentrations to determine linearity: 4.0, 8.0, 12.0, 16.0, 20.0 and 24.0 µg/ml for guaiphenesin; 5.0, 10.0, 15.0, 20.0,25.0 and 30.0µg/ml for and phenylephrine; respectively. Six replicates of each different working standard were prepared for each drug. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Analytical method validation

The method was validated for analytical procedures according to ICH guidelines to determine the linearity, sensitivity, precision and accuracy for the analyte. A system suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability standard and one injection of a test standard were made. Regression characteristics, validation and system suitability parameters for guaiphenesin and phenylephrine in their pharmaceutical dosage form are shown in Table 3.

Linearity

The method was linear from 4.0µg/ml to 24.0µg/ml for guaiphenesin and, 5.0 to 30.0µg/ml for phenylephrine. The calibration curve was plotted using area vs. the concentration each compound and had an R^2value of 0.9980 or greater.

Accuracy

A recovery study for Gilphex TR was carried out per ICH guidelines [37], where a known concentration of these two standards solutions (equivalent to 80, 100, and 120% of total drug content) was added to a pre analysed solution of the tablet formulation and the percentage of recovery was calculated.

Precision

Intra and interday precision studies for Gilphex TR were calculated by assaying the sample solution (marketed formulation) on the same day and different days at different time intervals, respectively. The assay was performed with at least six replicates of the sample solution. An amount of the sample powder equivalent to 100% of the label claim of guaiphenesin and phenylephrine was accurately weighed and assayed. Method repeatability was achieved by repeating the same procedure six times on the same day for intra-day precision. The intermediate (interday) precision of the method was checked by performing the same procedure on different days under the same experimental conditions.

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

For LOD and LOQ, 10.0µg/ml of all four standard solutions were prepared from each of the 100.0µg/ml standard stock solutions: 0.2, 0.4, 0.6, 0.8, 1.0, 1.2µg/ml working dilutions for guaiphenesin and phenylephrine; 0.3, 0.5, 0.7, 0.9, 1.1 and 1.3 µg/ml. LOD and LOQ values were calculated to assess the detection limit of the method using the following equation, per ICH guidelines: where σ is the standard deviation of *y*-intercepts of regression lines and *S* is the slope of the calibration curve.

$$\text{LOD} = 3.3 \times \sigma/S,$$

$$\text{LOQ} = 10 \times \sigma/S$$

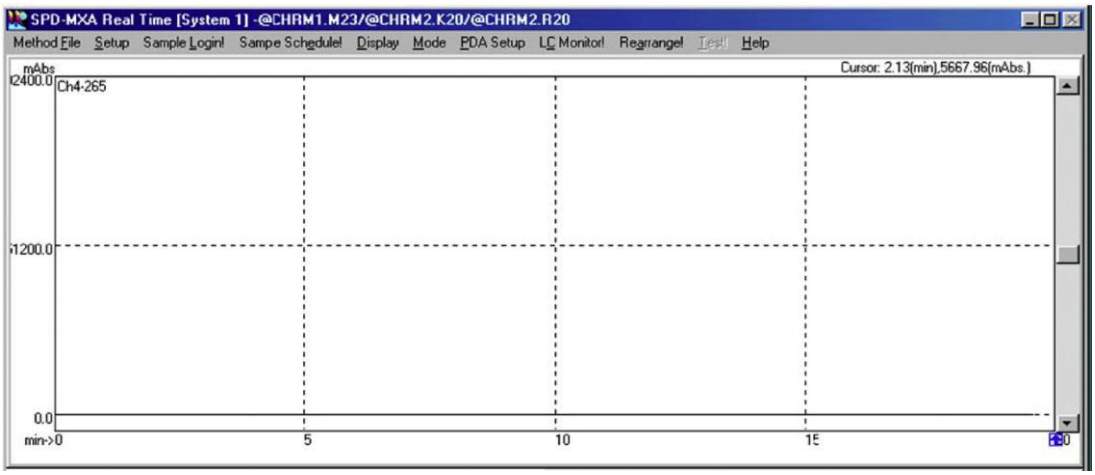


Fig.3: RP-HPLC chromatogram of placebo.

Selectivity and specificity

A combination of sodium dihydrogen phosphate buffer: Acetonitrile (30:70 v/v), pH 5.5 was used as a specific mobile phase, and 277.0 nm was selected as a specific analytical wavelength to simultaneously determine the levels of guaiphenesin and phenylephrine in a marketed formulation (Gilphex TR) using an HPLC method. Specificity was assessed by a qualitative comparison between chromatograms obtained from sample,standard, blank and placebo solutions. The diluent was injected as a blank. A placebo (Fig. 2) interference study was conducted by injecting a placebo solution prepared from the excipients most commonly used in pharmaceutical formulations, including starch, lactose monohydrate, and magnesium stearate.

RESULTS AND DISCUSSION

A new, rapid, sensitive and accurate RP-HPLC method was developed for the simultaneous estimation of guaiphenesin and phenylephrine in pharmaceutical formulations. After trying different columns, the final choice for the stationary phase that gave satisfactory resolution and runtime was the reverse phase Inertsil column,C18(150x4.6 ID) 5µm column. There were many mobile phases that were tested to resolve two chromatographic peaks, including methanol:water (80:20, v/v) and methanol: water (50:50, v/v), but the broadness of the peaks did not produce satisfactory results in these chromatograms. To improve the sharpness of the chromatographic peaks, we worked with slightly acidic acetonitrile and phosphate buffer. Finally, the mobile phase sodium dihydrogen phosphate buffer: Acetonitrile (30:70) v/v) (pH: 5.5) was found to be satisfactory as it gave two symmetric peaks for guaiphenesin and phenylephrine. The total run time was 20 min at a 1.0 ml min^{-1} rate and ambient temperature.The retention times of guaiphenesin and phenylephrine. were About 3.497 min for Guaifenesin and 2.353 min for Phenylephrine (Fig. 3), respectively. The best fit for the calibration curve (peak area vs. respective concentrations) could be achieved by separate linear regression equations, which were $y = 9040x + 9993$(Gua), $y = 9579x - 308$ (Phenyl). The proposed method was evaluated for formulations containing Guaifenesin and Phenylephrine. Four replicate determinations were performed using tablets and found 100.45%for Guaifenesin and 100.25% for Phenylephrine. Specificity was assessed by comparing the chromatogram of the tablet solution with the placebo solution and also with the chromatograms obtained from the standard drugs. The retention time of all four drugs was the same in the quaternary mixed standard solutions as well as the marketed formulation (Gilphex TR) solution, and there was also no interference from the excipients. This indicates the specificity of the method for quantitative estimation of Guaifenesin and Phenylephrine in a marketed formulation (Table 2).The recoveries of all of the components were between 99.0 and 102%. The LOD and LOQ values were 21.49 and 65.13 ng ml^{-1}, 29.17 and 88.39 ng ml−1, 11.65, respectively (Table 2). The excipients did not interfere with the peaks of interest. Hence, the proposed method is applicable for the routine simultaneous estimation of Guaifenesin and Phenylephrine in pharmaceutical dosage forms.

Table 2: Results from the assay of the marketed formulation.

Drug	Label claim (mg/tab) n = 6	Amount found (mg/tab)	Label claim (%)	S.D.	S.E	% COV
Gua	388	387.8965	100.45	0.5902	0.241	0.5875
Phenyl	10	9.5952	100.45	1.2891	0.5263	1.2858

Parameter	Gua	Phenyl
Linearity range	4–24	5–30
Slope	9040	9579
Interept (y)	9993	−308
R2	0.9987	0.9988
Accuracy (percentage of recovery)		
80%	99.67	100.052
100%	101.07	100.958
120%	100.15	101.042
Intraday precision		
%COV	0.1755–0.6333	0. 3050–0.6715
Interdays precision		
%COV	1018–0.2804	0.0675–0.1639
L.O.D. (ng ml−1)	21.49	29.17
L.O.Q. (ng ml−1)	65.13	88.39
Specificity/selectivity	No interference	
Robustness	Reliable results	
Ruggedness	Reproducible results	

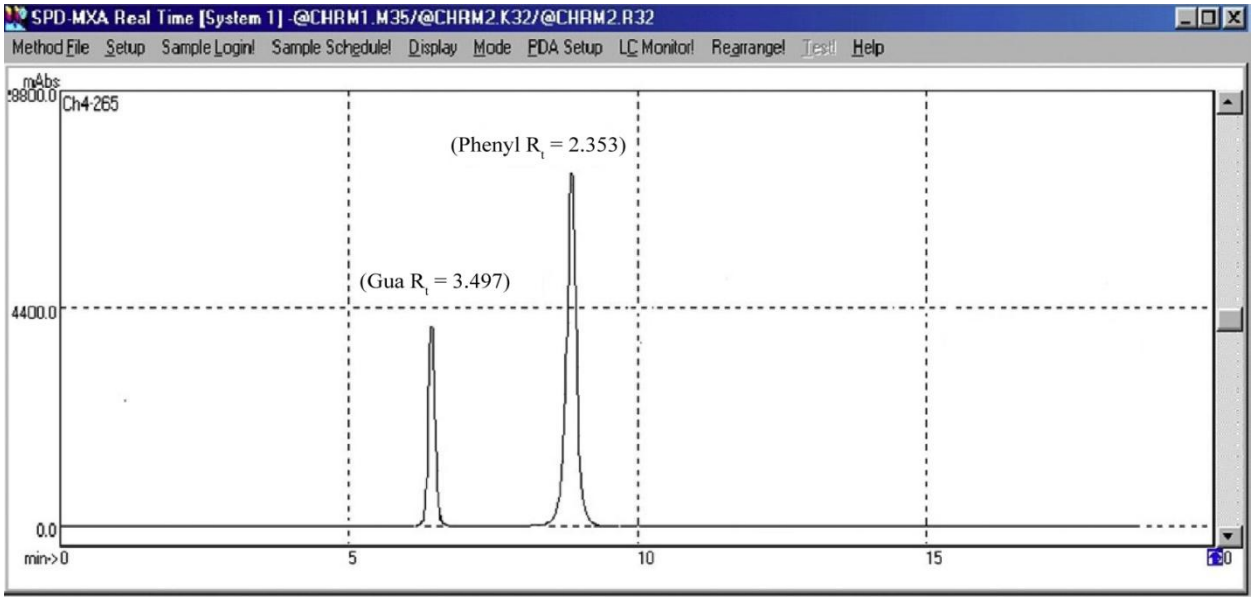
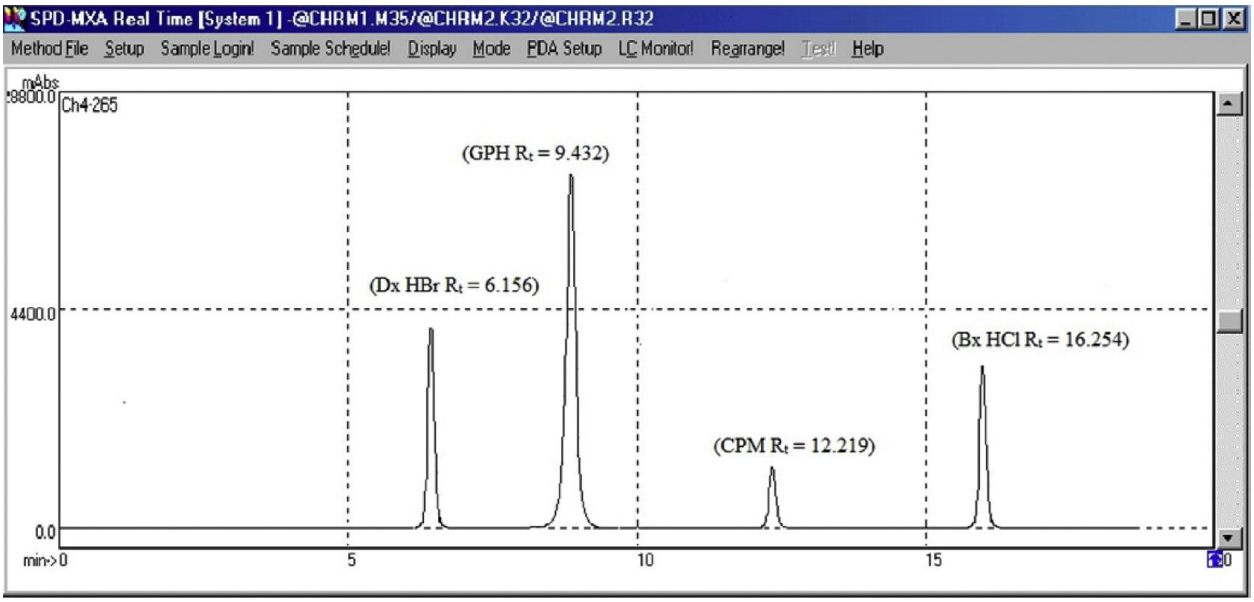


Fig. 4: HPLC chromatogram of marketed formulation.



CONCLUSIONS

In the present work, we successfully and simultaneously analyzed Guaifenesin and Phenylephrine in a marketed formulation (Gilphex TR) using an RP-HPLC method based on a literature survey. All of the critical steps for developing the method have been summarized and prioritized. To develop an effective RP-HPLC method, most of the effort should be spent in method development and optimization, as this emphasis will improve the final method performance. The method validation, however, should be treated as an exercise to summarize or document the overall method performance for its intended purpose. Thus, the present method is rapid, easy and accurate for the simultaneous estimation of Guaifenesin and Phenylephrine in a commercially available pharmaceutical formulation.

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