

A VALIDATED RP-HPLC METHOD FOR DETERMINATION OF GUAIFENESIN AND PSEUDOEPHEDRINE HYDROCHLORIDE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, rapid, precise, specific and cost effective reverse phase high performance liquid chromatography (RP-HPLC) method have been developed and subsequently validated for simultaneous estimation of Guaifenesin (GUA) and Pseudoephedrine hydrochloride (PSE) in pharmaceutical dosage forms. Chromatography is carried out isocratically at $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ on an ProntoSil C-18 column (4.6 x 250mm, 5 μ particle size) with a mobile phase composed of acetonitrile-methanol-phosphate buffer (pH-5.0) (72:8:20, v/v/v) at a flow rate of 1.2 mL/min. Detection was carried out using a PDA detector at 218 nm. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the International Conference on Harmonization guidelines. The retention times for GUA and PSE are 2.99 ± 0.5 min and 5.04 ± 0.5 min respectively. The linearity range for GUA and PSE are 15-75 μgml^{-1} and 6-30 μgml^{-1} respectively. The percentage recoveries of GUA and PSE are 98.72 and 98.35% respectively. The correlation coefficients for both components are close to 1. The relative standard deviations for three replicate measurements in three concentrations of samples in tablets are always less than 2%.

Keywords: RP-HPLC, Guaifenesin, Pseudoephedrine hydrochloride, Simultaneous estimation.

INTRODUCTION

Guaifenesin (Figure 1A) chemically named as (RS)-3-(2-methoxyphenoxy) propane-1, 2-diol. ^[1, 2] It is an only expectorant recognized as safe and effective by the FDA. Often it is used with antihistamines, decongestants and antitussives in combination product. ^[3] Pseudoephedrine hydrochloride (Figure 1B) chemically named as (S, S)-2-methylamino-1-phenylpropan-1-ol hydrochloride. ^[1, 2] It acts as a decongestant by stimulating alpha-adrenergic receptors of vascular smooth muscle, thus constricting dilated arterioles within the nasal mucosa and reducing the blood flow to the engorged area. ^[4] Various UV, ^[5-7] HPLC, ^[8, 9] Electrokinetic chromatography, ^[10] Voltammetric assay, ^[11] Capillary gas chromatography ^[12] and ion pair high performance liquid chromatography ^[13] methods are also reported in the literature for the estimation of Guaifenesin and Pseudoephedrine individually and in combination with other drugs. According to literature

survey there is no method reported for the simultaneous estimation of Guaifenesin and Pseudoephedrine by RP HPLC in combined tablet dosage forms.

Hence, an attempt has been made to develop and validate in accordance with ICH guidelines. ^[14, 15]

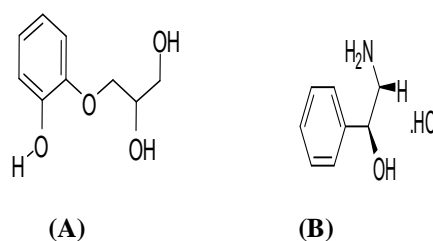


Figure1: (A) Structure of Guaifenesin, (B) Structure of Pseudoephedrine hydrochloride.

EXPERIMENTAL

Instrumentation: Chromatography was performed with Young Lin 9100 HPLC system provided with YL 9110 a quaternary pump, manual injector and Photodiode array detector. YL 9160 detector connected to software YL clarity for controlling the instrumentation as well as processing the data generated was used. The column used was a prontosil C-18 Column 5 μ m 4.6 \times 250 mm. The mobile phase was prepared daily, filtered through a 0.45 μ m membrane filter.

Reagents and chemicals: Pharmaceutically pure sample of guaifenesin was obtained from Global Pharma Mumbai and pseudoephedrine hydrochloride was obtained from Schon Pharmaceuticals Indore as gift samples along with their analytical reports. Acetonitrile and Methanol of HPLC grade was obtained from Merck chemical division, Mumbai and Commercial tablet of Guaifenesin (600mg), and Pseudoephedrine hydrochloride (60mg), Mucinex D (Reckitt Benckiser) were procured from the local drug market.

Chromatographic condition: The isocratic mobile phase consisted of acetonitrile: methanol: phosphate buffer (pH-5) in the ratio of 78:8:20 v/v/v at a flow rate of 1.2 ml min⁻¹. A Prontosil C-18 column (4.6 x 250mm, 5 μ particle size) was used as the stationary phase. Although the GUA and PSE have different λ max viz 272 and 257nm respectively, but considering the chromatographic parameter, sensitivity and selectivity of method for both drugs, 218 nm was selected as the detection wavelength for PDA detector.

Preparation of standard stock solution: Standard stock solutions were prepared by dissolving separately 100 mg of each drug (Guaifenesin and Pseudoephedrine Hydrochloride) into a clean and dry 100 ml volumetric flask in 80 ml of diluent, then volume was made up to 100 ml with diluent to get a concentration of 1000 μ g/ml (Stock-A) for both drugs.

Preparation of Sub Stock Solution (Stock-B): Aliquot of 10 ml was pipette out from stock-A of Guaifenesin and Pseudoephedrine hydrochloride and transferred into 100 ml volumetric flask separately and diluted up to 100 ml with diluent to give concentration of 100 μ g/ml (Stock-B).

Preparation of Working Standard Solutions: Aliquot of 1.5ml, 3.0ml, 4.5ml, 6.0ml and 7.5ml were pipette out from stock-B of Guaifenesin in 10 ml

volumetric flask separately and volume was made up to 10ml with diluent. This gives the solutions of 15 μ g/ml, 30 μ g/ml, 45 μ g/ml, 60 μ g/ml and 75 μ g/ml respectively for Guaifenesin. Aliquot of 0.6ml, 1.2ml, 1.8ml, 2.4ml and 3.0 was pipette out from stock-B of Pseudoephedrine hydrochloride in 10 ml volumetric flask separately and volume was made up to 10ml with diluent. This gives the solutions of 6 μ g/ml, 12 μ g/ml, 18 μ g/ml, 24 μ g/ml and 30 μ g/ml respectively for Pseudoephedrine hydrochloride. Aliquot of 3.75ml, 7.50ml, 11.25ml, 15.0ml and 18.75ml were pipette out from Stock B of Guaifenesin in 25ml volumetric flask separately and aliquot of 1.5ml, 3.0ml, 4.5ml, 6.0ml and 7.5ml from Stock B of Pseudoephedrine hydrochloride were also transferred in to the same volumetric flasks respectively and volume was made up to 25ml.

Sample preparation: Twenty tablets of Mucinex D (Reckitt Benckiser) containing GUA and PSE 600mg: 60mg respectively were weighed and crushed to fine powder. Powder equivalent to 60mg guaifenesin was weighed and dissolved in 100 ml diluent, sonicated for 10 min and filtered through whatmann filter paper No. 42, finally different concentrations of tablet sample were prepared by serial dilution technique.^[16]

METHOD DEVELOPMENT AND VALIDATION

Filtered and filled the standard solutions in syringe and set the chromatographic conditions and run the standard solutions. The chromatogram obtained was shown in the figure 2.

Chromatography: Initially reverse phase LC separation was tried to develop using methanol and water (70:30) as mobile phase, in which guaifenesin gave tailing of 2.5 although pseudoephedrine did not responded properly, and the resolution was also poor. The organic content of mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of mobile phase becomes important factor. At pH 5 the signal to noise ratio for GUA is less and RT was also 2.92 mins.

Thereafter, acetonitrile-methanol-phosphate buffer (pH-5) (72: 8: 20, v/v/v) was selected to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 215nm to 280nm. The wavelength at which both Guaifenesin and Pseudoephedrine hydrochloride showed maximum absorption (218nm) was selected for our proposed method.

System suitability: System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in table-1. The number of theoretical plates for GUA and PSE were 3645 and 7153 respectively.

Linearity: GUA and PSE showed a linearity of response between 15-75 $\mu\text{g ml}^{-1}$ (Figure 3 A) and 6-30 $\mu\text{g ml}^{-1}$ (Figure 3 B) and the linearity were represented by a linear regression equation as follows.

$$Y (\text{GUA}) = 16.56 \text{ conc.} + 3.91 \quad (r^2 = 0.9999)$$

$$Y (\text{PSE}) = 20.16 \text{ conc.} + 1.02 \quad (r^2 = 0.9999)$$

Accuracy: Recovery studies were performed to validate the accuracy of developed method. To preanalyzed sample solution, a definite concentration of standard drug was added and recovery was studied. These results are summarized in table- 2.

Repeatability: Five replicates in five concentrations were analyzed in same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in table- 3.

Intermediate Precision: Five replicates in five concentrations were analyzed on two different days and by two analysts for day to day and analyst to analyst variation and results were found within acceptable limits (RSD < 2) as shown in table- 3.

Robustness: As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase were made to check the method's capacity to remain unaffected. The change was made in the ratio of mobile phase, instead of ACN-Methanol-Phosphate buffer (pH-5) (72:8:20, V/V/V), ACN-Methanol-Phosphate buffer (pH-5) (68:12:20, V/V/V) was used as a Mobile Phase. Results of analysis were summarized in table- 4.

Stability of sample solution: The sample solution injected after 12 hr did not show any appreciable change. Results are shown in table- 5.

Tablet Analysis : Content of GUA and PSE found in the tablets by the proposed method are shown in Table- 6. The low values of R.S.D. indicate that the method is precise and accurate.

RESULT AND DISCUSSION

The regression value was found to be 0.9999 for both Guaifenesin and Pseudoephedrine Hydrochloride, which shows the response, is linear from 15-75 $\mu\text{g ml}^{-1}$ and 6-320 $\mu\text{g ml}^{-1}$ respectively. Selectivity experiment showed that there is no interference or overlapping of the peaks either due to excipients or diluents with the main peak of Guaifenesin and Pseudoephedrine Hydrochloride. The percentage RSD for precision is <2 which confirms that method is sufficiently precise and the total run time required for the method is only 8 mins for eluting both Guaifenesin and Pseudoephedrine Hydrochloride.

CONCLUSION

RP-HPLC method was developed and validated for simultaneous estimation of GUA and PSE in tablet dosage form. Proposed method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in industries.

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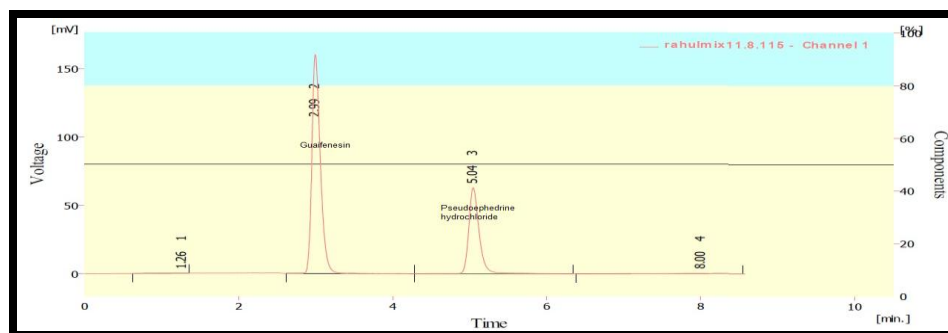


Figure 2: Representative chromatogram of guaifenesin and pseudoephedrine hydrochloride.

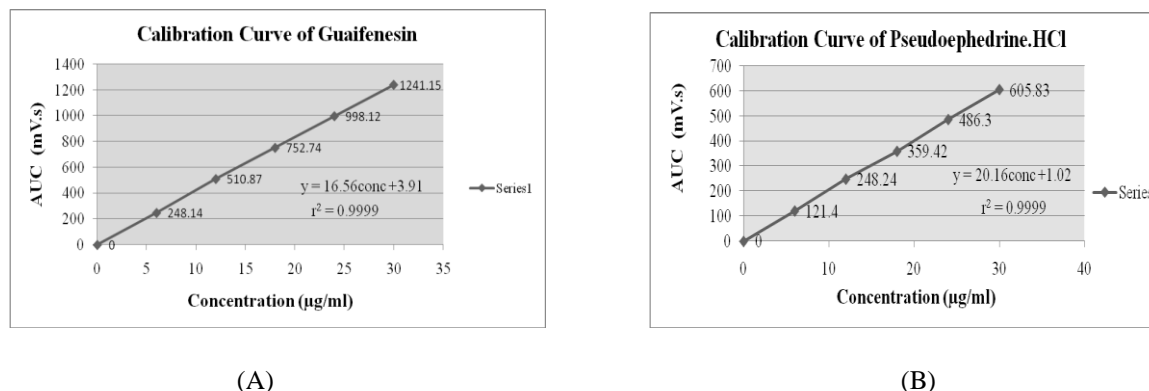


Figure 3: (A) Calibration curve of Guaifenesin (B) Pseudoephedrine hydrochloride

Table 1: System suitability of GUA and PSE

Parameters	GUA	PSE
No. of Theoretical plates	3645	7153
HETP	0.068	0.034
Tailing factor	1.30	1.25

Table 2: Results of Recovery Experiments of GUA and PSE

Conc of drug in preanalyzed samples (µg/ml)		Std. drug sol Added (µg/ml)		Recovered amount* (µg/ml)		% Recovered	
GUA	PSE	GUA	PSE	GUA	PSE	GUA	PSE
15	6	15	6	14.73	5.87	98.22	97.88
30	12	30	12	29.65	11.83	98.82	98.58
45	18	45	18	44.62	17.75	99.14	98.60
						MEAN	98.72
						SD	0.46
						% RSD	0.47

*Mean of three reading

Table 3: Results of precision of GUA and PSE

Validation Parameter	% Mean*		S.D.		% R.S.D.	
	GUA	PSE	GUA	PSE	GUA	PSE
Repeatability	99.75	99.43	0.13	0.12	0.38	0.84
Intermediate precision Day to Day	98.89	98.78	0.33	0.15	0.80	0.97
Intermediate precision Analyst to Analyst	99.80	98.98	0.32	0.13	0.78	0.92

* Mean of 25 determinations (5 replicates at 5 concentration level)

Table 4: Results of Robustness of GUA and PSE

Validation Parameter	% Mean*		S.D.		% R.S.D.	
	GUA	PSE	GUA	PSE	GUA	PSE
Robustness	98.54	99.46	0.26	0.10	0.620	1.28

* Mean of five determinations

Table 5: Stability data of GUA and PSE

Time (in hours)	AUC \pm %RSD	
	GUA 45 μ g/ml	PSE 18 μ g/ml
0	768 \pm 1.2	398 \pm 1.3
6	752 \pm 1.4	374 \pm 1.6
12	740 \pm 1.5	359 \pm 1.4

Table 6: Results of the HPLC analysis for tablets

Drug	% Mean*	SD	% CoV	SE σ .
GUA	99.76	0.15	0.15	0.12
PSE	98.96	0.69	0.69	0.56

* % Mean of nine determinations (3 replicates at 3 concentration level)

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