Development and validation of stability indicating the RP-HPLC method for the estimation of related compounds of guaifenesin in pharmaceutical dosage forms

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Abstract

Aim and background: A stability-indicating gradient reverse phase liquid chromatographic (RP-LC) method was developed for the quantitative determination of related substances of guaifenesin in pharmaceutical formulations. **Materials and methods:** The baseline separation for guaifenesin and all impurities was achieved by utilizing a Water Symmetry C18 (150 mm × 4.6 mm) 5 µm column particle size and a gradient elution method. The mobile phase A contains a mixture of 0.02 M KH_2PO_4 (pH 3.2) and methanol in the ratio of 90:10 v/v, while the mobile phase B contains 0.02 M KH_2PO_4 (pH 3.2) and methanol in the ratio of 10:90 v/v, respectively. The flow rate of the mobile phase was 0.8 ml/min with a column temperature of 25°C and detection wavelength at 273 nm. **Results:** Guaifenesin was subjected to the stress conditions of oxidative, acid, base, hydrolytic, thermal, and photolytic degradation. **Conclusion:** The developed method was validated as per ICH guidelines with respect to specificity, linearity, limit of detection and quantification, accuracy, precision, and robustness.

Key words: Development, degradation, guaifenesin, impurity, HPLC, stability-indicating, validation

INTRODUCTION

Guaifenesin, (+)-3-(2-methoxyphenoxy)-propane-1,2-diol, is a widely used expectorant, useful for the symptomatic relife of respiratory conditions [Figure 1]. Its empirical formula is $C_{10}H_{14}O_4$, which corresponds to a molecular weight of 198.21. It is a white or slightly gray crystalline substance with a slightly bitter aromatic taste. Its solid oral dosage form is available as extended release tables for oral administration.[1]

In the literature survey, there were several LC assay methods have been reported for determination of guaifenesin in pharmaceutical preparation[2-9] and in human plasma by LC-MS.[10-12] So far to our present knowledge, no stability indicating the HPLC method has been reported for the estimation of guaifenesin impurities and degradation products present in pharmaceutical formulation. Hence, we have developed a simple reproducible gradient stability indicating the reverse phase liquid chromatographic (RP-LC) method for the quantitative determination of degradation products and β-isomer and guaiacol impurities [Figure 1] present in guaifenesin pharmaceutical dosage forms. The developed LC method was validated with respect to specificity, limit of detection and quantification, linearity, precision, accuracy, and robustness. Force degradation studies were performed on the placebo and drug products to show the stability-indicating nature of the method. These studies were performed in accordance with established ICH guidelines.[13-15]

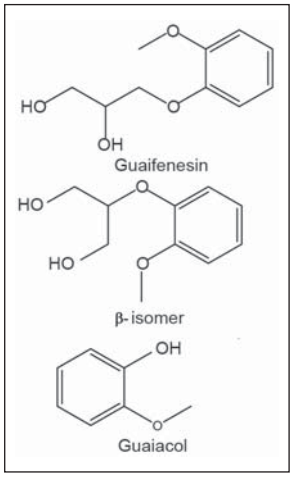

Figure 1: Structures of guaifenesin and its impurities

MATERIALS AND METHODS

Chemicals and reagents

The samples of guaifenesin-extended release tablets and its impurities were supplied by Dr. Reddy's laboratories limited, Hyderabad, India. The HPLC grade methanol and analytical grade KH_2PO_4 and ortho-phosphoric acid were purchased from Merck, Mumbai, India. High purity water was prepared by using Millipore Milli-Q Plus water purification system (Millipore, Milford, MA, USA).

Equipments

The chromatography analysis was performed using Waters Alliance 2695 separation module (Waters Corporation, Milford, USA) equipped with 2489 UV/visible detector or 2998 PDA detector (for specificity and forced degradation studies), degasser, quaternary pump, and auto sampler system. The output signals were monitored and processed using Empower 2 software. Cintex digital water bath was used for hydrolysis studies. Photo-stability studies were carried out in the photo-stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (Cintex, Mumbai, India). The pH of the solutions was measured by a pH meter (Mettler-Toledo, Switzerland).

Chromatographic conditions

The method was developed using a Waters Symmetry C18 (150 mm × 4.6 mm, 5 μm) column with the mobile phase containing a gradient mixture of solvent A (90:10 v/v mixture of 0.02 M KH_2PO_4, pH adjusted to 3.2 with orthophosphoric acid and methanol) and B (10:90 v/v mixture of 0.02 M KH_2PO_4 buffer of pH 3.2 and methanol). The gradient program (time (min)/%B) was set 0/0, 50/40, 52/0, and 60/0. The mobile phases were filtered through nylon 0.45 μm membrane filters and degassed. The flow rate of the mobile phase was 0.8 mL/min. The column temperature was maintained at 25°C, and the eluted compounds were monitored at the wavelength of 273 nm. The sample injection volume was 10 μl.

Preparation of standard solution

Milli-Q water and acetonitrile in the ratio of 20:80 v/v were used as diluent. A standard stock solution of guaifenesin was prepared by dissolving an appropriate amount of drug in diluent having a concentration of 0.24 mg/mL. The working standard solution containing 12 μg/mL was prepared from the above stock solution.

Preparation of sample solution

Tablet powder equivalent to 600 mg of guaifenesin was dissolved in diluent with sonication for 10 min to give a solution containing 2.4 mg/mL drug. This solution was centrifuged at 4000 rpm for 10 min.

RESULTS AND DISCUSSION

Method development and optimization

The aim of the study was to separate all known and unknown degradation products from guaifenesin and their simultaneous determination in pharmaceutical tablet forms. Various attempts were made to separate all degradation products with different pH of the mobile phase buffer and composition of methanol in the mobile phase using C-18 and C-8 stationary phase columns. To ensure great resolution between all known and unknown degradation compounds, the C-18 stationary phase with an end-capping was used. In this case, the optimized mobile phase was constituted by solvent A (90:10 v/v mixture of 0.02 M KH_2PO_4, pH adjusted to 3.2 with orthophosphoric acid and methanol) and B (10:90 v/v mixture of 0.02 M KH_2PO_4 buffer of pH 3.2 and methanol). The gradient program (time (min)/%B) was set 0/0, 50/40, 52/0, and 60/0. The flow rate of the mobile phase was 0.8 mL/min. The column temperature was maintained at 25°C, and the eluted compounds were monitored at the wavelength of 273 nm.

Validation of the method

The proposed method was validated as per ICH guidelines.[13-15] The following validation characteristics were addressed: Specificity, accuracy, precision, limit

of detection and quantification, linearity, range, and robustness.

System suitability

System suitability shall be checked for the conformance of suitability and reproducibility of chromatographic system for analysis. System suitability was determined before sample analysis from duplicate injections of the standard solution containing 12 µg/mL of guaifenesin. The acceptance criteria were USP tailing factor not more than 2.0 and the area similarity ratio between 0.9 and 1.1 for the guaifenesin peak from duplicate injections of standard preparation. All critical parameters tested met the acceptance criteria.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. Placebo interference was evaluated by analyzing the placebo prepared as per the test method. No peak due to placebo detected at the retention time of guaifenesin and its impurities. The specificity of the developed LC method for guaifenesin was carried out in the presence of its impurities and degradation products. Stress studies were performed at 2.4 mg/mL concentration of guaifenesin on tablet to provide an indication of the stability-indicating property and specificity of proposed method. The stress conditions employed for degradation study included acid hydrolysis (1 N HCl at 60°C for 12 h), base hydrolysis (1 N NaOH at 60°C for 12 h), oxidation (1% H_2O_2 at room temperature for 12 h), hydrolytic (water at 60°C for 12 h), thermal (105°C for 24 h), and photolytic degradation (drug product exposed to visible light for 240 h resulting an overall illustration 1.2 million lux hours and UV light for 250 h resulting an overall illustration 200 watt h/m^2 at 25°C). Sight degradation was observed under acid and base stress conditions [Figures 2 and 3]. Guaifenesin found stable under oxidative, hydrolytic, thermal, and photolytic stress conditions. The peak purity test was carried out for the guaifenesin peak by using the PDA detector in stress samples. The mass balance (% assay + % sum of all degradants + % sum of all impurities) results were calculated and found to be more than 95% [Table 1]. The purity of guaifenesin was unaffected by the presence of its impurities and degradation products, and thus confirms the stability-indicating power of the developed method.

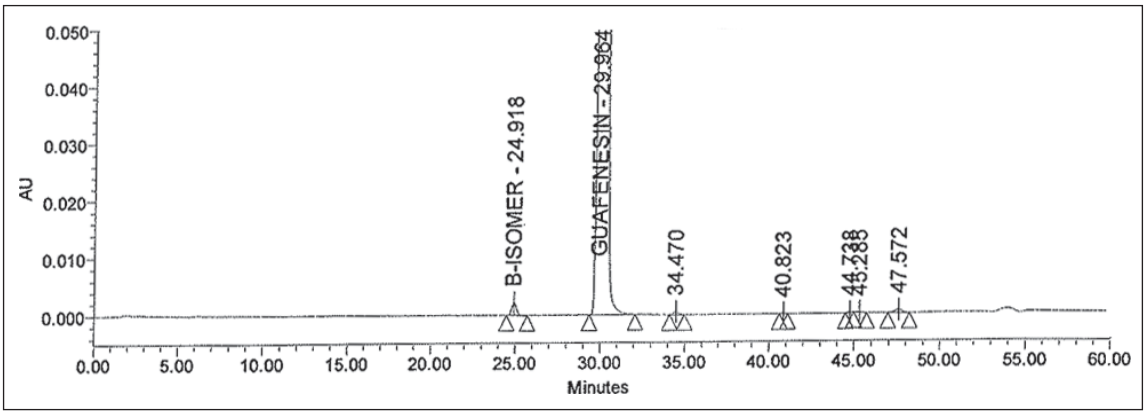

Figure 2: Typical chromatogram of the acid degradation sample

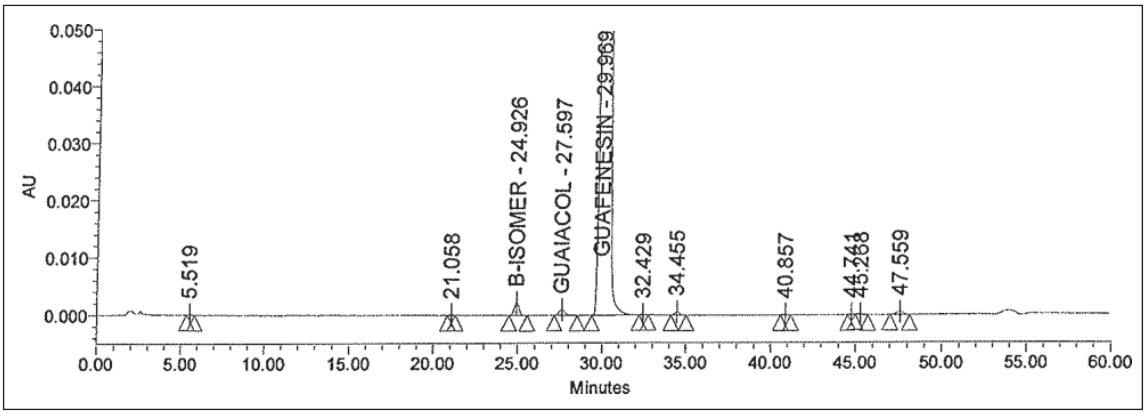

Figure 3: Typical chromatogram of the base degradation sample

Table 1: Summary of forced degradation results

Stress conditions	Purity angle	Purity threshold	% Degradation	% Assay of active substance	Mass balance (%)
Acid hydrolysis (1 N HCl at 60°C, 12 h)	1.536	2.304	0.1	98.2	98.3
Base hydrolysis (1 N NaOH at 60°C, 12 h)	1.688	2.418	0.2	98.4	98.6
Oxidation (1% H_2O_2 at room temp, 12 h)	1.625	2.379	0.0	99.2	99.2
Thermal (At 105°C, 24 h)	1.718	2.410	0.0	99.0	99.0
Hydrolytic (Water at 60°C, 12 h)	1.783	2.465	0.0	98.7	98.7
Photolytic (1.2 million lux h visible light and 200 Wh/m^2 UV light)	1.911	2.594	0.0	100.6	100.6

Table 2: Evaluation of LOD, LOQ, linearity, and precision data

Parameter	β-isomer	Guaiacol
LOD (µg/mL)	0.003	0.002
LOQ (µg/mL)	0.010	0.006
Correlation coefficient	0.999	0.999
Intercept (*a*)	2524.62	533.72
Slope (*b*)	8226.21	14147.65
Bias at 100% response	1.3	3.4
Precision (%RSD)	0.4	2.7
Intermediate precision (%RSD)	0.5	0.1
Precision at LOQ (%RSD)	0.2	0.5

Precision

The precision of method was verified by repeatability and intermediate precision. Repeatability was checked by injecting six individual preparations of guaifenesin tablets spiked with its two impurities, β-isomer and guaiacol at 1.0% and 0.05% level, respectively (1.0% and 0.05% of impurities with respect to 2.4mg/mL guaifenesin). The intermediate precision of the method was also evaluated using different analyst, different instrument, and performing the analysis on different days. The % RSD for the area of β-isomer and guaiacol in repeatability study was within 2.7% and in intermediate precision study was within 0.5%, which confirms the good precision of the method. The % RSD values are presented in Table 2.

Limits of detection and quantification

The LOD and LOQ for β-isomer and guaiacol were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. The precision study was also carried out at the LOQ level by injecting six individual preparations of β-isomers and guaiacol impurities and calculating the % RSD of the area [Table 2].

Table 3: Recovery study of the analytical method

Amount spiked[a]	% Recovery[b]	
	β-isomer	Guaiacol
LOQ	108.7 ± 4.5	90.9 ± 2.1
50%	97.2 ± 0.5	91.3 ± 4.6
75%	96.8 ± 1.5	92.8 ± 3.0
100%	95.7 ± 0.3	91.4 ± 1.3
200%	96.7 ± 0.4	96.1 ± 0.9
300%	96.9 ± 0.6	97.6 ± 0.7

[a]Amount of two impurities spiked with respect to specification (0.1% for β-isomer and 0.05% guaiacol impurity); [b]Mean ± % RSD for three determinations

Linearity

Linearity test solutions were prepared by diluting the stock solutions to the required concentrations. The solutions were prepared at six concentration levels ranging from 0.227 to 72.046 µg/mL for β-isomers and 0.163–4.8121 µg/mL for guaiacol impurites. Calibration curves were plotted between the responses of peak vs. analyte concentrations. The correlation coefficient obtained was greater than 0.999 and % bias at 100% response was within 5% [Table 2]. The above results show that an excellent correlation existed between peak area and concentration of β-isomer and guaiacol.

Accuracy

Accuracy of the method for β-isomer was evaluated in triplicate using six concentration levels at 0.235, 11.843, 17.765, 23.686, 44.412, and 54.281µg/mL and guaiacol at 0.163, 0.542, 0.854, 1.164, 2.871, and 3.492 µg/mL (i.e. LOQ, 50%, 75%, 100%, 200%, and 300% level of specification, respectively). The percentage recovery of β-isomer and guaiacol in guaifenesin samples varied from 88.3% to 108.7%. The LC chromatogram of the spiked sample at the specification level of both impurities in the guaifenesin tablet sample is shown in Figure 4. The recovery values for β-isomers and guaiacol are presented in Table 3.

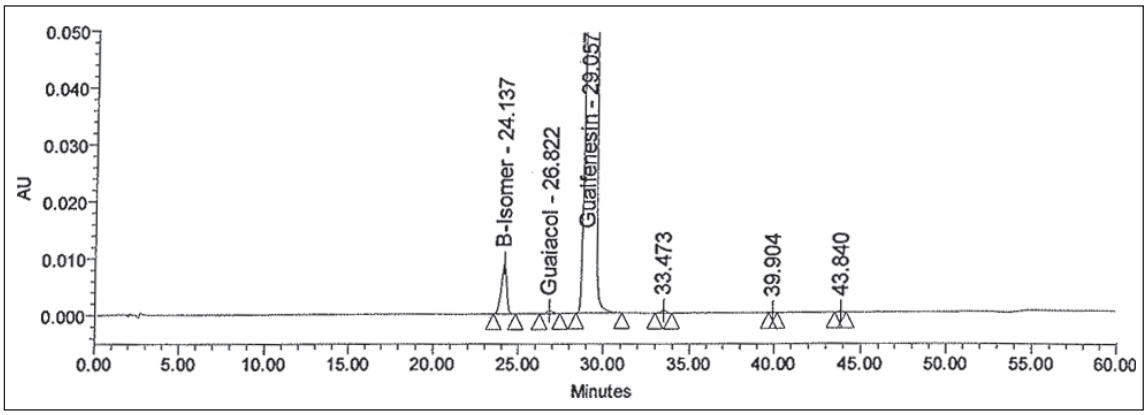

Figure 4: Typical chromatogram of sample spiked with impurities

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the relative retention time (RRT) of β-isomer and guaiacol with respect to guaifenesin; and system suitability parameters for guaifenesin standard was recorded. The variables evaluated in the study were pH of the mobile phase buffer (+0.2), column temperature (± 5°C), flow rate (± 0.2 mL/min), and % organic in the mobile phase (±10%). In all the deliberate varied chromatographic conditions, all analytes were adequately resolved and the elution order remained unchanged. The area ratio for the guaifenesin peak from standard solution was between 0.9 and 1.1 and the tailing factor was less than 1.1 [Table 4].

Table 4: Robustness results of the HPLC method

Variation in chromatographic conditions	Observed system suitability parameters			
	USP tailing < 2.0	Area similarity ratio >0.9 and <1.1	RRT[a]	
			β-isomer	Guaiacol
Column temperature 20°C	1.0	1.0	0.83	0.92
Column temperature 30°C	1.0	1.0	0.83	0.92
Flow rate 0.6 mL/min	0.9	1.0	0.85	0.95
Flow rate 1.0 mL/min	0.9	1.0	0.82	0.90
Methanol 90%	0.9	1.0	0.83	0.92
Methanol 110%	0.9	1.0	0.83	0.92
Mobile phase buffer pH 3.0	0.9	1.0	0.83	0.91
Mobile phase buffer pH 3.4	1.0	1.0	0.83	0.92

[a]Relative retention time of β-isomer and guaiacol impurity with respect to the guaifenesin peak

Stability of solution and the mobile phase

The solution stability of guaifenesin and its impurities were determined by leaving test solution and standard solutions in tightly capped volumetric flasks at room temperature for 48 h and measured the amount of both impurities at every 24 h against freshly prepared standard solution. The stability of the mobile phase was also determined by freshly prepared solutions of guaifenesin and its impurities at 24 h interval for 48 h. The mobile phase was not changed during the study. The variability in the estimation of β-isomers and guaiacol was within ± 10% during solution stability and mobile phase stability. The results from solution stability and mobile phase stability experiments confirmed that sample solution, standard solution and mobile phase were stable up to 48 h.

CONCLUSIONS

A simple and efficient reverse-phase HPLC method was developed and validated for quantitative analysis of guaifenesin in pharmaceutical dosage forms. The method found to be precise, accurate, linear, robust, and rugged during validation. Satisfactory results were obtained from the validation of the method. The method is stability indicating and can be used for routine analysis of production samples and to check the stability of the guaifenesin tablets.

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