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RESEARCH ARTICLE

Application of Validated Stability-Indicating HPLC Method in Stability Testing of Acetaminophen and Guaiphenesin Tablets

RAJYALAKSHMI CHAVAKULA[*1] and C. RAMBABU[2]

[*1]Department of Chemistry, Vishnu Institute of Technology, Vishnupur, Bhimavaram-534202, A.P, India

[2]Department of Chemistry, Acharya Nagarjuna University, Nagarjunanagar, Guntur, 522510, A.P, India

rajyalakshmi.ch@gmail.com

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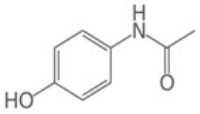
Abstract: The aim of the work is to develop and validate the simple, fast and precise stability indicating high performance liquid chromatography method for the separation and quantification of acetaminophen and guaiphenesin in pharmaceutical dosage form. The quantification was carried out using Symmetry C_{18} (4.6x150 mm, 3.5 µm) enhanced polar selectivity column and mobile phase comprised of potassium dihydrogen phosphate buffer pH 2.5 and methanol in proportion of ratio 65:35v/v. The flow rate was mL/min and the effluent was monitored at 228 nm. The retention time of acetaminophen and guaiphenesin were 2.6 and 4.6 min respectively. Linearity of acetaminophen and guaiphenesin were in the range of 50 to 90 µg/mL and 30 to 55 µg/mL respectively with correlation coefficients 0.999. The percentage recoveries were 99.80% and 99.85% for acetaminophen and guaiphenesin respectively. There was complete separation of degradation peaks and analyte peaks, which demonstrate the specificity of assay method in the presence of its degradation products; it can be employed as a stability indicating one. Due to simplicity, rapidity and accuracy of the proposed Stability Indicating High Performance Liquid Chromatography method, it is used for analysis of stability samples of Acetaminophen and Guaiphenesin in quality control laboratories.

Keywords: Acetaminophen, Guaiphenesin, RPHPLC method, Validation, Stability studies

Introduction

Paracetamol or acetaminophen (Figure 1) chemically named as *N*-acetyl-*p*-aminophenol is a widely used as analgesic and antipyretic. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. Literature survey reveals several spectroscopic[1-4], RP-HPLC[5-12] methods for the estimation of acetaminophen individually and in combination with other drugs. Guaiphenesin (Figure 2) also glyceryl guaiacolate is an expectorant drug and usually taken orally to assist the bringing up phlegm from the airways in acute respiratory tract infections.



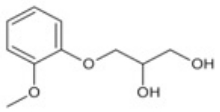


Figure 1. Acetaminophen **Figure 2.** Guaiphenesin

The principal use of guaiphenesin is in the treatment of coughing. Chemically it is an *(RS)*-3-(2-methoxyphenoxy) propane-1, 2-diol. The combination of acetaminophen and guaiphenesin are used to treat headache, aches and pains, fever and chest congestion caused by common cold or flu. It also loosens phlegm (mucus) in chest to help breathe more easily. A survey of the analytical literature for guaiphenesin revealed methods based on UV spectrophotometric[13,14] and HPLC[15-18] for its determination in biological fluids and in pharmaceutical formulations individually and in combination with other drugs.

In the literature few HPLC methods[19] were reported for simultaneous estimation of above mentioned drugs, besides the lack of stability indication and time consuming gradient elution. The authors have developed a new, simple and fast analytical method by RP-HPLC, which is stability indicating to quantify acetaminophen and guaiphenesin in bulk and its dosage forms. It has been shown that the method presented here is rapid, convenient and sufficiently sensitive for analysis of acetaminophen and guaiphenesin in pharmaceutical dosage forms with acceptable recovery and precision. This manuscript gives the first report for the application of validated stability indicating HPLC method in stability testing of pharmaceutical dosage forms in short analysis time. The novelty of the proposed method is simple, accurate, shows good resolution with shorter run time than existing methods and applicable to stability testing of pharmaceutical dosage forms.

Experimental

Acetaminophen and guaiphenesin were obtained as gift samples from Dr. Reddy's Laboratories, Hyderabad. Sample tablets (TYLENOL® Chest Congestion) with acetaminophen 325 mg & guaiphenesin 200 mg were purchased from local market. HPLC grade acetonitrile and analytical grade of potassium dihydrogen phosphate, hydrochloric acid, sodium hydroxide, hydrogen peroxide were obtained from Merck India Chemicals Ltd, Mumbai.

Instrumentation and chromatographic conditions

HPLC system (Waters 2695 LC) consisting of quaternary gradient pump, auto sampler, column oven and PDA detector (2996) was employed for analysis. The output of signal was monitored and integrated using Waters Empower software. Chromatographic analysis was performed on Symmetry Xterra C_{18} (150×4.6 mm, 3.5 µm) column. Separation was achieved using a mobile phase consisting of phosphate buffer with pH 2.5 and methanol in the ratio of 65:35v/v solutions at a flow rate of 1 mL/min and run time was 8 min. The column was maintained at ambient temperature and injection volume of 20 µL was used. The mobile phase was filtered through 0.45 µ filter prior to use. The eluent was monitored using UV detector at a wavelength 228 nm.

Preparation of standard solution

10 mg of acetaminophen and 10 mg of guaiphenesin accurately weighed and transferred into a 10 mL clean dry volumetric flask, about 7 mL of mobile phase was added and sonicated to dissolve it completely, the solution was cooled to room temperature and diluted up to volume with mobile phase and it was used as standard stock solution. 1.25 mL of acetaminophen and 1.0 mL of guaiphenesin standard stock solution was pipette out into a 10 mL volumetric flask and diluted up to volume with mobile phase and used as working standard solution.

Preparation of sample solution

Finely powdered not fewer than 20 tablets of acetaminophen and guaiphenesin were weighed 10 mg each and transferred into a 10 mL clean dry volumetric flask, about 7 mL of diluent was added and sonicated to dissolve it completely, the solution was cooled to room temperature and diluted to volume with diluent. The above sample solution was filtered through 0.45 µ membrane filter. 1.25 mL of the above filtered sample solution was pipetted out into a 10 mL volumetric flask and diluted to volume with diluent.

Validation of the proposed method

The developed method was validated with respect to various parameters such as linearity, accuracy, precision and robustness, ruggedness, limit of detection and limit of quantification as per the ICH guidelines.

System suitability

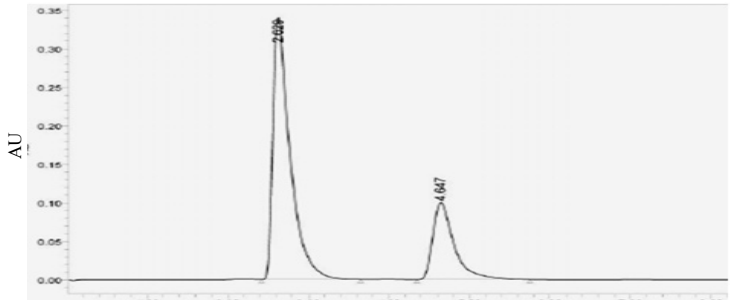
The system suitability was assessed by three replicate analyses of the drugs at concentrations of 125 µg/mL of acetaminophen and 100 µg/mL of guaiphenesin. The % RSD of peak area and retention time for the both drugs are within 2% indicating the suitability of the system. Results are shown in Table 1.

Table 1. System Suitability parameters for acetaminophen and guaiphenesin by the proposed method

System suitability parameter	Acetaminophen	Guaiphenesin
Retention time (min)	2.62	4.64
Theoretical plates	2874	3128
Tailing factor	1.6	1.2
Resolution	-	4.6

Specificity

The specificity of the method was performed by separate injections of the acetaminophen, guaiphenesin and the sample. The specificity chromatogram was shown in Figure 3, where the retention time of acetaminophen does not interfere with the retention time of the guaiphenesin. Also injecting diluent and placebo using the chromatographic conditions defined for the proposed method. The blank and placebo sample chromatogram showed no interference peaks at the retention time of acetaminophen and guaiphenesin respectively which demonstrates the specificity of the proposed method.



	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Tailing	USP Resolution	USP Plate Count
1	Acetaminophen	2.628	511921.6	345256	1.6		2874
2	Guaiphenacin	4.648	1647027	100686	1.2	4.6	3128

Figure 3. Typical chromatogram of acetaminophen and guaiphenesin

Linearity & calibration curve

Calibration curves were prepared by taking appropriate aliquots of acetaminophen and guaiphenesin standard stock solutions in different 10 mL volumetric flask and made up to the mark with mobile phase to obtain final concentrations of 50, 60, 70, 80 and 90 µg/mL of acetaminophen and 30, 36, 43, 49 and 55 µg/mL of guaiphenesin. Linearity curve was constructed by plotting average peak area against concentration and regression equation was computed. The statistical data calculated for acetaminophen and guaiphenesin found to be accurate and was given in Table 2 and Figure 4 & 5.

Table 2. Regression analysis of the calibration curves for the proposed method

Parameter	Acetaminophen	Guaiphenesin
Linearity Range, mcg/mL	50-90	30-55
Slope	99152	49210
Intercept	-1783677	-26256
Correlation coefficient R^2	0.999	0.999

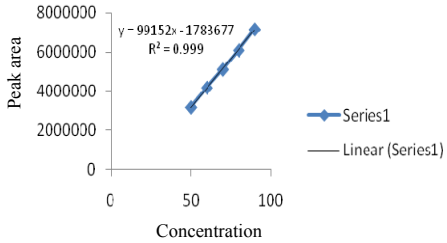


Figure 4. Linearity curve of acetaminophen

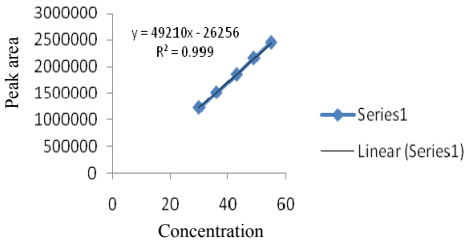


Figure 5. Linearity curve of guaifenesin

Accuracy (recovery studies)

Accuracy of the method was determined by recovery studies which were performed in triplicate by standard addition method at 50, 100 and 150%. The percentage recoveries found are in the range of 99.80 and 99.85% for acetaminophen and guaiphenesin respectively. From the data obtained, the proposed method was found to be accurate. The results are summarized in Table 3.

Table 3. Recovery data of acetaminophen and guaiphenesin

% of Concentration	Area average	Amount added, mg	Amount found, mg	% Recovery	Mean Recovery
		Acetaminophen			
50	2559157	5	4.99	99.8	99.80
100	5118314	10	9.98	99.8	
150	7677469	15	14.97	99.8	
		Guaiphenesin			
50	924012	5	4.99	99.85	99.85
100	1848025	10	9.99	99.85	
150	2772038	15	14.98	99.9	

Precision and intermediate precision

The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, five repeated injections of sample solutions were made in a day and the

response factor of drug peaks and percentage of RSD were calculated. In the interday variation studies, five repeated injections of sample solutions were made in different day with different make column of same dimensions. The repeatability of sample applications and measurement of peak area were expressed in terms of %RSD and found to be less than 2% (Table 4).

Table 4. Precision and intermediate precision data of acetaminophen (ACET) and guaiphenesin (GUAI)

	Intraday precision		Interday precision	
	ACET	GUAI	ACET	GUAI
Injection	Area	Area	Area	Area
Injection-1	4684466	1690048	4934667	1781527
Injection-2	4680730	1683628	4949886	1788651
Injection-3	4679619	1690783	4949375	1790742
Injection-4	4695633	1691465	4961354	1795711
Injection-5	4687948	1697529	4973945	1800165
Average	4685679	1690691	4958572	1793267
Standard Deviation	6459.7	4941.1	17515.6	7873.9
%RSD	0.14	0.29	0.35	0.44

Limit of detection and limit of quantification

The LOD is the smallest concentration of the analyte that gives a measurable response of signal to noise ratio of 3. The LOD for acetaminophen and guaiphenesin were found to be 0.02 & 0.05 respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified signal to noise ratio of 10. The LOQ was 0.091 & 0.18 of acetaminophen and guaiphenesin respectively.

Robustness of method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate and mobile phase ratio on the retention time and tailing factor were studied. On evaluation of the results, it can be concluded that the variation in flow rate and changes in mobile phase composition affected the method significantly. Hence it indicates that the method is robust even during change in the flow rate and mobile phase. The results are shown in Table 5.

Table 5. Robustness data of acetaminophen and guaiphenesin

Sample Name	Parameters	Variations	Retention time	Tailing factor	Plate count
Acetaminophen	Flow rate	0.9	2.7	1.6	2329
		1.1	2.40	1.7	2344
	Change in organic composition in mobile phase	10% less	2.63	1.5	2426
		10% more	2.51	1.7	2271
Guaiphenesin	Flow rate	0.9	4.6	1.8	2624
		1.1	3.97	1.9	2514
	Change in organic composition in mobile phase	10% less	4.64	1.7	2633
		10% more	3.84	1.8	2508

Application of method to dosage form

The proposed method was applied to the determination of acetaminophen and guaiphenesin in commercial tablet form (TYLENOL® contains acetaminophen 325 mg, and guaiphenesin 200 mg). The result of these assays yielded 100% and 99.2% for acetaminophen and guaiphenesin respectively with RSD <2%. The result of the assay (Table 6) indicates that the method is selective for the assay of acetaminophen and guaiphenesin without interference from the excipients used in these tablets.

Table 6. Analysis of formulation

Sample name	Label claim	Amount found	% Recovery
Acetaminophen	325	325	100
Guaiphenesin	200	198.4	99.2

Stability studies

Forced degradation studies were conducted indicating the stability of proposed method. The results of the degradation studies are presented in Table 7. The International Conference on Harmonization guideline entitled 'stability testing of new drugs and products' requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the acetaminophen and guaiphenesin using the proposed method.

Table 7. Degradation results for acetaminophen (ACET) and guaiphenesin (GUAI)

Degradation parameter	Results of degradation					
	Peak area of degraded product		% of recovery		% of Degradation	
	ACET	GUAI	ACET	GUAI	ACET	GUAI
Acid Degradation (0.1 N HCl)	4964763	1774104	98.4	96.8	1.6	3.2
Base Degradation (0.1 N NaOH)	4862396	1755624	96.4	95.8	3.6	4.2
Peroxide Degradation (3% H_2O_2)	4606481	1663223	91.3	90.8	8.7	9.2
Thermal Degradation (60 ^{0}C)	4760030	1700183	94.4	92.8	5.6	7.2

Forced degradation studies of both the drugs were carried out under conditions of acid/base hydrolysis, oxidation and thermal hydrolysis. The drugs were subjected to acid hydrolysis by using 0.1 M hydrochloric acid and base hydrolysis by using 0.1 N sodium hydroxide solution; oxidation by using 3% hydrogen peroxide solution and thermal hydrolysis at 60 ^{0}C. The stress conditions varied both in terms of temperature and time to achieve the appropriate degradation. All degradation studies in solution were carried out at a drug concentration at 1000 µg/mL. The purity of the main peaks was evaluated using photodiode array detector to verify that the degradation peaks are well resolved from the main peaks. After the degradation treatments were completed, the stress content solutions were allowed to room temperature and diluted with mobile phase up to the mark. Filter the solutions with 0.45 microns filters and injected to column under proposed conditions. The forced degradation studies prove the stability indicating power of the method and can be used to assess the stability of acetaminophen and guaiphenesin in the bulk drug and in pharmaceutical dosage forms.

Results and Discussion

The typical chromatogram of acetaminophen and guaiphenesin is shown in Figure 3, it was found that the retention times were 2.62 and 4.64 min. which are very short retention times than earlier reported method[19] (4.0 & 9.5 min). The mobile phase composition at a ratio of 65:35 (v/v) of buffer pH 2.5 and methanol was found to be most suitable to obtain peaks well defined and free from tailing. A good linear relationship (r=0.999) was observed within the concentration ranges 50, 60, 70, 80 and 90 µg/mL of acetaminophen and 30, 36, 43, 49 and 55 µg/mL of guaiphenesin. Low values of S.D are indicative of high precision of the method. The assay of Tylenol tablets was found to be 100% and 99.2% for acetaminophen and guaiphenesin respectively. From the recovery studies, it was found that 99.80% of acetaminophen and 99.85% of guaiphenesin recovered which indicates high accuracy of the method. The results of LOD and LOQ indicate that the method is reliable and also shows good resolution (4.6 which is better value than earlier reported method[19] value 9.2) with short separation time for analysis. The forced degradation studies were also carried out as per ICH guidelines. There was complete separation of degradation peaks and analyte peaks, which demonstrate the specificity of assay method for estimation of acetaminophen and guaiphenesin in the presence of its degradation products; it can be employed as a stability indicating one.

Conclusion

The proposed HPLC method is stability indicating one and less time consuming method and also satisfactory results were obtained for all validation parameters. Hence the proposed method is rapid, simple, accurate and precise. Moreover the degraded peaks were well resolved from analyte peaks. So the developed method may be used for analysis of stability samples of acetaminophen and guaiphenesin in quality control laboratories.

Competing interests

The authors declare that they have no competing interests.

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