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ESTIMATION OF GUAIFENESIN BY RP-HPLC METHOD IN PHARMACEUTICAL SUBSTANCE AND PHARMACEUTIAL PRODUCTS

Manasa Merugu*, B. Sai Teja, B. Kavya Sree,

B. Pravallika and CH. Navya Prashanthi

Department of Pharmaceutical Analysis, Pulla Reddy Institute of Pharmacy, Domadugu (V),Gummadidala(M),Sangareddy(D), Hyderabad, Telangana, India.

ABSTRACT

A simple, accurate and precise economic RP HPLC method was develop and validated for the estimation of Guaifenesin in Pharmaceutical substance and product. The drug was separated in CAPCELL C18 column (250×4.6mm, 5µ) using mobile phase consisting of methanol: water(50:50% v/v).The flow rate was kept constant at 1 mL/min and eluents were detected at 209 nm. In calibration curve experiments, linearity was found in concentration range50-150 µg/ml with regressions efficient R^2=0.999. The equation obtained was Y=16468x + 16614. In accuracy studies percentage recovery was found to be 99.55%W/W. By performing assay percentage purity was found to be 99.99%W/W. Hence the method can be applicable in routine determination of Guaifenesin in pharmaceutical formulations.

Keywords: Guaifenesin, methanol, water, ICH guidelines and Analytical method validation.

INTRODUCTION[1-18]

Guaifenesin is a medication used to try to help cough out phlegm from the airway it is unclear if it decreases coughing, it is an expectorant it will reduce chest congest caused by common cold, infection, allergies or illness.

Guaifenesinmolecular formula is $C_{10}H_{14}O4$. Other name is Glycerylguaiacolate and the trade name is Barkeit. It is metabolized in kidney. IUPAC is 3-(2-methoxyphenoxy) propane-1, 2-diol. Molecular mass of Guaifenesinis 198.216 g/mol. Chemical structure of Guaifenesin shown it fig no.1.

In this research work we have developed, optimized, and validated the method using RP-HPLC. Drug was assayed by validated method. The main objective of this method is to develop time saving and cost effective.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Reference standard was obtained from sigma Aldrich laboratories. The formulation used for assay is Guaifenesin is manufactured by Barkeit and solvents used in this method were methanol and HPLC grade water.

INSTRUMENTATION

Method development and validation was carried out by RP-HPLC (Shimadzu) with PDA detector module with auto-sampler. Column used was Agilent Eclips XBD (150*4.6 * 5µm), and data recorded using LC Solutions software.

DILUENT

Methanol: Water (50:50 % v/v)

PREPARATION OF STANDARD

Weigh accurately about 100mg of drug and transfer it into 10ml volumetric flask and make up the volume up to 10ml using diluent and sonicated for 5 minutes. Pipette out 1ml of above solution into 10ml volumetric flask, make up the volume with diluent .obtained standard concentration is 100µg/ml.

PREPARATION OF SAMPLE

Take 20 tablets and triturate it. Weigh accurately equivalent to 100mg of tablet powder and transfer it into 10ml volumetric flask and make up the volume up to 10ml using diluent and sonicatedfor 5 minutes. Pipette out 1ml of above solution into 10ml volumetric flask, make up the volume with diluent.

METHOD OPTIMIZATION

Based on the literature of Guaifenesin and its combination, one method was developed after conducting several trails and developed method optimized.

VALIDATION

The developed method was validated foe different parameters like system suitability, Linearity, accuracy, precision, LOD, LOQ, and Robustness as per ICH guidelines.

SYSTEM SUITABILITY

By injecting it six times into the system, the chromatograms of 100µg/ml were analyzed. Form chromatogram the system suitability parameters like plate count, tailing factor, capacity factor and reproducibility were determined.

LINEARITY

The solutions were prepared from stock solution at 5 concentration levels (50, 75, 100, 125, and 150 µg/ml).Take 10µlvolume from each concentration solution and injected trice into the HPLC system. Chromatogram were recorded under optimized equation. A graph was plotted considering peak areas on Y-axis and concentration on X-axis. Data treated by square linear regression analysis.Y-intercept, slope of regression constant (r^2) were calculated.

PRECISION

Repeatability or intra-day precision: The peak areas of 100 µg/ml were analyzed on the same day by injecting it six times into the system. Inter-day precision also performed in different days using same method. The chromatogram was recorded and RSD was calculated., % should be more than 2%.

ACCURACY

Recovery of the assay method for Guaifenesin was established by three determination of the test sample using tablets at 50%,100%and 150% of analyte concentration .Each solution was injected thrice (n=3) into HPLC system and the average peak area was calculated from which percentage recoveries were calculated. The % recovery should be between 98.0to102.0 as per ICH Q_2R_1 guidelines.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

LOD and LOQ can be calculated based on the signal to noise ratio approach, visual evaluation and standard deviation of the response and slope of the calibration curve. The slope (S) is calculated from the equation of straight line in calibration curve of the analyte. The standard deviation (σ) is calculated based on its blank response or they-intercepts of regression line. Formulas were given below.

LOD= (3.3X SD)/Slope
LOQ= (10 X SD)/Slope

ROBUSTNESS& RUGGEDNESS

Ruggedness of the method was determined by carrying out the analysis by three different analysis and the respective absorbance were noted The results was indicated by % RSD .The robustness of a method is its ability to remain unaffected under changes in parameters. Robustness was carried out by altering the flow rate (+/-0.2 ml/min), changing and mobile phase (60:50&50:60).The standard solution comprising of Guaifenesin (100µg/ml) was injected six times and %RSD was calculated for the resultant area of the peak.

ASSAY

Take twenty tablets of Guaifenesin and powdered it and weigh accurately equivalent about 10mg of label drug and take the drug into 100ml volumetric flask and add 30mlof diluent, sonicate for 5 minutes and finally make up the volume with diluent. The solution was then injected into the HPLC system .The sample was prepared in six replicates.

% Assay =

$$\frac{\text{(Area of unknown X Conc of standard)}}{\text{(Area of standard X Conc of unknown)}} \text{ X 100}$$

RESULTS AND DISCUSSION

OPTIMIZATION OF CHROMATOGRAPHIC

Developed method was optimized for different parameters. Parameters were given in Table.1 and Fig No 2.

SYSTEM SUITABILITY

The percentage area of Relative Standard deviation (RSD) from six replicate injections was found below 2.0% (diluted standard solution, 100 µg/ml of Guaifenesin). Low values of RSD of replicated injections indicate that the system is precise. The results are

presented in Table 2.

LINEARITY

The calibration curve was made by plotting the concentration on X-axis against peak area on Y-axis. A series of Guaifenesin standard solution were prepared in the range of 50 µg/ml-150µg/ml. The correlation coefficient of the curve was found to be 0.999 with a regression equation of Y=16468x + 16614. This is shown in figure no 3 and results were given in table no 3.

PRECISION

Repeatability or intra-day precision: The peak areas of 100ug/mL were analyzed on the same day by injecting it six times into the system. Inter-day precision: The peak areas of 100ug/ml were analyzed on other day by injecting it six times into the system. %RSD was calculated. The %RSD was found to be 0.0063&0.0029 respectively. Results were given in table no:4.

ACCURACY

Recovery of Guaifenesin was found to be 99.40% to 99.99%. The summary of % recovery of Guaifenesin was mentioned in Table 5.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

LOD and LOQ were calculated from linearity graph. The limit of Detection and limit of Quantification were found out to be 0.008µg/mL and 0.025µg/mL respectively.

ROBUSTNESS & RUGGEDNESS

Robustness studies were performed by changing the flow rate (±2 ml/min), column temperature (±5^{o}C) and mobile phase ratio. No significant effect was observed on system suitability parameters deliberate change such as resolution, RSD, tailing factor, or the theoretical plates of Guaifenesin. Thus, the method was found to be robust with respect to variability in applied conditions.

ASSAY

Tablet solution was injected into the HPLC system for six times and % assay of drug was found to be99.99%. These results were tabulated in Table No 6.

CONCLUSION

An easy, rapid and efficient Reverse-Phase HPLC method was developed for quantitative estimation of Guaifenesin in drug product and drug substance. The method was validated as per ICH Q2 (R1) guidelines. A precise, accurate, linear, robust and rugged method was found during validation. Limits of detection 0.008µg/mL and limits of quantification 0.025µg/mL also determined. By performing the assay of Barkeit tablet the percentage purity was found to be 99.99%. Hence it was concluded that this method is useful for the determination of both pharmaceutical substance and pharmaceutical product.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors have no conflict of interests to disclose other than what has been acknowledged above.

Table 1: Optimized conditions of Guaifenesin

S.NO	PARAMETER	RESULTS
1	Mobile phase	Methanol :water (50:50)
2	Stationary phase	CAPCELL C18 Column
3	Flow rate	1ml/min
4	Injection volume	20 µ lit
5	λmax	209nm
6	Running time	10
7	Retention time	5.449
8	Area	430559
9	Tailing factor	1.387

Table 2: System Suitability

Results of Guaifenesin

S.no	Area	Tailing Factor
1	661794	5.449
2	661763	5.449
3	661797	5.449
4	661752	5.449
5	661689	5.449
6	661795	5.449
Avg	**661765**	
SD	**41.74**	
%RSD	**0.0063**	

Table 3: Linearity Results of Guaifenesin

Conc	Area	Average
50 µg/ml	328248	328881.3
	329148	
	329248	
75 µg/ml	498247	495561.3
	489195	
	499242	
100 µg/ml	661725	661729.3
	661768	
	661695	
125 µg/ml	829373	829379.6
	829292	
	829474	
150 µg/ml	985374	985380.6
	985294	
	985474	

Table 4: Intra-day& Inter day Precision results of Guaifenesin

S.No	Intra-day Precision		Inter-day Precision	
	RT (min)	Area	RT (min)	Area
1	4.034	661794	4.034	661814
2	4.034	661763	4.034	661793
3	4.034	661797	4.034	661827
4	4.034	661752	4.034	661792
5	4.034	661689	4.034	661819
6	4.034	661795	4.034	661775
Avg		661765	661803.3	
SD		41.74	19.78	
%RSD		0.0063	0.0029	

Table 5: Accuracy results of Guaifenesin

Conc Level	Area	STD Area	Conc added	Conc Recovery	% Recovery	Avg
50%	328248	661765	50	49.60	99.20	99.55
	329148	661765	50	49.74	99.48	
	329248	661765	50	49.75	99.51	
100%	661725	661765	100	99.99	99.99	
	661768	661765	100	100.00	100.00	
	661695	661765	100	99.99	99.99	
150%	985374	661765	150	148.90	99.27	
	985294	661765	150	148.89	99.26	
	985474	661765	150	148.92	99.28	

Table 6: Assay Results of Guaifenesin

S.No	Sample Area	Standard Area
1	661684	661794
2	661813	661763
3	661792	661797
4	661622	661752
5	661789	661689
6	661835	661795
Avg	**661755.833**	**661765**
% Assay	**99.99**	

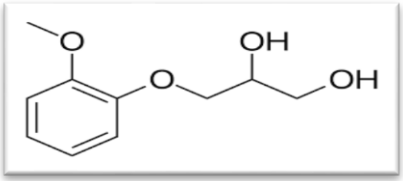

Fig. 1: Structure of Guaifenesin

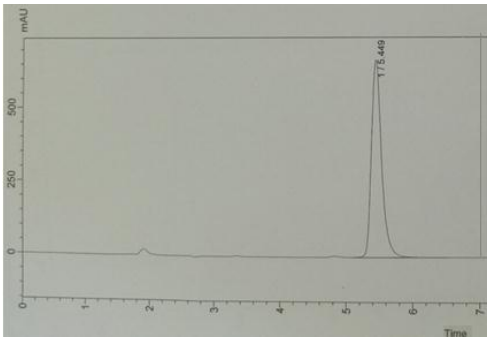

Fig. 2: Optimized Chromatogram of Guaifenesin

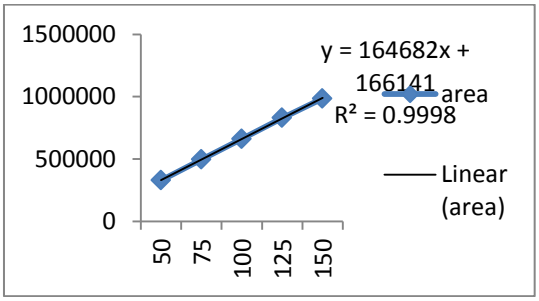

Fig. 3: Linearity Graph of Guaifenesin

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