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Method development and method validation of guaifenesin and dextromethorphan by RP-HPLC

P. Salomi*[1], D. Mahendra Nayak[1], T. Vimalakannan[1], K. Ravindra Reddy[2]

[1]Department of Pharmaceutical Analysis, P. Rami Reddy Memorial college of pharmacy, Kadapa, India.
[2]Department of Pharmaceutics, P. Rami Reddy Memorial college of pharmacy, Kadapa, India.

ABSTRACT

A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of bulk and pharmaceutical formulations. Separation of Guaifenesin and Dextromethorphan was successfully achieve THERMO, C18, 250X4.6mm, 5µm or equivalent in an isocratic mode utilizing 0.1M KH_2PO_4: Methanol (60:40) at a flow rate of 1.0ml/min and eluate was monitored at 280nm, with a retention time of 3.259 and 4.164 minutes for Guaifenesin and Dextromethorphan respectively. The method was validated and there response was found to be linear in the drug concentration range of 50µg/ml to150 µg/ml for Guaifenesin and 50µg/ml to150 µg/ml for Dextromethorphan. The values of the correlation coefficient were found to 0.999 for Guaifenesin and 1for Dextromethorphan. respectively. The LOD and LOQ for Guaifenesin were found to be 0.597 and 1.991 respectively. The LOD and LOQ for Dextromethorphan were found to be 0.1072 and 0.3572 respectively. This method was found to be good percentage recovery for were found to be 99 and 100 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Accuracy, Precision, Specificity and Robustness.

Keywords: Guaifenesin; Dextromethorphan; RP-HPLC.

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Corresponding Author

Name: P. Salomi
Email: rayofhope01@gmail.com
Contact: +916301233512

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INTRODUCTION

In order to develop a simple, reliable and an accurate method development and validation of Gauifenes in and Dextromethorphan in pharmaceutical dosage form by Reverse phase HPLC and validate the method for its repeatability and reproducibility

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.[8] Thus, it may increase the efficiency of the cough reflex and facilitate removal of the secretions.

Guaifenesin is an expectorant commonly used in performance horses to aid in the clearance of mucus from the airways. Guaifenesin is also a centrally acting skeletal muscle relaxant and as such is a prohibited drug with withdrawal necessary prior to competition. To the authors' knowledge, there are no reports in the literature describing single or multiple oral administrations of guaifenesin in the horse to determine a regulatory threshold and related withdrawal time.[9] Therefore, the objective of the current study was to describe the pharmacokinetics of guaifenesin following oral administration in order to provide data upon which appropriate regulatory

recommendations can be established. Nine exercised Thoroughbred horses were administered 2 g of guaifenesin orally BID for a total of five doses. Blood samples were collected immediately prior to drug administration and at various times post administration. Serum guaifenesin concentrations were determined and pharmacokinetic parameters calculated. Guaifenesin was rapidly absorbed (Tmax of 15 min) following oral administration. The Cmax was 681.3 ± 323.8 ng/mL and 1080 ± 732.8 following the first and last dose, respectively. The serum elimination half-life was 2.62 ± 1.24 h. Average serum guaifenesin concentrations remained above the LOQ of the assay (0.5 ng/mL) by 48 h post administration of the final dose in 3 of 9 horses.[18]

At therapeutic doses, dextromethorphan acts centrally (meaning that it acts on the brain) as opposed to locally (on the respiratory tract). It elevates the threshold for coughing, without inhibiting ciliary activity. Dextromethorphan is rapidly absorbed from the gastrointestinal tract and converted into the active metabolite dextrorphan in the liver by the cytochrome P450 enzyme CYP2D6. The average dose necessary for effective antitussive therapy is between 10 and 45 mg, depending on the individual. The International Society for the Study of Cough recommends "an adequate first dose of medication is 60 mg in the adult and repeat dosing should be infrequent rather than the qds recommended."[27]

DXM has an elimination half-life of approximately 4 hours in individuals with an extensive metabolizer phenotype; this is increased to approximately 13 hours when DXM is given in combination with quinidine.[20] The duration of action after oral administration is about three to eight hours for dextromethorphan hydrobromide, and 10 to 12 hours for dextromethorphan polistirex. Around one in 10 of the Caucasian population has little or no CYP2D6 enzyme activity, leading to long-lived high drug levels

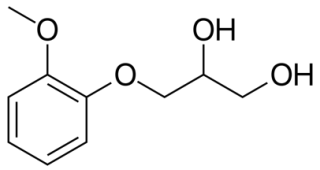

Figure 1: Structure of Guaifenesin

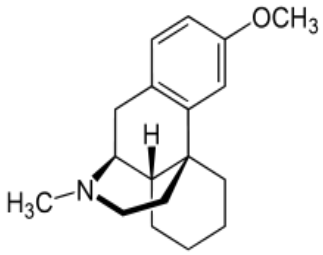

Figure 2: Structure of Dextromethorphan

MATERIALS AND METHODS

Chemical and Reagents

Guaifenesin and Dextromethorphan were kindly gifted by Pvt Ltd, Hyderabad certified to contain 99.9% and 99.7% purity respectively. The drugs were used without further purification. All the solvents used in analysis were of HPLC grade.

HPLC method

Instrument

Waters HPLC, Model: Waters 2695, Photo diode array detector (PDA), with an automated sample injector. The output signal was monitored and integrated using Empower 2 software. THERMO, C18, 250X 4.6mm, 5µm,column was used for separations.

Optimized Chromatographic conditions

Mobile Phase: KH_2PO_4: Methanol (60:40)
Column : THERMO, C18, 25cmx4.6mm, 5µm
Flow Rate : 1.0ml/Min
Temperature : 25˚C
Volume : 10µl
Run time : 10min
Detector : 280

Preparation of Mobile Phase

Transfer 1000ml of HPLC water into1000ml of beaker add 0.1M KH_2PO_4. Transfer the above prepared KH_2PO_4 buffer and Methanol is mixed in the proportion of (60:40). They are mixed and sonicated for 20min.

Preparation of the Guaifenesn and Dextromethorphan standard and sample solution

Standard Solution Preparation

Accurately weigh and transfer 400.00 of Guaifenesin and 20.00 Dextromethorphan 100ml of volumetric flask and add 10ml of Methanol and sonicate 10min (or) shake 5min and make with water. Transfers the above solution into 1ml into 10ml volumetric flask dilute to volume with water.

Preparation of sample stock solution

Commercially available 20 tablets ware weighed and powdered the powdered equivalent to the 980.00 mg of Guaifenesin and Dextromethorphan of active ingredients were transfer into a 100ml of volumetric flask and add 10ml of Methanol and sonicate 20min (or) shake 10min and makeup with water. Transfers above solution 1ml into 10ml of the volumetric flask dilute the volume with Methanol. And the solution was filtered through 0.45µm filter before injecting into HPLC system.

Recovery studies

To check the accuracy of sample by the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 50, 100 and 150% level. From the total amount of drug found, the percentage recovery was calculated.

RESULTS AND DISCUSSION

HPLC Method Validation

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, Specificity, precision, limit of detection, limit of quantitation

Specificity

Solution of standard, sample, blank and placebo were prepared as per test procedure and injected into the HPLC system.

Acceptance criteria: Chromatogram of standard and sample should be identical with near Retention time.

Blank interference: A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure.

Acceptance criteria: Chromatogram of blank should not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte. Hence the method is specific.

Linearity

Prepare a series of standard solutions and inject into HPLC system. Plot the graph of standard versus the actual concentration in µg/ml and determine the coefficient of correlation and basis for 100% response.

Acceptance criteria: Linearity regression coefficient of average peak area response of replicate injections plotted against respective concentration should not be less than 0.999. The % y-intercept as obtained from the linearity data (without extrapolation through origin 0, 0) should be within ±2.0.

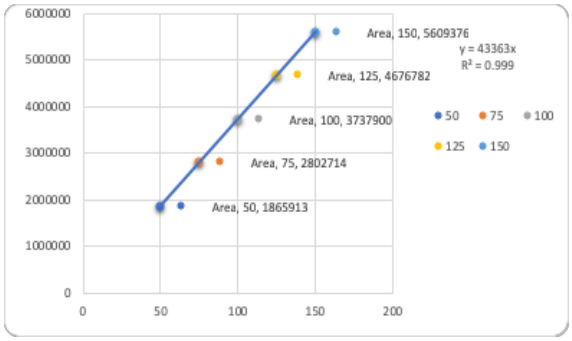

Figure 3: Linearity plot of Guaifenesin

Table 1: Linearity data for Dextromethorphan

S.no	Conc (µg/ml)	RT	Area
1	50	3.226	1865913
2	75	3.234	2802714
3	100	3.238	3737900
4	125	3.243	4676782
5	150	3.246	5609376
Correlation coefficient (r^2)			0.999

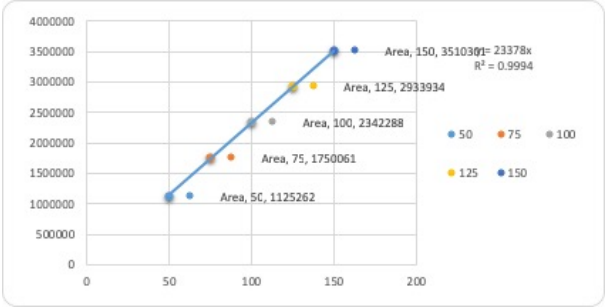

Figure 4: Linearity data of Dextromethorphan

Table 2: Linearity data of Dextromethorphan

S.no	Conc(µg/ml)	RT	Area
1	50	4.078	662944
2	75	4.086	9933167
3	100	4.095	1328163
4	125	4.099	1651197
5	150	4.098	1981291
Correlation coefficient (r^2)			1.0

Table 3: Linearity data for Guaifenesin

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5	150	4.098	1981291
Correlation coefficient (r^2)			1.0

Statistical Evaluation: A graph between the concentration and the average area was plotted. Points for linearity were observed. Using the method of least squares, a line of best fit was taken and the correlation Coefficient, slope and, y-intercept were calculated.

Precision

Preparation of sample: Transfer the 980mg of sample into a 100ml of volume at flask and add 10ml of Methanol and sonicate 20min and makeup with methanol. Transfer the above solution into 1ml into 10ml volume metric flask dilute to the volume with water.

The method precision parameters were evaluated from sample chromatograms obtained, by calculating the % RSD of peek areas from 6 replicate injections.

Table 5: Precision data for Guaifenesin

S.no	RT	Area	%Assay
Injection1	3.250	3739051	100
Injection2	3.250	3739650	100
Injection3	3.242	3732973	99
Injection4	3.242	3732125	99
Injection5	3.246	3737009	100
Injection6	3.243	3735485	100
Mean			100
Std. Dev.			0.8
% RSD			0.8

Acceptance criteria: The injection reproducibility requirements are met if the %RSD for peak areas is not more than 2.0 and for retention times is not more than 2.0.

Table 6: Precision data for Dextromethorphan

S.no	RT	Area	%Assay
Injection 1	4.134	1322868	100
Injection 2	4.132	1326738	100
Injection 3	4.116	1321671	100
Injection 4	4.118	1322735	100
Injection 5	4.122	1321325	100
Injection 6	4.113	1320900	100
Mean			100
Std. Dev.			0.16
%RSD			0.16

Recovery/accuracy

Recovery study can be performed in the concentration range of 80% to 120% of the target concentration of the test. Minimum 3 concentrations are recommended.

Acceptance criteria: The average percentage recovery was between 98-102% and Relative standard deviation of these recovery concentrations was less than 2%.

Table 7: Accuracy data for Guaifenesin

S.NO	Accuracy Level	Injection	Sample area	RT
1	50%	1	1866611	3.234
		2	1865356	3.233
		3	1865316	3.233
2	100%	1	3734210	3.244
		2	3738762	3.240
		3	3739706	3.241
3	150%	1	5607485	3.244
		2	5601456	3.248
		3	5605099	3.248

Table 8: Accuracy data for Dextromethorphan

S.NO	Accuracy level	Injection	Sample area	RT
1	50%	1	662941	4.102
		2	662147	4.099
		3	662043	4.095
2	100%	1	1325072	4.105
		2	1326464	4.100
		3	1326207	4.099
3	150%	1	1980940	4.102
		2	1980145	4.103
		3	1982320	4.105

Limit of detection

The sensitivity of measurement of Guaifenesin and Dextromethorphan by use of proposed method was estimated in terms of the limit of detection (LOD). The LOD was calculated by the use of signal to noise ratio. In order to estimate the LOD value, the blank sample was injected six times and peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level.

Table 9: LOD data for Guaifenesin and Dextromethorphan

S.no	Sample name	RT	Area
1	Guaifenesin	3.218	168283
2	Dextromethorphan	4.071	58818

Limit of quantitation

The sensitivity of measurement of Guaifenesin and Dextromethorphan by the use of proposed method was estimated in terms of limit of quantitation (LOQ). The LOQ was calculated by the use of signal to noise ratio. In order to estimate the LOQ value, the blank sample was injected six times and the peak area of this blank was calculated at noise level. The LOQ was calculated as ten times the noise value gave the LOQ.

Table 10: LOQ data for Guaifenesin and Dextromethorphan

S.no	Sample name	RT	Area
1	Guaifenesin	3.218	568444
2	Dextromethorphan	4.070	197597

Robustness

Effect of variation in flow rate: Prepare the system suitability solution as per the test method and inject into the HPLC system with ±0.2 ml of the method flow. Evaluate the system suitability values as required by the test method for both flow rates. Actual flow rate was 1.0 ml/min and it was changed to 0.8ml/min and 1.2ml/min and inject into HPLC and system suitability was checked.

Effect of variation in wavelength: Prepare the system suitability solution as per the test method and injected into the HPLC with ±2nm variation in wavelength. Evaluate the system suitability values as required by the test method for both wavelengths.

Table 11: System suitability data of Guaifenesin and Dextromethorphan

Parameter	Guaifenesin	Dextromethorphan	Acceptance criteria
Retention time	3.259	4.164	+-10
Theoretical plates	7596	5595	>2500
Tailing factor	1.45	1.53	<2.00
% RSD	0.2	0.5	<2.00

Table 12: Standard Results of Guaifenesin

S.no	Sampl name	RT	Area	USP plate count	USP tailing
1.	Injection1	3.255	3751177	7555	1.44
2.	Injection 2	3.256	3733409	7640	1.46
3.	Injection 3	3.254	3739270	7616	1.46
4.	Injection 4	3.252	3731263	7697	1.45
5.	Injection 5	3.250	3739890	7612	1.45

Table 13: Standard Results of Dextromethorphan

S.no	Sample name	RT	Area	USP plate count	USP tailing
1.	Injection 1	4.157	1335256	5643	1.53
2.	Injection 2	4.153	1319961	5603	1.52
3.	Injection 3	4.149	1325705	5618	1.53
4.	Injection 4	4.142	1325466	5738	1.53
5.	Injection 5	4.138	1317842	5706	1.53

Table 14: Specificity data for Guaifenesin and Dextromethorphan

S no	Sample name	Guaifenesin area	RT	Dextromethorphan Area	RT
1	Standard	3726649	3.359	1341704	4.164
2	Sample	3739051	3.250	1322868	4.134
3	Blank	-	-	-	-
4	Placebo	-	-	-	-

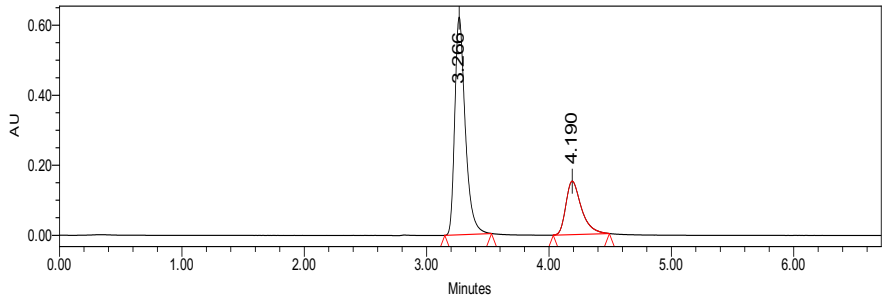

Figure 5: Typical chromatogram of optimized method

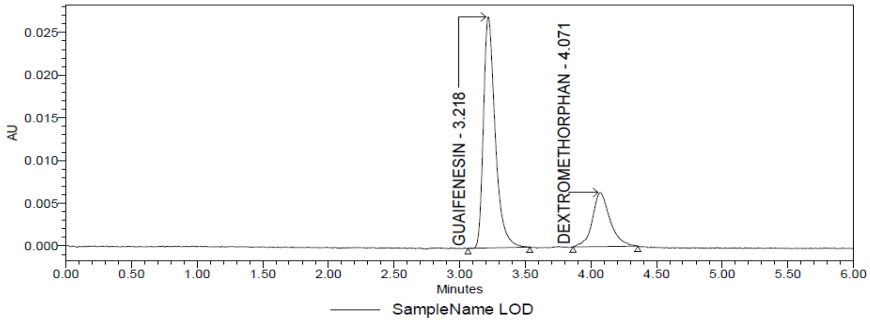

Figure 6: LOD data for Guaifenesin and Dextromethorphan

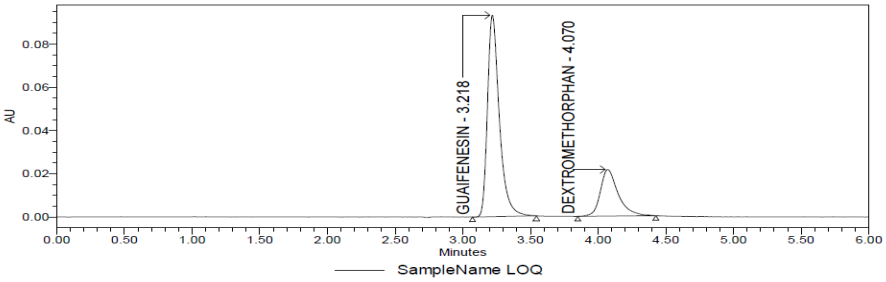

Figure 7: LOQ data for Guaifenesin and Dextromethorphan

Table 15: Robustness data for Guaifenesin

Parameter	RT	Theoretical plates	Tailing Factor
Decreased flow rate(0.8ml/min)	4.034	8447	1.49
Increased flow rate(1.2ml/min)	2.693	7155	1.46
Decreased temperature(20°c)	4.037	8570	1.49
Increased temperature(30°c)	2.701	7237	1.47

Table 16: Robustness data for Dextromethorphan

Parameter	RT	Theoretical plates	Tailing factor
Decreased flow rate (0.8ml/min)	5.054	5355	1.56
Increased flow rate (1.2ml/min)	3.404	5940	1.54
Decreased temperature (20°c)	5.058	5451	1.56
Increased temperature (30°c)	3.437	6019	1.55

CONCLUSION

The study is focused to develop and validate HPLC methods for estimation of Guaifenesin and Dextromethorphan in tablet dosage form. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Guaifenesin and Dextromethorphan.

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