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Determination of Guaifensen and Dextromethorphan by UPLC

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ABSTRACT

A new HPLC method was developed for selective and simultaneous determination of Guaifensen and Dextromethorphan HBr tablets 1200/60 mg. The chromatographic separation was achieved on a Acquity UPLC BEH, C18,100 x 2.1 mm, 1.7um column. The mobile phase consisted of buffer, acetonitrile and methanol delivered at a flow rate of 0.15 ml/min and dual wavelength at 271 nm for Guaifensin and 220 nm for Dextromethorphan with UV detector. Accuracy found by % recovery from 98.2 to 102.2 at 50 to 150% level and the linearity results for Guaifensen and Dextromethorphan HBr in the specified concentration calibration curves linear with coefficient of variation not less than 0.99. The proposed was found to be specificity, linearity, and precision, Intermediate precision and accuracy, stability of analytical solution and robustness. The validation was performed according to the ICH guide lines.

Keywords: Guaifensen, Dextromethorphan, UPLC.

INTRODUCTION

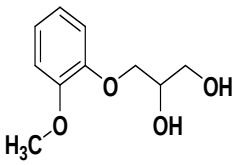


Fig-1: (RS)-3-(2-methoxyphenoxy)propane-1, 2- diol

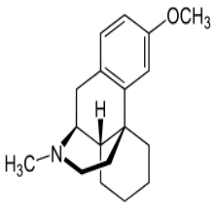


Fig-2: (+)-3-methoxy-17-methyl-(9a,13a,14a)-morphinan

Guaifenesin or guaiphenesin **(fig.-1)**, is an expectorant drug sold over the counter and generally taken orally to assist the bringing of phlegm from the airways in acute respiratory tract infections [1]. Guaifenesin was first approved by the Food and Drug Administration (FDA) in 1952. Guaifenesin is sold as pills or syrups under many brand names. Single-ingredient formulations of guaifenesin are available, and it is also included in many other over-the-counter cough and cold remedy combinations, for example it is generally conjugation with Dextromethorphan or acetaminophen. Guaifenesin was useful in the treatment of primary dysmenorrheal [2]. It was also used for treatment of coughing [3], asthma [4], gout [5] and fibromyalgia [6]. Consumption of guaifenesin in above-normal quantities has the potential to cause side-effects. Known side-effects include nausea, vomiting, and rarely the formation of kidney stones of uric acid [7].

Several methods describing the simultaneous determination of a wide variety of active compounds in various cough-cold formations have been reported. The commonly used cough syrup ingredients are a cough suppressant mean, an expectorant, a preservative and as part of the excipients sweeteners, acidulants and natural or artificial coloring and flavoring agents. These compounds are contained in the pharmaceutical form in very different proportions and present chemical forms of very different nature. All these aspects increase the analytical problem.

Previous HPLC methods have measured these compounds either individually or in combination. Simultaneous HPLC assays have been described for pseudoephedrinedextromethorphan [8–10], pseudoephedrine - codeine [11] and guaifenesin–pseudoephedrine–dextromethorphan [12] usually along with other components. The determination of guaifenesin–pseudoephedrine [13–15] and guaifenesin–codeine [16,17] was also reported, however, the procedures required the use of more than one column or mobile phase or an increased flow rate which can be time-consuming and too costly. In this report, first time we have introduced a new method for the simultaneous determination of Guaifenesin- Dextromethorphan by using HPLC with economically feasible and very convenient method.

MATERIALS AND METHODS

Reagents and Chemicals

Guaifenesin (**Fig-1**) and Dextromethorphan (**Fig-2**) procured from Pragathi organics Limited, Hyderabad, India. HPLC grade acetonitrile, Methanol (HPLC grade), Analytical grade Sodium perchlorate and analytical grade potassium Di-hydrogen phosphate purchased from Fine Chemicals, India. All the aqueous solutions including buffer for the mobile phase, were prepared with Milli Q grade water (milli-Q) from Millipore (USA) equipment.

Apparatus and Chromatographic conditions

An Waters UPLC is comprised of a degassing unit, ultra fast auto sampler, column oven, and a UV-VIS dual wavelength detector was used for chromatographic separation, a Acquity UPLC BEH, C_{18}, 100*2.1mm,1.7µ column and the detection of the compounds was monitored at 271 nm for Guaifensen and 220 nm for Dextromethorphan. A buffer consisting of 20 mM Potassium dihydrogen phosphate buffer and 8mM Sodium perchlorate (pH 3.0 $\pm$ 0.1). Mobile phase -A consisting of buffer and acetonitrile in a ratio of 90:10 v/v and Mobile phase-B consisting of buffer and methanol in a ratio of 20:80 v/v was used at a rate of 0.15mL/min.

Column oven temperature maintained at 45°C. Injection volume was 3 µL with partial loop with needle overfill. The gradient programme shown in **Table-1**.

Table-1: Gradient programme

Time in minutes	Mobile phase A	Mobile phase B
0	80	20
5	0	100
7	0	100
8	80	20
10	80	20

Preparation of diluent

Prepared a mixture of water: Methanol: Acetonitrile in the ratio of 60:20:20 ml v/v respectively and degased.

Preparation of Dextromethorphan Standard Stock

Transferred accurately weighed amount of about 50 mg of Dextromethorphan HBr working standard into a 250 ml volumetric flask, added 150 ml of diluent and sonicated to dissolve the material completely. Made up to the volume with diluent and mixed well.

Preparation of Guaifensin Standard Stock

Transferred accurately weighed amount of about 90 mg of Guaifensin working standard into a 50 ml volumetric flask, added 30 ml of diluent and sonicated to dissolve the material completely. Made up to the volume with diluent and mixed well.

Standard preparation
Transferred 5 ml of Dextromethorphan standard stock and 10 ml of Guaifensin standard stock in to 100 ml volumetric flask then dilute to volume with diluents and mixed well.

Test preparation for 1200/60 mg
Weighed 20 tablets and take the average weight. Crushed the tablets in mortar and pestle to a fine powder. Weighed and transferred the sample equivalent to 1800 mg of Guaifensin into a 250 ml volumetric flask added about 50 ml of methanol and sonicated for 5 minutes then added 100 ml of diluent and sonicated for 45 minutes (Until no lumps observed),dilute to volume with diluent and mixed well. Centrifuge a portion of the above solution with cap at 4000 RPM for 10 minutes.

Note: Maintained the temperature of water in sonicator bath between 20°C to 25°C.

RESULTS AND DISCUSSION

In this work we developed a simple, gradient accurate and sensitive UPLC method for the simultaneous determination of Guaifensin and Dextromethorphan in their fixed dose combination. Initially various mobile phase and stationary phase were tested to obtain the best separation and resolution between Guaifensin and Dextromethorphan. A mobile phase consisting of a mixture of buffer acetonitrile and Methanol and Acquity UPLC BEH, C18, 100*2.1 mm, 1.7µ column were found to be the most appropriate for the separation of both the components at the flow rate of 0.15 mL/min. Using the mentioned chromatographic conditions, well resolved sharp peaks can be obtained at retention time of 4.2 and 5.4 min for Guaifensin and Dextromethorphan respectively but these two Guaifensen and Dextromethorphan were determined at two different λ max. (Guaifensen at 271 nm and Dextromethorphan at 220 nm). The chromatograms of standard and tablet solutions of Guaifensin and Dextromethorphan are shown in **Fig-3, 4, 5**, and **6** respectively.

Method development was started with different mobile phase with changing concentrations of acetonitrile, (Less polar mobile phase 40% acetonitrile) and buffers however broadening peaks could be obtained. The chromatographic conditions were optimized for the simultaneous determination of Guaifensin and Dextromethorphan within a short analysis time (<10 min). To accomplish these objectives, the chromatographic column was first chosen based on peak shapes and resolution. In preliminary experiments, the sample retention time increased with a decrease in concentration of acetonitrile and resulted in peak overlap between them, in order to avoid long run times and overlaps. The polarity of the mobile phase was then increased by the increase in concentration of Acetonitrile, Methanol and addition of buffer. The optimum mobile phase composition was found to be in the ratio of 90:10, (v/v) for 20 mM potassium dihydrogen phosphate + 8 mM Sodium perchlorate (pH-3.0) and acetonitrile this is considered as mobile phase –A and A ratio of 20:80, (v/v) for 20 mM potassium dihydrogen phosphate + 8 mM of Sodium perchlorate (pH-3.0) and methanol this is considered as mobile phase – B resulted in good separation and sharp peaks.

Method Validation
The developed chromatographic method for the simultaneous determination of Guaifensin and Dextromethorphan was validated using ICH guidelines. Validation parameters performed include linearity, limit of detection /quantitation, specificity, accuracy, precission and robustness of solutions.

System suitability
The system suitability was assessed using three replicate analysis of drugs at concentration of 180ug/mL for Guaifensin and 10 ug/mL for Dextromethorphan. The system suitability parameters like number of theoretical plates, asymmetric factor were presented in **Table-2.**

Tabke-2: System suitability data for mixed standard solution

Parameter	Guaifensin	Dextromethorpahn
Theoretical plates	39878	68141
Tailing factor	1.1	1.2

Precision

The method precision (repeatability) and intermediate precision of the method was determined by analysis of six samples in terms of %RSD. Bench top stability of Guaifensin and detxtromethorphan HBR (Ruggedness) was determined by analysis of standard and test preparation (in duplicate) at initial after 1day, 2days and 5 days on bench top. The results of precision and intermediate precision results are given in **Table-3** & **4**.

Table-3: Method Precision Results

S.No.	%Assay of Guaifensin 1200mg	%Assay of Dextromethorphan HBr – 60 mg
1	98.5	102.1
2	98.6	102.2
3	98.2	101.8
4	99.0	102.7
5	98.3	101.9
6	99.0	102.6
Average	98.6	102.2
% RSD	0.4	0.4

Table–4: Intermediate Precision Results

S.No.	%Assay of Guaifensin 1200mg	%Assay of Dextromethorphan HBr – 60 mg
1	98.2	101.8
2	97.8	101.8
3	98.5	102.2
4	97.9	101.5
5	98.3	101.7
6	97.8	101.7
Average	98.1	101.8
% RSD	0.3	0.2

Bench top stability results of Guaifensen and Dextromethorphan are presented in **Table-5 &6**.

Table-5: Bench top stability results of Guaifensen

Time in days	Standard Similarity factor	% Assay of test preparation		Difference	
		Test - 1	Test - 2	Test - 1	Test - 2
Initial	NA	97.5	98.3	NA	NA
1	0.98	100.0	98.5	2.5	0.2
2	1.01	98.3	99.5	0.8	1.2
5 Days	0.99	97.1	98.0	0.4	0.3

Table-6: **Bench top stability results of Dextromethorphan**

Time in days	Standard Similarity factor	% Assay of test preparation		Difference	
		Test - 1	Test - 2	Test - 1	Test - 2
Initial	NA	101.1	101.9	NA	NA
1	0.98	102.8	101.2	1.7	0
2	1.01	100.8	101.7	0.3	0.2
5 Days	0.99	101.8	102.6	0.7	0.7

Linearity

Linearity was assessed by performing single measurement at different analyte concentration varying quantities of stock standard solution diluted with the mobile phase to give concentrations in the range of 63-297 µg/mL for Guaifensin and 3.0-15 µg/mL for Dextromethorphan HBr. Good linearity was observed over the range for both Guaifensin and Dextromethorphan. The calibration curve was made by using the concentration of the anlytes versus peak area. The linear coefficient of correlation for Guaifensin was 0.9996 and the linear coefficient of correlation for Dextromethorphan was 1.0000.

Accuracy (Recovery)

The accuracy of the test method was determined by the addition of known quantities of Placebo and API at various concentrations ranging from 50% of the target initial test concentration to 150% of the target initial test

concentration. The % recovery range and relative standard deviation for Guaifensin was found to be 98.2-99.3 and 0.1–0.7 respectively. The % recovery range and relative standard deviation for Dextromethorphan was found to be 99.7-102.2 and 0.5-0.8 respectively.

Fig-3: Standard chromatogram of Guaifensen

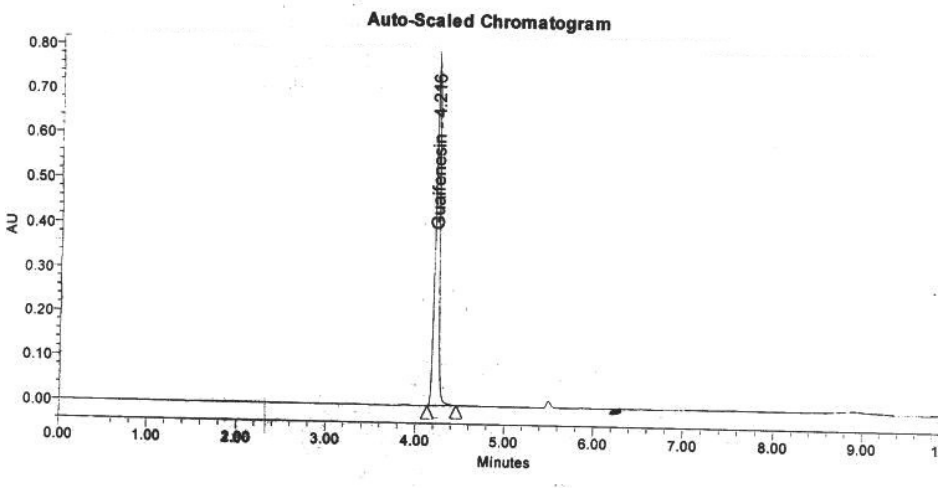


Fig-4: Sample chromatogram of Guaifensen

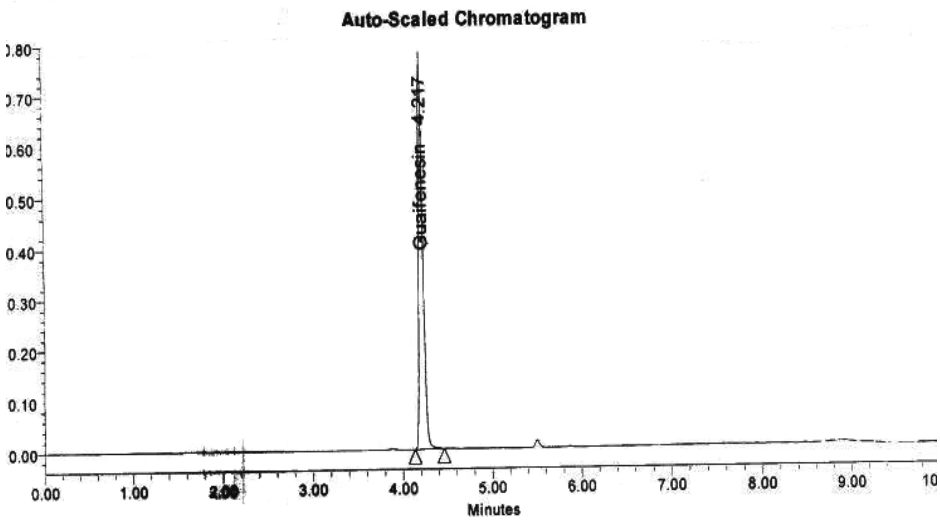


Table-7: Robustness study of Guaifensin

Conditions	Tailing factor	Theoretical plates
Flow rate (0.12 mL)	1.1	36489
Flow rate (0.18 mL)	1.1	37895
Temperature at 40°C	1.1	38975
Temperature at 50°C	1.1	36875
pH of mobile phase B (2.8)	1.1	36705
pH of mobile phase B (3.2)	1.1	38003
pH of mobile phase A (2.8)	1.1	34142
pH of mobile phase A (2.8)	1.1	37825
% Acetonitrile mobile phase A (90%)	1.1	41407
% Acetonitrile mobile phase A (110%)	1.1	34355
% Methanol mobile phase B (90%)	1.1	40289
% Methanol mobile phase B (110%)	1.1	33598

Table-8: Robustness study of Dextromethorphan HBr

Conditions	Tailing factor	Theoretical plates
Flow rate (0.12 mL)	1.2	69829
Flow rate (0.18 mL)	1.2	68870
Temperature at 40°C	1.2	65897
Temperature at 50°C	1.2	66895
pH of mobile phase B (2.8)	1.2	66789
pH of mobile phase B (3.2)	1.2	67280
pH of mobile phase A (2.8)	1.2	67889
pH of mobile phaseA (2.8)	1.2	69840
% Acetonitrile mobile phase A (90%)	1.2	66611
% Acetonitrile mobile phase A (110%)	1.2	67385
% Methanol mobile phase B (90%)	1.2	74839
% Methanol mobile phase B (110%)	1.2	60890

Fig.5: Standard chromatogram of Dextromethorphan

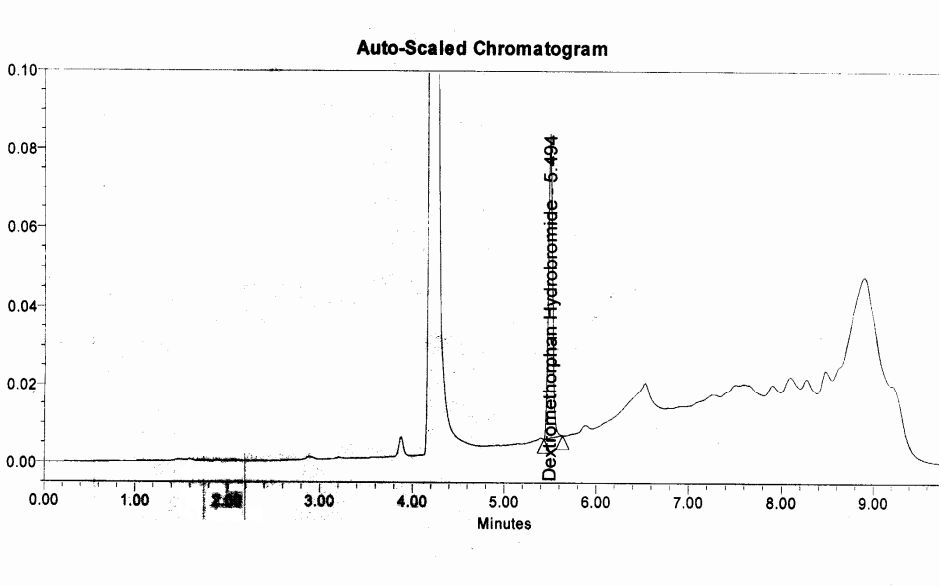
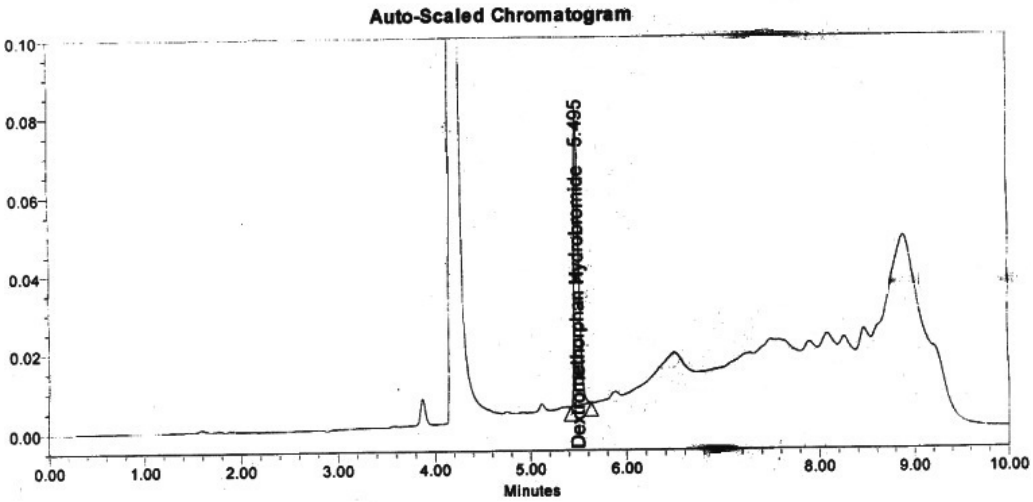


Fig.6: Sample chromatogram of Dextromethorphan



Robustness

Robustness of the method was performed by intentionally but slightly modifying the chromatographic conditions such as flow rate, pH of the mobile phase and organic solvent composition. The results showed that the slight

change in the chromatographic conditions had no pronounced effects on the chromatographic parameters. The robustness study results of Guaifensin and Dextromethorphan are given in **Table –7 & 8.**

CONCLUSION

A rapid, simple and specific reverse phase UPLC method has been developed for simultaneous determination of Guaifensin and Dextromethorphan from tablet dosage form. The method was validated for accuracy, precession, linearity, robustness and ruggedness. It is concluded that the new UPLC method was suitable for the simultaneous determination of Guaifensin and Dextromethorphan in the pharmaceutical formulations with low cost and less time (less than 10 min).

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