RP-HPLC Determination Of Paracetamol, Guaifenesin And Dextromethorphan In Liquid Dosage Form

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ABSTRACT

A simple, economical, rapid, reliable and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous estimation of paracetamol, guaifenesin and dextromethorphan in syrup dosage form. The separation was achieved on a Phenomenex C_{18} (250×4.6 mm) 5μ column in isocratic mode with water: methanol: glacial acetic acid (60:38.5:1.5 v/v) as mobile phase at a flow rate of 1.5ml/min. The detection was carried out at 276nm. The retention time of paracetamol, guaifenesin and dextromethorphan were found to be 3.20, 5.80 and 9.45 min, respectively. This developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for the estimation of these drugs in combined dosage forms in routine analysis. © 2007 Trade Science Inc. - INDIA

INTRODUCTION

Paracetamol[1] is used as an analgesic and antipyretic. Guaifenesin[2] is used as an expectorant. Dextro-methorphan[3] is methylmorphinan hydro bromide monohydrate is used as an antitussive. Many methods have been described in the literature for the determination of paracetamol, guaifenesin and dextromethorphan individually and in combination with other drugs[4-15]. However, there is no HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms. Fixed dose combination containing 250mg of paracetamol, 100mg of guaifenesin and 10 mg of dextromethorphan is available in syrup form in the market. The aim of this work was to develop RP-HPLC method with

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ultraviolet detection for the simultaneous determination of paracetamol, guaifenesin and dextromethorphan in pharmaceutical dosage forms. The present RP-HPLC method was validated following the ICH guidelines[16, 17].

Reagents and instruments

Methanol HPLC grade was procured from E Merck (India) Ltd, Mumbai. Glacial acetic acid AR grade was procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. Reference standard of paracetamol and guaifenesin were procured from Mede rich sterilab, Bangalore and dextromethorphan was procured from Divi's lab, Hyderabad.

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), SPD M-10AVP photo diode array detector, Rheodyne 7725i injector with 20 μl loop volume. Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). A Phenomenex C_{18} (250 × 4.6mm i.d., 5μ) was used for the separation.

Mobile phase and standard preparation

The mobile phase is a mixture of water, methanol and glacial (60:38.5:1.5 v/v). It was filtered through a 0.2 μ membrane filter and degassed. Standard stock solutions of 1 mg/ml of paracetamol, guaifenesin and dextromethorphan were prepared separately using mobile phase. From the standard stock solution, mixed standard solution was prepared to contain 250 μg/ml of paracetamol, 100μg/ml of guaifenesin and 10 μg/ml of dextromethorphan. The mobile phase was delivered at a flow rate of 1.5 ml/min with detection at 276 nm. The injection volume was 20 μl; Analysis was performed at ambient temperature.

Assay

Accurately weighed quantity of the syrup preparation containing 250mg of paracetamol, 100mg of guaifenesin and 10 mg of dextromethorphan (TUSSEX-Medreich Ltd, Bangalore) were dissolved and made up to 100 mL with mobile phase. Further dilutions were made to obtain final concentration of

250 μg of paracetamol 100 μg of guaifenesin and 10 μg of dextromethorphan.

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of paracetamol, guaifenesin and dextromethorphan was found to be 3.20, 5.80 and 9.45 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The percentage drugs content were estimated using following formula:

$$\% \text{drug content} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard weight}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight}} \times \frac{\text{Volume taken}}{\text{Lable claim}} \times 100 \times \frac{\text{Weight}}{\text{mL}}$$

The typical chromatogram of sample solution and 3D spectrum is given in figure 1 and figure 3. Detection was done at 276 nm. The assay procedure was repeated for six times and percentage of individual drugs found in formulation, mean, standard deviation were calculated and presented in TABLE 1. The results of analysis shows that the amount of drugs recovered were in good agreement with the label claim of the formulation.

Validation

The method was validated as per ICH guidelines. The accuracy of the method was determined by re-
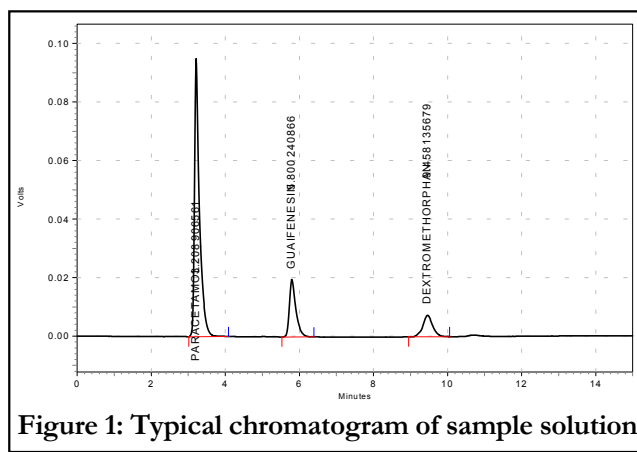

Figure 1: Typical chromatogram of sample solution

TABLE 1: Recovery of paracetamol

Label claim (mg)	Amount recovered(mg)*	%of recovery*
250(Paracetamol)	256.52±1.041	102.60±1.245
100(Guaifenesin)	102.49± 1.462	102.49±1.015
10(Dextromethorphan)	10.45 ± 1.724	104.5± 1.146

* Average of six determinations, mean ± standard deviation
TUSSEX Syrup -Medreich Ltd, Bangalore each 5ml containing 250 mg of Paracetamol,100 mg of Guaifenesin and 10 mg of Dextromethorphan hydro bromide
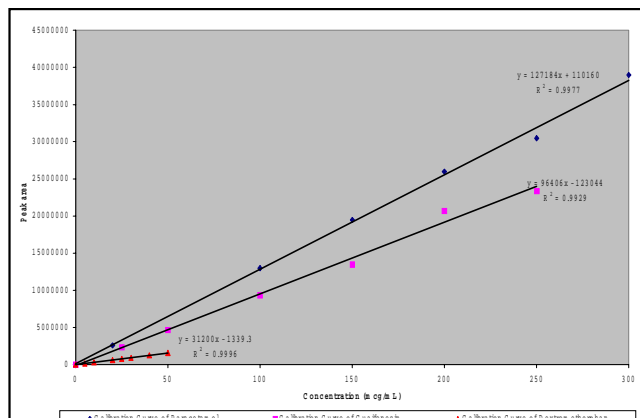
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Figure 2: Calibration curve of paracetamol, guaifenesin and dextromethorphan

Figure 3: 3d Spectrum of paracetamol, guaifenesin and dextromethorphan

covery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in TABLE 1. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated. From the data obtained, the developed HPLC method was found to be precise.

The linearity of the method was determined at different concentration levels ranging from 50 to 300 μg/ml for paracetamol, 25 to 250 μg/ml for guaifenesin and 5 to 50 μg/ml for dextromethorphan. The calibration curve was constructed by plotting peak area against concentration of drugs (Figure 2). The slope and intercept value for calibration curve was y =127184x+110160 (R^2=0.9977) for paracetamol, y=96406x+123044 (R^2=0.9929) for guaifenesin and y=31200x–1339.3 (R^2=0.9996) for dextromethorphan. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above.
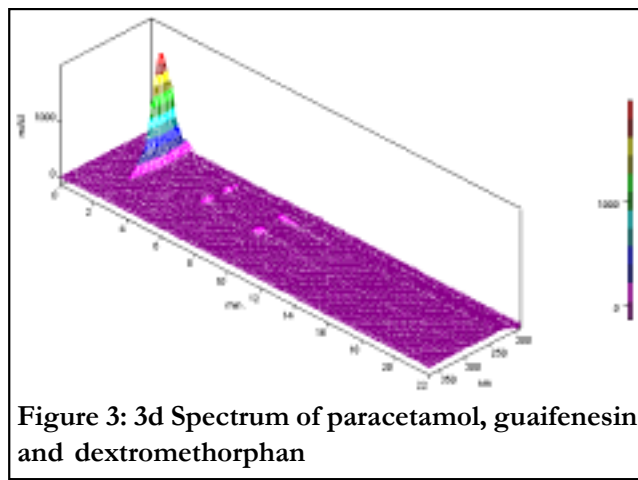
The ruggedness of the method was determined

by carrying out the experiment on different instruments like Shimadzu HPLC (LC-10AT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C$_{18}$, Phenomenex LUNA C$_{18}$ and Hichrom C$_{18}$. Robustness of the method was determined by making slight changes in the chromatographic conditions. No marked changes in the chromatograms demonstrated that the HPLC method developed is rugged and robust.

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h at room temperature. The results show that for both solutions, the retention time and peak area of paracetamol, guaifenesin and dextromethorphan remained almost unchanged (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 h, which was sufficient to complete the whole analytical process.

The system suitability studies were carried out to determine theoretical plate/meter, resolution factor, asymmetric factor and tailing factor. The results were given in the TABLE 2. The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

Thus the proposed RP-HPLC method for the simultaneous estimation of paracetamol, guaifenesin and dextromethorphan in combined dosage forms is

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TABLE 2: Validation and system suitability studies

S. No.	Parameters	Paracetamol	Guaifenesin	Dextromethorphan
1	Linearity range	50 to 300 μg/ml	25 to 250 μg/ml	5 to 50 μg/ml
2	Regression equation Y = mx + c*	y = 127184x +110160	y = 96406x -123044	y = 31200x -1339.3
3	Correlation coefficient	0.9977	0.9929	0.9996
4	Theoretical plate/meter	4562	5236	6841
5	Resolution factor	1.24	1.65	1.02
6	Asymmetric factor	0.89	1.05	1.15
7	Tailing Factor	1.2	1.0	1.0

accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

ACKNOWLEDGEMENT

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