

Quantization of Dextromethorphan and Levocetirizine in Combined Dosage Form Using a Novel Validated RP-HPLC Method

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Joshi, *et al.*: RP-HPLC for Dextromethorphan and Levocetirizine

The present study reveals a simple isocratic RP-HPLC method for the simultaneous determination of dextromethorphan hydrobromide and levocetirizine dihydrochloride in a cough syrup. The separation of these compounds was achieved within 10 min on a Phenomenex (USA) C₁₈ analytical column, 250×4.0 mm i.d., using an isocratic mobile phase consisting of potassium dihydrogen phosphate buffer (pH 2.5) - acetonitrile- tetrahydrofuran (70:25:5, v/v/v). The analysis was performed at a flow rate of 1.2 ml/min and at a detection wavelength of 232 nm. Percentage recovery and RSD were 100.36% and 0.05% for levocetirizine dihydrochloride, 100.35% and 0.27% for dextromethorphan hydrobromide respectively. Quantification of the components in syrup formulation was calculated against the peak areas of freshly prepared standard solutions. The method was validated as per ICH guidelines.

Key words: Cough syrup formulation, dextromethorphan hydrobromide, levocetirizine dihydrochloride, RP-HPLC, validation

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Dextromethorphan hydrobromide, the d-isomer of the codeine analog of levorphanol, is employed as an antitussive agent. Chemically it is 3-methoxy-17-methyl-(9 α ,13 α ,14 α) morphinan hydrobromide monohydrate. It controls cough spasms by depressing the cough center in the medulla. The oral administration of 30 mg to an adult provides effective antitussive activity over an 8 to 12 h period. Unlike codeine, it is devoid of analgesic properties and produces little or no depression of CNS. Levocetirizine dihydrochloride, the R-enantiomer of cetirizine is a second generation H₁ antagonist. It is a selective and potent compound for treatment of allergic rhinitis and chronic idiopathic urticaria. Chemically it is 2-[2-[4-[(R)-(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy] acetic acid. The recommended dosing of levocetirizine is 5 mg per day. It works by blocking histamine receptors. It does not prevent the actual release of histamine from mast cells, but prevents its binding to its receptors^[1].

Various methods have been reported for determination of dextromethorphan in drug formulation and in biological samples, which include spectrophotometry^[2], capillary electrophoresis^[3], gas chromatography^[4] and high performance liquid chromatography^[5-8].

Analytical procedures for determination of cetirizine in single and multi component formulation include spectrophotometry^[9], capillary electrophoresis^[10], HPLC^[11-14] and LCMS^[15]. However, no method has been reported for the simultaneous analysis of dextromethorphan with levocetirizine in counter cough formulation. Our earlier work on HPLC analysis of counter cough formulation^[16,17] and review article on HPLC analysis of formulated and unformulated samples of antibiotics^[18] drawn our attention towards developing rapid and effective HPLC methods for the simultaneous determination of commercially important formulation of dextromethorphan with levocetirizine. The method is validated as per ICH Guidelines^[19].

Reference standards were received as a gift sample from Roorkee Research and Analytical Labs Pvt. Ltd., Roorkee. Solvents employed were of HPLC grade while the chemicals were of analytical grade (Qualigens India, Mumbai). Commercial formulation viz. Viscodyne-D syrup containing dextromethorphan hydrobromide and levocetirizine

dihydrochloride manufactured in India by Wockhardt Ltd. was purchased from the local pharmacy.

The chromatographic system consisted of a gradient HPLC (Shimadzu, Prominence model) equipped with LC-20AT double reciprocating pump. All samples were injected (20 μ l) using a Rheodyne 7725i manual injector. Elutions of all analytes were monitored at 232 nm by using a SPD-20A UV/Vis detector. Each chromatogram was analyzed with LC Solutions software. Separations were achieved using a Phenomenex, USA, C₁₈ analytical column, 250 \times 4.0 mm i.d., with a 5 μ m particle size. The aqueous component of the mobile phase was 10 mM KH₂PO₄ buffer adjusted to pH 2.5 with dilute orthophosphoric acid prior to diluting to volume. The mobile phase for anticough formulation consisted of a buffer-acetonitrile-tetrahydrofuran (70:25:5, v/v/v) and filtered through a 0.45 μ m membrane filter to degas and pumped from the respective solvent reservoirs to the column at a flow rate of 1.2 ml/min. The run time was set at 10 min and column temperature was 40 \pm 1 $^{\circ}$.

Approximately 100 mg of dextromethorphan hydrobromide and 8 mg of levocetirizine dihydrochloride reference standards were accurately weighed and transferred to a 100 ml volumetric flask. The weighed sample was dissolved in methanol, sonicated for 10 min and made up to volume with methanol to produce a stock solution. Aliquots of the stock solution were diluted to produce dilutions of suitable concentration. Each of the drug solution was filtered through a membrane filter of 0.45 μ m, injected (20 μ l) in triplicate into the column and the peak area and retention time were recorded. The average value of the peak area was used for calculations after ensuring that the RSD was <2% (n = 6).

Sample solution of syrup was prepared by diluting it with methanol to achieve concentration of 100 μ g/ml of dextromethorphan hydrobromide and 8 μ g/ml of levocetirizine dihydrochloride.

Efforts were made to achieve best separation conditions with short analysis time. Literature suggests that buffer with organic modifier has been used for the RP-HPLC determination of dextromethorphan^[5-8] and cetirizine^[11-14] in single and combined dosage form with other drugs. After several trials, separation of peaks was observed with phosphate buffer and acetonitrile as the mobile

phase. However it was showing tailing in peaks. Therefore, to reduce peak tailing, tetrahydrofuran was added in the mobile phase. Thus mobile phase of 10 mM potassium dihydrogen phosphate buffer (pH 2.5)-acetonitrile-tetrahydrofuran in the ratio of (70:25:5 v/v/v) was found to be an appropriate mobile phase for adequate separation of dextromethorphan and levocetirizine in multi component formulation.

The method was validated as per ICH guidelines for specificity, system suitability, linearity, accuracy and precision. Specificity was investigated by analyzing HPLC chromatogram of standard solution of active ingredients (fig. 1) where peaks of the active components are well resolved in the presence of endogenous compounds. System suitability tests (retention time, selectivity, resolution and tailing factor) were performed for dextromethorphan and levocetirizine (Table 1).

The calibration curve was generated from six concentration levels of 1 to 20 $\mu\text{g/ml}$ for levocetirizine dihydrochloride and 10 to 200 $\mu\text{g/ml}$ for dextromethorphan hydrobromide and their corresponding peak areas. Linear correlation was obtained over the range studied, with correlation coefficients ≥ 0.9999 for each drug. (Table 1)

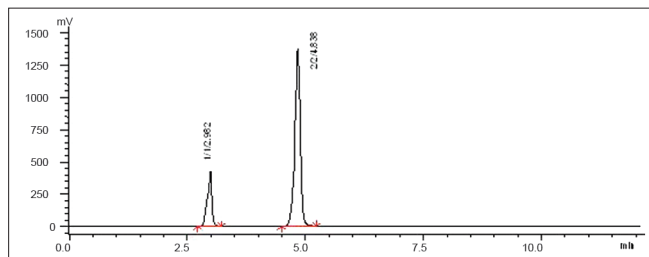


Fig. 1: Representative chromatogram of standard active ingredients. Dextromethorphan (RT- 2.98 min) and levocetirizine (RT- 4.84 min)

TABLE 1: SUMMARY OF VALIDATION PARAMETERS

Parameters	Levocetirizine	Dextromethorphan
Retention Time (Time)	2.98	4.84
Resolution (Rs)	-	2.18
Selectivity (α)	-	1.62
Tailing Factor	0.96	1.06
Multiple R	0.99998	0.99997
R Square	0.99996	0.99994
Standard Error	51628.48	661147.69
Slope	27579.0	407706.3
Intercept	25405.7	55746.9
Recovery (%)	100.36	100.35
Relative standard deviation (%)	0.05	0.27

Accuracy of method was evaluated with the help of recoveries of dextromethorphan hydrobromide and levocetirizine dihydrochloride determined by comparing the response of sample solutions with the response of identical standard solutions prepared in the diluent (Table 1). Percent recovery and RSD were 100.35% and 0.27% for dextromethorphan hydrobromide and 100.36% and 0.05% for levocetirizine dihydrochloride, respectively.

Precision of the method was established by replicate (n=6) analysis of pharmaceutical preparation. Interday precision was determined by analysis of the solution three times on same day and intraday precision was evaluated by analysis of the solution on three different days over a period of one week. All the relative standard deviations for both drugs were found to be less than 2%.

The proposed RP-HPLC method was developed and validated for the simultaneous determination of dextromethorphan hydrobromide and levocetirizine dihydrochloride in combined dosage form. The procedure is simple, rapid and results are reliable. Because of its simplicity and accuracy, the method is suitable for routine quality control analysis of these drugs in commercial formulation.

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