Short Communication

Development of RP-HPLC method for the analysis of levocetirizine. 2HCl and ambroxol. HCl in combination and its application

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ABSTRACT: A simple, sensitive isocratic and reproducible reversed phase High Performance Liquid Chromatographic (RP-HPLC) method was developed for the estimation of ambroxol hydrochloride (ABH) and levocetirizine dihydrochloride (LCD) in combination using PDA detector. The system consisted of RP-C18 column and the detection was performed at 230nm. The mobile phase was a mixture of acetonitrile : phosphate buffer solution (60:40) (pH 7.0) pumped at room temperature and a flow rate of 1 ml/min. ABH and LCD were eluted at 2.75 and 5.01 sec respectively. The mean absolute recoveries of ABH and LCD were about 98 % and 99 % respectively and the limit of detection of LCD and ABH in the mixture of given proportion is observed to be 0.1 µg/ml and 1.5 µg/ml and the limit of quantitation is 0.3 µg/ml and 4.5 µg/ml respectively. The calibration was linear over a concentration range of 4.5 µg/ml to 15.0 µg/ml with \( r^2 > 0.997 \) for ABH and 0.3 µg/ml to 1.0 µg/ml with \( r^2 > 0.999 \) for LCD. The intra (n = 5) and inter (n = 5) day assay variations in the linear range are < 4 % for ABH and < 6 % for LCD. Three pharmaceutical products containing this combination are analyzed to test the applicability of the new method. The percentage of ABH and LCD in three marketed capsule dosage form studied range from 99 to 102 % and 100 to 103 % respectively to the claimed value.

KEYWORDS: Ambroxol hydrochloride; HPLC; Levocetirizine; PDA detection

Introduction

Levocetirizine (as levocetirizine 2HCl) is a third generation non-sedative antihistamine acts by blocking histamine receptors. It is used in the treatment of several allergic reactions, viz., allergic rhinitis, idiopathic urticaria, hay fever etc., (Pasquali et al., 2006; Grob and Lacapelle, 2008; Dubuske, 2007). Ambroxol (as ambroxol, HCl) is an active mucolytic agent works by the breakdown of acidic mucopolysaccharide fibers, which makes the sputum thinner and less viscous and therefore more easily removed by coughing. It is used in the treatment of upper respiratory tract diseases (Nobata et al., 2006). LCD and ABH combination have been used clinically for their anti-allergic and expectorant properties. Earlier literature reveals analytical methods like UV, HPLC, and LC - MS (Shahed et al., 2008; Qi et al., 2004; Arayne et al., 2008; Morita et al., 2008) for the determination of these drugs individually and with other combinations. Lakshmana et al., reported an UV method for simultaneous determination of Levocetirizine and Ambroxol HCl in tablet dosage forms. To date there is no report available for the simultaneous determination of LCD and ABH by HPLC using diode array detection. This paper reports a simple, sensitive and reproducible HPLC method for the simultaneous determination of LCD and ABH and its application in the evaluation of three marketed capsule dosage forms.

Experimental

Materials and methods

Pure samples of LCD and ABH were gifted by Orchid Chemicals and Pharmaceutical Ltd., (Chennai, India). All the solvents were of HPLC and analytical grade purchased from Merck (Mumbai, India). Capsules of three brands, Airitis Plus (Brand 1; Nicholas Piramal India Ltd, Mumbai, India), Laveta – A (Brand 2; Alembic Ltd, Vadodara, India) and Levocet – Plus (Brand 3; Hetero Health Care Ltd, Hyderabad, India) were purchased from local market.
Preparation of standard solutions

LCD (100 mg) was accurately weighed into 100 ml calibrated volumetric flask, dissolved in mobile phase (acetonitrile: phosphate buffer solution (60:40) (pH 7.0)) (~20 ml), and the solution was diluted to volume with the same solvent to get a standard solution of 1 mg/ml. 1 ml of this solution was diluted 10 times in volumetric flask to prepare the working standard solution (0.1 mg/ml). Similarly ABH working standard solution of concentration 1.5 mg/ml was prepared using the same solvent. 10 ml of LCD working standard solution is mixed with 10 ml of ABH working standard solution and was used for the preparation of solutions for calibration curve.

Solutions for calibration curve

The LCD concentration in the mixture solution were 0.05, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.8, 1.0, 1.3, 1.5, 1.8, 2.0, 2.5, 3.0 and 4.0 µg/ml, and the concentration of ABH were 0.75, 1.2, 1.5, 3.0, 4.5, 6.0, 7.5, 12.0, 15.0, 19.5, 22.5, 27.0, 30.0, 37.5, 45.0 and 60.0 µg/ml

HPLC conditions

The samples prepared were analyzed by an isocratic HPLC method. The HPLC analysis was performed on LC-10AT (Shimadzu Corporation, Kyoto, Japan) system by injecting 20 µl of sample Hamilton Rheodyne syringe (Hamilton Bonaduz AG, Switzerland) into syringe loading sample injector (Model 7725i, Rheodyne LP, CA, USA). The column used was Luna C18, 5 µ, 250 x 4.6 mm i.d. (Phenomenex, USA). The mobile phase consisted of a mixture of acetonitrile:buffer(10mM diaminonlm hydrogen o-phosphate and pH adjusted to 7.0 with sodium hydroxide) in 60:40 v/v. The mobile phase was degassed using ultrasonic bath (Model Sonorex, Bandelin Electronic, Germany). The analysis was performed at ambient temperature with a flow rate of 1 ml/min using diode array detector (Shimadzu SPD M10Avp model, Shimadzu Corporation, Kyoto, Japan). Detection was carried out at a wavelength of 230 nm. The data analysis was performed by Class M10 software (Shimadzu Corporation, Kyoto, Japan).

Method validation

The standard solutions were chromatographed for inter and intraday assay variation (n = 5). The calibration curves were obtained by plotting the peak area against concentration for linearity of LCD and ABH in the above concentration range. The accuracy of the method was determined using external standard addition. Known amounts of standard drugs were added at four different levels, and each determination was carried out in triplicate. The limit of detection (LOD) and quantitation (LOQ) were obtained.

Application to the analysis of marketed Capsules

Three marketed Capsules of this combination (each containing 5 mg of LCD and 75 mg of ABH) was selected for the analysis using the procedure described above. Ten capsules of each brand were taken and the contents were weighed. Accurately weighed powder of three brands each equivalent to 5 mg of LCD and 75 mg of ABH was transferred to three 100 ml volumetric flask, dissolved in mobile phase (~20 ml), and shaken for 15 min. The solutions was then diluted to volume with the same solvent, mixed, and finally filtered through Whatmann No. 42 filter paper (Whatmann, middlesex, UK). A sample (1 ml) of each filtrate was serially diluted to get a concentration of 0.5 and 7.5 µg/ml of LCD and ABH respectively. These solutions were used for analysis. The analysis was done in triplicate, and the amount of LCD and ABH in these marketed preparations was calculated from the calibration curve.

Results and Discussion

The Rf for ABH and LCD using this method was found to be 2.75 and 5.01 sec respectively. The chromatograms ABH and LCD in combination using PDA detector at wave length 230nm is shown in Figure 1. The mean absolute recoveries of ABH and LCD were about 98 % and 99 % respectively and the LOD of LCD and ABH in the mixture of given proportion is observed to be 0.1 µg/ml and 1.5 µg/ml and the LOQ is 0.3 µg/ml and 4.5 µg/ml respectively. The calibration was linear over a concentration range of 4.5 µg/ml to 15.0 µg/ml (r^2 > 0.997) for ABH and 0.3 µg/ml to 1.0 µg/ml (r^2 > 0.999) for LCD. The intra (n = 5) and inter (n = 5) day assay variations in the linear range are < 4 % for ABH and < 6 % for LCD

Three pharmaceutical capsule products containing this combination were analyzed to test the applicability of the new method. The percentage of ABH and LCD in the capsules studied are shown in Table 1 and range from 99 to 102 % and 100 to 103 % and respectively to the claimed value.
Conclusion
The HPLC method developed for the determination of ABH and LCD in capsules is accurate, precise, rapid, and selective. It can, therefore, be easily and conveniently used for routine quality control analysis, particularly when large numbers of samples are encountered. The developed method was found to be specific as there was no interference of the excipients, which is confirmed by the absence of extra peaks.

References


