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Simultaneous estimation of levofloxacin and ambroxol hydrochloride from tablet dosage form using RP-HPLC

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ABSTRACT

A reversed phase high performance liquid chromatography (RP-HPLC) method was developed, validated and used for the quantitative determination of levofloxacin (LE) and ambroxol hrdrochloride (AM) from its tablet dosage form. Chromatographic separation was performed on a Intersil ODS-3u C18 column (250mmx4.6mm, 5μm) with a mobile phase comprising of a mixture of buffer: acetonitrile: methanol (75:10:15v/v/v) and pH adjusted to 3 with ortho phosphoric acid, at flow rate of 1ml/min, with detection at 215nm. As per International Conference on Harmonisation (ICH) guidelines the method was validated for linearity, accuracy, precision, limit of detection, limit of quatification and robustness. Linearity of LE was found to be in the range of 19.5-45.5μg/ml and that for AM was found to be 3-7μg/ml. the correlation coefficient were 0.9972 and 0.9999 for LE and AM respectively. The results of tablet analysis were found to be 101.1 and 102.6 for LE and AM respectively. Percentage recovery of LE was found to be 97.20% - 99.98% and that of AM was 98.05% - 99.9%. The assay experiment shows that the method is free from interference of excipients. This demonstrates that the developed HPLC method is simple, linear, precise and accurate and can be conveniently adopted for the routine quality control analysis of the tablet.

Keywords: Reversed-phase high performance liquid chromatography (RP-HPLC); levofloxacin (LE); ambroxol hydrochloride (AM); pharmaceutical tablet dosage form

INTRODUCTION

Levofloxacin (LE) is a fluoroquinolone antiinfective, is the optically active l-isomer of ofloxacin. LE is effective against gram positive and gram negative bacteria. LE inhibits bacteria type II topoisomerases, topoisomerases IV and DNA gyrase. LE like other fluoroquinolone, inhibits the A subunits of DNA gyrase, two subunit encoded by gyrA gene. This result in strand breakage on a bacterial chromosome, supercoiling and resealing, DNA replication and transcription is inhibited. Its empirical formula for LE is $C_{18}H_{20}OFN_3O_4.1/2H_2O$. Its structure is given in Fig.1.

Ambroxol hydrochloride (AM) is a mucolytic expectorant. It is metabolite of bromhexine and acts to reduce the viscosity of tenacious mucous secreations via fragmentation of long mucopolysaccharide chains.Its empirical formula for AM is $C_{13}H_{18}Br_2N_2O.HCl$. (British Pharmacopoeia., 2003). Its structure is given in Fig.2.

Fixed dose combination of LE and AM are indicated for the treatment and relief of symptoms of both upper and lower respiratory tract infections. AM also enhances penetration power of antibiotic.
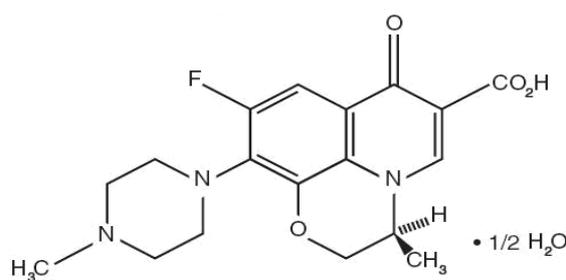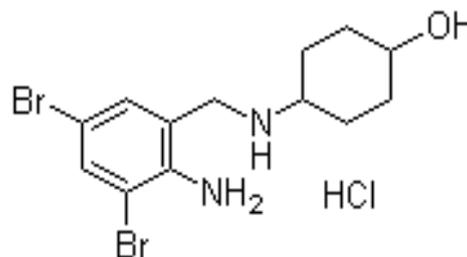

Figure 1: Structure of levofloxacin

Figure 2: Structure of ambroxol hydrochloride

Some analytical methods for the quantitative determination of fluoroquinolones in pharmaceutical formulations are described in literature like capillary electrophoresis, UV spectrophotometry and high performance

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liquid chromatography (HPLC). (Ines M et al.,2006, Sivasubramaniah et al., 2004, Hong AnnNguyen et al., 2004, Hairui Liang et al., 2002, Djabarouti et al., 2004, Yasogima Makoto et al., 2004).

Methods available for the determination of ambroxol hydrochloride include capillary electrophoresis and spectrophotometry, gas chromatography and LC with potentiometric detection, MS detection and UV detection.(Perz rviz T et al., 1997, Dincerz et al., 2003, Koundovrellis JE et al., 2000, Neela Manish Bhatia et al., 2008, Kuchekar BS et al., 2000, Shaikh KA et al., 2008, Lakshmana prabu S et al., 2008). However no references have been found for quantitative determination of Levofloxacin and Ambroxol hydrochloride in pharmaceutical preparations. The major advantage of the proposed method is that levofloxacin and ambroxol hydrochloride can be determined on a single chromatographic system with the same detection wavelength. In the present investigation an economical, precise, accurate revered phase HPLC method using an photo diode array (PDA) detector has been developed for the simultaneous quantitative determination of LE and AM from tablet preparation.

MATERIALS & METHODS

Chemicals and reagents

Bulk drugs LE and AM were procured from Biocon limited . Acetonitrile (HPLC grade, purity 99.80%), ortho phosphoric acid (AR grade, purity 93.00%) and potassium di hydrogen phosphate (AR grade, purity 99.50%) were all procured from Qualigens Fine Chemicals (Mumbai, India).

Instruments and Chromatographic conditions

Chromatographic separation was performed on a Water alliance 2695 HPLCwith 50μL loop and a PDA detector. The wavelength of detection chosen was 215nm. A reversed phase thermo Inertsil ODSu C_{18} column (250mm X 4.6mm, 5μm) was used for analysis. The mobile phase comprised of a mixture of potassium di hydrogen phosphate buffer pH 6, Methanol and Acetonitrile (75:10:15 v/v/v) at a flow rate of 1ml/min. the injection volume was 50μL.

Preparation of stock, working standard solutions

An accurately weighed quantity of 162.5mg of levofloxacin and 25mg of ambroxol was dissolved in diluents and volume was made up to 100ml with same solvent in separate volumetric flask. They were further for dilute to make final concentration of levofloxacin 32.5μg/ml and ambroxol 5μg/ml respectively.

Preparation of sample solutions

Weigh accurately tablets powdered equivalent to about 162.5mg of levofloxacin was transferred to 100ml volumetric flask. Add about 50ml of diluent (0.1N HCl) and sonicate it for 30 minute to dissolve and make up the volume with same solvent. Filter it

through 0.45μ HVLP nylon filter and made further dilution. The final solution containing 32.5μg/ml of levofloxacin and 5.0μg/ml of ambroxol was used as sample solution.

RESULTS AND DISCUSSION

Method validation

Every 50μl of the working standard solution of LE in the mass concentration range of 19.5 to 45.5μg/ml and that for AM in the mass concentration range of 3.0 to 7.0μ/ml was injected into the chromatographic system. The chromatograms were developed and peak area was determined for each drug solution. Calibration curves of LE and AM were obtained by plotting the peak areas versus the applied concentrations of LE and AM. The linear regression coefficients were found to be 0.9972 and 0.9999 for LE and AM (ICHQ2A, ICHQ2B, Sethi PD., 2001, Snyder L., 1997).

The limits of detection (LODs) and the limits of quantitation (LOQs) for LE and AM were determined by the standard deviation of the response and the slope based on the calibration curve. The results of LODs were found to be 0.325μg/ml and 0.005μg/ml for LE and AM respectively. The LOQ results were found to be 19.5μg/ml and 3.0μg/ml for LE and AM respectively.

The instrument precision was performed by injecting 50μl of both LE and AM in five replicates, into the chromatographic system under optimized chromatographic conditions. Parameters evaluated were repeatability of peak response of drugs. The relative standard deviations (RSDs) of the peak area were found to be 0.4786% and 1.01% for LE and AM, respectively.

Repeatability of the method was checked by injecting replicate injections of the combined solution (32.5μg/ml and 5.0μg/ml of LE and AM respectively). Variability of the method was studied by analyzing the solution on the same day (intra-day precision) and on three different days (inter-day precision). The result obtained for the intra-day precision (RSDs) were 0.4786% and 1.1179% respectively, at n=3, for both LE and AM. The inter-day precision (RSDs) were 0.8943% and 1.0391%, respectively at n=3 for both LE and AM.

Accuracy of the method was tested by carrying out recovery studies at three different spiked levels (80%, 100% and 120%) on the basis of the label claim. The estimation was carried out as described earlier. At each level, three determinations were performed and result obtained. The results from validation and system suitability studies are listed in Table1.

The specificity of the method was checked for the interference of impurities in the analysis of blank solution (without any sample) and then a drug solution of 50μg/ml was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both LE and AM from any of the impurities, if present. As there was no interference of impuri-
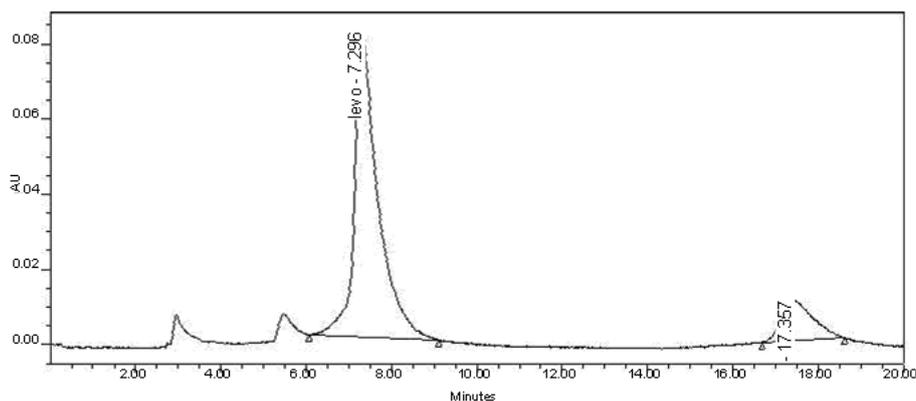
Figure 3: Chromatogram of tablet analysis

Table 1: Results from validation and system suitability studies

Method parameters	Levofloxacin	Ambroxol HCl
Theoretical plate	2183.2	2248.9
Resolution	0	8.488
Linearity range (µg/ml)	19.5-45.5	3-7
Percentage Recovery (%)	101.1	102.6
Correlation co-efficient (R^2)	0.9972	0.9999
Accuracy (%RSD)	0.6241	0.9821
Intraday Precision (%RSD)	0.4786	1.1179
Interday Precision (%RSD)	0.8943	1.0391
LOD (µg/ml)	0.325	0.005
LOQ (µg/ml)	19.5	3.0
Tailing factor	1.38	1.58

ties and also no change in the retention time, the method was found to be specific.

To determine the robustness of the method, experimental condition such composition of the mobile phase, pH of the mobile phase and flow rate of the mobile phase and wave length measurement were altered and the chromatographic characteristic were evaluated. No significant change was observed.

Tablet analysis

Twenty tablets of LE and AM in combination were weighed, their average weight was determined and finally they were crushed to a fine powder. The tablet powder equivalent to 162.5mg of LE was weighed and transferred to a 100ml volumetric flask, first dissolved in 50ml of diluents and then the volume was made up to the mark with same diluents. The content was ultra sonicated for 30min for complete dissolution. The solution was then filtered through a 0.2µm Nylon 6.6 (N66) 47mm membrane. The selection of the mixed sample solution 32.5µg/ml of LE and 5µg/ml of AM which was falling in the Beer's Lamberts range showed good results and was selected for the entire analysis. The results of tablet analysis (n=5) were found to be 97.20% to 99.98% and 98.05% to 99.90% for LE and AM respectively.

From the typical chromatogram of LE and AM (Fig.3) it was found that the retention time of LE was 7.296 and

AM was 17.357 min, which were well resolved peaks with a resolution factor of 8.488.

CONCLUSIONS

The developed method was validated in terms of accuracy, repeatability and precision. A good linear relationship was observed for LE and AM in the concentration ranges of 19.5 – 45.5µg/ml and 3 - 7µg/ml respectively. The correlation coefficient for LE was found to be 0.9972 and that for AM was 0.9999. The inter day and intraday precision results were good enough to indicate that the proposed method was precise and reproducible. The assay experiment showed that the contents of levofloxacin and ambroxol hydrochloride estimated in the tablet dosage form were free from the interference of excipients. This demonstrated that the developed HPLC method was simple, linear, precise and accurate and could be conveniently adopted for routine quality control analysis of LE and AM simultaneously from its pharmaceutical formulation and bulk drugs.

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