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"ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF LEVOFLOXACIN: REVIEW"

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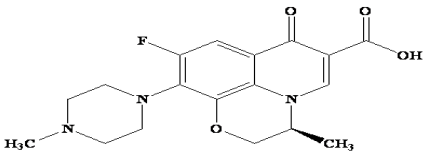
ABSTRACT

Analytical method development and validation are the continuous and interdependent task associated with the research and development, quality control and quality assurance departments. Analytical procedures play a critical role in equivalence and risk assessment, management. It helps in establishment of product specific acceptance criteria and stability of results. Validations demonstrate that the analytical procedure is suitable for its intended purpose. Literature survey reveals that the analytical methods based on UV spectrometry, RP-HPLC & HPTLC for the determination of Levofloxacin individually and in combination with other drugs. The methods were validated according to ICH guideline in terms of accuracy, precision, robustness, and other aspects of analytical validation. The developed methods are simple, sensitive and reproducible and can be used for the routine analysis of Levofloxacin in bulk and Tablet dosage form.

Key words: Levofloxacin, Literature Survey, Method Development, Validation, ICH Guidelines.

1. INTRODUCTION

Levofloxacin (LVFX) is a synthetic fluoroquinolone antibacterial agent that inhibits the supercoiling activity of bacterial DNA gyrase, halting DNA replication. It is used to treat a number of bacterial infections including acute bacterial sinusitis, pneumonia, urinary tract infections, chronic prostatitis, and some types of gastroenteritis[1,2].



Levofloxacin is chemically (S)-9-Fluoro-2,3-dihydro-3-methyl- 10-(4-methyl-1-piperazinyl)-7 oxo-7H-pyrido(1,2,3-de) -1,4 benzoxazine-6-carboxylic acid, is a new quinolone antimicrobial agent which exhibits broad-spectrum in vitro bactericidal activities against gram-positive and gram-negative aerobes[3]. It has a molecular formula of C18H20FN3O4 and molecular weight of

361.368g/mol. LVFX is freely soluble in Glacial acetic acid, chloroform, sparingly soluble in water. Brand name of Levofloxacin is Levaquin.

2. REVIEW OF LITERATURE

Pradeep Singh[3] *et al.,* All immediate release tablets are subjected to dissolution studies in 0.1 N HCl as recommended by SUPAC-IR guidelines or in specified dissolution medium as per their official monograph a simple, selective, rapid, and precise double beam UV-Visible spectrophotometer method has been developed and validated for the estimation of Levofloxacin in pharmaceutical dosage form. The standard solution of Levofloxacin in 0.1 N HCl showed maximum absorption at 293 nm. Beer-Lambert's law obeyed in the concentration range of 2-12 µg/ml, with regression, slope and intercept 0.9997, 0.058 and 0.086 respectively.

Swapna G[4] *et al.,* The present study was undertaken to develop and validate a simple, accurate, precise, reproducible and cost effective UV-Visible spectrophotometric method for the estimation of levofloxacin in bulk and pharmaceutical formulation in three different Brands of cipla lkem, pdpl throught the experiment the solvent used for uv method was water and the absorption spectra was carried out at 288nm and for visible method reagent used is 2,4 DNP in methanol and determination carried out at 510 nm. The concentration 'range is 2-10µg/ml. The method was shown linear in the mentioned concentrations with correlation coefficient of R20.9999.

Patel Dhara[5] *et al.,* The present study describes the stability indicating RP-HPLC method for simultaneous estimation of Cefixime trihydrate and Levofloxacin hemihydrate in pharmaceutical dosage forms. The proposed RP-HPLC method was developed by using Shimadzu (LC- 20 AD) system equipped with PDA detector and chromatographic separation was carried out on Phenomenex Luna C18 (250 x 4.6 mm x 5µ) column at a flow rate of 1 mL/min. The mobile phase consisted of 0.5 % Glacial acetic acid in water pH adjusted to 4.5 with ammonia solution: Methanol (45:55 % v/v) and eluents were scanned using PDA detector at 290 nm. The retention time of Cefixime trihydrate and Levofloxacin hemihydrate was found to be 3.07 and 5.40 min, respectively.

Xingjie Guo[6] *et al.*, A simple and rapid HPLC-UV method has been developed for determination of levofloxacin in human plasma. Chromatographic separation was performed on a Kromasil C18 column with the mobile phase consisting of acetonitrile, water, phosphoric acid, and triethylamine (14:86:0.6:0.3v/v/v/v) and flow rate was 1.0 mL/min. The method used ultraviolet detection set at a wavelength of 294 nm. The standard curves were linear over a concentration range of 0.05-5.0 µg/mL ($r > 0.99$).

Nuthalapati mamatha[7] *et al.,* The aim of the study is an attempt has been made to develop simultaneous determination methods for combined dose tablet formulation Azithromycin and Levofloxacin the by a simple, accurate, precise, sensitive, less expensive and less time consuming method by using RP-HPLC in pharmaceutical dosage form. The column efficiency as determined is not less than 3000 USP plate count and the tailing factor is not more than 2.0. The % relative standard deviation for the peak areas of the six replicate injections is not more than 2.0%. The % RSD of assay of six replicate injections was found to be within the limits. The recovery results indicating that the test

method has an acceptable level of accuracy. The correlation coefficient met the acceptance criteria of NLT 0.999. The LOD and LOQ values from the study demonstrate that the method is sensitive. The system suitability parameters found to be within the limits for a temperature change of 2000C, 2500C, 3000C. Similarly, sample solution was chromate graphed at 2000C, 2500C and 3000C temperature. Retention times were compared and were found that with the increase in temperature retention time decreases. A study was conducted to determine the effect of variation in flow rate and from the results it is concluded that the method is robust.

Mohammad A Rashid[8] *et al.,* A simple, fast and economic reversed phase high performance liquid chromatographic (HPLC) method has been successfully developed and validated for simultaneous determination of fluoroquinolone analogs namely levofloxacin and moxifloxacin in both pure form (as API) and in pharmaceutical dosage forms. For method development a C-18 bonded silica column (250 x 4.6 mm, 5µ, Phenomenex, Inc) was used with a mobile phase comprising of 10% aqueous solution of acetic acid and acetonitrile in a ratio of 80:20 v/v. The flow rate was 0.5 mL/min and effluents were monitored at 300 nm and the retention times were found to be at 7.0±0.1 min and 10.59±0.1 min for levofloxacin and moxifloxacin, respectively. The recovery was found to be more than 99% for each spiked sample of levofloxacin and moxifloxacin, demonstrating the accuracy of the protocol. Intra-day and inter-day precisions of the new method were less than the maximum allowable limit (RSD% ≤ 2.0) according to FDA. The method showed linear response with correlation coefficient value of 0.9975 in both the cases.

Krupa M Kothekar[9] *et al.,* The objective of this present work was to develop and validate analytical method for quantitative determination of Levofloxacin and Ambroxol hydrochloride in a new tablet formulation. Chromatographic separation of the two drugs were analysed on a Hypersil BDS C18 column (25cm X 4.6mm, 5µm). The mobile phase constituted of Buffer: Acetonitirile:Methanol (650:250:100) with triethylamine and pH adjusted to 5.2 with dilute orthophosphoric acid was delivered at the flow rate 1.0 mL min-1. Detection was performed at 220 nm. Separation was completed within 10 min. Calibration curves were linear with coefficient correlation between 0.99 to 1.0 over a concentration range of 7 to 22 µg mL-1 of Levofloxacin and 50 to 150µg/mL for Ambroxol hydrochloride respectively. The relative standard deviation (R.S.D) was found <2.0%.

Narasimha Raju BH[10] *et al.,* A simple, precise RP–HPLC method was developed for the estimation of levofloxacin and ornidazole in combined tablet formulation. The quantification was carried out using a Phenomenex C18 column 250 x 4.6 mm i.d, 5 µm particle size in isocratic mode, with mobile phase comprising of 0.1% v/v phosphate buffer pH 3.0 ± 0.05, acetonitrile, methanol in the ratio of 70:10:20 (v/v/v). The flow rate was 1 mL/min and the detection were carried out by UV detector at 295 nm. The retention times were 3.45min and 6.67 min for levofloxacin and ornidazole, respectively. The method produced linear response in the concentration range of 40-60 µg/mL and 80-100 µg/mL for levofloxacin and ornidazole.

Safila Naveed[11] *et al.,* A simple reversed phase HPLC method has been successfully developed and validated for the quantitative determination of Levofloxacin (LVX) in bulk material, pharmaceutical formulation and serum. Purospher STAR C18 (25 cm x 4.6 mm, 5 µ m) was used. The mobile phase MeOH: H2O (70:30, v/v) was delivered at a flow rate of 1 Ml min-1. The proposed method is specific, accurate with a recovery of 100 ± 0.02. The detection limits were 2 ng with an RSD ± 0.1 (n=6). The anticipated method is applicable to routine analysis of LVX in pharmaceutical formulations and human serum samples. The method was applied to study the In vitro availability of levofloxacin in presence of various elements essential to the human body, like magnesium, calcium, chromium, copper Zinc and iron. The availability of Levofloxacin in presence of these elements was depressed up to 21% in simulated gastric juice, while up to 5% in pH 7.4 and 27% in simulated intestinal juice.

Dhandhukiya Vipul R[12] *et al.,* A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of Levofloxacin hemihydrates and Cefixime trihydrates from tablet dosage forms using C18 column (Grace Smart (250mm × 4.6mm, 5µm). The sample was analysed using 0.08M Potassium dihydrogen phosphate: Acetonitrile in the ratio of 60:40 (% v/v) pH adjusted to 6.5 with triethylamine, as a mobile phase at a flow rate of 1.0ml/min and detection at 254nm. The retention time for Cefixime trihydrate and Levofloxacin hemihydrates was found to be 2.19min and 3.60min respectively. The method can be used for estimation of combination of these drugs in tablets. The method was validated as per ICH guidelines. The linearity of developed method was achieved in the range of 20-120µg/mL (r2=0.9995) for Cefixime trihydrates and 20-120µg/mL (r2=0.9995) for Levofloxacin hemihydrates and assay of tablets were between 98.0-102.0%. Due to these attributes, the proposed method could be used for routine quality control analysis of these drugs in combined dosage forms.

3. CONCLUSION

Literature survey suggested that various HPLC, UV, and few HPTLC methods were developed and reported. The published methods were validated for various parameters as per ICH guidelines. Statistical analysis proved that the published methods were reproducible and selective. Thus, it can be concluded that the reported and published methods can be successfully applied for the estimation of the Levofloxacin in pure and pharmaceutical dosage form.

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