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4.1 LAFUTIDINE AND DOMEPERIDONE

Prajapati et al. 2012 have developed Stability indicating Reversed Phase High Performance Thin Layer Chromatography / Densitometry method for estimation of Lafutidine in Bulk and Tablets. Chromatography was performed on aluminum-backed silica gel 60 RP-18 F254 S plates with Methanol: Water: Triethylamine 8: 2: 0.5 (v/v) as mobile phase. Densitometric scanning was performed at 276 nm. Stress testing of Lafutidine was done according to the International Conference on Harmonization (ICH) guidelines in order to validate the stability-indicating power of the analytical procedures. Stress testing demonstrated that Lafutidine underwent acid, alkaline, oxidative and dry heat degradation; on the other hand, it showed stability towards neutral and photolytic degradation.

Chen et al. 2006 have developed a sensitive and specific liquid chromatography electro spray ionization mass spectrometry (LC–ESI–MS) method for the identification and quantification of Lafutidine in human plasma. Lafutidine and internal standard were isolated from plasma samples by liquid–liquid extraction with diethyl ether. The chromatographic separation was accomplished on a stainless-steel column (C18 Shimpack 5µm 150 mm×2.0 mm i.d. Shimadzu) at a flow rate of 0.2 ml/min by a gradient elution. Detection was performed on a single quadrupole Mass Spectrometer by selected ion monitoring (SIM) mode via electron spray ionization (ESI) source. This method was proved to be sensitive and specific by testing six different plasma batches. Linearity was established for the range of concentrations 1.0–400.0 ng/ml. The method was validated as per the ICH guidelines. The proposed method enables the unambiguous identification and quantification of Lafutidine for pharmacokinetic, bioavailability or bioequivalence studies.

Sun et al. 2009 have developed a sensitive and universal LC–MS/MS method for the simultaneous determination of famotidine, cimetidine, ranitidine and lafutidine in human plasma. This was the first single LC–MS/MS method reported for the simultaneous analysis of these four H₂ antagonists in human plasma. Author has used a ethyl acetate for liquid–liquid extraction. The separation was performed on an Agilent Zorbax SB-CN (150mm×2.1 mm I.D., 5mm) column using a mobile phase methanol: 20mM ammonium acetate (55:45, v/v). The total run time was 7 min per sample. For the quantification was
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performed by electro spray ionization–triple quadrupole mass spectrometry by selected reaction monitoring (SRM) detection in the positive mode. All calibration curves showed good linear regression from 0.5 to 1000 ng/mL for famotidine and lafutidine, and 5–20,000 ng/mL for cimetidine and ranitidine. The method showed good precision and accuracy with overall intra- and inter-day variations of 1.37–9.29% and 3.51–9.40%, respectively. Author applied the assay method successfully to a bioequivalence study using ranitidine as the model compound.

Prajapati et al. 2011 have developed a simple, economical and rapid UV-Spectrophotometric first order derivative method using ‘Area Under Curve’ (AUC) technique for the quantitative determination of Lafutidine in bulk and tablets. In this author used methanol (20% v/v) as a solvent. Zero order spectrum of lafutidine was derivatized into first order using UV-probe software of the UV-Visible Spectrophotometer and the AUC was determined between the two selected wavelength 276.80 nm to 306.00 nm. Lafutidine followed linearity in the concentration range of 10 - 80 µg/ml with r² > 0.99. The quantity of drug estimated by this method was in good accord with label claimed. This method was found to be accurate, precise and ruggedness as shown by low values of % RSD.

Rani et al. 2012 have performed UV estimation of Lafutidine. The method was simple, accurate, cost efficient and reproducible. A mixture of water and methanol was used as a solvent. Stock solution was prepared in a mixture of water and methanol (1:1). The drug was determined at maximum wavelength 279 nm. Beers law was obeyed in the concentration range of 10-50 µg/ml having line equation y = 0.100x + 0.035 with correlation coefficient of 0.999. The result of analysis was validated as per ICH guidelines and this method may be used for the routine analysis of Lafutidine formulation.

Karunakaran et al. 2012 have developed a stability indicating RP-HPLC method for a combination drug product containing a high dose of Paracetamol and low doses of Domperidone and Tramadol HCL. Author used reverse phase column and an isocratic mobile phase consisting of 0.1 %v/v trifluoroacetic acid: acetonitrile: methanol in the ratio 70:25:5 (v/v) with a flow rate of 1.0 ml/min for separation. The analytes were well separated by the effluent was monitored at 272 nm. The drug products were subjected to stress conditions of acid, base, peroxide, thermal and photolytic degradation and peak homogeneity of were obtained using photo diode array detector. The degradation products
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were well resolved from analytes peaks, thus indicating the stability-indicating nature of the method. The assay was linear from 165 – 495 μg/ml for Paracetamol, 18.75 – 56.25 μg/ml for Tramadol, and 5 – 15 μg/ml for Domperidone. Although the tablet contained high and low doses of the drugs, the intra- and inter-day variations were < 2.0 %. The proposed method was validated according to the ICH guidelines and proved suitable for stability and homogeneity testing, as well as for quality control of the combined drugs in pharmaceutical preparations.

Singh et al. 2010 have developed a simple reverse phase HPLC method for the simultaneous estimation of Ranitidine hydrochloride and Domperidone present in combined tablet dosage forms. Chromatographic separation was achieved on Phenomenax C18 stationary phase (250 X 4.6 mm i. d., 5μ particle size) with simple mobile phase combination of phosphate buffer: acetonitrile: methanol 40: 30: 30 (V/V/V) in an isocratic mode at a flow rate of 1.5 ml per min at 210 nm. The retention times were 2.417 and 7.375 (±0.5) min for Ranitidine hydrochloride and Domperidone respectively. Author applied method the simultaneous analysis of Ranitidine hydrochloride and Domperidone in combined tablet dosage form with good accuracy and precision. Further, author concluded that the method can be employed for quality control and routine analysis of Ranitidine hydrochloride and Domperidone in pharmaceutical dosage form.

Shaikh and Patil 2010 have developed and validated a simple and precise High Performance Liquid Chromatographic method for the simultaneous determination of Drotaverine Hydrochloride, Domperidone and Paracetamol in a binary tablet formulation containing Drotaverine Hydrochloride-Paracetamol and/or Domperidone-Paracetamol. Chromatography was carried out at 25°C on a 4.6mm×150mm, 5μm Symmetry shield RP C18 column with the isocratic mobile phase of 0.02 M aqueous Potassium Dihydrogen Phosphate buffer (pH 7.5) and Acetonitrile (40:60, v/v) at a flow rate of 0.5 ml/min. Drotaverine Hydrochloride, Domperidone and Paracetamol were separated in less than 10 min with good resolution and minimal tailing, without interference of excipients. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met in all cases. The method was linear in the range of 192–448 μg/ml for Drotaverine Hydrochloride, 24–56 μg/ml for Domperidone and 12–28 μg/ml for Paracetamol.

Sharma et al. 2012 have developed a simple, accurate, reliable and reproducible HPLC method for the determination of Domperidone in solid dosage forms. The method
employed C18 column, water: methanol (55:45) as mobile phase and detection was made at 291nm. The retention times was found to be 4.5 min for DOM. The method was validated as per ICH guidelines. The method showed good linearity, accuracy, and precision, limit of detection and limit of quantification. The method was suitable for routine analysis of DOM individually and in combined dosage forms.

Sivasubramanian and Anilkumar (2007) have estimated the Omeprazole and Domperidone from tablet formulations by reverse phase HPLC. In present work determination was carried out on a Hypersil, ODS, C-18 (150×4.6 mm, 5 micron) column using a mobile phase of methanol:0.1 M ammonium acetate (pH 4.9) (60:40). The flow rate and runtime were 1 ml/min and 10 min, respectively. The eluent was monitored at 280 nm. The method was found reproducible, with good resolution between Omeprazole and Domperidone. The detector response was found to be linear in the concentration range of 10-60 µg/ml for Omeprazole and 5-30 µg/ml for Domperidone.

Rana et al. 2012 have developed a validated Spectrophotometric method for Simultaneous estimation of Lafutidine and Domperidone in combined dosage form by area under curve method. It involved measurement of area under curve in the range of 268-278 nm (For Lafutidine) and 282.2-292.2 nm (For Domperidone) for the analysis in methanol. The linearity was observed in the concentration range of 2-12 µg/ml for Lafutidine and 3-18 µg/ml for Domperidone. The method showed good reproducibility and recovery with % RSD less than 2. The method validation according to ICH guidelines was also carried out. Method was found to be rapid, specific, precise and accurate can be successfully applied for the routine analysis of Lafutidine and Domperidone in bulk and combined dosage form without any interference by the excipients.

Moon et al. 2012 have developed two simple, economical, precise and accurate methods are described for the simultaneous determination of Lafutidine and Domperidone in combined tablet dosage form UV Spectroscopy. The first method (Method A) was absorption corrected method and second method (Method B) was first order derivative Spectrophotometry. The amplitudes at 258.0 nm and 299.85 nm in the Absorption Corrected Method and 301.87 nm and 276.18 nm in the first order derivative Spectrophotometry were selected to determine Lafutidine and Domperidone, respectively in combined formulation. Beer’s law was obeyed in the concentration range of 2-10 µg/ml for Lafutidine and 6-30 µg/ml for Domperidone for both methods. The methods were validated by following the analytical performance parameters suggested by the
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International Conference on Harmonization. Author found all validation parameters were within the acceptable range. The % assay in commercial formulation was found to be in the range 99.12-100.2 % for Lafutidine and 98.52-99.25 % for Domperidone by the proposed methods. Recovery was found in the range of 98.12-100.24% for Lafutidine and 99.53-100.34% for Domperidone by absorbance corrected method and 98.32-99.24% for Lafutidine and 99.10-100.02% for Domperidone by First Order Derivative.

Moon et al. 2012 have developed and Validated RP-HPLC Method for Simultaneous Estimation of Lafutidine and Domperidone Maleate in Tablet Dosage Form. Author used Hypersil BDS C8 column (250 mm x 4.6 mm, 5μ) as stationary phase with a mobile phase comprising of phosphate buffer (pH adjusted to 4.5 with orthophosphoric acid): Methanol : Acetonitrile in the ratio 55:25:20 (v/v/v) at a flow rate of 1.0 mL/min. detection was carried out at 222 nm. Retention time was found to be 4.07 minutes for Lafutidine and 6.13 minutes for Domperidone. The method was found selective and peaks of Lafutidine and Domperidone were well separated (resolution 9.82). Author found method is linear for both Lafutidine and Domperidone. Author concluded that the method can be used for the analysis of Lafutidine and Domperidone in tablet dosage form.

Santhosha et al. 2012 have developed and validated a stability indicating RP-HPLC method for the simultaneous estimation of Domperidone and Lafutidine in bulk and pharmaceutical dosage form. Chromatography was carried on Thermo Hypersil BDS C_{18} (250mm x 4.6i.d, 5μm) column with mobile phase comprising of Dipotassium Hydrogen Phosphate (0.1M) buffer and Methanol in the ratio 60:40 v/v. The flow rate was adjusted to 1.0ml/min with UV detection at 280 nm. The retention times of Domperidone, Lafutidine were found to be 1.813 min, 8.949 min respectively. The different analytical parameters such as accuracy, linearity, precision, robustness, limit of detection(LOD), limit of quantification (LOQ) were determined according to the International Conference on Harmonization (ICH) Q2B guidelines. The detector response was linear in the range of 180-540 μg/ml, 60-180 μg/ml for Domperidone and Lafutidine, respectively. The method was highly sensitive, precise and accurate & hence was successfully applied for the reliable quantification of active pharmaceuticals present in the commercial formulations. Degradation products produced as a result of stress studies did not interfere with the detection of Domperidone and Lafutidine and the assay can thus be stability-indicating.

Kadam et al. 2012 have developed and validated a simple, precise, and accurate HPTLC method for simultaneous estimation of Lafutidine and Domperidone as the bulk drug and
in tablet dosage forms. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F254 as the stationary phase and the solvent system consisted of ethyl acetate: methanol : water (8:1:0.3) (v/v/v). The separated zones were densitometrically evaluated at 223 nm. The two drugs were satisfactorily resolved with Rf values 0.34 ± 0.02 and 0.64 ± 0.02 for Lafutidine and Domperidone, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (200-1000ng/spot for Lafutidine and 600-3600 ng/spot for Domperidone). The developed method is simple, sensitive and precise; it can be used for the routine testing of formulations in the quality control departments.

Jagadeeswaran et al. 2012 have developed a new, simple and precise HPLC method for the determination of Lafutadine and Domperidone in capsule dosage form. Researcher used Phenomenex, C18, 5μ particle size, 250 x 4.6 mm column as stationary phase and 0.02 M Potassium dihydrogen phosphate: Acetonitrile: Methanol in a ratio of 50:35:15 v/v/v as a mobile phase at the flow rate of 1 ml/ min. All the analytes were quantified at 285 nm. Statistical validation of the method included determination of linearity, accuracy and precision.

4.2 DARUNAVIR AND RITONAVIR

Rezk et al. 2009 have developed a novel LC-ESI-MS method for the simultaneous determination of Etravirine, Darunavir and Ritonavir in human blood plasma. The author has developed and validated a novel LC-MS method for the simultaneous quantification of the most recently FDA-approved protease inhibitor and non-nucleoside reverse transcriptase inhibitor. Author used a sub-2 micron particle column, which gave excellent chromatographic separation and peak shape for all three analytes and internal standard. The method was validated over the range of 0.002-2.0 µg/ml. Intra- and inter-day accuracy of all analytes ranged from 88 to 106%, and intra- and inter-day precision was <7%. Dilution of samples 2, 5, and 10-fold maintained accuracy and precision, using a sample volume as low as 10 µl. Finally, the applicability of the method was investigated with clinical samples and external quality assurance proficiency testing samples.

Huang et al. 2011 have developed a simple, rapid, and sensitive UPLC-MS/MS quantification of nano formulated Ritonavir, Indinavir, Atazanavir, and Efavirenz in mouse serum and tissues. In this study author mentioned that these antiretroviral drugs were currently the cornerstones of common therapeutic regimens for human immunodeficiency virus (HIV) infection. Chromatographic separation was achieved
using a gradient mobile phase (5% acetonitrile in methanol and 7.5 mM ammonium acetate (pH 4.0)) on an Acquity UPLC BEH Shield RP 18 column. All compounds eluted within a seven minute run time. Lopinavir was used as an internal standard. In this method detection was achieved by dual positive and negative ionization modes on a quadrupole linear ion trap hybrid mass spectrometer with an electron spray ionization (ESI) source. This method can be used to support the preclinical development studies of targeted- and sustained-release combination. Author found that 1.5–4 fold increase in serum and tissue AUC of nano formulated Ritonavir, Indinavir, Atazanavir and Efavirenz administered to mice when compared to native drug. In addition, the tested formulation enhanced exposure of the same anti-HIV drugs in mouse tissues.

**Antonio D’Avolio et al. 2007** have developed a new method using HPLC–MS method for the simultaneous quantification of the new HIV protease inhibitor Darunavir and 11 other antiretroviral agents in plasma of HIV-infected patients. A simple protein precipitation extraction procedure was applied on 50 µl of plasma aliquots and chromatographic separation of drugs and quinoxaline (IS) was achieved with a gradient mobile phase that was acetonitrile and water with formic acid 0.05% on C-18 reverse phase analytical column with 25 min of analytical run. Calibration curves were optimized according to expected ranges of drug concentrations in patients, and correlation coefficient was higher than 0.998 for all analytes. Mean intra- and inter-day precision (relative standard deviation %) for all compounds were 8.4 and 8.3%, respectively, and mean accuracy (% of deviation from nominal level) was 3.9%. Extraction recovery ranged within 93 and 105% for all drugs analyzed. Author concluded that the method can used to measure darunavir and ritonavir plasma concentration in HIV-positive patients as well as can be successfully applied for routine therapeutic drug monitoring and pharmacokinetics studies.

**Martin et al. 2009** have developed a liquid chromatography–tandem mass spectrometry assay for simultaneous determination of the plasma concentration of 11 antiretroviral agents that are nevirapine, indinavir, atazanavir, amprenavir, saquinavir, ritonavir, lopinavir, efavirenz, tipranavir, darunavir and maraviroc. In this method sample pre-treatment was done by protein precipitation with a mixture of methanol and zinc sulfate. After centrifugation the supernatant is injected in the chromatographic system, which consists of on-line solid phase extraction followed by separation on a phenyl–hexyl column. Author concluded that simple sample preparation provides sensitive, accurate
and precise quantification of the plasma concentration of antiretroviral drugs and can be used for therapeutic drug monitoring in patients infected with HIV.

D’Avolio Antonio et al. 2011 have developed a validated sensitive and accurate HPLC–MS method for the intracellular determination of 14 antiretroviral drugs in peripheral blood mononuclear cells (PBMC’s) for HIV+ patients. PBMCs were isolated by Ficoll density gradient centrifugation and cells count and the relative mean volume was performed with a Coulter instrument. Extraction of drugs from PBMCs pellets was obtained with methanol: water (70:30 v/v), with quinoxaline used as internal standard. Sonication was performed and supernatant was dried and then dissolved in water/acetonitrile (60/40 v/v), before injection into a 2.1mm×150mm AtlantisT3 3µ column. Chromatographic separations were performed using a gradient program with a mixture of water (0.05% formic acid), as mobile phase A and acetonitrile (0.05% formic acid), as mobile phase B. Author quantified the analytes by electrospray ionisation–single quadrupole mass spectrometry using the selected ion recording (SIR) detection mode. The positive ionization was used for the HIV the all drugs except efavirenz for whom the negative ionization was applied. The calibration curves were built using blank PBMCs spiked with antiretroviral drugs at concentrations ranging from 0.1 to 32 ng/ml (1–320 ng/ml for tipranavir) and fitted to a quadratic regression model weighted by 1/X. The mean extraction recovery for all PIs, II and NNRTIs was always above 82%. Author concluded that the method was precise, with a range of intra/inter-day percent standard deviation and accurate with mean of percent coefficient of variation from nominal values −7.85 to +9.7%. Each drug concentration evaluated was expressed in ng/ml and optimized using each patient medium corpuscular volume and cell number.

Else et al. 2010 have stated that the clinical pharmacokinetic studies of antiretrovirals require accurate and precise measurement of plasma drug concentrations. For the determination of the commonly used protease inhibitors (PI) and the non-nucleoside reverse transcriptase inhibitor (NNRTI), as well as the more recent antiretrovirals and the “second generation” NNRTI, a simple, fast and sensitive HPLC–MS/MS method was developed using quinoxaline as an internal standard which was added to plasma aliquots (100 µl) prior to protein precipitation with acetonitrile (500 µl) followed by centrifugation and addition of 0.05% formic acid (200 µl) to the supernatant. Chromatographic separation was achieved using a gradient (Acetonitrile and 0.05% formic acid) mobile phase on a reverse-phase C18 column. Detection was via selective reaction monitoring
(SRM) operating in positive ionization mode on a triple-quadrupole mass spectrometer. All compounds eluted within a 5 min run time. Calibration curves were validated over concentration ranges reflecting therapeutic concentrations observed in HIV-infected patients from pharmacokinetic data reported in the literature. Correlation coefficients ($r^2$) exceeded 0.998. Inter- and intra-assay variation ranged between 1% and 10% and % recovery exceeded 90% for all analytes. Further, author mentioned that the developed method can be successfully applied to measure plasma antiretroviral concentrations from samples obtained from clinical pharmacokinetic studies.

Penzak et al. 2001 have developed a rapid and sensitive high-performance liquid chromatographic method for the determination of ritonavir in human plasma. Author also described the pharmacodynamic relationships between plasma ritonavir concentrations and efficacy and toxicity. Ritonavir was precipitated with acetonitrile plus barium hydroxide and zinc sulphate. In this method the chromatographic separation was achieved using a C-18 base-deactivated (250 x 4.6 mm I.D., 5 micron particle size) analytic column with a mobile phase composed of acetonitrile: water (52:48, v/v). Quantification was performed at 239 nm. Calibration curves were linear from 0.5-25 µg/ml ($R^2 > 0.999$); percent errors, as a measure of accuracy, were < 12.7%. Intra and inter assay relative standard deviations (RSD) were below 12.8%. Author concluded that the present assay method requires neither the use of a buffered mobile phase adjusted to a specific pH, nor the addition of amine modifiers nor the method has been successfully used to determine plasma ritonavir concentrations in drug interaction studies.

Fayet et al. 2009 have developed a LC–tandem MS assay method for the simultaneous measurement of new antiretroviral agents: Raltegravir, maraviroc, darunavir, and travirine. In his research work author have developed a sensitive and accurate liquid chromatography-tandem mass spectrometry (LC–MS/MS) method for the determination of plasma drug levels. Single-step extraction from plasma (100µl) was performed by protein precipitation using 600µl of acetonitrile, after the addition of 100µl darunavir at 1000 ng/ml in methanol/H$_2$O 50/50 as internal standard (I.S.). The mixture was vortexed and centrifuged. An aliquot of supernatant (150µl) is diluted 1:1 with a mixture of 20mM ammonium acetate/methanol 40/60 and 10µl was injected onto Waters AtlantisTM-dC18 3µm analytical column. Separations were performed using a gradient program with 2mM ammonium acetate containing 0.1% formic acid and acetonitrile with 0.1% formic acid and quantification was performed by electrospray ionisation-triple quadrupole mass
spectrometry using the selected reaction monitoring detection in the positive mode. The method has been validated over a range of 12.5 to 5000 ng/ml, 2.5 to 1000 ng/ml, 25 to 10,000 ng/ml, 10 to 4000 ng/ml, and 5 to 2000 ng/ml for RAL, MRV, DRV, ETV and RTV, respectively. The extraction recovery for all antiretroviral drugs is always above 91%. Finally, author concluded that the method was precise and accurate. This was the first analytical method allowing the simultaneous assay of antiretroviral agents targeted to four different steps of HIV replication.

*Smith et al. 2012* have developed a simple, robust, selective and sensitive spectrophotometric method for the determination of Ritonavir in pharmaceutical formulations. The method was based on the scanning of methanolic solution of the drug and methanolic solution of formulation. The method showed high sensitivity with linearity range from 10 to 20 μg/ml. The lower limit of detection (LOD) was found to be 1.1 μg/ml and the limit of quantization (LOQ) was determined as the lowest concentration was found to be 3.3 μg/ml. Author have applied the proposed method successfully for the determination of Ritonavir in pharmaceutical formulations.

*Chiranjeevi et al. 2011* have developed and validated two Simple, precise and economical UV methods for the quantitative for the estimation of Ritonavir in bulk and pharmaceutical dosage forms. Ritonavir has the absorbance maxima at 239 nm (Method A), and in the first order derivative spectra, showed sharp peak at 232 nm (Method B). Beer’s law was found to be obeyed in the concentration range of 10-50 μg/mL for the Method A and B. The developed methods were validated according to ICH guidelines and were found to be accurate and precise. Author has successfully applied the developed methods for the estimation of Ritonavir in bulk and pharmaceutical dosage forms.

### 4.3 AZITHROMYCIN AND OFLOXACIN

*Rimawi and Kharoaf. 2010* have developed a simple, validated stability-indicating liquid chromatographic method is developed for the analysis of azithromycin in raw material and in pharmaceutical forms. Author used a reversed-phase C(18) stationary phase and detection was carried out at 210 nm. Isocratic elution is employed using a mixture of phosphate buffer-methanol (20:80). The method was validated in accordance with USP requirements for the parameters such as accuracy, precision, specificity, linearity, and range. The method demonstrated good linearity over the range of 0.3-2.0 mg/ml of azithromycin. The accuracy of the method was 100.5% with a relative standard deviation of 0.2%. The precision developed method reflected by relative standard
deviation of replicates was 0.2%. Author concluded that the method is sensitive and impurities and degradation products of azithromycin can be selectively determined with a good resolution in both raw material and pharmaceutical forms.

Kovacic-Bosnjak et al. 1988 have developed a RP-HPLC method for the determination of semisynthetic macrolide antibiotic Azithromycin. In this method author used reversed-phase octadecyl column, pH of 9.3–9.5 and isocratic mode at ambient temperature are the best conditions of analysis. The mobile phase phase used was ammonium or phosphate buffer, 2 – propanol and acetonitrile in various ratio with a flow rate of 1-1.5 per ml. The said analytical procedures enabled authors to get reliable assay of azithromycin and its intermediates, as well as other impurities are defined.

Miguel and Barbas. 2003 have determined impurities in azithromycin tablets by liquid chromatography using UV detector. Author used mobile phase as A - KH2PO4 10 mM (H2O) at pH 7.00, B - mixture methanol: acetonitrile 1:1 (v/v) with linear gradient elution was employed starting with 47% A and 53% B to reach 28% A and 72% B at 48 min. The detection was performed at 210 nm. The chromatographic column was Phenomenex Synergi MAX-RP 4 mm 250×460 mm kept at 50 ºC. Author separated six impurities and identified. In this study author has quantified five out of the six with reasonable accuracy and precision.

Senthil et al. 2010 have developed a simple reverse phase liquid chromatographic method and subsequently validated for simultaneous determination of azithromycin and ambroxol hydrochloride in combined dosage form. For the separation author has used acetonitrile and mono basic potassium phosphate buffer of pH 8.5 in the ratio of 65:35 v/v was carried as a mobile phase and C18 phenomenex Gemini 5m, 250cm x 4.6mm id column as a stationary phase with flow rate of 2 ml/min using PDA detection at 220 nm. For this method author observed linearity over a concentration range of 96-145mg/ml and 80-125mg/ml for azithromycin and ambroxol hydrochloride respectively. The retention times of ambroxol and azithromycin were found to be 3.7min and 6.1min respectively. Results of analysis were validated statistically and by recovery studies. Author concluded that developed RP-HPLC method is simple, rapid, precise and accurate which is useful for the routine determination of azithromycin and ambroxol hydrochloride bulk drug and in its pharmaceutical dosage form.

Sudheer et al. 2012 have developed a sensitive and specific isocratic RP-HPLC method for quantitative estimation of azithromycin and ambroxol HCl tablet formulation. In this
method author has used the mobile phase consisting of $\text{K}_2\text{HPO}_4$ (pH 6.5) : Acetonitrile (68 : 32) with isocratic programming, Hypersil, BDS, C8, column as stationary phase with a flow rate of 1.5 ml/minute by using $\lambda_{\text{max}}$ 215nm and PDA detector. Linearity of the proposed method was found to be in the range of 100.0 to 360.0 μg/ml for Azithromycin and 15.0 to 54.0 μg/ml for Ambroxol HCl respectively. Author validated the developed method and also performed the forced degradation studies and concluded that the drug peaks were well resolved from the degradants peaks.

**Shah and Raj. 2012** have developed simple, accurate, sensitive, reproducible, economical Second order derivative spectroscopy method for the determination of Azithromycin dihydrate and Cefixime trihydrate in combined dosage form. The method was validated for linearity, accuracy and precision as per ICH guidelines. The method obeys Beer’s Law in concentration ranges of 10-40 ppm for Cefixime trihydrate and 25-100 ppm of Azithromycin dihydrate. Author reported the zero crossing point for Azithromycin dihydrate and Cefixime trihydrate at 326.4 nm and 226.8 nm, respectively in water. The LOD and LOQ value were found to be 0.54 and 1.64 ppm for Cefixime trihydrate and 0.77 and 2.34 ppm for Azithromycin dihydrate respectively. Author applied method successfully the quantitative analysis of commercially available dosage form.

**Singh et al. 2010** have a developed and validated RP-HPLC method was for quantitative determination of azithromycin in pharmaceutical suspension dosage forms. The chromatography was carried out on a Phenomenex C18 (150 x 4.6 mm i.d.,5μ) column with Acetonitrile: 0.5 % Formic acid as mobile phase (Isocratic A: B = 40: 60 % v/v), at 215 nm detector wave length with a flow rate of 1 ml/min. in this work author used Clarithromycin as an internal standard. The linearity was established in the range of 20 - 600 ng/ml for HPLC. Author concluded that the HPLC method was accurate and precise for azithromycin suspension and can be used for estimation of azithromycin in pharmaceutical suspension.

**Wongsinsup et al. 2009** have developed a specific, selective, sensitive and precise reversed-phase high-performance liquid chromatographic method for the determination of ofloxacin in human plasma. In this study author used Pipemidic acid was used as an internal standard. The simple extraction method using protein precipitation with acetonitrile was employed for sample preparation. Good chromatographic separation was achieved by using ODS hypersil (5 μm, 250 x 4.0 mm) column and a mobile phase
consisting of Acetonitrile: 25 mM Phosphoric acid + 2.5 mM N-Cetyl-N,N,N-trimethylammonium bromide (CTAB) pH=7.0 (20: 80) at a flow rate of 1.2 ml/min. detection was carried out by using Ofloxacin and pipemidic acid were detected with fluorescence detector at 285 nm and 460 nm. Author has observed that no endogenous substances were found to interfere. The linearity range for ofloxacin was 25–4000 ng/ml. The method was successfully used in pharmacokinetic and bioequivalence study of ofloxacin in healthy volunteers.

**Anton Smith et al. 2011** have developed a validated HPLC method for the determination of Ofloxacin eye drop. In this study author used C8 (250 cm × 4.6 mm i.d., 5 μm) column with a mobile phase consisting of Acetonitrile: Buffer in the ratio 35:65 v/v with a flow rate of 1.5 ml/min as Chromatographic condition. Detection was carried out at 315 nm . Validation of developed method was carried out as per ICH guideline. Author found the linearity over the concentration range of 50 – 300 μg/ml and % recoveries was found to be in the range of 99.8 – 103.73%. Author has concluded that the proposed method was precise, accurate, selective and rapid for the determination of ofloxacin in quality control and in assay.

**Premanand et al. 2010** have developed a validated reverse phase high performance liquid chromatography method for the simultaneous determination of Nitazoxanide and Ofloxacin in combined dosage form using cosmosil 5 C18 (4.6 mm × 250 mm, 5 μm) and acetonitrile: 0.005 M triethylamine Buffer in the ratio of 55:45 (v/v) as the mobile phase at a flow rate of 1.0 mL/min and eluents were monitored at 240 nm. The average retention time of Dexrabeprazole and Domperidone was found to be 6.051 min and 2.106 min respectively. Author found linearity over the range of 160-240 μg/mL for Nitazoxanide and 400-600 μg/mL for Ofloxacin.

### 4.4 CEFPODOXIME PROXETIL AND OFLOXACIN

**Singh et al. 2010** have developed a simple, accurate and sensitive spectrophotometric and RP- HPLC methods for the simultaneous determination of Cefpodoxime Proxetil and Clavulanate Potassium in combined dosage form. Detection was carried at 270 nm and 235 nm in SEM. Author found the linearly range of 15 – 150 μg/ml and 5- 50 μg/ml for Cefpodoxime Proxetil and Clavulanate potassium respectively. For HPLC method detection was carried out at 220 nm using PDA detector on Luna C18 column with mobile phase consists of methanol: acetonitrile: water: THF in the ratio of 40:30:20:10. Linearity was found in the range of 15- 200 μg/ml for Clavulanate Potassium and 5-50
μg/ml for Cefpodoxime Proxetil. Author has performed the validation of method as per ICH guidelines. Recoveries by both method was found well within the acceptable limits. Author applied both the method for determination of these drugs in pharmaceutical dosage forms.

**Fukutsu et al. 2006** have develop a method for the determination of trace amounts of cefmetazole and cefpodoxime proxetil contaminants in pharmaceutical manufacturing environments by LC/MS/MS. The necessary sensitivity of this method was estimated based on the detection limit for Penicillin G required by the FDA and the total surface area and volume of the manufacturing facility. The detection limits of this method were estimated to be 10 pg/ml for cefmetazole and 5 pg/ml for and cefpodoxime proxetil from the signal to noise ratio. In this study author found the linearity in a concentration range from 0.20 to 3.20 ng/ml. The accuracy and precision were verified by the determination of the amount of standard drug added to the sampling materials which were glass plate and silica fiber filter. Author concluded that the method can be successfully applied to their contaminants determination in samples collected from an actual manufacturing environment.

**Patel and Patel 2011** have developed a Q-absorbance ratio spectrophotometric method for determination of cefpodoxime proxetil and ofloxacin in tablets. In this method Cefpodoxime proxetil and ofloxacin showed an isoabsorptive point at 273.2 nm in methanol. The second wavelength used is 297 nm, which is the λ-max of ofloxacin in methanol. The linearity was obtained in the concentration range of 2-14 μg/ml for both cefpodoxime proxetil and ofloxacin. This method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found and the results of analysis have been validated statistically and by recovery studies.

**Patel and Patel 2011** have developed a dual wavelength spectrophotometric method for Simultaneous Estimation of Ofloxacin and Cefpodoxime Proxetil in Tablet Dosage Form. The method was based on determination of ofloxacin at the absorbance difference between 224 nm and 247.4 nm and cefpodoxime at the absorbance difference between 278.2 nm and 320 nm. The linearity was obtained in the concentration range of 2-12 μg/ml and 4-24 μg/ml for ofloxacin and cefpodoxime proxetil respectively. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients. The developed method was validated as per ICH guidelines and can be used for the routine analysis.
Malathi et al. 2007 have developed a method for the Simultaneous RP-HPLC Estimation of cefpodoxime proxetil and clavulanic acid in tablet dosage forms. The method was carried out on a Zorbax Eclipse XDB 5 µ C 18 (150×4.6 mm) column with a mobile phase consisting of acetonitrile: 50 mM potassium dihydrogen phosphate buffer (pH 3.0, 70:30 v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 228 nm. Aspirin was used as an internal standard. The retention time of clavulanic acid, cefpodoxime proxetil and aspirin was 4.43, 6.44 and 5.6 min, respectively. Author has validated the method for the parameter such as accuracy, precision, linearity, limit of detection, limit of quantification and solution stability. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Kumar et al. 2012 have developed a validated HPLC method for simultaneous estimation of cefpodoxime proxetil and ofloxacin in bulk drugs and in pharmaceutical dosage forms. The separation was accomplished on a Waters C-18 column with a mobile phase consisting of acetonitrile: methanol : 0.02M potassium dihydrogen ortho phosphate buffer pH adjusted to 3.0 with ortho- p phosphoric acid (54 : 6 : 40 v/v) at a flow rate of 0.6 ml/min. at 270 nm by using PDA detector. The retention times for Ofloxacin and cefpodoxime proxetil were found to be 1.92 and 2.98min, respectively. Author has validated the method for the parameter such as accuracy, precision, linearity, limit of detection, limit of quantification and solution stability. The cefpodoxime proxetil and ofloxacin showed linearity in the concentration range of 5-50 µg/ml. The amount of both these drugs estimated by proposed method was found to be in good agreement with label claim. Finally author concluded that the proposed method can be used for the estimation of these drugs in combined dosage forms.

Mathew et al. 2013 have developed new stability indicating RP-HPLC method for Cefpodoxime Proxetil. Both R and S isomers of the drug were separated using Phenomenex (250 × 4.6 mm, 5 µm particle size) ODS column with a flow rate of 1ml/min 252 nm. The isocratic method used a mobile phase consisting of methanol and phosphate buffer of pH4.0 in the ratio 65:35. The linearity was observed in the range of 5–100 µg/ml. sensitivity of the method was justified by the lower value of LOD and LOQ. In this study sample was subjected to stress degradation using acid, alkali, hydrogen peroxide, dry heat, wet heat, and UV light and observed that the standard drug peaks were well resolved from the degradation products’ peaks with significantly different retention time and the resolution factor for the R and S isomers was greater than 2.
Abdel fattaha et al. 2013 have developed three UV Spectrophotometric method and one HPLC stability indicating methods for the determination of cefpodoxime proxetil in the presence of its acid and alkaline degradation products. Second derivative spectrophotometry (261 nm) third derivative spectrophotometry (282 nm), first derivative of ratio spectrophotometry (215 nm, 255 nm and 243 nm) and ratio subtraction Spectrophotometry (232 nm) were the three UV Spectrophotometric method developed for the determination of Cefpodoxime in presence of their degradants in acidic and alkaline condition. The fourth method is an isocratic reversed -phase HPLC with Zorbax C8 column using acetonitrile: water: triethylamine (60:40:1, v/v/v) as a mobile phase and detection at 232 nm was used to separate cefpodoxime proxetil from its acid and alkaline degradation products. These methods can be applied for the analysis of cefpodoxime proxetil in bulk powder and in pharmaceutical preparations.

Rote and kande 2011 have developed a HPTLC method for determination of cefpodoxime proxetil and ambroxol hydrochloride in human plasma by liquid–liquid extraction using paracetamol as an internal standard. The plasma sample was extracted by a mixture of methanol and acetonitrile. A concentration range from 500 to 3500 ng/spot of cefpodoxime proxetil and 1000 to 7000 ng/spot of ambroxol hydrochloride were used for the calibration curve, respectively. The Rf values for cefpodoxime proxetil and ambroxol hydrochloride and paracetamol were found to be 0.69 ± 0.005, 0.49 ± 0.0057, and 0.31 ± 0.0054, respectively. Author validated the proposed method was validated statistically and by performing a recovery study.

Miniyar et al. 2012 have developed a validated RP-HPLC method for simultaneous determination of ofloxacin and ornidazole in infusion. For the separation author have used ethanol and buffer (equal proportion of 0.01M orthophosphoric acid and 0.01M sodium phosphate monobasic dihydrate) with pH 4.00 adjusted by 20% of triethylamine in the ratio of 60:40 v/v with HiQ Sil C18 column with flow rate of 1 ml / min at 300 nm. Linearity was observed over a concentration range of 1.25-10 μg/ml for Ofloxacin and 3.12-25 μg/ml for ornidazole. Separation was achieved within 5 min. The mean % recovery was found to be 99.94% for ofloxacin and 100.27 % for ornidazole. The LOD and LOQ were also determined for both drugs. Author in his conclusion said that the proposed RP-HPLC method is simple, rapid, precise, accurate and cost effective which is useful for the routine determination of ofloxacin and ornidazole in bulk drug and in its infusion.
Dharuman et al. 2009 have developed a validated RP-HPLC method for the simultaneous estimation of ofloxacin and tinidazole in tablets. In this author achieved the separation using a mobile phase consisting of 0.5% v/v triethylamine buffer of pH 3.0 and acetonitrile in the ratio of 73:27. The column used was Kromasil C8, 5μ, 15 cm × 4.6 mm id with flow rate of 1.2 ml/min using PDA detection at 303 nm. The described method was linear over a concentration range of 10-50 μg/ml and 30-150 μg/ml for the assay of ofloxacin and tinidazole respectively using ambroxol (50 μg/ml) as internal standard. The retention times of ofloxacin, tinidazole and ambroxol were found to be 2.3, 4.1 and 5.1 min respectively. Results of analysis were validated statistically and by recovery studies. Author have concluded that the proposed RP-HPLC method is simple, rapid, precise and accurate can be useful for the routine determination of ofloxacin and tinidazole bulk drug and in its pharmaceutical dosage form.

Hassan et al. 2012 have developed stability indicating spectrophotometric methods for the determination of ofloxacin and ceftriaxone and their degradation products. Absorption difference and derivative difference Spectrophotometric methods were developed for the determination of ofloxacin and ceftriaxone in presence of their degradation products. The linear calibration graphs for ofloxacin were obtained from D1 at 281 nm for the acid solutions, D2 at 288.5 nm for the alkaline solutions, ΔA at 298.6 nm, ΔD1 at 292-304.2 nm, and ΔD2 at 298 nm, while those for its corresponding degradation product were obtained from D2 at 281.5 nm of the acid solutions, and D1 and D2 at 288.5 nm and 297 nm, respectively, for the alkaline solutions. On the other side, ceftriaxone was quantified using D2 amplitudes of the solutions in water at 241.3 nm, D1 at 268.3 nm and 238.5 nm, D2 at 257 nm and 283.4 nm, and D3 at 269.7 nm and 298.3 nm, for the acid solutions, while its corresponding degradation product was determined by measuring the D1 and D2 of the solutions in water at 241 nm and 249.8 nm, respectively, D1 and D3 of the acid solutions at 263 nm for both, D1 at 242.2 nm and 270.3 nm, and D2 at 248.7 nm of the alkaline solutions. The proposed methods were successfully applied to the determination of ofloxacin and ceftriaxone in synthetic mixtures with the corresponding degradation products. The results obtained indicated the high accuracy, calculated as mean percentage recovery, RSD% and Er% and precision; evaluated in terms of inter-day and intra-day precision. In addition, ofloxacin capsules and ceftriaxone ampoules were assayed using the proposed methods and the results obtained were compared with reference spectrophotometric methods.

Fabre et al. 1994 have developed reproducible, simple and sensitive A high-performance liquid chromatographic method with fluorometric detection was developed for the
analysis of ofloxacin in plasma and lung tissues. Author used 280 nm for excitation and 500 nm for emission. Linear detector response was observed for the calibration curve standards in the range of 0.1-5 μg/ml for plasma and 0.025-2.5 μg/ml for lung tissue. The limit of quantitation is 5 ng/ml -1 or 5 ng/g. The accuracy of the method is good; that is, the relative error is < 10%. Author has applied this method to the pharmacokinetic study of ofloxacin in 24 chronic obstructive pulmonary disease patients.

**Shah et al. 2012** have reported a new, simple, rapid, accurate, precise and sensitive method for the simultaneous estimation of Cefpodoxime proxetil and Ofloxacin in their combined dosage form. The method was carried out on a Hiber C18 column (250 mm×4.6 mm, i.d.5 μm) with a mobile phase consisting of acetonitrile: phosphate buffer pH 3 (pH adjusted with orthophosphoric acid) (75:25) at a flow rate of 1 ml/min and the detection was carried out at 271 nm. The retention time of Cefpodoxime proxetil and Ofloxacin was 3.24 and 2.16 min respectively. Linearity for Cefpodoxime proxetil and Ofloxacin were found in the range of 5-25 μg/ml. The developed method was validated in terms of linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ). The proposed method can be used for estimation of both drugs in their combined dosage form.

**Gandhi et al. 2011** have developed a validated, simple, specific, precise stability indicating HPTLC method for determination of Ofloxacin and Ketorolac Tromethamine in Combination. Author described the degradation of Ofloxacin and Ketorolac under ICH [Q1A (R2)] prescribed stress conditions (hydrolysis, oxidation, dry heat, wet heat and photolysis) and establishment of a stability-indicating HPTLC assay method. Different degradation peaks were observed for both Ofloxacin and Ketorolac when each was exposed to different stress condition. For HPTLC, aluminium plate precoated with Silica Gel 60 F254 and mobile phase consisting of n - butanol: ethanol: ammonia 6: 1: 3 v/v/v was used to achieve separation. Quantitation was done at 310 nm. The method was validated as per ICH Q2 R1 guidelines and results were in limit.

### 4.5 CEFPODOXIME PROXETIL AND LEVOFLOXACIN

**Desai et al. 2011** have developed a validated UV Spectrophotometric method for the determination of Levofloxacin in dosage formulations. Determination was carried out at 290 nm with 0.1M HCl as diluent. Author has validated the method for linearity, accuracy, precision, reproducibility, and specificity as per International Conference on Harmonization (ICH) guidelines and determined the content of levofloxacin in two
commercial brands of levofloxacin in the Nigerian market. The developed method showed good linear relationship over a concentration range of 0.25 – 12.0 μg/ml. The Mean recovery was 98.7 % in recovery study. Author further reported that the method was specific for levofloxacin in the presence of common excipients, and when it was applied to two marketed brands, levofloxacin content was 99.69 ± 2.38 and 102.65 ± 3.64 %, respectively, of labeled claim. Author concluded that the method is precise, accurate and reproducible, and hence can be employed for routine analysis of levofloxacin in bulk and commercial formulations.

Manish Kumar et al. 2011 have developed a Simple, rapid, selective and sensitive validated HPLC- UV method for the estimation of levofloxacin in human plasma. In this method author carried the extraction with ethyl acetate. Levofloxacin was measured in plasma using a validated HPLC method with UV detector at 235nm chromatographic peaks were separated on 5μm intensil, C18 column (4.6x250mmx5μm) using 80:20 v/v Phosphate buffer pH 2.5, Acetonitrile as mobile phase at a flow rate of 1 ml/min. The chromatograms showed good resolution and no interference from plasma. The retention time of levofloxacin and internal standard were approximately 5.9±0.05 min and 10.1±0.03 min respectively. The method was found linear over the concentration range of 0.1 to 10μg/ml. Author have applied the developed method was successfully applied to pharmacokinetics studies.

Shirkhedkar and Surana. 2009 have developed the UV-Spectrophotometry and first order derivative methods for Quantitative determination of levofloxacin hemihydrates in bulk and tablets. The determination was carried out at 288 nm and the same spectrum was derivatized into first order derivative, using UV probe software of instrument at Δλ = 4. In this study the amplitude of the trough was recorded at 297 nm. Author observed the linearity in the concentration range 2 – 12 μg/ml. In this study author found that the assay results in good agreement with label claim. The methods were validated statistically and by recovery studies. The relative standard deviation was found to be less than 2% with excellent precision and accuracy.

Chepurwar et al. 2007 have developed and validated HPTLC method for simultaneous estimation of levofloxacin hemihydrate and ornidazole in pharmaceutical dosage form. The method is based on the HPTLC separation of the two drugs followed by densitometric measurements of their spots at 298 nm. The separation was carried out on Merck TLC aluminium sheets of silica gel 60 F254 using n-butanol-methanol-
ammonia (5:1:1.5, v/v/v) as mobile phase. The linearity is found to be in the range of 50-250 and 100-500 ng/spot for levofloxacin hemihydrate and ornidazole, respectively. The method was validated as per ICH guidelines. The author has applied the developed method to pharmaceutical formulation because no chromatographic interferences from the tablet excipients are found.

**Avhad and Bonde. 2009** have developed a validated uv spectrophotometric method of simultaneous for the determination of levofloxacin and ambroxol in tablets. In method author used 219 (isoabsorptive point) and at 287 nm to get Q-absorbance equation using distilled water as a solvent. The linearity was found in the range of 2-14 μg/ml for levofloxacin and 5-35 μg/ml for ambroxol. The % recovery was found to be 100- 101% and 101-102% for levofloxacin and ambroxol respectively.

**Tamilarasi et al. 2014** have developed a Spectrophotometric method for the estimation of levofloxacin hemihydrate and cefpodoxime proxetil in tablet. Author developed a absorbance ratio method. The absorbance measurements were made six times for the formulations at 266 nm, 295.4 nm. The amounts of levofloxacin hemihydrate and cefpodoxime Proxetil were determined by constructing Q-analysis equation method. The absorbance of the solutions was measured and the percentage recovery was calculated. Author found percentage recovery in the range of 99.87 ±0.2869 for Levofloxacin hemihydrate and 100.26 ± 0.1418 for Cefpodoxime Proxetil. The percentage label claim present in tablets was found to be 100.26 ± 0.9483 and 100.28 ± 1.9449 for Levofloxacin hemihydrate and Cefpodoxime Proxetil respectively.

**Kole et al. 2014** have developed and validated an isocratic method for the simultaneous estimation of levofloxacin and cefpodoxime proxetil by using RP-HPLC in Combined Tablet Dosage form. Author used Alliance -Waters System with Agilant Zorbax Eclipse XBD-C8, (150mm×4.6; 5μm) column was used as a stationary phase. Mobile phase containing water with Ortho phosphoric acid: Methanol in the ratio of (80: 20, v/v) was used with a flow rate was 0.5 ml/min at 40°C column temperature and monitored at 270 nm. In this study the retention times was found to be 3.096 min for levofloxacin and 4.559 min for Cefpodoxime Proxetil. The correlation co-efficient for levofloxacin and cefpodoxime proxetil was found to be 1.0 and 1.0, respectively. Author validated the method for the parameters such as linearity, accuracy, precision, specificity, and robustness. Recovery of levofloxacin and cefpodoxime proxetil in formulations was found to be in the range of 97-103% and 97-103% respectively. Author concluded that
due to simplicity, rapidness and high precision, the method was successfully applied for the estimation of Levofloxacin and Cefpodoxime proxetil in combined dosage form.

**Srinivas et al. 2014** have developed a simple, reproducible and efficient reverse phase high performance liquid chromatographic method for simultaneous determination of levofloxacin and cefpodoxime proxetil in tablets. A column having 250 x 4.6 mm, 5μ i.d. in isocratic mode with mobile phase containing Orthophosphoric acid: Methanol (70:30) was used. The flow rate was 1.0 ml/min and effluent was monitored at 230 nm. Author has further reported that the retention times for levofloxacin and cefpodoxime proxetil were 3.7, 4.8 min and linearity was found to be in the range of 25-75 and 20-60 ppm. Author concluded that the developed method was found to be accurate, precise and selective for simultaneous determination of levofloxacin and cefpodoxime proxetil in tablets.

**Vaidya et al. 2014** have developed a Simple, precise and accurate HPTLC method for simultaneous determination of levofloxacin hemihydrate and cefpodoxime proxetil in synthetic mixture and tablet dosage form. The method was based on HPTLC separation of the two drugs followed by the densitometry measurements of their spots at 237 nm for both drugs. The separation was carried out on silica gel 60GF254 using mobile phase Butanol: Ethyl Acetate: hexane: triethylamine (2:6:4:0.1). The linearity for Levofloxacin hemihydrates and Cefpodoxime proxetil were found to be 125-750 ng/spot and 100-600 ng/spot. In this research work author found the Rf values were 0.66 and 0.33 for levofloxacin hemihydrates and cefpodoxime proxetil respectively and validated the method for ICH parameters. The author applied the developed method successfully for the determination of the two drugs in synthetic mixture and pharmaceutical dosage form.

**Patel and Shaikh. 2014** have developed a simple, rapid, precise and accurate stability-indicating reversed phase (RP) HPLC and validated the same for simultaneous estimation of Cefpodoxime proxetil and Levofloxacin in their combined tablet dosage form. The method has shown adequate separation of Cefpodoxime proxetil and Levofloxacin from their degradation products. Separation was achieved on a C18(100 mm x 4.6 mm,i.d., 3.5μm) column, kept at ambient temperature, using a mobile phase consisting of 50mM Ammonium acetate buffer : acetonitrile : triethylamine (60:40:0.2 v/v/v), pH 6.0 adjusted with glacial acetic acid at a flow rate of 1.0 ml/min and UV detection at 258 nm. The average retention times for Cefpodoxime Proxetil isomer A, Cefpodoxime Proxetil isomer B and Levofloxacin were found to be 5.70, 6.304 and 1.29 min respectively.
Further, author exposed the Cefpodoxime Proxetil, Levofloxacin and their combination drug product to various stress conditions such as acid hydrolysis, base hydrolysis, oxidation, thermal and photolytic. Also carried the Validation of the method as per ICH guidelines. Linearity was established for Cefpodoxime Proxetil, Levofloxacin in the range of 8-24 and 10-30 μg/ml respectively. Author carried out the accuracy by performing the recovery study and the percentage recovery of Cefpodoxime Proxetil and Levofloxacin was found to be in the range of 100.58-101.32 % and 99.91-100.32 % respectively. Author has concluded that the method is specific and stability indicating as no interfering peaks of degradants and excipients were observed.