SATRANIDAZOLE

Satranidazole (CG-10213-Go), a novel nitroimidazole possessing a C-N linkage at C₂ of the imidazole ring has been examined (during reduction), for its ability to damage DNA. Satranidazole (SAT) is not included in any official pharmacopoeias such as IP, USP and BP. Literature survey revealed that there are various methods for estimation of Satranidazole individually as well as in combination with Ofloxacin.

Zahoor A et al., 1986 stated that the drug produces extensive DNA damage characterized by helix destabilization and strand breakage. Its comparison with other 2- and 5-nitroimidazoles indicates it may be more active towards anaerobes than many 5-nitroimidazoles. It is due to its relatively high redox potential which may make it more resistant to inactivation by oxygen. It is recently introduced as an anti/protozoal agent in tablet dosage form. It is a highly potent, well-tolerated, and clinically useful agent against common protozoa. It is rapidly absorbed and exhibits higher plasma and liver concentration than metronidazole.

Pargal, A et al., 1993 studied the pharmacokinetic properties of metronidazole and satranidazole in the golden hamster (Mesocricetus auratus) at a dose of 80 mg/kg. Blood and liver samples were collected at frequent time intervals and assayed for metronidazole and satranidazole by HPLC. Satranidazole exhibited significantly higher plasma concentrations than metronidazole at 1 and 2 hr post-dose, but the comparative C(max) values were not significantly different. The satranidazole plasma elimination half-life of 1.01 hr was significantly shorter than the corresponding metronidazole half-life of 3.62 hr. The comparative liver pharmacokinetic parameters C(max), T(max) and T( 1/2 ) did not differ significantly. Satranidazole however exhibited significantly higher liver concentrations at 1 hr post-dose and C(max) and AUC(0-∞), values were approximately 35% higher. The in-vivo amoebicidal activity of both compounds was evaluated in the acute hamster hepatic model of amoebiasis. Both metronidazole and satranidazole were administered as single graded doses, and their dose-response profiles were characterized. Satranidazole demonstrated significantly greater amoebicidal activity than metronidazole with an ED₅₀ value of 19.5 mg/kg, compared to an ED₅₀ value of 45 mg/kg for metronidazole. These data suggest that higher plasma and liver concentrations of
satranidazole and greater intrinsic potency probably contribute to superior amoebicidal activity in the hamster model of hepatic infection.

Mruthyunjayaswamy BHM et al., 2001 developed two spectrophotometric methods (I and II) under visible region for the estimation of Satranidazole in bulk drug and pharmaceutical formulations. Methods I and II are based on the reaction of reduced satranidazole with p-dimethylamino benzaldehyde (PDAB) and p-dimethylaminocinnamaldehyde (PDACA) in acidic conditions to form orange red and purple coloured chromogens with absorption maxima at 511 nm and 568 nm respectively. The reduction of satranidazole was carried out with zinc granules and 4 N hydrochloric acid at room temperature in ethanol. Beer's law was obeyed in the concentration range of 10-50 μg/ml for both the methods. The results of analysis have been validated statistically and by recovery studies. The methods were found to be simple, rapid, accurate, reproducible and economic. The results are comparable with those obtained using UV spectrophotometric methods in alcohol at 315 nm.

Raju SA et al., 2002 developed two simple and sensitive spectrophotometric methods for the quantitative estimation of satranidazole. In method I, a simple UV spectrophotometric method has been developed for satranidazole in bulk drug and its formulations. Satranidazole is taken in alcohol and absorbance measured at 315 nm and Beer's Law is obeyed in the concentration range 2-10 μg/mL. In method II, the presence of amino group in reduced satranidazole enables the formation of complex with 1,10-phenanthroline and Fe(III) exhibiting absorption maximum at 495 nm and Beer's law is obeyed in the concentration range 5-25 μg/mL.

Rajnarayana K et al., 2002 developed a high performance liquid chromatographic (HPLC) method for the determination of tinidazole in human serum using metronidazole as internal standard (IS). Protein precipitation is used for the preparation of sample. Mobile phase consisting of 0.002 M phosphate buffer, methanol and acetonitrile mixture (85:7.5:7.5/v/v/v) was used at a flow rate of 1 ml/min on a C18 column. The eluate was monitored using an UV/Vis detector set at 320 nm. Ratio of peak area of analyte to IS was used for quantification of serum samples. The absolute recovery was greater than 95% over a concentration range of 0.5 to 30 micrograms/ml and the limit of quantitation was 0.05 microgram/ml. The intra-day relative standard deviation (RSD) measured at 0.5,
5, 15 and 30 micrograms/ml ranged from 0.36 to 6.14%. The inter-day RSD ranged from 1.14 to 4.21%. The method is simple, sensitive and has been successfully used in a pharmacokinetic study conducted in healthy human volunteers.

Lalla J et al., 2003 determined satranidazole in its dosage form by High-performance thin-layer chromatographic method. The HPTLC method involves use of silica gel 60F_{254} as the stationary phase, metronidazole was used as internal standard and chloroform-methanol, 10: 0.5 (v/v), as mobile phase. The separated bands were detected at \( \lambda = 317 \) nm. The response was found to be linearly dependent on amount of satranidazole between 200 and 1000 ng. The method was validated to determine its accuracy and precision. System suitability tests were conducted to verify that the resolution and reproducibility were adequate for the analysis.

Tavakoli N, et al., 2007 developed a simple, rapid, sensitive and robust reversed phase-HPLC method to measure simultaneously the amount of amoxicillin and metronidazole at single wavelength (254 nm) in order to assess drug release profiles and drug–excipients compatibility studies for a new floating-sustained release tablet formulation and its subsequent stability studies. An isocratic elution of filtered sample was performed on C18 column with buffered mobile phase (pH 4.0) and UV detection at 254 nm. Quantification was achieved with reference to the external standards. The linearity for concentrations between 0.15 and 600 \( \mu \)g/ml for amoxicillin and 0.13 and 300 \( \mu \)g/ml for metronidazole were established. Intra and inter-day precision were less than 2.5%. The limits of detection (LOD) and quantification were 0.05 and 0.15 \( \mu \)g/ml for amoxicillin and 0.10 and 0.13\( \mu \)g/ml for metronidazole. The determination of the two active ingredients was not interfered by the excipients of the products. Samples were stable in the release media (37 °C) and the HPLC injector at least for 12 h.

Patel MB et al., 2007 developed and validated a stability-indicating high performance thin layer chromatography (HPTLC) method for analysis of satranidazole both as a bulk drug and in formulations. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene/acetonitrile (60:40, v/v). Densitometric analysis of satranidazole was carried out in the absorbance mode at 314 nm. This system was found to give compact spots for satranidazole (Rf value of 0.53±0.02, for six replicates). Satranidazole was subjected to
acid and alkaline hydrolysis, oxidation, and photo degradation. The drug undergoes degradation under acidic and basic conditions, oxidation, and photo degradation. Also, the degraded products were well resolved from the pure drug with significantly different Rf values. Linearity was found to be in the range of 100-500 ng/spot with a significantly high value of correlation coefficient r²=0.9979±0.66. The LOD and LOQ were 50 and 85 ng/spot, respectively. The proposed HPTLC method was utilized to investigate the kinetics of the alkali degradation process.

Natarajan S et al., 2008 determined satranidazole in bulk and pharmaceutical dosage forms by HPLC. The stability indicating capability of the method was proven by subjecting the drugs to ICH stress conditions of alkaline and acidic hydrolysis, oxidation, photolysis and thermal degradation and resolution of the degradation products formed therein. The specificity was confirmed by spiking process related impurities of satranidazole and their separation from the main peak. The separation was obtained using a mobile phase of mixture of pH 3.0 buffer and acetonitrile in the ratio 600:400 with final pH of 3.6 on a ODS column (4.6 mm x 250 mm, 5 μ) with UV detection at 250 nm at 1 mL/min flow rate. For stress studies, a diode array detector was used. The elution of satranidazole was at 4.77 min. The linear dynamic range was 60 - 240 mcg/mL for satranidazole. A percentage recovery for satranidazole was 99.64 %.

Wankhede SB et al., 2008 determined simultaneous spectrophotometric estimation of ofloxacin and satranidazole in tablet dosage form. First method is first order derivative spectroscopy, wavelengths selected for quantitation were 259.0 nm for Ofloxacin (zero cross for Satranidazole) and 227.0 nm for Satranidazole (zero cross for Ofloxacin). Second method is area under curve method; area under curve in the range of 292.5-282.5 nm (for Ofloxacin) and 325.0-315.0 nm (for Satranidazole) were selected for the analysis. In both the methods linearity for detector response was observed in the concentration range of 5-40 μg/ml for Ofloxacin and Satranidazole, both. The proposed methods were successfully applied for the simultaneous determination of both drugs in commercial tablet preparation.

Gandhi S.V et al., 2008 developed ultraviolet spectrophotometric and reversed-phase high-performance liquid chromatographic (RP-HPLC) methods for simultaneous estimation of escitalopram oxalate (ESC) and clonazepam (CLO) in combined tablet
dosage form. The spectroscopic method employs an absorbance correction method using 238.6 and 308 nm as 2 wavelengths for estimation with methanol and water as solvents. Beer's law is obeyed in the concentration range of 10.0-50.0 and 0.5-3.0 μg/mL for ESC and CLO, respectively. The RP-HPLC method uses a Jasco HPLC system with HiQ SiL C18 column (250 × 4.6 mm id) acetonitrile-0.005 M tetrabutylammonium hydrogen sulfate (55 + 45, v/v) as the mobile phase, and satranidazole as an internal standard. The detection was carried out using an ultraviolet detector set at 287 nm. For the HPLC method, Beer's law is obeyed in the concentration range of 10.0-60.0 and 0.5-3.0 μg/mL for ESC and CLO, respectively.

Bansal K. et al., 2009 developed satranidazole-containing mucoadhesive gel for the treatment of periodontitis. Different mucoadhesive gels were prepared, using various gelling agents like sodium carboxymethylcellulose (SCMC), poloxamer 407, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, and the mucoadhesive polymer carbopol 934P. The selected formulations (based on the mucoadhesive force) were studied for different mechanical properties, such as mucoadhesive strength, hardness, compressibility, adhesiveness, and cohesiveness through Texture Profile Analyzer. In vitro satranidazole release from the prepared formulations was also determined and compared with marketed preparation of metronidazole (Metrogyl® gel). The formulation SC30 (containing SCMC 3% w/v) showed maximum mucoadhesive strength (167.72 ± 3.76 g) and adhesiveness (-46.23 ± 0.34 N∈mm), with low hardness (9.81 ± 0.04 N) and compressibility (40.05 ± 0.48 N∈mm) and moderate cohesiveness (0.87 ± 0.01). SC30 formulation exhibited long-term release. Thus, SC30 gel was evaluated for its clinical effectiveness along with marketed metronidazole gel. This study confirmed the acceptability and effectiveness of satranidazole gel for treatment of periodontitis.

Mital A, 2009 provides a brief account of various biological activities exhibited by synthetic nitroimidazole derivatives as well as their structure-mutagenicity relationships. Nitroheterocyclic drugs have been available since the early 1960s for the treatment of anaerobic protozoa. The application of these drugs has widened and they are presently used to treat anaerobic pathogenic bacteria and protozoa. 5-nitroimidazoles are a well-established group of antiprotozoal and antibacterial agents that inhibit the growth of both
anaerobic bacteria and certain anaerobic protozoa, such as Trichomonas vaginalis, Entamoeba histolytica and Giardia lamblia. The important antibacterial and antiprotozoal activities of nitroimidazoles are associated with reductive metabolism that has led to considerable interest in nitroimidazole reduction chemistry and the synthesis of new, highly effective drugs.

**Patel A.B. et al., 2009** developed and validated a RP-HPLC method for the estimation of satranidazole and gatifloxacin simultaneously in combined dosage forms. A Lichrosopher 100 C-18 and mobile phase comprises of Water: Acetonitrile: Triethylamine (75:25:0.35, v/v/v) were used for separation. Final pH was adjusted to 3.2 ± 0.02 with 10% v/v orthophosphoric acid. Measurements were made at the effluent flow rate of 1.0 ml/min with injection volume 20 μl and ultraviolet (UV) detection at 320 nm, as both components show reasonable good response at this wavelength. The retention times of satranidazole and gatifloxacin were 6.0 min and 3.44 min, respectively. Linearity of satranidazole and gatifloxacin was in the range of 1-70 μg/ml and 1-70 μg/ml, respectively. Average percentage recoveries obtained for satranidazole and gatifloxacin were 99.80 % and 100.20 %, respectively. The limit of detection and limit of quantification were found to be 0.3 and 1.0 μg/ml for satranidazole, respectively and for gatifloxacin were 0.5 and 1.0 μg/ml, respectively.

**Wankhede S.B et al., 2009** developed a simple, selective, rapid and precise reverse phase HPLC method for the simultaneous estimation of Ofloxacin and Satranidazole in tablets. The analyte was resolved by using a mobile phase 0.05 M phosphate buffer and acetonitrile in the ratio of 65:35 v/v at a flow rate of 1.0 ml/min on an isocratic HPLC system at a wavelength of 320 nm. The linearity was obtained in the concentration range of 5-40 μg/ml for Ofloxacin and Satranidazole, respectively.

**Boopathy D et al., 2010** developed and validated a method for simultaneous determination of Ofloxacin and Satranidazole in pharmaceutical dosage form by RP-HPLC. A Phenomenex Luna C18 (4.6*250 mm, 5μ) column was used for the separation. The mobile phase was Phosphate buffer: Acetonitrile (70: 30 % v/v) and pH 6.0 at a flow rate of 1.2ml/min with detection at 300nm. The retention time of Ofloxacin and Satranidazole was 3.720 and 6.130 min, respectively.
**Ganesh M. et al., 2010** determined Letrozole, an anti-cancer drug in tablet formulations by reversed phase high performance liquid chromatography using a new mobile phase of acetonitrile:water (50:50, v/v). The eluent was monitored at 265 nm. The optimized conditions developed showed a linear response from 160 to 240 μg/mL, with a correlation coefficient (R2) of 0.999. The limit of detection (LOD) and limit of quantification (LOQ) were 136 and 160 μg/mL, respectively. The assay values for the two branded letrozole tablets tested were 99.2 and 100.2 %, respectively with % relative standard deviation (RSD) of 0.781 and 0.568, respectively. The bench top stability data of the drug in the mobile phase indicate that the drug was stable in the mobile phase for 24 h. Recovery data were good. Placebo study for specificity and interference of common excipients showed that the method was specific and free from interfering substances.

**Pasha K. et al., 2010** developed a reverse phase – HPLC method for the analysis of tinidazole in pharmaceutical dosage form & bulk drug. C18, 250 x 4.6mm, 5μm column with mobile phase composition of acetonitrile and phosphate buffer 3:1 (PH 5), flow rate of 1.0 ml/min and UV detection at 295nm was selected. Linearity was observed over concentration range of 10‐80 mcg/ml. The accuracy of the proposed method was determined by recovery studies and found to be 101-103% the proposed method was validated and results conformed with ICH parameters.

**Sachin RS et al., 2010** estimated satranidazole and ofloxacin simultaneously in tablet dosage form by high performance liquid chromatography. Chromatographic separation of these drugs were performed on Kromasil C18 column (250 x 4.6 mm, 5 μ) as stationary phase with a mobile phase comprising of 20 mM potassium dihydrogen phosphate: acetonitrile in the ratio of 60:40 (v/v) containing 0.1% glacial acetic acid at aflow rate of 1 mL/min and UV detection at 318 nm. The linearity of satranidazole and ofloxacin were in the range of 1.5 to 3.6 μg/mL and 1.0 to 2.4μg/mL respectively. The recovery was calculated by standard addition method. The average recovery was found to be 100.63% and 100.02% for satranidazole and ofloxacin respectively.

**Sherje A.P. et al., 2010** developed two simple, accurate and precise spectrophotometric methods for simultaneous determination of satranidazole and ofloxacin in pharmaceutical fixed dosage form. The method A involves simultaneous equation using 297.3 and 317.0 nm as the wavelengths of detection while method B is two wavelength method where
281.5nm, 309.0nm were selected as $\lambda_1$ and $\lambda_2$ for determination of satranidazole and 300.0 nm, 333.1nm were selected as $\lambda_1$ and $\lambda_2$ for determination of ofloxacin. The Beer's law limits for each drug individually and in mixture was within the concentration range of 5-25 $\mu$g/ml. Linearity of satranidazole and ofloxacin were in the range of 80-120% of the label claim. 

Bhoir SI et al., 2011 developed a simple, rapid, and accurate reversed phase high-performance liquid chromatographic method and subsequently validated for the simultaneous determination of ofloxacin (OFL) and satranidazole (SAT) in combination. The separation was carried out using a mobile phase consisting of 10mM phosphate buffer and methanol in the ratio of 50:50. The pH of the mobile phase was adjusted to 3.0 with 10% o-phosphoric acid. The column used is Kromasil-100 C$_{18}$ (250 × 4.6 mm, 5 $\mu$m) with flow rate of 1.0 mL/min using UV detection at 294nm. The total run time was 5 min and the retention time of OFL and SAT was 2.59 min and 4.0 min respectively. The described method was linear for the assay of OFL and SAT over a concentration range of 10-24 $\mu$g/mL and 15-36 $\mu$g/mL respectively. The results of the studies showed that the proposed RP-HPLC method is simple, rapid, precise, and accurate, which is useful for the routine determination of SAT and OFL in bulk drug and its pharmaceutical dosage form.
PEFLOXACIN

It is a second group of fluorinated quinolone possessing excellent gram negative activity and moderate to good activity against gram positive bacteria [Katzung B.G., 2004]. Quinolones comprise a carboxylic group in position 3, and a carbonyl group in position 4, hence they are often referred to as 4-quinolones. Their antibacterial activity is greatly increased by the addition of 6-fluoro and 7-piperazinyl groups to the molecule and named fluoroquinolones. It possesses some favorable pharmacokinetic properties, such as complete absorption after oral administration, long half-life (10-12 hr) permitting infrequent dosage and rapid penetration into the intra-cellular and extracellular spaces. It is metabolized in the liver to an N-oxide derivative desmethyl-pefloxacin (norfloxacin), and excreted in the urine. Suitable and sensitive analytical methods for determination of drug are essential for successful evaluation in their dosage form. Several analytical methods for quantitative determination of pefloxacin in pharmaceutical formulations and in biological fluids are described in the literature.

Montay G et al., 1983 describe a high-performance liquid chromatographic (HPLC) method for the analysis of pefloxacin, a new antibacterial agent, in plasma and urine following administration of a therapeutic dose in humans. HPLC assay of pefloxacin and its two main active metabolites in urine was also described. The applicability of the methods to pharmacokinetic studies of pefloxacin in humans was demonstrated.

Jelikic-SM et al., 1989 established that pefloxacin (Abaktal) reacts with Fe(III) at pH 1.00-8.00 to form a water-soluble complex with maximum absorbance at 360 nm. The composition of the complex, determined Spectrophotometrically by the application of Job's, molar-ratio and Bent-French's methods, was pefloxacin: Fe (III) = 1:1 (pH = 2.50; lambda = 360 nm; mu = 0.1 M). Beer's law was followed for pefloxacin concentrations of 2.15-85.88 μg/ml. The relative standard deviation (n = 10) was 0.57-1.07%. The method can be applied to the simple determination of pefloxacin in aqueous solutions and tablets.

Abanmi N et al., 1996, determined pefloxacin and its main active metabolite in human serum by high performance liquid chromatography.

Gratteri P et al., 1999 used Partial least squares regression (PLS1 and PLS2) and GOLPE variable selection procedures for the treatment of differential pulse polarographic
and UV Spectrophotometric data obtained from the analysis of the therapeutic combination of metronidazole and pefloxacin. The analytical method used for the determination was set up using experimental design strategies (Doehlert's design, full factorial design, fractional face centre cube design) and by involving the simultaneous optimization of several responses (desirability function). Method validation was also performed, determining accuracy, precision, linearity and range, detection and quantification limits and robustness. The quantitative prediction abilities in determining metronidazole and pefloxacin plasma levels of the PLS1 and PLS2 models were tested on spiked plasma samples and good results were obtained (metronidazole, 97.5%, RSD = 4.8%, n = 3; pefloxacin, 100.6%, RSD = 3.6%, n = 3). The use of multivariate calibration was particularly useful for spectrophotometric quantification because of the highly overlapping spectra of the binary mixture.

Simmy O. Thoppil and P.D. Amin, 2000, developed and optimised a stability indicating method of ciprofloxacin as bulk drug and in pharmaceutical formulations. The mobile phase was modified from that reported in the USP so as to resolve the degraded products from the drug. The mobile phase consisting of water acetonitrile-triethylamine (80:20:0.6, v:v:v) of pH 3.0 adjusted with ortho-phosphoric acid resulted in a retention time of 3.61 min for ciprofloxacin. The chromatogram of the acid degraded sample showed two additional peaks at 1.36 and 2.36 min. The chromatogram of the base degraded sample showed three additional peaks at 1.55, 2.87, and 5.33 min. The peaks of the degraded products were well resolved from the ciprofloxacin peak (3.61 min).

Mostafa S et al., 2002 described a spectrophotometric method for the determination of the antibacterial quinolone derivatives ciprofloxacin, enrofloxacin and pefloxacin through charge transfer complex formation with three different acceptors. Chloranilic acid (CL) was utilized for their determination, forming charge transfer complex with lambda max 520 nm. The proposed method was applied for determination of Ciprocin tablets, Enroxil oral solution, Peflacin ampoules and Peflacin tablets, with mean percentage accuracies, 99.58±1.25, 99.94±0.96, 100.91±1.59 and 99.86±1.003. Also, tetracyanoethylene (TCNE) was utilized in the determination of the concerned compounds forming charge transfer complexes with maximum absorbance at 335 nm for ciprofloxacin and at 290 nm for both enrofloxacin and pefloxacin. The procedure was applied for determination of
Ciprocin tablets, Enroxil 10% oral solution, Peflacine tablets and Peflacine ampoules with mean percentage accuracies 99.40+/−1.27, 99.95+/−0.90, 98.98+/−1.565 and 99.88+/−0.998, respectively. Also, 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) was utilized for determination of pefloxacin forming charge transfer complex with maximum absorbance at 460 nm. The procedure was applied for determination of peflacine tablets and peflacine ampoules with mean percentage accuracies 100.40+/−0.76 and 99.91+/−0.623, respectively. Statistical analysis of the obtained results showed no significant difference between the proposed method and other official and reported methods as evident from the t-test and variance ratio.

**Beltagi AM., 2003** developed a fully validated square wave cathodic adsorptive stripping voltametric procedure for the determination of the pefloxacin drug in bulk form, tablets and human serum.

**Basavaiah et al., 2004** performed two methods for quantitative determination of pefloxacin mesylate by residual-base neutralisation method. In the first method involving titrimetry, the drug solution is treated with a measured excess of sodium hydroxide followed by back titration of the residual base with hydrochloric acid using a phenol red-bromothymol blue mixed indicator. The second spectrophotometric method involves treatment of a fixed amount of sodium hydroxide – phenol red mixture with varying amounts of the drug, and measuring the decrease in the absorbance of the dye at 560 nm. In the titrimetric method, a reaction stoichiometry of 1:1 was found in the quantification range of 4–20 mg of drug. The spectrophotometric method allows the determination of PFM in the 5–40 μg/ml range. The molar absorptivity is 5.91 x 10³ l mol⁻¹ cm⁻¹ and the Sandell sensitivity is 56.37 ng cm⁻².

**Maria Ines RM et al., 2006** developed and validated a simple and rapid chromatographic method for quantitative determination of four quinolone antibiotics in tablets and injection preparations. The fluoroquinolones studied were gatifloxacin (GAT), levofloxacin (LEV), lomefloxacin (LOM) and pefloxacin (PEF). The quinolones were analyzed by using a LiChrospher® 100 RP-18 column (5 μm, 125 mm × 4 mm) and a mobile phase constituted of water:acetonitrile (80:20, v/v) with 0.3% of triethylamine and pH adjusted to 3.3 with phosphoric acid. The flow rate was 1.0 mL/min and the analyses were performed using UV detector with wavelengths varying from 279 to 295 nm. The
analyses were performed at room temperature (24 ± 2 °C). All fluoroquinolones were separated within 5 min. The calibration curves were linear ($r \geq 0.9999$) over a concentration range from 4.0 to 24.0 μg/mL. The relative standard deviation (R.S.D.) was <1.0% and average recovery was above 99.54%.

Santoro MIRM et al., 2006 developed and validated a simple chromatographic method for quantitative determination of four quinolone antibiotics in tablets and injection preparations. The fluoroquinolones studied were gatifloxacin (GAT), levofloxacin (LEV), lomefloxacin (LOM) and pefloxacin (PEF). The quinolones were analyzed by using a LiChrospher 100 RP-18 column (5 microm, 125 mm x 4 mm) and a mobile phase constituted of water:acetonitrile (80:20, v/v) with 0.3% of triethylamine and pH adjusted to 3.3 with phosphoric acid. The flow rate was 1.0 mL/min and the analyses were performed using UV detector with wavelengths varying from 279 to 295 nm. The analyses were performed at room temperature (24 +/- 2 degrees C). All fluoroquinolones were separated within 5 min. The calibration curves were linear ($r \geq 0.9999$) over a concentration range from 4.0 to 24.0 microg/mL. The relative standard deviation (R.S.D.) was < 1.0% and average recovery was above 99.54%.

Basavaiah K et al., 2007 described a spectrophotometric method for assay of pefloxacin mesylate (PFM) in bulk drug and in tablets. The method is based on back extraction of the bromophenol blue dye at pH 5.2 from the dye-drug ion pair followed by measurement of the dye absorbance at 590 nm. Beer's law plot showed a good correlation in the concentration range of 0.15-1.25 mcg/ml. Intra-day and inter-day precision, and accuracy of the methods were established according to the ICH guidelines. The method was successfully applied to the assay of PFM in tablet preparations with recoveries varying from 97.5 to 101.9%, with standard deviation in the range of 0.6 to 1.9. Accuracy evaluated by means of the spike recovery method, range from 97.0 to 106.0%, with precision better than 3%.

Salem et al., 2007 determined certain fluoroquinolones in pharmaceutical dosage forms and in biological Fluids. They described the method for the quantitative determination of ten fluoroquinolones (amifloxacin, enoxacin, enrofloxacin, ciprofloxacin hydrochloride, difloxacine hydrochloride, lomefloxacin hydrochloride, levofloxacin, norfloxacin, ofloxacin and pefloxacin mesylate). The methods are based on precipitation of the ion
associates formed from the reaction of the cited drugs with silver nitrate, copper acetate and ferric chloride. The formation and solubility of the solid complexes at the optimum conditions of pH and ionic strength values have been studied. The methods depend on direct determination of the ions in the precipitate or indirect determination of the ions in the filtrate by atomic absorption spectroscopy. The optimum conditions for precipitation were carefully studied. Rectilinear calibration graphs were obtained in the range of 10-100 ng/ml for each of the investigated drugs and the limits of detection and quantitation ranged from 1.125 to 2.26, 0.937 to 2.754 and from 3.425 to 5.986 ng/ml, respectively.

**Vadera N., et al, 2007**, developed a simple, selective, precise and stability-indicating high-performance thin-layer chromatographic method of analysis of imatinib mesylate both as a bulk drug and in formulations was developed and validated. The method employed HPTLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of chloroform:methanol (6:4, v/v). The system was found to give compact spot for imatinib mesylate (Rf value of 0.53±0.02). Densitometric analysis of imatinib mesylate was carried out in the absorbance mode at 276 nm. The linear regression analysis data for the calibration plots showed good linear relationship with \( r^2 = 0.9966±0.0013 \) with respect to peak area in the concentration range 100–1000 ng per spot. The mean value ± S.D. of slope and intercept were 164.85±0.72 and 1168.3±8.26 with respect to peak area. The method was validated for precision, recovery and robustness. The limits of detection and quantitation were 10 and 30 ng per spot, respectively. Imatinib mesylate was subjected to acid and alkali hydrolysis, oxidation and thermal degradation. The drug undergoes degradation under acidic, basic, oxidation and heat conditions. This indicates that the drug is susceptible to acid, base hydrolysis, oxidation and heat. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of said drug. The proposed developed HPTLC method can be applied for identification and quantitative determination of imatinib mesylate in bulk drug and dosage forms.

**Ni Y et al., 2008** described a kinetic spectrophotometric method for the simultaneous determination of pefloxacin and its structurally similar metabolite, norfloxacin, for the first time. The analysis is based on the monitoring of a kinetic spectrophotometric reaction of the two analyte with potassium permanganate as the oxidant. The
measurement of the reaction process followed the absorbance decrease of potassium permanganate at 526 nm, and the accompanying increase of the product, potassium manganate, at 608 nm. It was essential to use multivariate calibrations to overcome severe spectral overlaps and similarities in reaction kinetics. Calibration curves for the individual analytes showed linear relationships over the concentration ranges of 1.0-11.5 mg/L at 526 and 608 nm for pefloxacin, and 0.15-1.8 mg/L at 526 and 608 nm for norfloxacin. Various multivariate calibration models were applied, at the two analytical wavelengths, for the simultaneous prediction of the two analytes including classical least squares (CLS), principal component regression (PCR), partial least squares (PLS), radial basis function-artificial neural network (RBF-ANN) and principal component-radial basis function-artificial neural network (PC-RBF-ANN). PLS and PC-RBF-ANN calibrations with the data collected at 526 nm, were the preferred methods--%RPE(T) approximately 5, and LODs for pefloxacin and norfloxacin of 0.36 and 0.06 mg/L, respectively. Then, the proposed method was applied successfully for the simultaneous determination of pefloxacin and norfloxacin present in pharmaceutical and human plasma samples. The results compared well with those from the alternative analysis by HPLC. Fierens et al and Flurer used capillary electrophoresis method for the estimation of pefloxacin.

**Okeri H. A. and Arhewoh I. M., 2008** reviewed the series of the fluoroquinolone antibacterial agents with focus on the analytical profile of ofloxacin. Ofloxacin and indeed all the other fluoroquinolones are synthetic antibacterial agents structurally related to nalidixic acid. This article examines the synthesis, physico-chemical properties and analytical methods that have been used for the determination of ofloxacin in pharmaceutical dosages forms and biological fluids.

**Lalitha Devi M. and Chandrasekhar K.B., 2009** developed a validated stability-indicating RP-HPLC method for levofloxacin in the presence of degradation products, its process related impurities and identification of oxidative degradant. Forced degradation studies were performed on bulk sample of levofloxacin as per ICH prescribed stress conditions using acid, base, oxidative, water hydrolysis, thermal stress and photolytic degradation to show the stability indicating power of the method. Significant degradation was observed during oxidative stress and the degradation product formed was identified
by LCMS/MS, slight degradation in acidic stress and no degradation was observed in other stress conditions. The chromatographic method was optimized using the samples generated from forced degradation studies and the impurity spiked solution. Good resolution between the peaks corresponds to process related impurities and degradation products from the analyte were achieved on ACE C18 column using the mobile phase consists a mixture of 0.5% (v/v) triethyl amine in sodium dihydrogen orthophosphate dehydrate (25mM; pH 6.0) and methanol using a simple linear gradient. The detection was carried out at 294 nm. The limit of detection and the limit of quantitation for the levofloxacin and its process related impurities were established. The stressed test solutions were assayed against the qualified working standard of levofloxacin and the mass balance in each case was in between 99.4 and 99.8% indicating that the developed LC method was stability indicating. Validation of the developed LC method was carried out as per ICH requirements. The developed LC method was found to be suitable to check the quality of bulk samples of levofloxacin at the time of batch release and also during its stability studies (long term and accelerated stability).

Shahnaz G et al., 2009 developed and validated a HPLC method for the quantitative determination of pefloxacin in bulk material, tablets and in human plasma. A shim-pack CLC-ODS column and a mobile phase constituting acetonitrile: 0.025 M phosphoric acid solution (13:87 v/v, pH 2.9 adjusted with KOH) were used. The flow rate was 1 ml/min and the analyses performed using ultraviolet (UV) detector at a wavelength of 275 nm using acetaminophen as an internal standard. The percentage of coefficient variation (CV) of the retention times and peak areas of pefloxacin from the six consecutive injections were 0.566% and 0.989%, respectively. The results showed that the peak area responses are linear within the concentration range of 0.125 μg/ml - 12 μg/ml (R^2 = 0.9987). The limits of detection (LOD) and limits of quantitation (LOQ) for pefloxacin were 0.03125 μg/ml and 0.125 μg/ml. The intra-day and inter-day variation, RSD were 0.376-0.905 and 0.739-0.853 respectively; also, inter-day variation with relative standard deviation (RSD) were 0.1465-0.821 in plasma. The accuracy results of 70%, 100%, and 130% drugs were 100.72%, 100.34%, and 100.09%, respectively.

Yassien M et al., 2009 studied the voltammetric behaviour of antibacterial danofloxacin (DANO) and orbifloxacin (ORBX) in a wide pH range from 5 to 12 using phase sensitive
alternating current, cyclic voltammetry and differential pulse cathodic adsorptive stripping voltammetric (DPCASV). The adsorption behaviour of both biological compounds at the hanging mercury drop electrode was investigated in order to achieve an increase in sensitivity and a possibility of the antibacterial DANO and ORBX determination by applying the adsorptive stripping voltammetric method.

Xie et al., 2010 applied the near-infrared spectroscopy (NIRS) to quantitatively analyze on 108 injection samples, which was divided into a calibration set containing 89 samples and a prediction set containing 19 samples randomly. In order to get a satisfying result, partial least square (PLS) regression and principal components regression (PCR) have been utilized to establish quantitative models. In addition, the process of establishing the models, parameters of the models, and prediction results were discussed in detail. In the PLS regression, the values of the coefficient of determination (R(2)) and root mean square error of cross-validation (RMSECV) of PLS regression are 0.9263 and 0.00119, respectively. The values of the standard error of prediction set (SEP) of PLS and PCR models were 0.001480 and 0.001140. The result of the prediction set suggests that these two quantitative analysis models have excellent generalization ability and prediction precision. However, for this PFLX injection samples, the PCR quantitative analysis model achieved more accurate results than the PLS model. The experimental results showed that NIRS together with PCR method provide rapid and accurate quantitative analysis of PFLX injection samples. Moreover, this study supplied technical support for the further analysis of other injection samples in pharmaceuticals.
**BALSALAZIDE DISODIUM**

Balsalazide is a new innovative, mesalamine-containing, anti-inflammatory prodrug that is used in the treatment of inflammatory bowel disease. It is usually administered as the disodium salt. It is an orally administered prodrug of 5-ASA in which an inert carrier molecule, 4-aminobenzoyl-alanine, has been substituted for the sulfapyridine moiety of sulfasalazine. After administration, colonic bacterial azoreductases split balsalazide into 5-ASA and 4-aminobenzoyl-alanine, releasing the active 5-ASA into the colon with minimal systemic absorption of balsalazide, 5-ASA, or 4-aminobenzoyl-alanine (*Chan RP et al 1983*). Balsalazide has been shown to deliver 5-ASA to the colon with a reduced side effect profile relative to that observed with sulfasalazine and olsalazine (3,4). In addition, balsalazide has been shown to be as effective and well tolerated as delayed-release mesalamine in the chronic treatment of ulcerative colitis with better relief of nocturnal symptoms in chronic treatment than mesalamine (*Green JR, B 1993*). In acute treatment of active, mild-to-moderate ulcerative colitis, balsalazide has been reported to provide more rapid relief of symptoms and induce complete remission in a greater percentage of patients than pH-dependent, delayed-release mesalamine (*Green JRB. et al, 1998*). Sulfasalazine is accepted therapy for active ulcerative colitis, but side effects and intolerance are common. Balsalazide is an azo-bonded pro-drug which also releases 5-aminosalicylic acid into the colon, but uses an inert carrier molecule. Eight weeks of treatment with balsalazide (6.75 g daily) is significantly more effective, well tolerated than balsalazide (2.25 g daily) and more rapid in onset than mesalamine (2.4g daily) in improving signs and symptoms of acute ulcerative colitis (*Douglas SL. et al, 2002*).

*Mansfield JC. et al, 2002*, compared the safety and efficacy of sulfasalazine (3 g) with balsalazide (6.75 g) in the initial daily treatment of mild to moderate ulcerative colitis. Balsalazide is not included in any official pharmacopoeias. Literature survey revealed that there are various methods for estimation of drug.

*Anandakumar K. et al., 2008* developed three simple, precise and accurate UV-Visible spectrophotometric methods for the estimation of Balsalazide in bulk drug and in pharmaceutical formulations. In method A, UV spectra of Balsalazide in double distilled water exhibits the absorption maxima at 358 nm. Method B is based on the formation of a
dark red colored chromogen in alkaline medium at pH 12 exhibits λ max at 456 nm. Method C is the derivative spectroscopic method. The first derivative spectra were recorded between 200 and 500 nm at N=1 and the assay was carried out by measuring the absorbance at 383 nm. Beers law obeyed in the concentration range of 2-20 μg/ml for methods A and B and 5-25 μg/ml for method C. The accuracy of the method was determined by recovery studies. The method were validated statistically. The methods showed good reproducibility and recovery with % RSD less than 2. The methods were found to be simple, economical, accurate and reproducible and can be used for routine analysis of balsalazide in bulk drug and in pharmaceutical formulations.

Naveen GS. et al, 2008, developed two simple and sensitive visible spectrophotometric methods (A and B) for the quantitative estimation of balsalazide in bulk drug and dosage form. Method A is based on the oxidation followed by complex formation reaction of balsalazide with 1, 10-phenanthroline in presence of ferric chloride to form blood red colored chromogen with absorption maximum at 509 nm and Beer’s law is obeyed in the concentration range of 6-18 μg/mL. Method B is based on the oxidation followed by complex formation reaction of balsalazide with potassium ferricyanide in presence of ferric chloride to form a bluish green colored chromogen with absorption maximum at 790.0 nm and Beer’s law is obeyed in the concentration range of 2-12 μg/mL. The developed methods were found to be precise and accurate.

Anandakumar K. et al., 2009 developed a simple, precise and accurate difference spectroscopic method for the estimation of balsalazide in bulk and in pharmaceutical dosage form. The proposed method is based on the principle that balsalazide can exhibit two different chemical forms in basic and acidic medium that differ in the absorption spectra in basic and acidic medium. Since the drug was freely soluble in distilled water, a stock solution (1 mg/mL) was prepared with distilled water. Further dilution was made by using 0.1 M sodium hydroxide and 0.1 M hydrochloric acid separately. The maxima and minima in the difference spectra of balsalazide were at 460 nm and 354 nm, respectively. Difference in absorbance between these maxima and minima was calculated to find out the amplitude. This amplitude was plotted against concentration. Beer’s law is valid in the concentration range of 2-20 μg/mL.
Ravi Kiran Kaja et al., 2009, developed a novel, sensitive, stability-indicating gradient RP-LC method for quantitative analysis of balsalazide disodium and its related impurities both in the bulk drug and in pharmaceutical dosage forms. Efficient chromatographic separation was achieved on a C18 stationary phase with a simple mobile-phase gradient prepared from methanol and phosphate buffer (10 mM potassium dihydrogen orthophosphate monohydrate, adjusted to pH 2.5 by addition of orthophosphoric acid). The mobile-phase flow rate was 1.0 mL min⁻¹. Quantification was achieved by use of ultraviolet detection at 240 nm. Under these conditions resolution of balsalazide disodium from its three potential impurities was greater than 2.0. Regression analysis resulted in a correlation coefficient greater than 0.99 for balsalazide disodium and all three impurities. This method was capable of detecting the three impurities at 0.003% of the test concentration of 0.3 mg mL⁻¹, using an injection volume of 10 μL. Inter-day and intra-day precision for all three impurities and for balsalazide disodium was within 2.0% RSD. Recovery of balsalazide disodium from the bulk drug (99.2–101.5%) and from pharmaceutical dosage forms (99.8–101.3%), and recovery of the three impurities (99.1–102.1%) was consistently good. The test solution was found to be stable in 70:30 (v/v) methanol–water for 48 h. When the drug was subjected to hydrolytic, oxidative, photolytic, and thermal stress, acidic and alkaline hydrolysis and oxidizing conditions led to substantial degradation. The RP-LC method was validated for linearity, accuracy, precision, and robustness.

Mayank Mandhanya et al., 2011 developed and validated a simple, accurate, precise and sensitive ultraviolet spectrophotometric and RP-HPLC methods for simultaneous estimation of paracetamol (PCM) and Balsalazide Disodium Dihydrate (BAL) in combined tablet dosage form. Linearity of PCM and BAL was found in concentration range of 2-30 and 1–12 μg/mL in methanol at 249 and 230 nm, respectively for spectroscopic method. Instrument used for RP-HPLC method was Shimadzu LC 10 AT VP system with Luna C18 column and methanol: acetonitrile: water (55:30:15 v/v/v) as the mobile phase. The detection was carried out using a diode array detector set at 239 nm. Linearity of the LC method was in the concentration range of 5.0–100.0 and 5-60 μg/mL for PCM and BAL respectively. The recoveries were in the range of 99.70 ± 0.38 and 99.38 ± 0.36 for PCM and 99.85 ± 0.09 and 99.31 ± 0.23 for BAL in simultaneous
equation method and HPLC method respectively. Both methods have been successfully applied for the analysis of the drugs in a pharmaceutical formulation. Results of analysis were validated statistically.

Shaik Shakeela et al., 2011, developed three novel spectrophotometric methods for the determination of mesalazine in bulk and pharmaceutical dosage forms. The proposed methods are based on the oxidative coupling of mesalazine with orcinol (method A), resorcinol (method B) and cresol (method C) in the presence of hydrogen peroxide and horseradish peroxidase to produce a colored complex having absorption maxima at 490 nm, 470 nm and 480 nm, respectively. The Beer’s law is obeyed in the concentration range of 5-30 μg/ml of the drug for all the three methods. The methods were validated statistically and by recovery studies. The proposed methods are applied to marketed tablet formulations. The results obtained from tablet formulations compared well with those obtained by the official method and demonstrated good accuracy and precision.

Senthil Kumar et al, 2012 developed a rapid and reproducible RP-HPLC chromatic graphic method for the estimation of Balsalazide in its pure form as well as in pharmaceutical formulation. Chromatography was carried out on a C18 column using a mixture of water and acetonitrile as the mobile phase at flow rate of 0.8 ml/min and detection was done at 368 nm. The retention time of the drug was 3.685. The results obtained with the proposed methods are in good agreement with labelled amounts when marketed pharmaceutical preparations are analyzed. The recovery in the present method was in the range of 99.96 - 100.6.

Naveen et al, 2013 developed a UV spectrophotometric method for the estimation of Balsalazide in bulk and capsules by overlay spectra. Balsalazide in water exhibited absorption maximum at 357 nm in UV region and obeyed Beer’s law in the concentration range of 2-10 μg/ml with correlation coefficient 0.999.