CHAPTER-II
SURVEY OF LITERATURE OF SELECTED DRUGS
The various techniques adopted for the estimation of paracetamol are H.P.L.C method<sup>1-6</sup>, Titrimetric method<sup>7</sup>, Spectrofluorimetric method<sup>8</sup>, Flow-Injection stopped-flow spectrofluorimetric<sup>9</sup>, Electro chemical<sup>10</sup>, RP-HPLC<sup>11</sup>, Titrimetric method<sup>12</sup>, Rapid Liquid Chromatographic method<sup>13</sup>, Polarographic method<sup>14</sup>, Spectrophotometric method<sup>15-41</sup>, Colorimetric method<sup>42,43</sup>, Chromatography method<sup>44</sup>. Bouhsain et al<sup>15</sup>, proposed a new spectrophotomeric method for the estimation of paracetamol in pharmaceuticals. The method is based on the alkaline hydrolysis of paracetamol to P-Amino Pnenol and the reaction with 8-quinolinol in the presence of potassium periodate as oxidant to form a blue indphenol dye which absorbs at 608 nm.

Boushain et al<sup>16</sup>, developed spectrophotometric method for the determination of paracetamol in pharmaceuticals. The method is based on the solubilization of paracetamol in a 10% v/v ethanol in chloroform solution and direct absorbance measurement at 1515 cm<sup>-1</sup> using the baseline established at 1900 cm<sup>-1</sup> for measurement correction. The procedure can be carried out in both the stopped flow and flow injection modes. In both instances the sensitivity is
approximately 0.09 ml/mg. The limits of detection being 8 mg/ml in the stopped flow mode and 33 mg/ml in the flow injection mode.

Damiani et al., proposed a rapid first derivative UV absorption spectroscopy method for the determination of paracetamol in human blood serum. The method involves no sample free treatment, extraction or derivatization procedures, other than a standard d-proteinizing technique with tri-chloro acetic acid. The results can be applied to both therapeutic and toxic levels of paracetamol.

Menon et al., proposed a method for the analysis of paracetamol in drugs samples by measuring the absorbance at 248 nm in methanol. The method has been applied to paracetamol tablets.

Prasad and Durga, have determined paracetamol in solid dosage form by using oxidation reagent potassium ferricyanide in the presence of IN sodium hydroxide and measurement of the absorbance of the oxidized product at 480 nm.

Chowdary and Rao, determined paracetamol by reacting the hydrolysed product of paracetamol with potassium dichromate to form a stable violet coloured chromogen and measuring the absorbance at 500 nm.

Erk et al., developed a method for the determination of paracetamol and hyoscine-n-butyl bromide in film-coated tablets by precipitating hyoscine n-
butyl bromide with ammonium reineckate at pH 6.0 and reading the absorbance of the solution of the precipitate in acetone at 520 nm.

Orbery et al\textsuperscript{22}, proposed a simple method for the estimation of paracetamol and aspirin in dosage forms by using second order derivative spectroscopy method. Gangwal et al\textsuperscript{23}, determined paracetamol and mefenamic acid in their combined dosage forms by using simultaneous spectrophotometric method based on the native UV absorbance maxima of mefenamic acid and paracetamol in 0.02 M sodium hydroxide. Mefenamic acid has two absorption maxima, at 285 nm and 333 nm., paracetamol has absorption maximum at 257 nm.

Anwar et al\textsuperscript{24}, proposed a simple and rapid spectrophotometric method for the determination of aspirin and paracetamol in pharmaceuticals. This method was based on the oxidation of aspirin or paracetamol with iron (III) ions. The resulting iron (II) formed a coloured complex with 1,10-phenanthroline. The absorbance of this iron complex was measured at 510 nm.

Ravisankar et al\textsuperscript{25}, developed for simultaneous determinations of paracetamol and chlormezanone in combined tablets formulations. The maximum absorbance of paracetamol measured at 243.6 nm and chlormezanone measured at 228 nm.
Mashru et al. proposed a simple and sensitive UV spectrophotometric method for simultaneous determination of chlorzoxazone and paracetamol based on absorption ratio technique.

Bogachyk et al., proposed a simple UV spectrophotometric method for determination of paracetamol and mefenamic acid in tablets formulations.

Nagaraja et al., proposed a rapid, sensitive and simple spectrophotometric method for the determination of paracetamol and phenacetin by using sodium 1, 2 naphthoquinone-4-sulfonate and acetyltrimethyl ammonium bromide in alkali medium. The absorbance was measured at 570 nm and 500 nm.

Rami Reddy and Chakravarthy, proposed a new spectrophotometric method for determination of Paracetamol in pharmaceutical preparations. This method is based on the bromination of the Paracetamol with brominating mixture. After bromination the excess brominating mixture is treated with KI. The yellow colour developed was measured at 350 nm against distilled water as blank.

Usifoh et al., developed a method for the determination of Paracetamol in raw material and in pharmaceutical dosage form. The method is based on measuring the intensity of the yellow colour developed when acetaminophen is allowed to react with p-dimethyl amino benzaldehyde in 2M HCl after heating. The colour absorbs in the visible region of 450 nm.
Amin and Alaa\textsuperscript{31}, have developed a spectrophotometric method for the estimation of Paracetamol. The method is based on its reaction with Pyrocatechol violet under basic conditions to form ion-pair complex. The absorption maximum of the coloured ion–pair complex formed is observed at 652nm. Beer’s law is obeyed over the concentration range 0.5-34 mg/ml. 

Nameh et al\textsuperscript{32} proposed a sensitive method for the determination of paracetamol in pharmaceutical preparations. The reaction of paracetamol with per sulfate was carried out in alkali medium at 40\textdegree c, for 10 minutes then determined at 315 nm. 

Toral et al\textsuperscript{34}, developed a method for the determination of mefenamic acid and paracetamol in pharmaceutical formulations by using a direct and simple first derivative spectrophotometric method. 

Ozgur et al\textsuperscript{35}, estimated paracetamol and dipyrone in combined dosage form using derivative spectrophotometric method using ‘zero crossing’ technique of measurement at 229.2 nm and 217.9 nm for paracetamol and dipyrone.

Bhatia et al\textsuperscript{36}, developed economical procedure for simultaneous estimation of diclofenac sodium chlorzoxazone and paracetamol in three component tablet formulations. This method employ first derivative UV spectrophotometry and simultaneous equations for the simultaneous estimation of the three drugs by using 0.02M sodium hydroxide, diclofenac sodium has an absorbance maxima
at 276 nm, chlorzoxazone has an maxima at 244 nm and 288 nm and paracetamol has an absorbance maxima at 257 nm.

Bhatia etal\(^3\) developed simple procedures for simultaneous determination of paracetamol and chlorzoxazone in two component tablet formulations. This method employed first-derivative, UV spectrophotometry and simultaneous equation for the determinations of the two component drugs by using 0.02M sodium hydroxide, chlorzoxazone showed two absorption maxima at 244 and 288 nm, and paracetamol had an absorption maxima at 257 nm.

Indrajeet Singhvi\(^3\), proposed a simple four simple, accurate, economical and reproducible UV spectrophotometric for simultaneous estimation of two component drug mixture of nimesulide and paracetamol in combined tablet dosage forms. First developed method employs multiwavelength spectroscopy using 395.0 nm and 257.0 nm as two wavelength for estimation. Second method involves first derivative spectroscopy using 369.0 nm and 296.0 nm as zero crossing points. Third method involves direct estimation of nimesulide at 395.0 nm and formation and solving of molar absorptivity equation at 257.0 nm for estimation of paracetamol. Fourth developed method involves two wavelength spectroscopy for estimation of paracetamol, two wavelengths selected for this method were 257.0 nm and 338.5 nm. All developed methods obey Beer's law
in concentration range employed for respective methods. Results of analysis were validated statistically and by recovery studies.

Knochen, M et al\textsuperscript{40} proposed a flow injection spectrophotometric method for the determination of paracetamol in pharmaceutical dosage forms. The method is based on the nitration of paracetamol with sodium nitrite, and the absorption of the reaction product is measured at 430 nm in alkaline medium. Unlike other colorimetric methods used for determination of paracetamol, this method does not require the use of heat. The influence of several operating parameters is studied. The method was applied to the determination of paracetamol in oral solutions and in tablets, alone or associated with caffeine. When the results were compared with those obtained by the official HPLC method (USP 24) the relative differences found were from 0.4 to 2.3\%, with relative standard deviations below 1\%.

Sena M.M and Poppi R. J et al\textsuperscript{41}, proposed a simple and rapid spectrophotometric method for simultaneous determination of acetylsalicylic acid (ASA), paracetamol (PRC, also known as acetaminophen) and caffeine (CAF) in pharmaceutical formulations. The method is based on multivariate calibration and UV spectrophotometric measurements (210-300 nm). The calibration set was constructed with nine solutions in the concentration ranges from 10.0 to 15.0 \( \mu \text{g} / \text{ml} \) for ASA and PRC and from 2.0 to 6.0 \( \mu \text{g} / \text{ml} \) for CAF,
according to an experimental design. The procedure was repeated at four different pH values: 2.0, 3.0, 4.0 and 5.0. Partial least squares (PLS) models were built at each pH and used to determinate a set of synthetic mixtures. The best model was obtained at pH 5.0. An N-way PLS model was applied to a three-way array constructed using all the pH data sets and enabled better results. This calibration model provided root mean squares errors of prediction (RMSEP) from 11.5 to 35% lower than those obtained with PLS at pH 5.0, depending on the analyte.
(B) RITODRINE HYDROCHLORIDE

The literature survey reveals Spectrophotometric method\textsuperscript{45-53} and HPLC method\textsuperscript{54} for estimation of ritodrine hydrochloride. Chilukuri et al\textsuperscript{45}, proposed a three simple and accurate and sensitive visible spectrophotometric methods for the estimation of ritodrine hydrochloride in pure and in formulations. The method is based on the coupling of RTH with diazotized dapsone under alkali. Conditions (method A) to yield an orange coloured chromophore ($\lambda$ max 460 nm) or by the formation of inner mol. Complex between the drug and pentacyano nitrosyl ferrate (method B, $\lambda$ max 720 nm) or by the reduction of Folin-Ciocalteau reagent (method C, $\lambda$ max 750 nm). Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges 2.0-10.0, 4.0-24.0 and 2.0-12 mg/ml for methods A,B,C, respectively. The results of analysis have been validated statistically and by recovery studies.

Bakry et al\textsuperscript{46}, have developed a simple and rapid method for the determination of etilefrine hydrochloride and ritodrine hydrochloride, either in pure form or in pharmaceutical formulations. The method is based on the development of red product in presence of sodium nitrite and cobalt (II) salt in acid medium. The reaction is thought to proceed via preliminary nitrosation of the phenolic nuclues followed by formation of the chelate in presence of cobalt (II) salt. The
highly coloured chelates showed wavelengths of maximum absorption of 570 and 560 nm for etilefrine hydrochloride and ritodrine hydrochloride respectively. The reaction product showed apparent molar absorptivities of 938 and 2930 Lmol⁻¹cm⁻¹ for etilefrine hydrochloride and ritodrine hydrochloride, respectively. A linear correlation was found between absorbance and concentration in ranges of 0.68-0.20 and 0.04-0.10 mg/ml for etilefrine hydrochloride ritodrine hydrochloride respectively. At the same time, the resulting colours were well developed within 25 min at boiling water temperature and were stable for more than 1 hour.

Bakry et al.⁴⁷ proposed a spectrophotometric procedure for the estimation of three phenolic sympathomimetic drugs: etilefrine hydrochloride, prenalterol hydrochloride and ritodrine hydrochloride. The method involved the used of 2,6-dichloro and 2,6-dibromoquinone chlorimides as chromogenic reagents. The phenolic drugs produced a blue colour, peaking form 610-630 nm. The colours produced obeyed Beer’s law and were suitable for the quant. Determination of the named compounds. The molar ratio’s of the reactions were established and a proposal for the reaction pathway is given.

Shalaby et al.⁴⁸ have developed a two spectrophotometric procedures for assay of fenoterol and ritodrine. The method based on coupling of the drugs with diazotized o-nitro aniline or p-amino benzoic acid. In the procedure chelation
with copper (II) and subsequent extraction with chloroform is performed. The results obtained are compared with those obtained by the official methods.

Razak OA, developed two simple sensitive and accurate methods for the determination of ritodrine hydrochloride in bulk and pharmaceutical preparations. The first method involves the direct measurement of the native fluorescence of the drug in the concentration range 4-9 µg/ml, the second method is based on the oxidation of ritodrine HCl with cerium(IV) followed either by spectrophotometric or fluorimetric measurement in the concentration ranges 0.5-1.0 and 0.05-0.1 µg/ml respectively. The interference of various formulation excipients was examined. The described methods have been applied to the determination of ritodrine HCl in tablets and ampoules. The assay results showed insignificant difference with those of the official USP HPLC method.

Revanasiddappa HD and Manju B.G, proposed a simple, accurate, and rapid spectrophotometric method for the quantitative determination of ritodrine hydrochloride (RTH) and isoxsuprine hydrochloride (ISH) in both pure and dosage forms, is described. The method is based on the development of pink colored product as a result of the condensation of 4-aminoantipyrine with phenols in the presence of an alkaline oxidizing agent.
Revanasiddappa HD and Manju B.G, have developed two simple and sensitive spectrophotometric methods are described for the determination of ritodrine hydrochloride (RTH) in both pure and dosage forms. The methods are based on the interaction of diazotised p-nitroaniline (DPNA) and sulphanilic acid (DSNA) with RTH in an alkaline medium. The resulting azo dyes are measured at 480 nm (for the DPNA method) and at 440 nm (for the DSNA method) and are stable for more than 1 hour. The optimum reaction conditions and other analytical parameters are evaluated. A study of the effect of commonly associated excipients and additives do not interfere with the determinations. Statistical analysis of results indicates that the methods are precise and accurate.

Padmarajaiah et al., proposed a simple, rapid and sensitive spectrophotometric method for the determination of isoniazid (INH) and ritodrine hydrochloride (RTH) in pure form as well as dosage forms is described. The method is based on the diazotisation of dapsone, followed by a coupling reaction with either INH or RTH in sodium hydroxide medium. Beer's law is obeyed in the concentration range of 0.5-20 μg/ml for INH at 440 nm and 0.5-18 μg/ml for RTH at 460 nm. The method is successfully employed for the determination of INH/RTH in pharmaceutical preparations and the results agree favourably with the official and reported methods. Common excipients used as additives in pharmaceuticals do not interfere in the proposed method.
Bhanu Prasad et al., proposed a simple, accurate and economical procedure for estimation of ritodrine hydrochloride in pharmaceutical formulation by spectrophotometrically. In this method ritodrine hydrochloride was brominated with brominating mixture under acidic medium. The excess brominating mixture was reacted with potassium iodide to produce yellow colour. The absorbance of the yellow colour was measured at 350 nm against
Various techniques adopted for the estimation of Dapsone are Spectrophotometric method\textsuperscript{55-64}, Colorimetric method\textsuperscript{65}, Iskender et al\textsuperscript{55}, have developed a method for the determination of dapsone in tablets by spectrophotometrically using 1,2-Naphthoquinone-4-sulfonic acid sodium salt. The method was based on the chromophore formation after reaction with sodium 1,2-naphthoquinone-4-sulfonate (NQ). The reaction proceeded quantitatively at pH 2 and $60^\circ$ within 30 minutes when the molar ratio of the reagent to dapsone was 10. After completion of the reaction, the derivative formed; dapsone-NQ, was extracted from the aqueous solution with chloroform/butyl alcohol (3:1). dapsone-NQ, showed maximum absorbance at 440 nm.

Zarapker et al\textsuperscript{56}, proposed a simple spectrophotometric method for the estimation of dapsone from pharmaceutical preparation. The method based on coupling of the diazotized dapsone with 8-aminolino-1-naphthalene sulfonic acid, resorcinol or $\beta$-naphthol in alkali medium and measurement of absorbance at 550, 440 or 490 nm respectively.

Shoukrallah et al\textsuperscript{57} determined dapsone by spectrophotometric method using 9-chloroacridine as a chromomeric reagent. The method was based on condensation reaction of 9-chloro acridine and the amino groups of dapsone.
The reaction variables were investigated and optimized. The resultant coloured product was measured at 443 nm.

El-Dolkiny et al.\textsuperscript{58}, have developed two methods for the micro determination of dapsone in bulk and dosage forms by spectrophotometric method. In the first method, sodium 1,2- naphthoquinone-4-sulfonate was used as a reagent for the colorimetric determination of the drug. The second method used coupling of the diazotized primary aromatic amino groups in dapsone with barbituric acid.

Rao B.C et al.\textsuperscript{59}, proposed a simple and sensitive spectrophotometric method for the estimation of dapsone. The method was based on schiff’s base formation with 4-dimethyl amino cinnamaldehyde in the presence of methanolic sulfuric acid. The red coloured chromogen had absorption maxima at 540 nm.

Rami Reddy et al.\textsuperscript{60} proposed a new spectrophotometric method for the estimation of dapsone. The method was based on the bromination of dapsone with brominating mixture. The excess brominating mixture was reacted with potassium iodide to produced yellow colour. The absorbance of yellow colour solution is measured at 350 nm.

Rami Reddy et al.\textsuperscript{61}, developed a simple spectrophotometric method for estimation of dapsone form pharmaceutical preparations. The method is based on the diazotisation of dapsone followed by the addition of ammonia solution. The yellow colour developed was measured at 445 nm.
Toral, M.I et al.\textsuperscript{62}, proposed a simple and fast spectrophotometric method for the simultaneous determination of dapsone and pyrimethamine by first-order digital derivative spectrophotometry. Acetonitrile was used as a solvent to extract the drugs from the pharmaceutical formulations, and the samples were subsequently evaluated directly by digital derivative spectrophotometry. The simultaneous determination of both drugs was performed by the zero-crossing method at 249.4 and 231.4 nm for dapsone and pyrimethamine, respectively. The best signal-to-noise ratio was obtained when the first derivative of the spectrum was used. The excipients of commercial pharmaceutical formulations did not interfere in the analysis. Chemical and spectral variables were optimized for determination of both analytes. A good level of repeatability, 0.6 and 1.7\% for dapsone and pyrimethamine, respectively, was observed. The proposed method was applied for the simultaneous determination of both drugs in pharmaceutical formulations.

Nagaraja, P et al.\textsuperscript{63}, proposed a rapid, simple and sensitive spectrophotometric method for the determination of some sulfa drugs. The method is based on the formation of orange yellow colored azo product by the diazotization of sulfonamides, viz., dapsone (DAP), sulfathiazole (SFT), sulfadiazine (SFD), sulfacetamide (SFA), sulfamethoxazole (SFMx), sulfamerazine (SFMr), sulfaguanidine (SFG) and sulfadimidine (SFDd) followed by a coupling
reaction with 3-aminophenol in aqueous medium. Absorbance of the resulting orange yellow product is measured at 460 nm and is stable for 6 days at 27 degrees C. Beer's law is obeyed in the concentration range of 0.05-8.0 μg/ml at the wavelength of maximum absorption. The method is successfully employed for the determination of sulfonamides in various pharmaceutical preparations and common excipients used as additives in pharmaceuticals do not interfere in the proposed method.

Wang HYetal\textsuperscript{64}, have developed Spectrophotometric method for determination of dapsone. The dapsone reacts with sodium 1,2-naphthoquinone-4-sulfonic in pH 6.98 buffer solution to form a salmon pink compound, and its maximum absorption wavelength is at 525 nm, $\varepsilon_{525}=3.68 \times 10^4$ mol$^{-1}$ cm$^{-1}$. The absorbance of dapsone from 0.40 to 10 μg/ml obeys Beer's law. The linear regression equation of the calibration graph is $C=0.2334 A + 0.01288$, with a linear regression correlation coefficient of 0.9998, the detection limit is 0.24 μg/ml and recovery is from 99.2 to 102.4%. Effects of pH, surfactant, organic solvents, foreign ions, and standing time on the determination of dapsone have been examined. This method is simple and can be used for the determination of dapsone in injection solution of dapsone. The results obtained by this method agreed with those by the official method.
Shetty et al\textsuperscript{65}, have developed a specific colorimetric assay for dapsone in biological fluids. The original Bratton and Marshall method for sulfanilamide assay was modified for differential assay of dapsone (DDS) even in the presence of other diazotizable compounds. The property of the dapsone diazo derivative to precipitate out upon into coupling with N-(1-naphthyl) ethylene diamine was used to differentiate this sulfone from that of other diazotizable compounds.
Various techniques adopted for the estimation of Sulfamoxole are Spectrophotometric method, Colorimetric method, and a proposed sensitive and rapid colorimetric method by Raghuveer, S et al., for the determination of sulfamoxole in its dosage forms. The method is based on the reaction of the drug with 4-dimethylamino cinnamaldehyde in the presence of orthophosphoric acid in methanolic medium. The red coloured chromogen had an absorption maximum at 545 nm.

Rami Reddy and I.E. Chakravarthy proposed a simple spectrophotometric estimation of sulfamoxole in pharmaceutical formulations. In this method, the drug treated with sodium nitrite and hydrochloric acid at 0-5°C. The diazotised drug is treated with ammonia solution to form yellow colour solution, which was measured at 425 nm.
Various methods are reported for the estimation of cisapride such as colorimetric method 68,69, HPLC method 70,71, spectrophotometric and HPLC method 72, and spectrophotometric method 73.

Parimo et al. 68, proposed a method for the determination of cisapride in dosage forms by colorimetric. This method is based on the reaction of cisapride with p-dimethyl amino cinnamaldehyde (PDAC) in presence of trichloroacetic acid in presence of methanol to form a very stable red schiff's base which has a $\lambda_{\text{max}}$ at 525 nm and obey Beer's–Lambert law in the range concentration of 50-10 mg/ml.

Krishna kumar, K.R and R.Raju 69, proposed a colorimetric method for the estimation of cisapride and its dosage forms. This method is based on the diazotisation of cisapride with HCl and NaNO₂ and then coupled with 1-naphthyl ethylenediamine to give a red complex. The maximum absorbance is recorded at 539 nm. Beer's law was obeyed over a concentration range of 1 to 11 mg/ml.

Hassan, E.M et al. 72, have developed derivative spectrophotometric and high performance liquid chromatographic methods (HPLC) for the determination of cisapride in pharmaceutical preparations. Spectrophotometrically, cisapride was
determined by measuring the 1D-values at 264, 300 nm and 2D-values at 276, 290 and 276-290 nm. Beer's Law was obeyed in the range 2-12 µg/ml. The HPLC method depends upon using micropack-Si-10 column at ambient temperature with a mobile phase consisting of methanol-concentrated ammonia (99.25:0.75) at a flow rate of 1 ml/min. Quantitation was achieved by UV detection at 272 nm using quinine as internal standard. Calibration curve was linear over the concentration range 2-10 µg/ml. Both derivative spectrophotometry and HPLC methods showed good linearity, precision and reproducibility. No interference was found from tablet or suspension matrices at the selected derivative wavelengths and chromatographic conditions. The proposed methods were successfully applied to the assay of commercial tablets and suspension. The procedures were rapid, simple and suitable for quality control applications.

S.P. Vyas et al, proposed a simple UV Spectrophotometric method for the determination of cisapride in pharmaceutical dosage forms. In this method cisapride is dissolved in DMF to give a clear solution having λ max at 274.4 nm. The solution obeyed Beer's law in the concentration range 2 to 20 mg/ml.

Revanasiddappa, H.D et al, have developed Spectrophotometric method for determination of some therapeutic agents using acetyl acetone.
Suitable portions of standard drug solutions were diazotized with 2 ml of 0.1% sodium nitrite solution and 1 ml 1M-hydrochloric acid solution. After 3 minute, unconsumed nitrous acid was removed with 1 ml of 3% sulfamic acid. Portions (4 ml) of 5% acetylacetone and 4 ml of 4M NaOH were added and the solutions were diluted to 25 ml with methanol. The absorbances of the diluted solution were measured at 460, 445, 430 and 430 nm for dapsone, cisapride, metoclopramide and p-aminobenzoic acid, respectively.

Barbhai, A.J etal\textsuperscript{75}, proposed a Spectrophotometric estimation of cisapride using para dimethyl amino cinnamaldehyde and ortho phosphoric acid. The described spectrophotometric procedure was based on the reaction of cisapride with p-dimethyl amino cinnamaldehyde and ortho phosphoric acid. For preparation of a calibration curve, a stock solution of 1 mg/ml of cisapride in methanol was prepared. Powdered tablets equivalent to 50 mg cisapride were extracted with methanol and filtered. Measured volumes of the standard solutions were placed in 10 ml volumetric flasks and methanolic solutions of 2 ml 0.1% p- dimethyl amino cinnamaldehyde and 2 ml 50% ortho phosphoric acid were added. The flasks were shaken for 1 min and made up to the mark with methanol. The absorbance was measured after 15 min at 546.5 nm. The sample solutions were measured similarly. The orange colour was stable for at least 2 h and Beer's law
was obeyed from 160-650 µg/ml. The standard deviation was +/-0.04988256, the coefficient of variation +/-0.49811 and recoveries were 98-100.7%.

Sastry, C.S.P et al76, proposed a new visible Spectrophotometric method for the Assay of cisapride in pharmaceutical formulations. Pharmaceuticals in CHCl₃ or aqueous acetic acid solutions (preparation described) were: (i) diluted to 4 ml with H₂O, mixed with 2 ml 3-methyl-2-benzothioazolinone hydrazone hydrochloride and 1.5 ml Fe(III), the mixtures diluted to 8.5 ml with H₂O, equilibrated for 40 min and then diluted to 25 ml prior to measurement of absorbance at 565 nm; (ii) mixed with 1.5 ml Fe(III) in 1M HCl and 2 ml 1,10-phenanthroline, the mixtures diluted to 10 ml with H₂O, heated in a boiling-water bath for 30 min, cooled to room temperature, mixed with 2 ml H₃PO₄, diluted to 25 ml with H₂O and equilibrated for 5 min prior to measurement of absorbance at 520 nm; or (iii) mixed with 0.5 ml chloranilic acid in CHCl₃/propan-2-ol (4:1) and diluted to 10 ml with CHCl₃ prior to measurement of absorbance at 555 nm. For the three methods, respectively, Beer's law was obeyed from 2-32, 0.4-6.4 and 25-450 µg/ml cisapride, absorptivities were 14300, 63000 and 1030, and the RSD (n = 6) were 0.54%, 0.33% and 0.76%. Recoveries were 98.9-101.9%. The effects of interferents were investigated
Sastry, C.S.P etal\textsuperscript{77}, have developed a simple spectrophotometric method for estimation of cisapride in pharmaceutical formulations. Sample equivalent to 100 mg cisapride (I) was extracted with 3 x 20 ml CHCl\textsubscript{3}, filtered and the extract was diluted to 100 ml with CHCl\textsubscript{3}. Portions were analysed with use of 0.2\% Suprachen Violet 3B, 0.4\% Erioglaucine A, 0.2\% Naphthalene Blue 12 BR or 0.2\% Tropaeolin 000 (methods A, B, C and D, respectively). For methods A and B, sample was mixed with glycine hydrochloride buffer of pH 1.3 (buffer A) and dye solution, the organic phase was dried over anhydrous Na\textsubscript{2}SO\textsubscript{4} and the absorbance was measured at 595 and 640 nm, respectively. For methods C and D sample was mixed with buffer A of pH 1.5 (method C) or 0.1M-HCl (method D) and dye solution, the organic phase was dried over Na\textsubscript{2}SO\textsubscript{4} and the absorbance was measured at 620 and 500 nm, respectively.

Meena, S etal\textsuperscript{78} have developed an extractive photometric method for determination of cisapride. A standard solution of cisapride was mixed with mentanil yellow (0.025\% w/v) in pH 2.4 buffer. The resulting ion-pair was extracted in CHCl\textsubscript{3}. The absorbance of the organic layer was measured on a Shimadzu UV spectrophotometer-1201 at 408 nm. The calibration graph was linear from 4-16 \(\mu\)g/ml. The recovery was 98.5-100.2\%.
Various methods are reported for the estimation of sparfloxacin such as Spectrophotometric method$^{79-95}$ and HPLC and derivative UV spectrophotometric method$^{96}$.

Hu, C.Q et al.$^{79}$ have developed Specificity of chloranil charge-transfer reaction for the determination of enoxacin, ciprofloxacin (I), sparfloxacin, pefloxacin, fleroxacin and levofloxacin. Sample solution (5 ml) was diluted with 20 ml borax buffer of pH 9 before adding 5 ml 3mM-chloranil and H$_2$O to 50 ml and heating at 40°C for 40 minute. After cooling, absorption was scanned between 300 and 450 nm. Fluoroquinolone antibiotics studied were enoxacin, ciprofloxacin (I), sparfloxacin, pefloxacin, fleroxacin and levofloxacin. Effect of structures of the compounds on their charge-transfer reaction for use in determination was investigated, particularly with more detailed studies on I and its photo degradation mixtures. The piperazine group as well as substituents at positions 3, 4 and 5 interfered.

Ahmed, S.M and Pai, P.N.S$^{80}$, developed Spectrophotometric method for the determination of sparfloxacin. A standard solution was prepared by dissolving 10 mg of pure sparfloxacin in 100 ml 0.1M-NaOH. Various volumes (1-5 ml)
of the standard solution were then placed in 10 ml volumetric flasks, 1.6 ml of 1N-HCl and 0.8 ml of 1% sodium nitrite solution added. The temperature was maintained at \(<5^\circ\text{C}\) during addition of reagents. A 1.2 ml portion of 1% resorcinol was added, the volume was made up to 10 ml with \(\text{H}_2\text{O}\) and the absorbance was measured at 428.5 nm. For the determination ofsparfloxacin in tablets, the tablets were weighed and powdered and an amount equivalent to about 100 mg sparfloxacin was placed in a 100 ml volumetric flask. The powder was dissolved in 0.1N-NaOH, filtered and diluted with 0.1N-NaOH to give a concentration of about 100 \(\mu\text{g/ml}\) and then analysed as above. The coloured solution obeyed Beer's from 1-50 \(\mu\text{g/ml}\) and was stable for up to 4 hours. The method was shown to be reproducible and accurate.

Chaudhuri, J.J etal\(^8\) proposed a comparative study of spectrophotometric and microbiological determination of sparfloxacin from tablet dosage form. The existing spectrophotometric method (i) for the determination of sparfloxacin is compared to a new microbiological method (ii) Sparfloxacin (I) tablets were powdered, and the equivalent of 200 mg sparfloxacin was used. For method i, powder was dissolved and diluted to 200 ml with 0.1N-NaOH, filtered, diluted to 10 \(\mu\text{g/ ml}\) with 0.1N-NaOH and the absorbance measured at 291 nm. For method ii, sample was treated similarly using phosphate buffer (pH 6, no other details given) instead of NaOH, with shaking (45 min) to dissolve the sample,
and dilution to final concentrations of 2 and 4 micrograms/ml in buffer. Petriplates were prepared containing 0.1 ml cups of samples and standards, inoculated with Bacillus pumilus ATCC 14884, and maintained at 25+/-.2°C/50+/-.5% r.h. for 45-60 min. After incubation at 35-37°C for 16-18 h, zones of inhibition were measured. The microbiological approach compared well to the spectrophotometric method: growth inhibition of the organism was linear from 1-16 µg/ml of sparfloxacin, whilst Beer's law was obeyed from 4-16 µg/ml. Recoveries ranged from 99-100.5% and 98.6-100.8% for methods i and ii, respectively.

Chowdary, K.P.R et al. propose new spectrophotometric methods for the determination of sparfloxacin in bulk and in dosage forms. Powdered tablets (40 mg sparfloxacin) were added to aqueous HCl (50 ml H2O/0.1 ml 0.5N-HCl). For method (i), the stock solution (16 µg/ml) was treated with 3-methylbenzothiazolinone hydrazine hydrochloride in the presence of FeCl3 to yield a magenta coloured chromogen (absorption at 560 nm). For method (ii), the stock solution (40 µg/ml) was treated with Folin-Ciocalteau reagent in the presence of sodium carbonate solution to form a blue coloured chromogen (absorption at 750 nm). The absorbances were measured on an Elico SL-150 UV-Visspectrophotometer.
Meyyanathan, S. N etal\textsuperscript{83} have developed Spectrophotometric method for
determination of sparfloxacin in its dosage forms using either 0.2% w/v cerric
ammonium sulfate (I) in 2N-H\textsubscript{2}SO\textsubscript{4} or 0.3% w/v sodium 12,2-naphtho-
quinoone-4-sulfonate (II) as the reagent. A sample solution was mixed with 1.2
ml I and diluted to 10 ml with 0.1N-NaOH. A reddish brown chromogen, stable
for 40 min, was formed having an absorption maximum at 484 nm. Beer's law
was obeyed from 10-80 \(\mu\)g/ml. In the second method, a sample solution was
mixed with 1 ml II, the mixture was heated on a boiling water bath for 15 min,
cooled and made up to 10 ml with 0.1N-NaOH. A reddish-orange chromogen,
stable for 70 min, was formed having an absorption maximum at 458 nm.
Beer's Law was obeyed from 20-100 \(\mu\)g/ml. Additives and excipients did not
interfere.

Chetna,T\textsuperscript{84}, have developed Spectrophotometric method for determination of
sparfloxacin in dosage forms. The chromogenic reagent was 0.2% w/v FeCl\textsubscript{3}
solution which was standardized titrimetrically. Tablets were dissolved in H\textsubscript{2}O
to give a 200 \(\mu\)g/ml solution of sparfloxacin. To estimate sparfloxacin, 0.25-4
ml of drug solution was treated with FeCl\textsubscript{3} reagent and after 10 minute, the
solution was made up to 10 ml with H\textsubscript{2}O. A yellowish orange chromogen was
formed having an absorption maximum at 510 nm. Beer's Law was obeyed
from 0.7-160 \(\mu\)g/ml of sparfloxacin and the reproducibility of the method was
The formation of the chromogen, a 2:1 complex of sparfloxacin and ferric iron, was examined at pH 1.5-2.3 in equimolar solutions at 30°C.

Marona, H.R.N and Schapoval, E.E.S\textsuperscript{85}, proposed a Spectrophotometric method for determination of sparfloxacin in pharmaceutical formulations using bromothymol blue. Methanolic extracts of dried tablets were diluted with water (nominal drug concentration 0.6 mg/ml). A 1 ml portion of extract was mixed with 4 ml phthalate buffer (pH 3.2) and 4 ml 0.5% bromothymol blue and the mixture was extracted with 6×5 ml dichloromethane. The combined organic extracts were diluted to 100 ml with the solvent and the absorbance was measured at 385 nm. The calibration graph was linear from 2-12 μg/ml of sparfloxacin in dichloromethane and the mean recovery of 100-300 mg of sparfloxacin added to the initial extract was 96.9%. The RSD (n=3) on a one tablet batch was 6.67%.

Rizk, M., etal\textsuperscript{86}, proposed a derivative UV-spectrophotometric analytical procedure for determination of three 4-quinolone anti-bacterials: norfloxacin (NFX), ciprofloxacin (CFX), and sparfloxacin (SFX). The method depends on the complexation of Cu (II) with the studied compounds in aqueous medium. A third order, measurement was applied for their quantification. A linear correlation was established between the amplitude of the peak and
concentration for all the studied drugs in the range of 15-80, 35-120, and 200-700 mg/ml, with minimum detectability (S/N=2) of 1.0, 1.3 and 5.1 μg/ml for NFX, CFX, and SFX, respectively. The method was successfully applied for accurate, sensitive, and selective determination of the studied drugs in bulk and tablets formulation with average percentage recoveries of 99.22±0.55 to 100.33±1.60. The results obtained were favourably compared with those of the reference method. The method was also used to determine sparfloxacin in spike human plasma and urine. The results obtained were satisfactory, accurate, and precise.

Chowdary, K.P.R and Ravi Kumar, have developed Spectrophotometric methods for the estimation of sparfloxacin in bulk and in dosage forms. For method A, an aqueous solution containing 2-10 μg/ml. sparfloxacin (SPF) in 1 ml was prepared and the absorbance was measured at 304 nm. For method B, 1 ml methanolic SPF solution containing 10-100 μg/ml of the drug was reacted with 1 ml 0.02% 1-fluor-2, 4-dinitro benzene at 100°C for 30 minute. After cooling and dilution, the absorbance was measured at 375 nm. Beer's law was obeyed for 1-10 μg/ml in method A and 2-20 μg/ml in method B. The RSD (n =6) for the determination of 4 μg/ml SPF (method A) and 40 μg /ml SPF (method B) were 0.48 and 1.89%, respectively.
Wen, J.W and Zheng, Z.D, have developed Ultra-violet spectrophotometric method for determination of sparfloxacin in tablets. Twenty tablets were ground and a portion, equivalent to 40 mg sparfloxacin, was dissolved in a suitable portion of 0.4% NaOH and the solution was diluted to 250 ml with more solvent and filtered. The absorbance of the filtrate was measured at 291 nm. An equation is given for calculating the amount of sparfloxacin. The calibration graph for sparfloxacin was linear (r=0.999). The average recovery of sparfloxacin in commercial tablets was 100.2%, (based on labelled values), with an RSD of 0.61%.

Ahmed et al, developed a spectrophotometric method for estimation of sparfloxacin. Sparfloxacin tablets were dissolved in 0.1 N sodium hydroxide solution and absorbance of the solution was measured at 428.5 nm.

Ahmed et al, developed a spectrophotometric method for the estimation of sparfloxacin. The method was based on the reaction of sparfloxacin with P-benzoquinone in the presence of pH 7.8 phosphate buffer. Beers law was obeyed in the concentration range of 0.1-25 mg/ml.

Preet et al, have developed a spectrophotometric method for the determination of sparfloxacin in tablet dosage form. The method was based on the UV spectroscopy of the 25% sulphuric acid of the drug at 296 nm.
Rajasekaran et al. proposed a spectrophotometric method for the determination of sparfloxacin in pharmaceutical formulations. The stable yellow colour of sparfloxacin solution in 25% sulphuric acid has an absorption max, at 405 nm. The Beers law was obeyed in the concentration range of 20-100 mg/ml.

Meyyanathan et al. have developed a spectrophotometric method for the determination of sparfloxacin in dosage form. The method was based on the reaction of the diazotization drug with 0.02% N-(1-naphthyl) ethylene diamine-2HCl to form a yellow coloured chromogen, which exhibited absorption maximum at 427 nm. The chromogen was stable for 30 minutes.

Gupta et al. have developed a spectrophotometric method for the determination of fluoroquinolone derivative in pharmaceutical formulations. In this method the interaction between ferric chloride and sparfloxacin and ofloxacin were used for their spectrophotometric estimation at 510 nm.

Gupta et al. have developed a spectrophotometric method for the determination of sparfloxacin and ofloxacin using the Iron (III) as a reagent. In this method sparfloxacin and ofloxacin provided a wine red colour formed with Iron (III) in both aqueous and alcoholic medium.

Kowalczyk, D et al. have developed HPLC and derivative UV spectrophotometric method for determination of sparfloxacin in the pure bulk
substance and in tablets. HPLC and derivative UV spectrophotometry (first-, second-and third-order) employing the "peak-zero" and "peak-peak" techniques. The methods were compared with respect to their accuracy, precision and selectivity.
Various methods have been reported in literature, which include Spectrophotometric method97-114, Colorimetric method115, spectrophotometry and Reverse-phase (RP)-LC116, polarographic and UV spectrophotometric method117, Thin-layer chromatography118 and HPLC method119-120.

Wei, youxia etal97, have developed a method for the determination of metronidazole cream by orthogonal function spectrophotometry. The method could eliminate the interference from ethylparaben and base. The scanning range was 200-360 nm.

Jadhav, G.P etal98, proposed a simple, rapid, and reproducible method for simultaneous determination of nalidixic acid and in pharmaceuticals by using a multicomponent mode of Jasco V-530 UV/Visible spectrophotometer. The wavelength range between 400 to 220 nm was used for the determination of both drugs.

Paliwal, R etal99, have developed a spectrophotometric method for the simultaneous determination of metronidazole and nalidixic acid in 2-component tablet and suspension formulations using multicomponent mode of analysis. No prior separation was required. The method was based on the measurement of UV absorbance maxima of the 2 drugs in 0.1N sodium hydroxide. Employing 7
mixed standards and three sampling wavelengths of 250, 319, and 334 nm reduced the interference among the components.

Mandal et al.\textsuperscript{100}, proposed a method of interaction of L-amino acids with metronidazole and tinidazole. The amino acid have been found to form fairly stable complexes with metronidazole and tinidazole as apparent from the association constants of AA+MDZ+TDZ complexes in water determined spectrophotometrically using an interactive procedure. The AA+MDZ complexes have been found to be more stable compared to AA+TDZ complexes.

LU, Guo-Bin et al.\textsuperscript{101}, improved UV determination of metronidazole in its glucose injection. The method was used for the determination of metronidazole in water as solvent at 318 nm.

Nagaraja, P et al.\textsuperscript{102}, developed a Sensitive and simple spectrophotometric methods for the determination of metronidazole (MNZ) and tinidazole (TNZ) in either pure form or in its pharmaceutical formulations are described. The first method is based on the interaction of 3-methylbenzothiazolin-2-one hydrazone (MBTH) with MNZ/TNZ (reduced drug) in presence of copper sulfate and pyridine in acidic medium. The resulting yellowish orange products have $\lambda_{\max}$ of 500 and 490 nm, respectively, for MNZ and TNZ and are stable for about 4 hours. The second method describes the reaction between reduced
diazotized drugs with N-(1-naphthyl) ethylenediamine dihydrochloride (NEDA) in neutral medium to yield pink products which have $\lambda$ max of 520 and 505 nm, for MNZ and TNZ, respectively. The products are stable for more than 24 hours.

Sola, N$^{103}$, have developed Quantitative analysis of metronidazole in intravenous admixture with ciprofloxacin by first derivative spectrophotometry. Metronidazole in parenteral admixture with ciprofloxacin was analysed by first-derivative spectrophotometry using the zero-crossing point.

Erk, N and Altun, M.L et al have developed Spectrophotometric resolution of metronidazole and miconazole nitrate in ovules using ratio spectra derivative spectrophotometry and reverse-phase (RP)-LC. The first method depends on ratio spectra first derivative spectrophotometry, by utilizing the linear relationship between substance concentration and ratio spectra first derivative peak amplitude. The ratio first derivative amplitudes at 242.6, 274.2, 261.8, 273.5 and 281.5 nm were selected for the assay of I and II, respectively. The second method is based on HPLC on a reversed-phase column using a mobile phase of methanol/H$_2$O/H$_3$PO$_4$ (150:350:1, v/v/v; pH (2.8) with programmable detection at 220 nm. The minimum concentration detectable by HPLC was 0.9 $\mu$g/ml I and 0.3 $\mu$g/ml II and by ratio derivative spectrophotometry 4 $\mu$g/ml I and 0.5 $\mu$g/ml II.
Huang, X.R et al\textsuperscript{104} proposed a new solution of simultaneous equations in spectrophotometry for the determination of metronidazole and vitamin B6 in tablets. Sample (166.88 mg), equivalent to 131.5 mg metronidazole (I) and 13.15 mg vitamin B\textsubscript{6} (II), obtained from 10 ground tablets was dissolved in 100 ml and the solution was filtered. Portions (10 ml) of filtrate were further diluted to 100 ml with more 0.1M-HCl and the absorbance was scanned between 200 and 400 nm. The absorbances were measured at 277 and 291 nm respectively, for I and II. Data were used for the calculation of I and II contents with the given simultaneous equations in which the $\alpha$ value for I and $\beta$ value for II are obtained from standard mixtures of I and II. Beer's law was obeyed from 4-38 $\mu$g /ml for both I and II. The recoveries were 100.6-101.4\%, with RSD of 0.97-1.31\%.

Gratteri, P and Cruciani, G developed polarographic and UV spectrophotometric method for the simultaneous plasmatic determination of the therapeutic metronidazole-perfloxacin combination. Polarographic (DPP) and a UV spectrometric method for the cited determination is reported. The experimental design strategies employed to develop and set-up the DPP method are described. Partial least-squares regression and the GOLPE variable selection procedure were used to treat the DPP and UV spectrophotometric data obtained in order to quantify metronidazole and pefloxacin. Validation of the
DPP method was also performed by determining accuracy, precision, linearity and range, detection and quantification limits.

Jadhav, G. P et al\textsuperscript{105}, have developed Simultaneous estimation of nalidixic acid and metronidazole by using UV/visible spectrophotometer. Two methods using simultaneous equations (Cramer's Rule and Matrices) and Q- analysis for the determination of nalidixic acid (I) and metronidazole (II) in combined tablet formulations are described. In the first method, the sample was analysed at two selected wavelengths and the second method involved using an isobestic point. In 50\% acetonitrile, the absorbance maxima of I was at 256 nm that of II at 320 nm and the isobestic point was 279 nm, respectively. Beer's law was obeyed up to 22.5 and 15 \( \mu \)g/ml of I and II, respectively.

Zhang, S.F et al\textsuperscript{106} have developed Simultaneous determination of metronidazole and vitamin B6 by derivative spectrophotometry. Tablets equivalent to 100 mg metronidazole (I) and \( \sim \)10 mg vitamin B6 (II) were dissolved in 50 ml 0.1M-HCl with stirring, set aside and a 2 ml portion of the supernatant was diluted to 50 ml with 0.1M-HCl. A 4 ml portion of the resulting solution was further diluted to 50 ml with 0.1M-HCl before scanning between 270-330 nm. The third-derivative spectrum was recorded and the amplitudes at 290.6 and 313.6 nm were substituted into given regression equations for determination of I and
II, respectively. The calibration graph was linear from 1.6-22.4 micrograms/ml of I or II. The detection limits of I and II were 0.81 and 0.16 micrograms/ml, respectively.

Paliwai, R et al. 107 developed Simultaneous spectrophotometric method for the estimation of metronidazole and nalidixic acid in combined pharmaceutical dosage forms. Tablets or pharmaceutical suspensions were dissolved in 1N-NaOH and the absorbance of the solution was measured from 200-400 nm. The concentration of metronidazole (I) and nalidixic acid (II) was determined by comparison of the spectral data with that obtained from seven mixed standard solutions using three sampling wavelengths of 250, 319 and 334 nm. Beer's law was obeyed up to 15 and up to 10 µg/ml of I and II, respectively. Recoveries were 97.3-101.6%. In the analysis of tablets, RSD were 0.6% for I and 2.3% for II; corresponding RSD in the analysis of suspensions were 0.9% and 0.3%.

Lopez-de-Alba, P. L et al. 108, proposed the bivariate spectrophotometric method for the determination of metronidazole, furazolidone and diiodohydroxyquinoline in pharmaceutical formulations. Bivariate calibration was performed by preparing two series of solutions containing 3-16.5 mg/l metronidazole (I)/2-12.4 mg/l furazolidone (II) and 1.5-20 mg/l I/1-16 mg/l diiodohydroxyquinoline (III). Portions were mixed with 2.5 ml DMF and 2.5 ml
Tris hydrochloride buffer of pH 7 for I/II or ammonium buffer of pH 9.5 for I/III and diluted to 25 ml with H$_2$O. Spectra were measured for 260-450 nm and 200-400 nm for the I/II solutions and I/III solutions, respectively, and first derivative spectra were also calculated by the Savitsky-Golay method (details given). I and II were determined at 317 and 365 nm, respectively, in I/II mixtures and I and III were determined at 268 and 320 nm, respectively, in I/III, mixtures. Linear calibration graphs were obtained. Using derivative spectrometry the corresponding wavelengths were 301.33 and 318.30 for III and 355.83 and 263.58 nm for I/III. Using bivariate calibration mean recoveries were 98.2+/-1.2% and 100.4+/-0.8% for I and II, respectively in I/II mixtures and 95.9+/-1.1% and 100.3+/-1.9% for I and III, respectively, in I/III mixtures. The method was applied to capsule contents and tablets after dissolution in DMF.

Erk, N have developed Simultaneous determination of metronidazole and miconazole nitrate in ovules by spectrophotometric methods. Five capsules were melted together on a steam bath, the product was cooled and weighed, and the equivalent of one capsule was dissolved to 100 ml in methanol; this solution was then diluted 500-fold with methanol. In the first method, metronidazole (I) and miconazole nitrate (II) were determined from their measured dA/dλ values at 328.6 and 230.8 nm, respectively, in the first-derivative spectrum. The
calibration graphs were linear for 6.2-17.5 μg/ml of I and 0.7-13.5 μg /ml of II. In the second (absorbance ratio) method, the absorbance was measured at 310.4 nm for I, at 272.0 nm for II and at 280.6 nm (isoabsorptive point). The calibration graphs were linear over the same ranges as in the first method.

Kamalapurkar, O.S and Priolkar\textsuperscript{110}, proposed a spectrophotometric method for the estimation of metronidazole and its dosage forms. Metronidazole (I) was extracted with CHCl\textsubscript{3} (3 \times 25 ml) from syrup, or from powdered tablets dissolved in H\textsubscript{2}O, equivalent to 100 mg of I. The combined extracts were evaporated to dryness, the residue was dissolved in 25 ml of water, and 10 ml of conc. HCl and 3 g of zinc dust were added. The reaction was allowed to proceed for 15 min, the mixture was then cooled to room temperature and filtered, and the residue was washed until free from acidity. The filtrate and washings were combined and diluted to 100 ml with water. To a portion (0.25 to 4.6 ml) of this solution was added 2 ml of aqueous 1% furfuraldehyde, the mixture was heated for 25 min on a boiling water bath, then cooled to room temperature and diluted to 25 ml with water, and the absorbance was measured at 395 nm. Beer's law was obeyed in the range 10 to 160 μg /ml of I.

Parimoo, P etal\textsuperscript{111}, have developed Simultaneous quantitative determination of metronidazole and nalidixic acid in tablets by difference spectroscopy.
Powdered sample equivalent to 30 mg nalidixic acid (I) and 20 mg metronidazole (II) was dissolved in 50 ml methanol and filtered. Portions (10 ml) of the filtrate were adjusted to ~15 μg/ml I and ~10 μg/ml II with 0.1M-HCl and 0.1M NaOH, respectively. The differences in absorbance of the resulting solutions were measured (acidic solutions in reference compartment, basic solutions in sample compartment) at 292 and 325 nm for determination of I and II, respectively.

Liu, S. D et al. proposed a spectrophotometric method for the Determination of metronidazole disodium phosphate by pH indicator absorbance ratio method with combined-use of a computer. Sample (62 mg) was mixed with 1 ml 0.1% bromocresol green indicator in 20% ethanol and 20 ml 0.01M-HCl and the mixture was diluted to 50 ml with H2O. The absorbance of the solution was measured at 444 and 617 nm and the r value was calculated which in turn was employed for calculating the x value. Equations were given for calculating the concentration of metronidazole disodium phosphate (the metronidazole precursor), with use of a computer. The average recovery was 99.98% with RSD of 0.18%. Results were comparable with those obtained by non-aqueous titration.
Chatterjee, P et al\textsuperscript{113}, have developed Simultaneous spectrophotometric method for the estimation of di-iodohydroxyquinoline and metronidazole or their analogous derivatives in combined dosage forms. A solution containing 10 \( \mu \)g ml\textsuperscript{-1} each of di-iodohydroxyquinoline and metronidazole (I) in DMF-methanol -20mM-NaOH (1:3:96) was subjected to spectrophotometry at 267 and 320 nm vs. a blank of the same solvent.

Fabayo, A. B and Grudzinski, S. K\textsuperscript{114}, have developed Quantitative determination of metronidazole in tablets by UVabsorption spectrophotometry. Samples of metronidazole (I) tablets were extracted by shaking with 0.1M-HCl, the extract was diluted with 0.1M-HCl to a concentration of 10 \( \mu \)g/ml and I was determined spectrophotometrically at 277 nm. Beer's law was obeyed for 5 to 20 \( \mu \)g/ml of I. No interference of the adjuvants was observed. The results obtained for samples of three commercial Nigerian tablets are tabulated and statistically evaluated.

Gandhi, T. P et al\textsuperscript{115} proposed a Colorimetric method for the determination of metronidazole in bulk and dosage forms. Metronidazole is reduced with zinc and HCl to an amino-derivative, which on diazotization and coupling with 2-naphthol gives a red dye (\( \lambda \) max 480 nm) suitable for spectrophotometry. Beer's law is obeyed for 10 to 80 \( \mu \)g/ml of the drug.
Various methods have been reported for the determination of Ofloxacin which include Spectrophotometric method and spectrofluorimetric determination\textsuperscript{121}, Spectrofluorimetric\textsuperscript{122}, Spectrofluorimetric and microbiological method\textsuperscript{123}, Spectrophotometric and Polarographic method\textsuperscript{124}, Chromatographic method\textsuperscript{125}, HPLC method\textsuperscript{126-128}, Colorimetric method\textsuperscript{129} and Spectrophotometric method\textsuperscript{130-147}, HPLC and Spectrophotometric method\textsuperscript{148}, potentiometry and conductometry\textsuperscript{149} and polarography\textsuperscript{150}.

El-Yazbi, F.A\textsuperscript{121}, proposed a simple spectrophotometric and spectrofluorimetric determination of ofloxacin. Two methods are presented for the analysis of ofloxacin. The first method is based on the application of $\lambda_{\text{max}}$, first and second derivative Techniques for its determination in bulk, tablet and urine. The second one depends on the fluorescence characteristics of ofloxacin I acidic solutions. The spectrofluorimetric method is 10-fold more sensitive than the spectrophotometric one.

Eboka etal\textsuperscript{129}, proposed a colorimetric method for determination of the ciprofloxacin, ofloxacin and norfloxacin. The ciprofloxacin, ofloxacin and norfloxacin formed an amber coloured complex with Iron (III) nitrate monohydrate. The complex, which formed instantaneously at room
temperature was stable. The solution of the complex obeyed Beer's law at 370 nm.

Feng et al.\textsuperscript{130}, proposed a spectrophotometric method based on the charge transfer reaction for the determination of ofloxacin. The molar absorptivity of the complex at 409 nm. The complex was found to be 1:1 by slope ratio and Bent-French methods.

Wang et al.\textsuperscript{131}, developed the spectrophotometric determination of ofloxacin in tablets. The method was based on the formation of an orange complex with ferric chloride in DMSO-MeOH, which exhibited maximum absorbance at 420 nm. The linear concentration range was 25-150 mg/ml.

Zhao et al.\textsuperscript{132}, describes the spectrophotometric determination of ofloxacin. The method based on the charge transfer complex formed between ofloxacin as the donor and 7,7,8,8-tetra-cyanoquinodimethane (TCNQ) as the acceptor in methanol-acetone medium has been studied. Beer's law is obeyed in the range of 0-15 mg/ml of ofloxacin. The charge transfer complex is found to be 1:1 by Bent-French and curved intersection methods.

Mashru et al.\textsuperscript{133}, proposed a simple and sensitive spectrophotometric method for the determination of perfloxacin and ofloxacin. These drugs involved in the reaction with ferric nitrate in nitric acid medium and have an absorption maxim at 462 nm and 445 nm, respectively for PFL and OFL.
Gandimathi et al. have developed three accurate and economical procedures for simultaneous estimation of tinidazole and ofloxacin in two-component tablet formulations. These methods employ third derivative spectroscopy. Simultaneous equation and a program in multicomponent mode of analysis of the instrument used for the simultaneous estimation of two drugs in methanol. Tinidazole has an absorbance maxima at 304 nm and ofloxacin at 291 nm.

Gupta et al. have developed spectrophotometric estimation of sparfloxacin and ofloxacin. The anti-macrobial drugs sparfloxacin and ofloxacin because of potential oxygen donor atoms, provided wine with a red coloration with iron (III) in both aqueous and alcoholic medium.

Guptal et al. proposed a simple spectrophotometric estimation of fluoroquinoline derivatives in pharmaceutical formulations with iron (III) chloride. Interaction between iron (III) chloride and sparfloxacin and ofloxacin were used for their spectrophotometric estimation at 510 nm.

Suslu, I and Tamer proposed a simple, rapid, and extractive spectrophotometric methods for the determination of ofloxacin in bulk and pharmaceutical dosage form. These methods are based on the formation of yellow ion-pair complexes between the basic nitrogen of the drug and bromophenol blue and bromocresol purple assulfophthalein dyes in phthalate.
buffer pH 3.0 and pH 3.1, respectively. The formed complexes were extracted with chloroform and measured at 414 nm for ofloxacin-bromophenol blue and 408 nm for ofloxacin-bromocresol purple. The analytical parameters and their effects on the reported systems are investigated. The reactions were extremely rapid at room temperature and the absorbance values remains unchanged at 48 hour for ofloxacin-bromophenol blue and 72 hour for ofloxacin-bromocresol purple. Beer's law was obeyed in the ranges 0.87-17.35 and 0.58-14.46 µg/ml for ofloxacin-bromophenol blue and ofloxacin-bromocresol purple, respectively. The proposed methods have been applied successfully for the analysis of the drug bulk form and its dosage form. The results obtained by the proposed methods were compared and statistical analysis showed no significant difference between the proposed methods.

Zhang, H et al., have developed spectrophotometric method for the Determination of ofloxacin. Sample of finely powdered granules, equivalent to 25 mg ofloxacin (I), was dissolved in 0.1M-HCl and diluted to 50 ml with more solvent A and filtered. Portions (3 ml) of filtrate were diluted to 50 ml with solvent A and a 10 ml portion was taken and diluted to 50 ml with the same solvent. The absorbance of the solution was measured at 293 nm vs. a solvent a blank.
Shao, S.J\textsuperscript{139}, Proposed a simple spectrophotometric method for the determination of ofloxacin in ointment. Sample equivalent to 30 mg of ofloxacin was dissolved in 30 ml of 0.1M-HCl with heating and the cooled solution was diluted to 100 ml. A 2 ml portion of the solution was further diluted to 100 ml with 0.1M-HCl and after filtration the absorbance was measured at 293 nm.

Sastry, C.S.P et al\textsuperscript{140}, developed two Extractive spectrophotometric methods for the determination of some fluoroquinolone derivatives in pure and dosage forms. Two methods are presented for the determination of norfloxacin (I), ciprofloxacin (II), ofloxacin (III) and enrofloxacin (IV). In the first method, 0.5-4 ml (100 µg/ml) of I, 0.5-6 ml (50 µg/ml) or 0.5-5 ml (50 µg/ml) of III and IV were added to 2 ml Supracene Violet 3B and 6 ml buffer (pH 1.3; 7.507 g of glycine and 5.85 g of NaCl dissolved in 11 ml H\textsubscript{2}O and 774 ml 0.1M-HCl), and the solution made up to 15 ml with H\textsubscript{2}O. The mixture was shaken with 10 ml CHCl\textsubscript{3} for 2 min then the absorbance of the CHCl\textsubscript{3} layer was read at 575 nm.

In the second method, 0.5-6 ml (50 µg/ml) of III or 0.5-4 ml (5 µg/ml) IV were added to 6 ml 0.1M-HCl and 2 ml troaeolin and the volume adjusted to 15 ml with H\textsubscript{2}O. The mixture was shaken with 10 ml CHCl\textsubscript{3} for 2 min and the absorbance of the CHCl\textsubscript{3} layer was read at 485 nm. Beer’s law was obeyed and the detection limits were 5 µg/ml for I and 2.5 µg/ml for II in the first method.
and 2.5 μg/ml for III and IV in both methods. The methods were applied to the analysis of the drugs in various formulations (extraction details given).

Kapetanovic, V₄¹, et al developed Spectrophotometric and polarographic investigation for the determination of ofloxacin-copper(II) complexes. Sample containing ~200 mg ofloxacin (I) was mixed with 500 ml H₂O. A portion (0.25-2.5 ml) was mixed with 0.5 ml copper(II) nitrate, 5 ml Britton- Robinson buffer of pH 4.5 and 1 ml 2M-NaNO₃, diluted to 10 ml with H₂O and analysed by spectrophotometry at 360 nm. Beer's law was obeyed from 18-180 mg/ml I. The effects of pH on spectra from 350-450 nm were investigated. Maxima were observed at pH 4 (360 nm), pH 7.02 (363 nm) and pH 8.3 (365 nm) corresponding to the formation of 1:1, 1:2 and 1:3 Cu (II): I complexes, respectively. The stability constants are tabulated. The complex was also investigated by differential pulse polarography using a three-electrode cell (dropping Hg electrode/SCE/Pt auxiliary electrode) with a drop time of 2 s, modulation amplitude of 25 mV, scan rate of 2 mV/s and Hg column height of 81 cm. The results agreed closely with those obtained by spectrophotometry.

Sastry, C.S.P et al¹⁴², have developed Spectrophotometric method for the determination of enrofloxacin and ofloxacin in pharmaceutical formulations. Powdered tablets equivalent to 25 mg enrofloxacin (I) or ofloxacin (II) were
treated with 10 ml 0.1N-HCl and diluted to 100 ml with HCl. Any solid was separated by filtration and the filtrate was diluted to a final concentration of 50 micrograms/ml. The solution was treated with 3 ml 3-methyl-2-benzothiazolinone hydrazone hydrochloride and Ce(IV) (7 ml for I, 6 ml for II). The reaction mixture was left to stand for 20 (I) or 10 min (II). The solution was up to 25 ml with H₂O and the absorbance was measured at 630 or 640 nm for I or II, respectively, within 20 min of colour development (20-40 min for I; 10-30 min for II). Injection solutions (1 ml) containing 2 mg drug was diluted to a final concentration of 50 μg/ml and analyzed similarly. Beer's law was obeyed from 1-10 μg/ml.

Issa, Y. M et al.¹⁴³, developed Spectrophotometric method for the determination of ofloxacin and lomefloxacin hydrochloride with some sulphonphthalein dyes. Powdered tablets equivalent to 50 mg ofloxacin (I) were dissolved in 10 ml 0.05M- NaOH, the solution was filtered and the filtrate was diluted to 100 ml with H₂O. Lomefloxacin (II) tablets were similarly treated but H₂O was used in place of 0.05M- NaOH. Prepared samples were diluted 10-fold and portions (0.5-1.5 ml) of the resulting solution were mixed with 3 ml 0.05% dye [bromophenol blue (BPB), bromothymol blue (BTB) or bromocresol purple (BCP)] in 0.1M-NaOH. The mixtures were buffered with 3 ml acetate buffer of pH 4 (for I, or II using BPB or BCP) or 3 ml phosphate buffer in the case of
BTB (pH 5.7 for I; pH 7 for II). The mixtures were then diluted to 10 ml with H₂O, extracted with 5 ml CHCl₃ and the absorbances of the CHCl₃ extracts were measured at 410, 415 and 410 nm for BPB, BTB or BCP respectively. The calibration graphs were linear from 5-25, 2-15 and 2-20 μg/ml for both I and II, using BPB, BTB and BCP as the reagents, respectively.

Zhao, F.L et al.¹⁴⁴ propose a new Spectrophotometric method for the determination of ofloxacin based on a charge-transfer reaction. Portions of a methanolic solution of ofloxacin (I), containing 75 μg/ml I, were mixed with 2 ml 7, 7,8,8-tetracyanoquinodimethane in acetone and the mixture was diluted with methanol to 5 ml. The methanolic solution was heated to 30°C for 30 minute, cooled to room temperature, and the absorbance was measured at 743 nm (ε= 35 800) vs. a reagent blank. Beer's law was obeyed up to 15 mg/l of I. The method was applied to the analysis of I in tablets. The average recovery of I was 101.2% with an RSD (n = 4) of <3% based on labelled values.

Quan, H., et al.¹⁴⁵ have developed Spectrophotometric method for the determination of ofloxacin with a charge-transfer complex a new charge-transfer complex was formed by reaction of ofloxacin with p-nitrophenol (4-nitrophenol). The complex had strong absorbance of 302 nm while ofloxacin had no absorbance at the same wavelength. The absorbance coefficient, the
molar ratio, and the dissociation constant of the complex were studied. The linear calibration range was 2 to 80 μg/ml for ofloxacin.

Feng, Y et al\textsuperscript{146}, proposed a spectrophotometric method for the estimation of ofloxacin. Portions of a standard ofloxacin (I) solution were treated with 2 ml 0.04% tetracyanoethylene in acetone and the mixture was diluted with acetone to 5 ml. The solution was heated at 50\degree C for 30 minute and the absorbance was measured at 409 nm (ε = 28 000) vs. a reagent blank. Beer's law was obeyed for to 12 micrograms/ml of I. The method was applied to the analysis of I in a commercial tablet. The recovery was 99.5\% (based on listed value) with an RSD (n=10) of 0.72\%. Results were compared with those obtained by UV spectrophotometry. Ivankiv, I.L et al\textsuperscript{147}, have developed Ultra-violet spectrophotometric method for the determination of ofloxacin. A tablet (≈0.2 g) was dissolved in 250 ml of 0.1M-NaOH and the solution was filtered. A 2 ml portion of the filtrate was diluted to 100 ml with 0.1M-NaOH and the absorbance of the solution was measured at 287 nm.

Carlucci, G et al\textsuperscript{148}, developed high-performance liquid chromatography and derivative UV-spectrophotometric methods for the determination of ofloxacin in pharmaceutical forms. Powdered tablets and ointments were extracted with 0.1M-NaOH and eye washes were diluted with 0.1M-NaOH before analysis.
The resulting solutions were analyzed on an anionic exchange Vydac column with a mobile phase of 0.05M-phosphate buffer of pH 7/acetonitrile (1:4) at 2 ml/minute and detection at 297 nm, norfloxacin was used as internal standard. The solutions were also analyzed by second derivative espectrophotometry with Δλ = 6 nm and measurement of the peak-trough amplitude between 303 and 315 nm. The detection limit was 10 mg/ml of ofloxacin with use of the HPLC method and 20 μg/ml with the spectrophotometric method, the respective RSD were 1.2 and 2.0%.
Various methods are reported in literature for the estimation of nimesulide which include, Spectrophotometric method\textsuperscript{151-157}, colorimetric method\textsuperscript{158}, HPTLC method\textsuperscript{159,160}, Fluorimetric method\textsuperscript{161}, electrophoretic method\textsuperscript{162}.

Mrinalini, D et al\textsuperscript{151}, have developed Simultaneous spectrophotometric method for the estimation of nimesulide and chlorzoxazone in tablet dosage form. The method is based on the use of simultaneous equations (given) to calculate nimesulide (I) and chlorzoxazone (II) from absorbances of solutions of sample in 0.1M-NaOH measured at 244 and 395 nm. Beer's law was obeyed for 2-10 and 25-125 $\mu$g/ml of I and II, respectively. The method was applied to tablets containing 100 and 250 mg of I and II, respectively, giving results which agree with claimed values. Recoveries of 1 or 2 $\mu$g/ml I and 2.5 or 3.5 $\mu$g/ml II added to sample solutions were 99.31-101.41%.

Chandran, S et al\textsuperscript{152}, proposed a new ultra-violet spectrophotometric method for the estimation of nimesulide. Nimesulide was determined in its bulk and dosage forms by dissolving 10 mg drug in 100 ml aqueous 50\% or 100\% acetonitrile (methods A and B, respectively) to give a final concentration of 100 $\mu$g/ml. The absorbance of various dilutions was measured, the $\lambda$ max was observed at 300 nm in both media. Beer's law was obeyed in the range 10-50 $\mu$g/ml and both
methods were linear in the range 0-50 μg/ml. Detection limits were 0.46 and 1.04 μg/ml by methods A and B, respectively, and quantitation limits were 1.52 and 3.48 μg/ml. The RSD were 0.11 and 0.09%, respectively (n=5). The methods were applied to the analysis of nimesulide in tablets, recoveries were near 100%.

Altinoz, S and Dursun, O.Ö¹⁵³, have developed second order derivative UV spectrophotometry method for the determination of nimesulide in pharmaceutical dosage forms. In this study, nimesulide which has been used as an analgesic, antipyretic and anti-inflammatory agent, was analyzed by using second order derivative UV spectrophotometry. The solvent, the degree of derivation, ranges of wavelength and n-value were chosen in order to optimize the conditions. The concentration of nimesulide in its solutions in ethanol and chloroform were determined between the wavelength ranges of 200 and 500 nm (n = 6, Δλ=21) and in the linearity ranges of 2.0-90.0 μg/ml in ethanol and 2.0-50.0 μg/ ml in chloroform by using the values obtained from the second derivative UV spectrum of the substance. The developed second derivative UV spectrophotometric method was applied to the pharmaceutical preparations such as tablet, sachet (granule) and suspension. Tablet and sachet were analysed in ethanol while the suspension was analysed in chloroform. The results obtained from derivative UV spectrophotometry were compared with those obtained by
using HPLC. It was found that the difference was not statistically important between these methods. It was concluded that developed derivative UV spectrophotometric method was accurate, sensitive, precise, reproducible and could be applied directly and easily to the pharmaceutical preparations.

Lakshimi, C.S.R et al.\textsuperscript{154}, developed Spectrophotometric method for the estimation of nimesulide and its formulations. Four simple and sensitive visible spectrophotometric methods (A-D) have been described for the assay of nimesulide (NMD) either in pure form or in pharmaceutical formulations. Methods A and B are based on the oxidative coupling between the reduced product of NMD (RNMD) and p-N,N-dimethyl phenylenediamine dihydrochloride (DMPD) in presence of chloramine-T (CAT) or 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in presence of ferric chloride (Fe III) to form coloured products with $\lambda$ max at 540 nm and 600 nm, respectively. Method C is based on the diazotization of RNMD with excess nitrous acid (HNO$_2$) and estimating the consumed HNO$_2$ with cresyl fast violet acetate (CFVA). Method D is based on the formation of the coloured charge-transfer complex, when RNMD is treated with metol (p-methyl aminophenol sulphate; PMAP) in presence of potassium dichromate. All variables have been optimized and the reaction sequence is presented. The concentration
measurements are reproducible within a relative standard deviation of 1.0%. Recoveries are 98.6-100.2%.

Chowdary, K.P.R et al\textsuperscript{155}, have developed Spectrophotometric method for the determination of nimesulide using 3-methyl-2-benzothiazolinone hydrazone hydrochloride reagent. A pharmaceutical equivalent to 50 mg nimesulide (I) was dissolved in 20 ml methanol and treated with 5 g of Zn dust and 4 ml concentrated HCl. After standing for 1 h, the mixture was filtered and the residue was washed with 3 x 5 ml methanol. The filtrate was adjusted to 100 ml with H\textsubscript{2}O then further diluted to a concentration of 100 \(\mu\)g/ml. Portions (0.5-2.0 ml) of the reduced I solution were mixed with 1.5 ml 3-methyl-2-benzothiazolinone hydrazone hydrochloride (0.2\% w/v in H\textsubscript{2}O) and left for 2 minute, then 2 ml ferric chloride solution (0.7\% w/v in 0.5N-HCl) was added. The mixture was left for 10 min, diluted to 10 ml with H\textsubscript{2}O, and its absorbance measured at 600 nm (\(\varepsilon = 12\ 270\)). Beer's law was obeyed from 5-20 \(\mu\)g/ml (I). The RSD (\(n = 6\)) was 0.65\%. The method was applied to tablets and suspensions; results compared favourably with a UV method (details not given). Recoveries ranged from 99-101\%. Common excipients did not interfere.

Chowdary, K. P.R et al\textsuperscript{156}, proposed a new spectrophotometric method for the determination of nimesulide. Powdered tablets dissolved in 20 ml methanol
were mixed with 5 g Zn dust, 4 ml concentrated HCl, left to stand for 30 min, filtered and the volume made up to 100 ml with 0.1N-HCl. A 1 ml portion was mixed with 1 ml 5N-HCl, 1 ml NaN02, left to stand for 5 min, mixed with 1 ml 0.5% ammonium sulfamate and left to stand for 2 minute. N-(1-naphthyl) ethylenediamine dihydrochloride (1 ml of 0.1%) was added, the tube was heated at 100°C for 30 min in a water bath, cooled and the volume made up to 10 ml with H2O. The purple colour was measured at 560 nm. Beer's law was obeyed from 0-40 μg/ml and the colour was stable for >12 hours.

Chandran, S et al\textsuperscript{157}, proposed a New Ultraviolet spectrophotometric method for the estimation of nimesulide. Two simple and accurate ultraviolet (UV) spectrophotometric methods with better detection range for estimation of nimesulide in pure form and in solid dosage form were developed in the present studies using 50% v/v and 100% v/v acetonitrile as the solvent system. The linearity range of nimesulide in both the methods was found to be 10-50 micrograms/ml at a \( \lambda \) max of 300 nm. The linear regression equations obtained by the least-square regression method are \( \text{Abs}=1.33 \times 10(-1) \). Conc + 1.89 \times 10(-1) in 50% v/v acetonitrile and \( \text{Abs}=1.05 \times 10(-1) \). Conc + 1.14 \times 10(-1) in 100% v/v acetonitrile. The detection limit as per the error propagation theory was found to be 0.46 microgram/ml and 1.04 μg/ml, respectively, in 50% v/v and 100% v/v acetonitrile. The developed methods were employed with high
degree of precision and accuracy for the estimation of total drug content in three commercial formulation of nimesulide.

Navalgund, S.G et al\textsuperscript{158}, proposed a simple colorimetric method for the determination of nimesulide from its pharmaceutical preparation. Tablets containing 100 mg nimesulide were dissolved in acetonitrile (50 ml). The filtrate obtained was diluted to 100 ml and 10 ml of the solution was further dissolved to 100 ml. A sample (2.5 ml) was mixed with acetonitrile (2.5 ml) and 2N-NaOH and, finally, diluted to 10 ml. The absorbance was measured at 399 nm. The calibration graph was linear from 5-50 µg/ml. The recovery was 100.09%.
Various methods are reported in literature for the estimation of isoniazid in pharmaceutical formulations which includes, Spectrophotometric method\textsuperscript{163-166} and High performance liquid chromatography\textsuperscript{167}.

Gowda, B.G etal\textsuperscript{163}, proposed a simple, sensitive and accurate spectrophotometric method for the determination of isoniazid in pharmaceutical formulations. The method is based on the oxidation of iron by potassium periodate followed by coupling with isoniazid in alkaline medium leading to the formation of a red coloured product having maximum absorbance at 505 nm. The reaction conditions were optimized to obtain the maximum colour intensity. The absorbance was found to increase linearly with increase in concentration of isoniazid, which was corroborated by the calculated correlation coefficient (0.9991). The system obeyed Beer's law in the concentration range of 1.5-18 \( \mu \text{g/ml} \). The common excipients and additives used in pharmaceutical formulations do not interfere with the proposed method. The method was successfully applied for the assay of INH in various pharmaceutical formulations and the results compare favorably with those of the official method.
Devani, M.B et al\textsuperscript{164}, proposed a spectrophotometric method for the determination of isoniazid in the presence of its hydrazones. The method involves the reaction between isoniazid and 2,3-dichloro-1,4-naphthoquinone in the presence of ammonia in an ethanolic medium. The colored product has an absorbance maximum at 640 nm. The Lambert-Beer law is obeyed in the 1-14-\mu g/ml range. The proposed method was applied to the analysis of isoniazid tablets. In commercial tablets, hydrazone formation due to the reaction between isoniazid and lactose was detected by TLC. The analysis of lactose-containing isoniazid tablets showed 10--22\% lower recovery than that obtained by the official method.

Safavi, A et al\textsuperscript{165}, proposed a simple, rapid, sensitive and accurate indirect spectrophotometric method for the micro determination of isoniazid (INH) in pure form and pharmaceutical formulations. The procedure is based on the reaction of copper (II) with isoniazid in the presence of neocuproine (NC). In the presence of neocuproine, copper (II) is reduced easily by isoniazid to a Cu (I)-neocuproine complex, which shows an absorption maximum at 454 nm. By measuring the absorbance of the complex at this wavelength, isoniazid can be determined in the range 0.3-3.5 \mu g/ml. This method was applied to the determination of isoniazid in pharmaceutical formulation and enabled the determination of the isoniazid in \mu g quantities (0.3-3.5\mu g/ml).
Goicoechea, H.C and Olivieri, A.C\textsuperscript{166}, have developed Simultaneous determination of rifampicin, isoniazid and pyrazinamide in tablet preparations by multivariate spectrophotometric calibration. The use of multivariate spectrophotometric calibration is presented for the simultaneous determination of the active components of tablets used in the treatment of pulmonary tuberculosis. The resolution of ternary mixtures of rifampicin, isoniazid and pyrazinamide has been accomplished by using partial least squares (PLS-1) regression analysis. Although the components show an important degree of spectral overlap, they have been simultaneously determined with high accuracy and precision, rapidly and with no need of nonaqueous solvents for dissolving the samples. No interference has been observed from the tablet excipients. A comparison is presented with the related multivariate method of classical least squares (CLS) analysis, which is shown to yield less reliable results due to the severe spectral overlap among the studied compounds. This is highlighted in the case of isoniazid, due to the small absorbances measured for this component.
Various methods are reported for the estimation of diloxanide furoate such as Spectrophotometric method\textsuperscript{168-177} and HPLC method\textsuperscript{178} and PMR spectrophotometry\textsuperscript{179}.

Prasad, C. V. N et al\textsuperscript{167} proposed a second derivative spectrophotometric method for the simultaneous determination of tinidazole, furazolidone and diloxanide furoate in a combined tablet preparation by second-derivative spectrophotometry. A second-derivative spectrophotometric procedure has been developed for the simultaneous determination of tinidazole (TD), furazolidone (FD) and diloxanide furoate (DF) in a commercial preparation. Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of TD (5-20 $\mu$g/ml), FD (2.5-10 $\mu$g/ml) and DF (7.5-15 $\mu$g/ml).

Galal, S. M et al\textsuperscript{169} have developed derivative spectrophotometric method for determination of antiprotozoal drugs in two-component tablet preparations. Diloxanide furoate (I) and metronidazole (II) were determined in two-component tablets by derivative spectrophotometry.
Das, T.K and Halder, D\textsuperscript{170}, have developed Simultaneous spectrophotometric method for the analysis of binary mixtures of metronidazole diloxanide furoate and tinidazole-diloxanide furoate in pharmaceutical dosage forms. A 1-ml portion of the filtrate was diluted to 50 ml with H\textsubscript{2}O and the absorbance of the resulting solution was measured at 320 and 262 nm for I-diloxanide furoate (III) or 318 and 262 nm for II-III vs.

Podder, G et al\textsuperscript{171}, proposed a spectrophotometric method for the determination of tinidazole and diloxanide furoate in combined pharmaceutical formulation. The absorbance of a methanolic solution of sample, containing 20 to 40 \textmu g/ml of each drug, was measured from 254 to 310 nm. Tinidazole exhibits an absorption max. at 310 nm, and diloxanide at 254 nm.

Sastry, C. S. P et al\textsuperscript{172}, have developed spectrophotometric method for the determination of diloxanide furoate with iodine and isonicotinic acid hydrazide (isoniazid). Powdered tablets or syrup of diloxanide furoate (I), equivalent to 50 mg of I, were dissolved in methanol (10 ml) and hydrolysed with 5M-HCl (20 ml) under reflux for 1 hour. The mixture was cooled and excess of HCl was removed under vacuum (1.5 ml) and isoniazid solution. (1.0 ml) were added successively at 2-minute intervals to K H phthalate-HCl buffer solution.
Srinath, V and Bagavant, G\textsuperscript{173}, have developed Spectrophotometric method for the analysis of binary mixtures of tinidazole - diloxanide furoate and metronidazole-diloxanide furoate. Binary mixtures of tinidazole (I)-diloxanide furoate (II) and metronidazole-II in spectroscopic ethanol were analysed at 258 and 310 nm. Beer's law was obeyed for 10 to 25 μg/ml of I or II.

Sane, R. T et al\textsuperscript{174}, proposed a simple spectrophotometric method for the determination of diloxanide furoate from pharmaceutical preparations. Diloxanide furoate was extracted from ground tablets into ethanol or was filtered from a suspension and dissolved in ethanol 6% NaOH and Folin-Ciocalteu reagent or with a 1% solution of sodium nitroprusside in aqueous solution. Beer's law was obeyed in the respective ranges 4 to 28 μg/ml and 5 to 25 μg/ml.

Sadana, G. S and Gaonkar, M. V\textsuperscript{175}, have developed simultaneous derivative spectroscopic determination method for the estimation of diloxanide furoate and tinidazole in pharmaceutical dosage forms. Powdered tablets equivalent to 250 mg of diloxanide furoate (I) and 150 mg of tinidazole (II) were dissolved in 50 ml of methanol and the solution was set aside for 30 min with frequent shaking. In both instances, the extracts were further diluted 1:10, and then 1:25 with methanol and the second-derivative spectra were recorded from 190 to 400 nm.
Al-Ghanam SM and Belal F\textsuperscript{176}, proposed a simple and sensitive spectrophotometric method for the determination of diloxanide furoate in its dosage forms. The method is based on the reaction of the drug with potassium permanganate in the presence of sodium hydroxide to produce a bluish green coloured species measurable at 610 nm. The absorbance-concentration plot is linear over the range 2.5-20 \( \mu \)g/ml with correlation coefficient \((n = 8)\) of 0.9998 and minimum detectability of 0.2 \( \mu \)g/ml \((6.1 \times 10^{-7} M)\).

Hasan, N.Y etal\textsuperscript{177}, have developed five new selective, precise and accurate methods for the determination of diloxanide furoate (DI) in presence of its degradation products. Method A utilizes the first and second derivative spectrophotometry at 270 and 280 nm, respectively. Method B is a RSD(1) spectrophotometric method based on the simultaneous use of the first derivative of ratio spectra and measurement at 270 nm. Method C is a pH-induced difference spectrophotometry using UV measurement at 295 nm. Method D is a densitometric one, after separation on silica gel plate using chloroform: methanol as mobile phase and the spots were scanned at 258 nm. Method E is reversed phase high performance liquid chromatography using methanol: water \((80:20\% \, v/v)\) as mobile phase at a flow rate of 1 ml/min and UV detection at 258 nm.
spectrophotometric methods\textsuperscript{180-188} and HPLC method\textsuperscript{189-196} have been reported in literature for the estimation of furosemide in pharmaceutical formulations.

Gotardo, M.A etal\textsuperscript{180} have developed for Determination of furosemide in pharmaceutical formulations by diffuse reflectance spectroscopy. In this report an analytical method to determine furosemide by using diffuse reflectance spectroscopy is presented. This study shows that this technique can give quantitative results using spot test analysis, particularly in the case of pharmaceuticals containing furosemide. The color spot test could be obtained by reaction between furosemide with p-dimethyl amino cinnamaldehyde, in acid medium and the effect of common excipients on the reflectance measurements was evaluated. The method was applied to determine furosemide in commercial brands of pharmaceuticals.

Ferraro, M. C. F etal\textsuperscript{181}, proposed a spectrophotometric-partial least squares (PLS-1) method for the simultaneous determination of furosemide and amiloride hydrochloride in pharmaceutical formulations. The results for synthetic mixtures of 8-13 mg/l of furosemide and 1-1.6 mg/l of amiloride hydrochloride gave RSD of 0.5-1.37\% and recoveries of 99.59 and 101.04\%, respectively.
Sevillano-Cabeza, A et al.\textsuperscript{182}, have developed an extractive-spectrophotometric method for the determination of furosemide with sodium 1,2-naphthoquinone-4-sulphonate in pharmaceutical formulations. Sample (0.1-1 ml) containing 200 mg/ml furosemide (I) was heated at 700°C for 30 minutes with 1 ml NaH$_2$PO$_4$/Na$_2$HPO$_4$ buffer of pH 7.5 and 7.7 mm$^{-1}$, 2 naphthoquinone-4-sulfonate.

Agatonovic-Kustrin, S et al.\textsuperscript{183}, proposed a spectrophotometric method for the determination of furosemide and its palladium (II) complex. A portion (2 ml) of the solution was treated with 50 ml PdCl$_2$ in HCl (2 ml), 2M-KCl (1 ml), Britton-Robinson buffer solution. Beer's law was obeyed from 0.25 to 3.5\mu g/ml frusemide (I), the lower limit of sensitivity was 8.41 \mu g/ml.

Zivanovic, L et al.\textsuperscript{184}, have developed a spectrophotometric method for the determination of furosemide (frusemide) as its iron (III) complex in pharmaceutical preparations. A 5-ml portion was mixed with 0.1M-FeCl$_3$ (1.0 ml) and 1M-KCl (1.0 ml) and the pH adjusted to 5.7 with 0.1M-HCl.

Ines Toral M, Pope S et al.\textsuperscript{185}, have developed simultaneous determination of amiloride and furosemide in pharmaceutical formulations by first digital derivative spectrophotometry. This work presents a simple and fast method for the simultaneous determination of amiloride and furosemide by digital derivative spectrophotometry. HCl 1 \times 10^{-2} mol/ml dissolved in ethanol was
used as solvent and to extract drugs from formulations. Subsequently the samples were evaluated directly by first digital derivative spectrophotometric method, using a smoothing factor of 8 and scale factor of $1 \times 10^{-4}$. The simultaneous determination of furosemide and amiloride can be carried out at 241.4 and 343.6 nm, respectively. In both cases, the zero crossing approach was used. When both compounds are present together in a sample, it is possible to quantify one in the presence of the other, without mutual interference.

Garcia MS et al.\textsuperscript{186}, have developed two sensitive and fast flow-injection spectrophotometric determination of furosemide or sulphathiazole in pharmaceuticals. Two sensitive and fast flow-injection spectrophotometric methods are proposed for the determination of furosemide or sulphathiazole based on the formation of coloured complexes between these compounds and Pd (II) at pH 5.0 and $55^0$C. Using the peak height as a quantitative parameter, furosemide or sulphathiazole was determined at 410 nm over the range $2.0 \times 10^{-5}-4.0 \times 10^{-4}$ M or $5.0 \times 10^{-5}-3 \times 10^{-4}$ M, respectively. The methods were applied to the determination of these sulphonamides in pharmaceuticals.

Gangwal, S and Trivedi, P\textsuperscript{187}, have developed Comparative evaluation of two different spectrophotometric methods for simultaneous estimation of spironolactone and furosemide from combined dosage forms. Crushed tablets were dissolved in aqueous methanol and analysed by multiwavelength
spectrophotometry from 200-300 nm. Frusemide (I) and spironolactone (II) were determined using their absorbance maxima at 228 and 242 nm, respectively. Alternatively, first-derivative spectrophotometry was carried out and the determination wavelengths were switched, the resulting calibration graphs were linear up to 20 µg/ml (I) and 30 µg/ml (II).

Ferraro, M.C et al. proposed a spectrophotometric-partial least squares (PLS-1) method for the simultaneous determination of furosemide and amiloride hydrochloride in pharmaceutical formulations. A numerical method, based on the use of spectrophotometric data coupled to PLS-1 multivariate calibration, is reported for the simultaneous determination of furosemide and amiloride hydrochloride in synthetic samples and commercial tablets. Its accuracy and precision were determined, and it was validated by the analysis of synthetic mixtures of both drugs. The method was successfully applied to the quantitation of furosemide and amiloride hydrochloride in three different pharmaceutical formulations, providing results in agreement with those obtained by HPLC. It allowed the rapid, accurate and precise simultaneous estimation of the concentration of both analytes of interest in spite of their important spectral overlap, high concentration relationship and the presence of small amounts of different, unmodelled, absorbing excipients.
Various methods are reported in the literature for the estimation of rifampicin in pharmaceutical formulations which include Spectrophotometric method\textsuperscript{197-203}, Thin Layer Chromatography method\textsuperscript{204}, High Performance Liquid Chromatography method\textsuperscript{205-208}, RP-LC method\textsuperscript{209} and Micellar electrokinetic capillary chromatography\textsuperscript{210}.

Goicoechea, H.C and Olivieri, A.C\textsuperscript{197}, have developed Simultaneous determination of rifampicin, isoniazid and pyrazinamide in tablet preparations by multivariate spectrophotometric calibration. Twenty tablets, containing all 3 cited drugs were ground, an amount of powder corresponding to 20 mg rifampicin was dissolved in 1 l of H\textsubscript{2}O, and the solution was sonicated for 5 min, filtered, and diluted twofold (final pH <=7.5). A calibration set of 15 solutions containing 0-10\(\mu\)g/ml rifampicin, 0-40\(\mu\)g/ml-isoniazid and 0-0.158 \(\mu\)g/ml pyrazinamide.

Benetton, S. A etal\textsuperscript{198}, proposed a visible spectrophotometric and first-derivative UV spectrophotometric method for the determination of rifampicin and isoniazid in pharmaceutical preparations. Rifampicin was determined by visible spectrophotometry by measuring the absorbance at 475 nm in phosphate
buffer at pH 7.4. A linear response was obtained for 20-70 μg/ml rifampicin. Isoniazid was determined in 0.12M-HCl by first derivative UV spectrophotometry using the amplitude of the signal at 257 nm. The methods were applied to determine rifampicin and isoniazid in pharmaceutical preparations.

Dahibhate, P. P\textsuperscript{199}, have developed Simultaneous spectrophotometric method for the estimation of rifampicin, isoniazid and pyrazinamide from combined dosage forms. Capsules were also analysed in this way (preparation details given) along with mixed standards with various compositions of I, rifampicin (II) and isoniazid (III).

Panzade, P. D etal\textsuperscript{200}, have developed Simultaneous spectrophotometric determination of rifampicin and isoniazid from combined dosage forms. Ground capsule contents equivalent to 10 mg of rifampicin were dissolved in acidic H\textsubscript{2}O to a final concentration of 10 μg/ml of rifampicin, and the absorption spectrum of the solution was scanned over the range 300-200 nm and compared with those for two-component standard solutions containing 6, 0, 6, 12 and 15 μg/ml of rifampicin and 0, 4, 4, 8 and 10 μg/ml of isoniazid, respectively.

Hui, R. H and Hou, D. Y\textsuperscript{201}, have developed Quantitative determination of rifampicin by three-wavelength spectrophotometry. Portions (0.5 ml) of a
standard solution of rifampicin (1 mg/ml, I) were diluted to 25 ml with phosphate buffer of pH 7 and the absorption of the solution was scanned between 300 and 600 nm.

Sharma, S.C et al\textsuperscript{202}, proposed a spectrophotometric method for the simultaneous determination of rifampicin and isoniazid in dosage form is described. By using a mixture of methanol and water (2+3) as solvent, the absorbance of the mixtures of rifampicin and isoniazid is measured at 264 nm and 335 nm.

Mahalanabis, K.K et al\textsuperscript{203}, proposed a least-squares method in the matrix for the simultaneous determination of rifampicin and isoniazid in a mixture. The method allows the rapid analysis of binary pharmaceutical formulations with minimum error. The concentration of each component in the mixture has been determined spectrophotometrically by measuring the absorbance of the mixture at 5-nm intervals from 230 to 290 nm. To calculate the matrix of the proportionality constant a standard mixture was used for each component. All data analyses were performed on a personal computer.
Various spectrophotometric methods\textsuperscript{211-214} and HPLC method\textsuperscript{215-217} are reported in the literature for estimation of spironolactone in tablets formulations.

Dinc, E and Ustundag, O\textsuperscript{211}, have developed Spectrophotometric method for the simultaneous determination of hydrochlorothiazide and spironolactone in tablets. The method was performed by classical least-squares (CLS), inverse least-squares (ILS), principal component regression (PCR) and partial least-squares (PLS). The methods of the chemometric analysis do not require sample pretreatment procedure. A training set of 25 standard mixtures containing both drugs was prepared in the concentration range of 2-20 μg/ml according to mixture design. The multivariate calibrations were obtained by measuring the zero-order and first-derivative absorbances at 15 points from 220 to 290 nm using the training set. The validation of the multivariate methods was realised by analysing the synthetic mixtures of hydrochlorothiazide and spironolactone. The result obtained on the synthetic mixture and tablets were statistically compared by the one-way ANOVA test.

Gangwal, S and Trivedi, P\textsuperscript{212}, have developed Comparative evaluation of two different spectrophotometric methods for simultaneous estimation of spironolactone and frusemide from combined dosage forms. Crushed tablets
were dissolved in aqueous methanol and analysed by multiwavelength spectrophotometry from 200-300 nm. Frusemide (I) and spironolactone (II) were determined using their absorbance maxima at 228 and 242 nm, respectively. Alternatively, first-derivative spectrophotometry was carried out and the determination wavelengths were switched, the resulting calibration graphs were linear up to 20 µg/ml (I) and 30 µg/ml (II).

Erram, S. V and Tipnis, H. P\textsuperscript{213}, proposed a simple spectrometric method for the analysis of spironolactone and frusemide from combined pharmaceutical dosages. Powdered tablets or capsule contents equivalent to 20 mg of frusemide were dissolved in 50 ml of methanol. After dilution to 100 ml with methanol, 0.1N-NaOH or 0.1N-HCl, the absorbance of the solution was measured at 255, 288 or 258 nm, respectively (for total content of spironolactone and frusemide) and at 330, 360 or 345 nm, respectively (for frusemide content). Beer's law was obeyed with use of all three solvents. RSD were 1.4-2.8% and recoveries were 98.6-99.9%.

Luis ML et al\textsuperscript{214} have developed simultaneous determination of chlorthalidone and spironolactone with univariate and multivariate calibration. Univariate calibration was performed by the zero-crossing and derivative ratio spectrum methods. Extensive spectral overlap and the scarcity of wavelengths in derivative spectra allowing one analyte to be distinguished and quantitated in 95
the presence of the other gave rise to poor results that called for multivariate calibration. Partial least-squares regression was used in combination with a suitable method for selecting the optimum wavelength range and number of factors for analysis.
Various spectrophotometric methods\textsuperscript{218-219}, Infrared Spectrophotometric method\textsuperscript{220} and Electro chemical method\textsuperscript{221} are reported in the literature for estimation of ornidazole in tablets formulations.

Hassan, M.M.A et al\textsuperscript{218}, have developed spectrophotometric method for the determination of ornidazole and its formulations. Ornidazole (I) was determined by direct spectrophotometry at 312 nm in ethanol and by 1H NMR in ethanol-free CHCl\textsubscript{3} or (2H\textsubscript{6})DMSO with use of acetanilide (II) as internal standard and tetramethylsilane as shift lock.

U.N. Kale et al\textsuperscript{219}, have developed two simple spectrophotometric methods for the determination of ornidazole and norfloxacin in pharmaceutical preparations. First method is based on simultaneous equations. In the second method, derivative spectroscopy is used to eliminate spectral interference. Both drugs obey Beer's law in the concentration range employed for the analysis. The results of analysis have been validated statistically and by recovery studies.
Colorimetric method\textsuperscript{222} and Spectrophotometric method\textsuperscript{223} are reported in literature for the estimation of mosapride citrate in pharmaceutical formulations.

Appala Raju and Shobha M et al.\textsuperscript{222} proposed a simple and sensitive UV spectrophotometric method for the estimation of mosapride citrate in bulk drug and its formulations. Mosapride citrate exhibits absorption maximum at 272 nm. Beer’s law obeyed in the concentrate on range of 2-10 $\mu$g/ml.

B.S. Kuchekar et al.\textsuperscript{223}, proposed a simple colourimetric method for the estimation of mosapride citrate in solid dosage forms. Estimation of mosapride citrate is based on diazotization of mosapride and coupling of the diazonium salt with N-(1-napthyl) ethylene diamine dihydrochloride to form a stable purple colored chromogen. With absorbance maxima at 540 nm the chromogen obeyed linearity over 20 to 160 $\mu$g/ml.