REVIEW ON ANALYTICAL METHOD VALIDATION OF NITROIMIDAZOLES

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ABSTRACT
Imidazole is a planner five membered ring system with 3 carbon and 2 nitrogen atom in 1 and 3 position. It is soluble in water and other solvents. It presents in two equivalent tautomeric forms i.e the hydrogen atom is located on one of the two nitrogen atoms. Imidazole is entirely soluble in water and highly polar compound as evidenced by a calculated dipole of 3.61 D. It is colorless liquid having high boiling point of 256°C than all other 5 membered heterocyclic compound due to intermolecular H bonding where there is linear association of molecule. Imidazole drug have broadened scope in remedying various dispositions in clinical medicines. Medicinal properties of imidazole include anticancer, β-lactamase inhibitors, carboxypeptidase inhibitor, antiaging agent, anticoagulants, anti-inflammatory, antibacterial, antiviral, antifungal antitubercular, antidiabetic and antimalarial. Nitroimidazoles class of drugs are a well-established group of antiprotozoal and antibacterial agents that have ability to inhibit the growth of anaerobic bacteria and certain anaerobic protozoa, for example Trichomonas vaginalis, Entamoeba histolytica and Giardia lamblia. This review article presents determination of Ornidazole and Tinidazole was done by UV spectrophotometer, HPLC, potentiometry, calorimetry, titrimetry and HPTLC.

Keywords: Imidazole, Nitroimidazole, Ornidazole and Tinidazole.

INTRODUCTION
Imidazole (1,3 diazo-2,4 cyclopentadiene) is a planner five membered ring system with 3 carbon and 2 nitrogen atom in 1 and 3 position. The simplest member of the imidazole family is imidazole itself, a compound with molecular formula C₃H₄N₂. The systemic name of
compound is 1,3 diazole, one of the annular N bear a H atom and can be regarded as pyrole type N. It is soluble in water and other solvents. It presents in two equivalent tautomeric forms i.e the hydrogen atom is located on one of the two nitrogen atoms. Imidazole is entirely soluble in water and highly polar compound as evidenced by a calculated dipole of 3.61 D. Due to presence of sextet of \( \pi \) electrons, consisting of pair of electrons from the protonated nitrogen atom and from each of the remaining four atoms of the ring the imidazole is classified as aromatic.

Imidazole is amphoteric in nature i.e it can function like both acid and a base. As an acid, the \( pK_a \) of imidazole is 14.5, making it less acidic than carboxylic acid phenols and imides but slightly more acidic than alcohols. The acidic proton is located on N-1. As a base the \( pK_a \) of the conjugate acid is approximately 7 making imidazole’s approximately sixty times more basic than pyridine the basic site is N-3.

![Structure of Imidazole](image)

Imidazole is included into many important biological molecules. The most pervading is the amino acid “histidine” which has an imidazole side chain. Many proteins and enzymes contains histidine because it plays a vital part in the structure and binding function of haemoglobin. Histamine is the decorboxylated product of histidine, which is also a common biological compound. Imidazole’s significance is in the purification of his tagged proteins in immobilised metal affinity chromatography. Imidazole’s have become an important part of many pharmaceuticals. Imidazoles were prepared in 1858 from glyoxal and ammonia. Several approaches are available for synthesis of imidazole as Radiszewski synthesis, dehydrogenation of imidazolines from alpha halo ketones, Wallach synthesis from aminonitrile and aldehyde and markwald synthesis.

**Physical Properties:** It is colorless liquid having high boiling point of 256\(^0\)C than all other 5 membered heterocyclic compound due to intermolecular H bonding where there is linear association of molecule. Imidazole shows a large value of dipole moment of 4.8 D in
dioxane. The electrophilic substitution occurs frequently in imidazole and nucleophilic substitution happens in the presence of electron withdrawing group in its nucleus. Imidazole have melting point 90°C.it is a weak base and tautomeric substance. Since position 4 and 5 are equivalent.

Its spectroscopic parameters are λ max of 207 nm. IR= 1550.1492(cm-1) T=2.30, 2.86 mass spectroscopy is studied for heterocyclic compound containing one hetero atom , in detail, not in case containing two or more heteroatom1.

In the field of five membered heterocyclic structures imidazole nucleus shows various properties. The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel therapeutic agents. Imidazole drug have broadened scope in remedying various dispositions in clinical medicines. Medicinal properties of imidazole include anticancer , b-lactamase inhibitors, carboxypeptidase inhibitor ,antiaging agent, anticoagulants , anti-inflammatory, antibacterial, antiviral, antifungal antitubercular, antidiabetic and antimalarial. This group presents in azoles antifungal which inhibit the accumulation of methylated sterols destroy the composition of lipid bilayer of membranes. Some imidazole drugs at high concentration could exert direct inhibitory action on membranes without interference with sterols and sterol esters. Imidazole and its derivatives are reported to be physiologically and pharmacologically active and find application in the treatment of several diseases2.

The significance of imidazole is high because large number of drugs in use now a days contain this moiety and several 5-nitroimidazole derivatives such as metronidazole, tinidazole, ornidazole, ronidazole, used for the treatment of critical infections caused by protozoa and anaerobic bacteria for long time. They have many other biological activities of therapeutic importance such as radiosensitizers in treatment of cancer, control of fertility and use as antitubercular agent. 5-nitroimidazole derivatives have also been tested in cell-based assays and in enzyme assays against HIV-1 recombinant reverse transcriptase.

Nitroimidazoles are synthetic antibacterial preparations. They are highly sensitive against anaerobic microorganisms and protozoal infections in humans. Nitroimidazoles class of drugs are a well-established group of antiprotozoal and antibacterial agents that have ability to inhibit the growth of anaerobic bacteria and certain anaerobic protozoa, for example Trichomonas vaginalis, Entamoeba histolytica and Giardia lamblia3.
Mechanism of action
Nitroimidazole have selective bactericidal action against the microorganisms in that enzymatic systems reduce nitro group. Active reductive forms of medications inhibit DNA replication and protein synthesis in microbial cell and inhibit their respiratory chains (cellular respiration). Nitroimidazoles are active against the majority of gram-negative and gram-positive anaerobes: bactericides (including B.fragilis), clostridium (including C.difficile), Fusobacterium spp., Eubacterium spp., Peptostreptococcus spp., P.niger,G.vaginalis. P.acnes are resistant to imidazoles.T.vaginalis, E.histolytica, G.lamblia, L.intestinalis, E.coli, Leishmania spp. are also found to be resistant to nitroimidazoles. These drugs are used in the treatment of Vaginitis, Bacterial vaginosis, Acne, Seborrheic eczema, Acne rosacea.

The nitroimidazole classifieds into different categories such as 2-nitroimidazole 4-nitroimidazole, 5-nitroimidazoles. 5-Nitroimidazole contains a nitro group at 5 position. Several derivatives of nitroimidazole contains the class of nitroimidazole antibiotics that have been used to compete anaerobic bacterial and parasitic infections. Example of some nitroimidazole is ornidazole, tinidazole, metronidazole, secnidazole, ronidazole, nimorazole, satranidazole etc.

Analytical methods for drug determination
**Ornidazole:** IUPAC NAME: α-(chloromethyl)-2-methyl-5-nitro-1H-imidazole-1-ethanol.
Structure of Ornidazole:

![Structure of Ornidazole](image)

Melting point: 77-78^0 C   pKa=2.4±0.1 Therapeutic category: Antiinfective, antiprotozoal.

**Rana Mazumdar et al:** was developed a simple precise and accurate spectrophotometric method for validation of ornidazole in its bulk and solid dosage form. Ornidazole shows maximum absorbance at 310.5 nm in ethanol as a solvent and obeys beer’s law in the concentration range of 5-25 mcg/ml. the methods was validated statistically and by recovery
studies and it was found to be accurate, precise and reproducible for determination of ornidazole in its bulk and solid dosage form. Sarode M.R et al: was developed rapid, sensitive and specific RP-HPLC method involving U. V. detection and validated for the estimation of Cefixime and Ornidazole in tablet dosage form. The mobile phase used acetonitrile: water in the ratio of 30:70 and pH adjusted to 3.4 with Orthophosphoric acid. The detection of combined dosage form was carried out at 237nm at constant flow rate of 1ml/min. The retention time of Cefixime and Ornidazole were found 3.9 min and 6.9 min respectively. Linearity was observed in 5µg/ml-30 µg/ml for both the drugs. The proposed method was successfully applied for the quantitative determination of Cefixime and Ornidazole in tablet dosage form.

Venkateswarlu et al: was developed two simple, sensitive, rapid and reproducible spectrophotometric methods for the determination of Ornidazole in pure form or in their tablets. The reduction of Ornidazole was done with the help of Zn powder and 5N HCL at room temperature in Methanol. The resulting amine was then subjected to two methods. Method A was based on the extraction product with Potassium Ferricyanide-Fe(III) reagent to form bluish green coloured chromogen exhibiting on absorption maxima at 570 nm with apparent molar absorptivity of $0.395 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ and obeyed Beer’s law in the concentration range 5-55 µg/ml. Method B was based on the oxidation followed by complex with 2,2bipyridyl-Fe(III) to form orange coloured chromogen exhibiting absorption maxima at 510nm with apparent molar absorptivity of $0.710 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ and obeyed Beer’s law in the concentration range of 5-50 µg/ml. the sandell’s sensitivity, limit of detection (LOD) and quanification(LOQ) values have been reported for both the methods. The accuracy and precision of the methods were evaluated on intra-day and inter-day basis. The realibility and the performance of the proposed are established through recovery studies. Nanda et al: was developed two accurate, precise, rapid and economical methods for the estimation of Cefixime and Ornidazole in tablet dosage form. The first method was simultaneous equation method and second was absorbance ratio method i.e principle of Q-Analysis. The methods were linear for both Cefixime and Ornidazole in the concentration range of 10-50 µg/ml. the results of analysis have been validated and statistically and by recovery studies.

Rekha et al: was developed a reverse phase high performance liquid chromatography method for simultaneous estimation of Levofloxacin Hemihydrate and Ornidazole in tablet formulation the mobile phase used for separation contains triethylamine(0.5% v/v adjusted...
pH3 using Orthophosphoric Acid) Acetonitrile, Methanol (40:30:30) at a flow rate of 0.5ml/mi. the detection was carried out at 310 nm. The stationary phase was Phenomenex Luna C18 column (5µ.150x4.6mm.I.D) retention time was 3.42 and 5.65 min for Levofloxacin Hemihydrate and Ornidazole respectively. Mean recovery obtained for Levofloxacin Hemihydrate and Ornidazole were 100.58% and 99.68% respectively. The developed method was found to be accurate, precise selective and rapid for simultaneous estimation of Levofloxacin Hemihydrate and Ornidazole in tablets. Shirkhedkar A.A. et al: was developed simple, rapid, and accurate high-performance thin-layer chromatography (HPTLC) method for the simultaneous determination of Levofloxacin Hemihydrate and Ornidazole in tablet dosage form. The method was depend on the HPTLC separation of the two drugs followed by Densitometric measurements of their spots at 298 nm. The separation was done on Merck TLC aluminium sheets of silica gel 60 F254 using nbutanol–methanol–ammonia (5:1:1.5, v/v/v) as mobile phase. The linearity is found to be in the range of 50–250 and 100–500 ng/spot for Levofloxacin Hemihydrate and Ornidazole, respectively. The method is successively applied to pharmaceutical formulation because no chromatographic interferences from the tablet excipients are found. The suitability of this HPTLC method for the quantitative determination of the compounds is proved by validation in accordance with the requirements laid down by International Conference on Harmonization (ICH) guidelines.

T. Ramangi et al: was developed two simple, sensitive, accurate, rapid spectrophotometric methods for the estimation of Ornidazole in parenteral dosage forms. Method A is based on the reaction of Ornidazole with PDAB, in presence of zinc dust and acidic environment, giving a orange colour chromogen, which shows maximum absorbance at 390 nm against reagent blank, while method B is based on the reaction with Bromophenol blue, in zinc dust and acidic environment absorbance at 430 nm. Beer’s law was obeyed in the concentration range of 25-162.5 µg/ml in method A, and 5-20 µg/ml in method B. Results of the analysis were validated statistically and by recovery studies. G. Mubeen et al: was developed two simple, precise and accurate colorimetric methods and validated for determination of Ornidazole in bulk and tablet formulation. These methods involves formation of complex diazonium salt of reduced Ornidazole with Metacresol reagent and Resorcinol reagent which shows absorption maxima (λmax) at 425 nm and 435 nm respectively. The linearity was observed in the concentration range of 5-40 µg/mL and 8-20 µg/mL for method A and method B respectively. The assay result was found to be in good agreement with label claim.
The recovery studies were carried out at three different levels. The methods were validated statistically and by recovery studies and they were found to be accurate, precise and reproducible for determination of Ornidazole in bulk and solid dosage form\textsuperscript{13}.

**Tinidazole:** IUPAC NAME: 1-[2-(ethylsulphonyl)-ethyl]-2-methyl-5-nitro-1H-imidazole.

Molecular formula: C\textsubscript{8}H\textsubscript{13}N\textsubscript{3}O\textsubscript{4}S. Molecular weight: 247.27. Elemental Analysis: C=38.86% H=5.30% N=16.99% O=25.88% S=12.97% Melting point: 127-128\textdegree C\textsuperscript{14}.

Structure of Tinidazole:

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\includegraphics[width=0.5\textwidth]{tinidazole_structure.png}
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Therapeutic category: Antiprotozoal (Trichomonas Giardia) Antibacterial, Antiamoebic.

**Prathyusha V et al:** was developed two simple, accurate, precise, reproducible and economical UV spectrophotometric methods for simultaneous estimation of Ciprofloxacin and Tinidazole in tablet dosage formulation. The first method used was simultaneous equation for which solvent used was 0.1 N NaOH. The second method used was absorbance ratio method i.e principle of Q-Analysis for which solvent used was 0.1 N HCL. Ciprofloxacin and Tinidazole shows linearity at all selected wavelength and obeys Beer’s law in the concentration range of 2-7 mcg/ml for Ciprofloxacin and 4-24 mcg/ml for Tinidazole. The intraday and interday precision results within acceptable limits. The correlation coefficient(r\textsuperscript{2}) was 0.9998 and 0.9996 for Ciprofloxacin and Tinidazole respectively\textsuperscript{15}. **Venugopal Darak et al:** was developed a simple rapid sensitive and precise spectrophometric method for simultaneous determination of Doxycycline and Tinidazole in bulk and its tablet dosage formulations. The solvent used was double distilled water. The two methods used were simultaneous equation method and absorbance ratio method. The linearity was found to be in the concentration range of 5-25 mcg/ml for both drugs. The S.D was found to be 4.11\times10\textsuperscript{-4} and 5.18\times10\textsuperscript{-4} for Doxycycline and Tinidazole respectively on their respective \textlambda max. The LOQ and LOD were 0.1343, 0.1419 and 0.0443, 0.0468 for Doxycycline and Tinidazole respectively. The accuracy and precision results were within acceptable limits\textsuperscript{16}.

**Salvi V.S et al:** was developed method simple and precise method for the estimation of Tinidazole and Ciprofloxacin in single formulation by using Differential Pulse Polarography.
The Tinidazole and Ciprofloxacin produces a cathodic wave at -0.38 V and -1.30 V respectively against Saturated Calomel Electrode in Britton Robison buffer having pH 6.5. The dynamic range for Tinidazole is 0.50 to 279.31 mcg/cm$^3$ and for ciprofloxacin its 24.39 to 245.28 mcg/cm$^3$. The quantitative determination of the both the analytes has been done by both calibration and standard addition method. L. Okunrobo et al: was developed simple, sensitive, rapid reproducible and economical method for determination of Tinidazole in its tablet dosage form. He carried out recrystallization of Tinidazole tablet before analysis. Ultraviolet absorption analysis corresponds the result obtained with non-aqueous titration. The solvent used was acetic anhydride and crystal violet indicator for non-aqueous titration. He done titrimetric and spectrophotometric determination of Tinidazole tablets.

Prahlad V. Rege et al: was developed a simple sensitive and validated HPLC method for determination of Norfloxacin and Tinidazole in its combined pharmaceutical formulation. The Norfloxacin and Tinidazole was separated by using chromatographic C-18 column with the help of mobile phase containing Water:Acetonitrile:Methanol:Triethylamine in the ratio of 700:200:100:2. The wavelength for detection was 315nm. The method found to be linear for Norfloxacin and Tinidazole in the concentration range of 8-80 mcg/ml and 12-120 mcg/ml respectively with correlation coefficient greater than 0.999 for both the analytes. The method was validated in terms of linearity, accuracy, precision, robustness, ruggedness, LOD, LOQ.

Nirav Patel B et al: was developed a simple, accurate, precise and reproducible RP-UPLC method for quantification of Ciprofloxacin and Tinidazole in tablet dosage form. The sample was analysed by reverse phase C-18 column (PUROSHERE STAR 100×2.1 mm, 2µm) as stationary phase and phosphate buffer and acetonitrile (80:20) as mobile phase and pH 3.0 was adjusted by Orthophosphoric Acid at a flow rate of 0.3 ml/min. determination was achieved of Ciprofloxacin and Tinidazole at 278.5nm and 317.5 nm respectively with PDA detector. The retention time for Ciprofloxacin HCL and Tinidazole were found to be 1.71 and 2.22 min respectively. The linearity of Ciprofloxacin HCL and Tinidazole was obtained in the concentration range of 3.125-43.75 mcg/ml and 3.75-52.5mcg/ml with mean accuracies 99.77% and 99.75% respectively. Ciprofloxacin HCL and Tinidazole API and market formulation were subjected to acid and alkali hydrolysis, oxidation, thermal and photolytic forced degradation. The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention time values.
Dhavani K et al: was developed a simple, rapid, precise and accurate RP-HPLC method and validated for simultaneous estimation of Ciprofloxacin Hydrochloride and Tinidazole in tablet formulations. HPLC Waters e2695 system equipped with Empower 2 Software with PDA Detector and INERSIL ODS C18 column (4.6x 250*5µm particle size) was operated in isocratic mode using water and methanol (60:40v/v) as mobile phase and flow rate of 0.8ml/min and eluents were monitored using UV-Visible detector at 316nm .The linearity was found in the range of 50-150 µg/ml and shows a correlation coefficient of 0.999. The retention time of Ciprofloxacin Hydrochloride and Tinidazole was noted to be 5.6 and 4.6 min, respectively. This study concluded that the proposed method was found to be accurate, reproducible, and consistent and could be effectively used for the routine analysis of these drugs in marketed formulations21.

Umadevi K et al: was developed a new, simple, precise and accurate method for the estimation of Tinidazole in bulk and pharmaceutical dosage forms. 0.1N HCl was used as the solvent system. The λ max was found to be 278nm. The method found to be linear in the range of 10-80µg/ml. The regression equation of the calibration graph and correlation coefficient were found to be y = 0.026x - 0.042 and 0.999 respectively. Validation of the method was done in order to demonstrate accuracy, precision, interday and intraday assay, robustness and ruggedness of the proposed method. The % RSD values for both intraday and interday precision were less than 1%. The recovery of the drug from the sample was ranged between 99.12% and 100.96%. Commercial tablets containing 500mg and 300mg of Tinidazole (COZIT and TINA respectively) were analyzed by the proposed method and the results were well within the claimed limits22.Khaja Pasha et al: was developed simple rapid and reproducible high performance reverse phase liquid chromatographic method for the estimation of Tinidazole in bulk drug sample and pharmaceutical dosage form. Separation done by using swakosil II. C18, 250 x 4.6mm, 5µm column with mobile phase composition of acetonitrile and phosphate buffer 3:1 (PH 5), flow rate of 1.0 ml/min and UV detection at 295nm linearity was observed over concentration range of 10-80mcg/ml. The accuracy of the proposed method was determined by recovery studies and found to be 101-103% the proposed method was validated and results conformed with ICH parameters23.

L.Singh et al: He developed three simple, rapid, selective, precise and accurate spectrophotometric methods for the determination of Tinidazole in tablet formulation. The first method was based on the direct absorbance measurements of Tinidazole in 0.5 N NaOH
with a λmax of 368.6 nm and linearity range of 20 – 150 µg/ml. The second method was based on direct measurement of absorbance at 279.2 nm for Tinidazole in 0.5 N HCL whereby the linearity range was 50-150 µg/ml. The third method was based on the differential spectra between Tinidazole solution in 0.5 N NaOH and 0.5 N HCl. The maxima recorded was 368.8 nm while the minima was 276 nm with a linearity range of 20 – 120 µg/ml. The methods were validated by determining accuracy, precision, limit of detection, limit of quantitation and performing recovery studies. The developed methods were successfully applied in the analysis of commercial samples of Tinidazole and could therefore be used in the routine analysis of Tinidazole formulations.

Saurabh Pandey et al: Two derivative spectrophotometric methods have been developed for simultaneous determination of Tinidazole and Fluconazole in pharmaceutical formulations. The first method depends on utilization of first derivative UV spectrophotometry, with zero-crossing and peak-to-base measurement at 260.57 & 264.23 nm for fluconazole and 263.86 & 318.85 nm for Tinidazole. The second method, compensation technique depends on first derivative of the ratio-spectra by measurements of the amplitudes for Tinidazole and Fluconazole. Calibration graphs were established for Tinidazole and Fluconazole in the range of 10-100 µg ml-1 and 2-20 µg ml-1, respectively. All proposed methods have been extensively validated. The results were found to be precise and free from interferences. The described methods can be readily utilized for analysis of pharmaceutical formulations. There was no significant difference between the performances of all of the proposed methods regarding the statistical values.

Sowjanya Gummadi et al: was developed two simple, simple accurate, precise, and economical UV spectroscopic methods for simultaneous determination of Ciprofloxacin and Tinidazole in tablet dosage form. Method A includes the simultaneous equation method on measurements of absorbance at two wavelength. Method B involves the absorbance ration method i.e principle of Q-Analysis where the absorbance was measured at iso-absorptive point and wavelength of drug which nearest to Isoabsorptive point. Ciprofloxacin and Tinidazole shows linearity at all selected wavelength and obey’s Beers law in the concentration range of 10-35 µg/ml and 10-80 µg/ml respectively. Recovery studies for Ciprofloxacin and Tinidazole were performed and the percentage recovery for both the drugs was obtained in the range of 99.1-99.7%(method A) and 98.0-100.4% (method B)confirming the accuracy of proposed method. Method A and B showed good reproducibility and recovery with % RSD less than 2.

Rajesh kumar et al: was developed method for
spectrophotometric estimation of Tinidazole tablets by using application of mixed solvency techniques. Sodium benzoate , Niacinamide used as hydrotropic agents, PEG 300, glycerine propylene glycol as cosolvents and PEG 6000 as a water soluble solid have been tried for solubilizing Tinidazole according to mixed solvency technique\textsuperscript{27}.

**Manaswi patil et al:** was developed new simple, rapid and novel spectrophotometric methods for simultaneous estimation of Ciprofloxacin and Tinidazole in tablet dosage form. Ciprofloxacin and Tinidazole was determined by three methods such as simultaneous estimation, absorption ratio method and first order derivative spectroscopy method. Beer’s law was obeyed in the concentration range of 2-10 µg/ml and 2.4-12 µg/ml for Ciprofloxacin and Tinidazole respectively by all the methods\textsuperscript{28}.

**Kholoud Ahmed et al:** A sensitive and precise thin layer chromatographic method has been developed and validated for simultaneous determination of Omeprazole, Tinidazole and Clarithromycin in bulk powder; laboratory prepared mixture and combined dosage form. The technique adopted for quantification is coupled TLC-densitometry. The mobile phase used was a mixture of Methylene Chloride, Isopropyl Alcohol, Acetonitrile and Ammonia (11: 1.2: 5: 0.2, v/v/v/v). The detection of spots was carried out Densitometrically using a UV detector at 300 nm in absorbance mode. This system was found to give compact spots for Omeprazole (Rf 0.45), Tinidazole (Rf 0.67) and Clarithromycin (Rf 0.89). The method was linear in the range of 1-20µg spot-1, 1-10µg spot-1, and 1-20µg spot-1 for Omeprazole, Tinidazole and Clarithromycin respectively with significantly high value of correlation coefficient (r\textsuperscript{2} > 0.99). The selectivity of the proposed method was checked using laboratory prepared mixtures. The proposed method was found to be accurate, precise, reproducible and specific and can be applicable for the simultaneous determination of Omeprazole, Tinidazole and Clarithromycin in tablet dosage form without interference from other additives\textsuperscript{29}.

**Noura H. Abou-Taleb et al:** was developed a simple and rapid difference spectroscopic method for the simultaneous determination of binary mixture of Norfloxacin (NF) and Tinidazole (TZ) without prior separation. The proposed method depends upon measuring the absorbance of NF at 291.6 nm which is the zero crossing point on the difference spectra of TZ in 0.1 N NaOH vs. 0.1 N HCl. Similarly, the absorbance of TZ was measured at 344.4 nm which is the zero crossing point on the difference spectra of NF. Beer’s law was obeyed in the concentration range of 2-20 and 5-50 µg/mL for NF and TZ, respectively. The lower limits of detection (LOD) of NF and TZ are 0.23 and 0.36 µg/mL, respectively, while the
lower limits of quantification (LOQ) of NF and TZ were 0.70 and 1.08 µg/mL, respectively. The precision of the method was satisfactory; the maximum value of relative standard deviations did not exceed 1.5% (n=10). The accuracy, expressed as recovery is between 98.25 and 101.8% with relative error of 0.29 and 0.23 for NF and TZ, respectively. The proposed method was successfully applied for the determination of both drugs in bulk powder, laboratory prepared mixture and commercial dosage forms such as tablets without interference from the commonly encountered excipients and additives. The results obtained are in good agreement with those obtained by the reference methods.

Monal S. Salvi et al: was develop and validate novel, accurate, sensitive, precise, rapid and isocratic Reverse Phase HPLC (RP-HPLC) method for the simultaneous determination of Doxycycline hyclate (DOX) and Tinidazole (TIZ) in bulk and combined tablet dosage form. The separation was achieved on Zorbax C8 column (250mm × 4.6mm, 5µm) with mobile phase consisting of 20mM Potassium Dihydrogen Ortho Phosphate (pH 6, adjusted with Triethylamine): Acetonitrile (60:40 % v/v) at a flow rate of 1 ml/min. UV detection at 293nm. DOX and TIZ obeyed linearity in the concentration range of 10-50 µg/ml (r² = 0.9993) and 10-50 µg/ml (r² = 0.9987) respectively. The asymmetric factors were found to be 1.12 for DOX and 0.97 for TIZ. The developed method was validated as per ICH guidelines. It was concluded that the method can be used for routine analysis of DOX and TIZ in combined formulations.

Suad Muslih Al-Deen et al: was developed UV-spectrophotometric method for the effect of superficial X-rays on the Tinidazole compound in two forms, solid and aqueous solution. Both acceleration potentials of 120 kVp and 160 kVp exert a pronounced effect on aqueous solution of Tinidazole at different concentrations represented by changes in optic density of Tinidazole. The effect of superficial X-rays on the solid form of Tinidazole is less than that observed with the aqueous preparation. It concluded from these that Tinidazole is radiosensitive compound and several protection measurements should be used to prevent its hydrolysis.
HPLC Method development of Ornidazole and Tinidazole

<table>
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<tr>
<th>Drugs</th>
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<th>Column</th>
<th>Mobile phase</th>
<th>Flow rate</th>
<th>Detection</th>
<th>Ref</th>
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<tr>
<td>Cefixime and ornidazole</td>
<td>Tablet</td>
<td>Kromasil 100 C8 (150×0.46mm id)</td>
<td>Water:Acetonitrile (75:25 % v/v)</td>
<td>1.0 ml/min</td>
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<td>310 nm</td>
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<td>Levofloxacin and Ornidazole</td>
<td>Tablet</td>
<td>Hypersil ODS C18 (150mm×4.6 mm id×5µm)</td>
<td>Buffer: Acetonitrile (75:25%)</td>
<td>1.0 ml/min</td>
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<td>Ofloxacin and Ornidazole</td>
<td>Formulation</td>
<td>Inertsil C-18 (150mm×4.6 mm id×5µm)</td>
<td>Water:Acetonitrile: Triethylamine</td>
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<td>230 nm</td>
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<td>Norfloxacin and Ornidazole</td>
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<td>Prontosil AQ ODS (250mm×4.6 mm id×5µm)</td>
<td>NaH₂PO₄:ACN:MeOH(15:70:15 % v/v)</td>
<td>1.0 ml/min</td>
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<td>Phenomenex C18 (250mm×4.6 mm id×5µm)</td>
<td>2mM Phosphate buffer : Acetonitrile (70: 30 % v/v)</td>
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<td>293 nm</td>
<td>B. Dhandapani³⁸</td>
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<td>Combin ed Dosage Formulation</td>
<td>Xterra RP 18 (250mm×4.6 mm id×5µm)</td>
<td>Acetonitrile: Mixed Phosphate buffer (40:60 %v/v)</td>
<td>1.0 ml/min</td>
<td>294 nm for OFL and 305 nm for ORN</td>
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<td>25mM KH₂PO₄ buffer : Acetonitrile (40: 60 % v/v)</td>
<td>1.0 ml/min</td>
<td>238 nm</td>
<td>V. Bojara Ju⁴⁰</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin and Tinidazole</td>
<td>Tablet</td>
<td>Ambroxol Kromasil C8 (15 cm×4.6mm id 5µm)</td>
<td>Triethylamine buffer: Acetonitrile (73: 27 % v/v)</td>
<td>1.2 ml/min</td>
<td>303 nm</td>
<td>J. Dharumanj⁴¹</td>
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<tr>
<td>Ofloxacin and Tinidazole</td>
<td>Tablet</td>
<td>Ambroxol Phenomenex C18 (250mm×4.6</td>
<td>2mM Phosphate buffer :</td>
<td>1.0 ml/min</td>
<td>303 nm</td>
<td>M Rama Kotaiah⁴²</td>
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<tr>
<td>Compound</td>
<td>Sample matrix</td>
<td>Solvent</td>
<td>Detection $\lambda_{\text{max}}$</td>
<td>Method</td>
<td>Name of Author</td>
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<td>Fluconazole and Tinidazole</td>
<td>Tablet</td>
<td>Kromasil Stainless steel C18 (250x4.6mm i.d)</td>
<td>Acetonitrile: Water (55:45 % v/v)</td>
<td>1.0 ml/min 260 nm</td>
<td>Chiranjee eviBode pudi&lt;sup&gt;43&lt;/sup&gt;</td>
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<tr>
<td>Fluconazole and Tinidazole</td>
<td>Tablet</td>
<td>Hypersil ODS C18 (150mmx4.6 mm i.d x 5µm)</td>
<td>Acetonitrile: KH&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt; Buffer: Acetonitrile 82:18 % v/v</td>
<td>1.5 ml/min 210 nm</td>
<td>D.B. Meshram&lt;sup&gt;44&lt;/sup&gt;</td>
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<tr>
<td>Tinidazole and Ciprofloxacin</td>
<td>Bulk</td>
<td>AligantZorba Rx-C18 (150mmx4.6 mm i.d x 5µm)</td>
<td>Orthophosphoric acid: methanol (70: 30 % v/v)</td>
<td>1.5 ml/min 225 nm</td>
<td>Rahul Reddy. C&lt;sup&gt;45&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>Ofloxacin and Ornidazole</td>
<td>Infusion</td>
<td>HiQSil C18 (150 mm x 4.6 mm i.d, 5 µm)</td>
<td>0.01M ortho - phosphoric acid and 0.01M sodium phosphate monobasic dihydrate 60:40 % v/v.</td>
<td>1 ml / min 300 nm</td>
<td>Pankaj B.Miniy&lt;sup&gt;46&lt;/sup&gt;</td>
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<tr>
<td>Cefixime and Ornidazole</td>
<td>Tablet</td>
<td>Hypersil ODS C-18 (150mmx4.6 mm)</td>
<td>Triethyl amine buffer: Acetonitrile 75:25 % v/v</td>
<td>1 ml / min 295 nm</td>
<td>Vasanth P. M.&lt;sup&gt;47&lt;/sup&gt;</td>
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UV Method development of Ornidazole and Tinidazole

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sample matrix</th>
<th>Solvent</th>
<th>Detection $\lambda_{\text{max}}$</th>
<th>Method</th>
<th>Name of Author</th>
</tr>
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<tbody>
<tr>
<td>Ofloxacin and Ornidazole</td>
<td>Liquid oral dosage form</td>
<td>Methanol</td>
<td>310.8 nm</td>
<td>Simultaneous equation method</td>
<td>V.M Gandhi&lt;sup&gt;48&lt;/sup&gt;</td>
</tr>
<tr>
<td>Norfloxacin and Tinidazole</td>
<td>Combined Dosage Form</td>
<td>Acetate buffer</td>
<td>313-323 nm 318 nm</td>
<td>Area Under Curve Method</td>
<td>A.J. Shinde&lt;sup&gt;49&lt;/sup&gt;</td>
</tr>
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<td>Ornidazole and Ciprofloxacin</td>
<td>Tablet dosage form</td>
<td>Distilled water</td>
<td>302.5 and 335 nm 332 nm</td>
<td>Dual wavelength method</td>
<td>K.S. Natraj&lt;sup&gt;50&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
CONCLUSION

Imidazole have been most frequently studied. Pharmacological properties of imidazole include anticancer ,b-lactamase inhibitors, carboxypeptidase inhibitor ,antiaging agent, anticoagulants , anti-inflammatory, antibacterial, antiviral, antifungal antitubercular, antidiabetic and antimalarial. This group presents in azoles antifungal which inhibit the accumulation of methylated sterols destroy the composition of lipid bilayer of membranes. Thus can say imidazole is a moiety which had been exploited in the past years for synthesizing various compounds having diverse pharmacological activities, and still it can be further utilized for future prospective against various pathological conditions and other uses.

ACKNOWLEDGEMENT

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