Analytical techniques for Posaconazole, an effective treatment option for mucormycosis: A Comprehensive Review

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ABSTRACT

A broad–spectrum, second-generation triazole antifungal drug called Posaconazole is efficient towards a greater variety of mostly recognized causatives like Aspergillus, Candida, Cryptococcus neoformans, Fusarium species, and Zygomycetes species, for preventive therapy of mucormycosis in today's trends. A comparative study reveals that Posaconazole safety and efficiency in comparison with Itraconazole is higher using nine pharmacokinetic models. Posaconazole, a basic, poorly soluble, high molecular weight drug, is cyp450 dependent. It acts by inhibition of lanosterol 14-demethylase responsible for ergosterol synthesis hung on membranes of cells to prevent multiplicity and achieve its antifungal effect. A cumulative review of Posaconazole as of literature reports, analyzed by HPLC equipped with Fluorometric, DAD/ UV/ PDA, MS/MS. HPTLC, UHPLC - UV, UPLC - UV MS/MS. Stability analysis of forced degradation by P – HPLC, IR, NMR, LC-MS (ESI – QTOP). Bioanalytical samples were studied by RP – HPLC,and impurities upon degradation were also analyzed.

Keywords: Posaconazole, analytical methods, degradation products, impurities, Black fungus, Mucormycosis**,** stability methods, bioanalytical methods.

INTRODUCTION

Posaconazole is a triazole antifungal drug derivative it was permitted for usage by the United States Food and Drug Administration in 2006. chemically 4-f4- [4– (4-f [(3R,5 R)-5–(2,4-difluorophenyl)-5-(1H-1,2,4-triazol-1-ylmethyl)-tetrahydrofuran-3yl]methoxyphenyl) piperazin-1-yl] phenyl-2-[(1S,2S)-1-ethyl-2-hydroxy-propyl]-2,4-dihydro-3H-1,2,4-triazol-3-one, to treat invasive Aspergillus and Candida infections, Posaconazole is utilized.OPC,which is unaffected by itraconazole and/or fluconazole therapy, is another condition that it is used to treat. Additionally, in the treatment of invasive Candida, Mucor, andAspergillus species infections in individuals with severe immunosuppression.

A group of molds called mucoromycetes is the source of the deadly but uncommon fungal infection known as mucormycosis (formerly known as zygomycosis).Mucormycosis – a potentially fatal mucormycotic infection that usually affects underlying immune system-impairing comorbidities[1]

While immunosuppressive patients (tumors in the bloodstream and organ or fluid receivers as a transplant) usually present with respiratory involvement and widespread infection, rhino-orbital-cerebral involvement is utmost commonly found in those with ailing managed diabetes mellitus.After breathing in fungal spores from the air, it most frequently affects the sinuses or the lungs. It can also develop on the QWCX 0PPOEB1HGskin following a burn, cut, or other skin damage.**Figure *1***, **Figure *2***.

Therapies

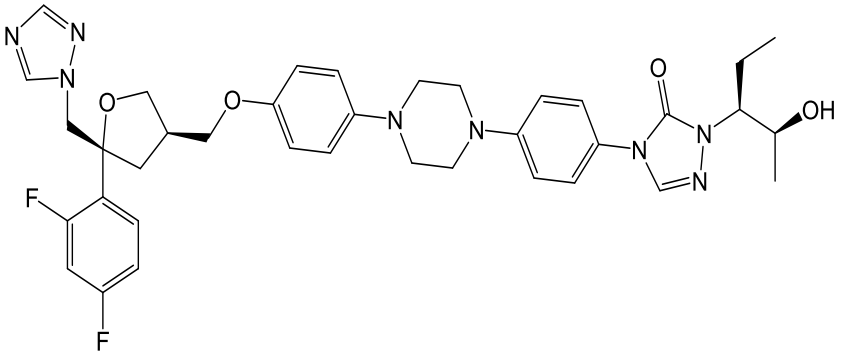
Posaconazole and Isavuconazole have recently been added to our arsenal against mucormycosis, along with amphotericin B products, but there are still many unknowns regarding the supervision of this unusualresourceful infection because there is data unavailability from forthcoming randomized scientific trials to direct respective treatment. We outline the state of therapy alternatives in this brief review. Due to the disease's heterogeneity (various affected hosts, point of infection, and infecting Mucorales), mucormycosis organized calls for an individual control strategy that considers the host's overall compromise in immunology as well as comorbidities, diagnosis certainty, the location of the infection, and the pharmacological characteristics of antifungal medications[2–4]

Effect

Posaconazole was shown to be equally efficient as fluconazole for treating all aggressivemycological infections (ratio; 5.3% and 9.0%, respectively; odds ratio, 0.56; 95 percent confidence interval [CI], 0.30 to 1.07; P = 0.07) and higher to fluconazole in avoiding proved or suspected invasive aspergillosis (2.3% vs. 7.0%; odds ratio, 0.31; 95% CI, 0.13 to 0.75; P = During the working period, the Posaconazole group had fewer breakthrough invasive fungal infections (2.4% vs. 7.6%, P = 0.004), notably invasive aspergillosis (1.0% vs. 5.9%, P = 0.001). When the two groups' overall mortality rates were compared, the number of fatalities from aggressive mycological infections in the Posaconazole group was lower (1% vs. 4% in the fluconazole group; P = 0.046). The frequency of therapy-associated ADEswas comparable in the two groups (36% in the Posaconazole group and 38% in the fluconazole group), with 13% and 10%, respectively, oftherapy-associatedmajor ADEs[5,6]

This review is a brief description of the Posaconazole determination in terms of its physical properties like molecular weight, solubility, crystallinity, and flow properties. Posaconazole analysis by the well-established methods of chromatographic techniques,
with different combinations of detectors. Also, impurity profiling studies are taken into consideration upon degradation of the drug by photolysis, oxidation, elevated temperatures, and acidity followed by alkalinity to identify novel potential degradants. Estimation of two unknown degradants (N – oxide/ N - dioxide) was found by oxidative degradation with meta – Chloroperoxybenzoic acid in delayed-release tablets by Preparative HPLC.A cumulative bioanalytical analysis was reviewed along with degradation pathways.

Structure of Posaconazole drug



Posaconazole is structural analogue of itraconazole

IUPAC name:	4-[4-[4-[4-[[(3*R*,5*R*)-5-(2,4-difluorophenyl)-5-(1,2,4-triazol-1-ylmethyl)oxolan-3-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-[(2*S*,3*S*)-2-hydroxypentan-3-yl]-1,2,4-triazol-3-one.
CAS no	171228 – 49 - 2
Molecular Formula	C37H42F2N8O4
Synonym	Noxafil

PHYSICAL PROPERTIES OF THE POSACONAZOLE DRUG

Physical properties of Posaconazole include solubility, pH, pKa, melting point, Log P, crystallinity, dissolution, and flow properties.Posaconazole and its adduct were discovered to have pKa values of 4.6 and 0.19, respectively.Thermal analysis was captured on a DSC (Shimadzu DSC-60, Tokyo, Japan). Beforethe usage of the cell was heated, DSC was calibrated using indium as standard. The enthalpy rate was 10 ºC per min by N_2supply. With a reference aluminum pan that is empty, a 5–10 mg powder sample was placed in an aluminum pan, heated to a temperature between 0 and 300 ºC, and then evaluated at 168.4°C, Posaconazole showed an endothermic peak.The capacity of solubilization and degree of dissolution arecrucialconstraints for a drug's pharmacological efficacy to attain the desired plasma drug concentration in the bloodstream for a characteristic therapeutic effect.It is categorized as weakly dibasic by the Biopharmaceutical Classification System (BCS) under class II i.e., little solubility and greater permeability.Posaconazole has a low aqueous solubility of 1 g/mL, a greater lipid solubility of Log P 4.6, and a greater molecular mass of 700.8 g/mol[7]

To increase the solubility of Posaconazole one of the crystal engineering techniques of co-crystallization is performed, to create co-crystals of Posaconazole with 4-aminobenzoic acid (4AMB), Supercritical C_{O2} was used as an antisolvent in GAS with ACN and as a solvent inCo-crystallization with supercritical solvent[37].The crystalline nature of Posaconazole and co-crystals was evaluated using thePosaconazoleX-ray diffraction pattern and its co-crystals which are analyzed using

an x-ray diffractometer, Shimadzu module XRD 7000 whose x-ray tube velocity isoperated at 40 kV, 2θ=10-80 ºC, scan speed=2 deg/min) using Cu kα radiations.The co-crystal powder XRD pattern revealed intense peaks at 17.67^{o}, 19.37 ^{o}, and 9.77 ^{o}, indicating a shift in 2θ values[7]

Flow properties are validated by the fixed funnel method and Carr's index was used to compute the following equations, used to estimate Hausner's ratio from the bulk density and tapped density.Formulations available in the market are oral unit dosage forms, oral suspensions, intravenous injections, and novel drug delivery systems of delayed-release tablet form but the oral dosage forms and intravenous injection formulations only show increased plasma drug concentration than oral suspensions and delayed-release tablets as per the simulated PK profile.[8]**Table 1**, **Table 2**,

Figure*3*

PHARMACOLOGY OF POSACONAZOLE DRUG

Posaconazole isa lipophilic triazole class of drugs, which is widely used in individuals due tocritical leukemia experiencing chemotherapy and allogenic transplantation of stem cells in an individual. It is cytochrome P450 (cyp450) isoenzyme substrates and inhibitors, as well as membrane transporter inhibitors such as p-glycoprotein (P-gP). Posaconazole inhibits the enzyme-dependent lanosterol which is metabolized by 14-alpha-demethylase, resulting in damage to the cell membrane that leads to inhibition of cellular growth/ replicationfollowed bythe fatality of the cell. Posaconazole also inhibitsthe enzyme class of cyp450 in the efficientrespiring chain. It is active against all common Aspergillus species invitro and invivo[8]

PHARMACOKINETICS OF POSCONAZOLE DRUG

Posaconazole activity is evaluated in alive replicas as well as in the safety phase, mono to multidose studies, principally in hale and hearty individuals. The pharmacokinetic profile is distinguished by a considerable impact of edible items on the process of absorption, greater estimation of $t^{1/2}$, huge apparent volume of distribution, upon intake by oral route, and very modest breakdown by cytochrome P450 (CYP)[10]. **Table *3***

CYP= cytochrome P450; t½ = elimination half-life.

ANALYTICAL METHODS- DRUG ANALYSIS

HPLC analysis

According to the literature records, HPLC is majorly used in methodsfor the determination of the concentration. HPLC with UV detector, HPLC with fluorescence detector, and HPLC MS/MSare the major methodology used in the determination of concentration in human plasma with a single or a mixture of antifungal drugs.

In addition to these methods, capillary electrophoresis, HPTLC, and sweeping-micellar electrokinetic chromatography are also used. But spectrometry and fluorometric analysis of the Posaconazole was found to be the least, only one report can be identified by literature. The Main intention of this study is to introduce three different methods for Posaconazole concentration analysis in bulk powder and suspension formulation. HPTLC, spectrofluorimetric, and electrochemical analysis are three methods developed.

During the development, several types of reverse phase columns are used in method analysis best sharper peaks are considered using zorbax C18 column for HPLC and kinetic C18 column and suitable mobile phase i.e., ACN: 15 mM potassium dihydrogen n orthophosphate (30:70 to 80:20) has linearity around 7 minutes, and ACN:15 mM potassium dihydrogen orthophosphate (45:55) respectively. TLC plates are used to separate Posaconazole good results are reported with the choice of acetone and chloroform as mobile phase compared to other combinations of mobile phase like chloroform and methanol or acetone or methanol.

HPLC is most widely employedfor the investigation of Posaconazole in the organic template to ensure the safety and efficacy of the Posaconazole by therapeutic drug monitoring there are many kinds of literature available on HPLC using various detectors from basic UV detectors to sophisticated MS/MS detectors. Columns like C18 and C8 with various types are used for the quantification and the flow rate is used at about 0.8 to 1.0 ml per min in the estimation of the chemical entity is carried out with different types of the mobile phase.**Table *4***

Spectral analysis

A variety of solvents can be selected for spectrofluorometric analysis based on their reproducibility and sensitivity towards blank for estimation of Posaconazole 0.1 m of sulfuric acid as shown better and sharp sensitivity compared to that of solvents like ethanol and isopropyl alcohol. **Error! Reference source not found.**

Differential pulse voltammetry

Using differential-pulse frequency at the hanging mercury drop electrode (HMDE) produced a very well cathodic wave for Posaconazole in Britton-Robinson bufferpH 6.5.). pulse used 0.12V, scan rate is at 25mV/s versus Ag/AgCl reference electrode[13].

STABILITY METHODS

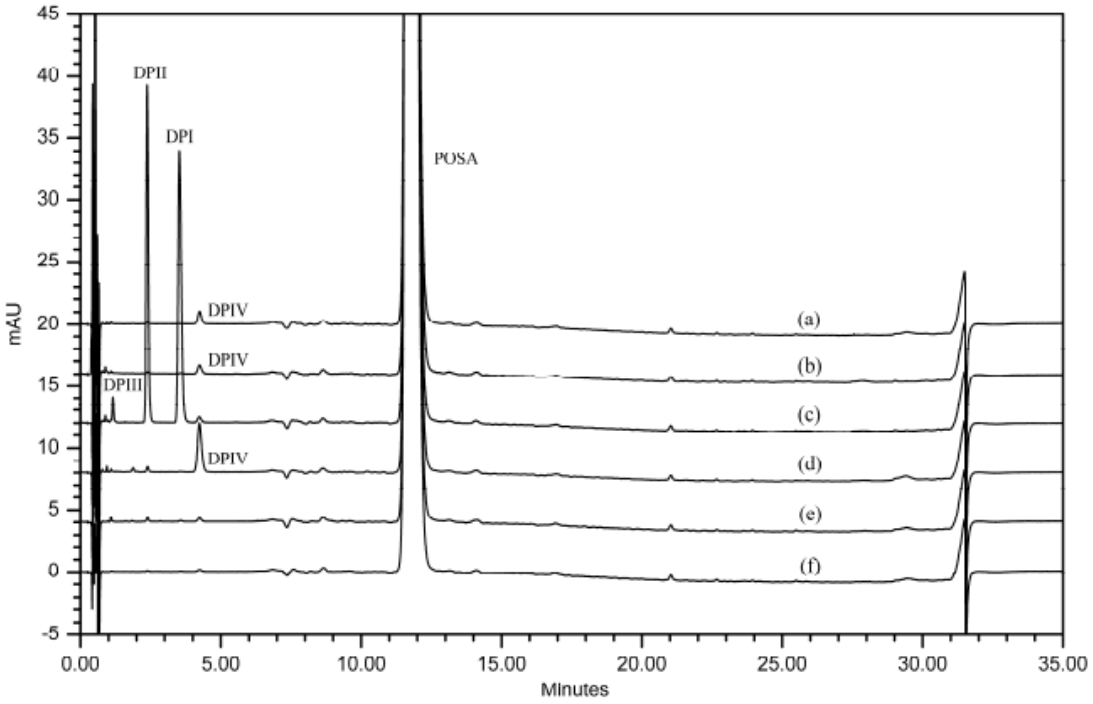
Preparative High-Performance Liquid Chromatography (p – HPLC)

Posaconazole is subjected to stress to undergo degradation in the presence of H_2O_2(oxidation), UV energy (Photolysis), heat 85^oC (thermal), and altered pH-like acidity/alkalinity respectively to determine the intrinsic stability upon forced degradation as per ICH Q2(R1). It forms a few unidentified novel potential degradants, three DPI, DP II, DP III (N – oxide derivatives of piperazine:H_2O_2) under oxidative stress, and DP IV at elevated temperature, isolated by new stability–indicating preparative HPLC method followed by identification, characterization, and quantification with LC – TOF/MS,LC-MS/MS,^{1}H NMR, ^{13}C NMR, and 2D NMR. Effect of low pH at 1 molar/5 N hydrochloric acid– effect of high pH at 1 molar/ 5 N sodium hydroxide, the effect of air at 3% (w/w) H_2O_2, the effect of lighton tablet powder in anentireradiance of NLT 1.2 million lux in time that isunified near UV energy of NLT 200watt supply per hour/ meter square. HPLC analysis by using solvents like water-acetonitrile (organic modifier) – phosphoric acid (80:20:1) v/v/v, rate of flow 1.5ml per min at 260nm, injection volume 10µl of 1mg/ml concentration, finally detected by PDA results were recorded.[19] **Figure 4**.

Figure 5, **Figure 6**,

Table *5*

HPLC overlay chromatogram of sample degradation under acidic (a), and alkaline (b). oxidative (c), Thermal (d), Photolytic (e), and finally Posaconazole (f).



Also, in another approach of PreparativeHPLC, two unknown degradants (N – oxide/ N - dioxide) were found by oxidative degradation with meta – Chloroperoxybenzoic acid in delayed-release tablets. In addition to, quantification by IR too. There is a specific method for the preparation of standards for checking impurities/ degradants that are formed upon degradation. 4 PPM concentration of Posaconazole, the diluent used is water: acetonitrile (40:60) v/v. as per equivalent weight Posaconazole is weighed, dissolved, sonicated, and made up to mark[19].

As a developmental research study, the finite HPLC chromatography was performed by mass balance study.in the initial degradation process, the % area of Posaconazole and DP is found to be 85.4% and 13.5% respectively at which the mass balance is 98.9%. By the end of the forced oxidative kinetic study, the % area of Posaconazole and DP are found to be 61% and 26.6% respectively of mass balance is 87.6%. Whereas uncounted degradation products in the absence of any chromophore groups are 12.4% that are unidentified by this method[21].**Figure*7***

Infrared ray spectroscopy

The delayed-release tablets of Posaconazole are finely ground and, made into a fine transparent pellet by a properly pressed pellet technique, placed into the module, and spectra are passed through that into a small amount of sample. Removal of background spectral noise by using KBr pellet for calibration of the instrument, then around 8 scans are executed in anarray of 4000 1/cmand 450 1/cm[21].

Figure *8*, **Figure *9***.

A total of 8 scans data for the IR spectrum of degradation products (N – mono oxide and dioxide) formed in oxidative degradation as per functional groups under investigation of delayed-release tablets[21].

Spectra of NMR

^{1}H NMR, and ^{13}C NMR of mono-dimensionand Bi-dimensional spectroscopic techniques by using this structural elucidation a detailed description is given in previous papers. It is a bit tuff to determine the structures of DP chemically with only Proton (^{1}H) NMR, so Carbon (^{13}C) NMR of 1D and 2D, DEPT, $H^{1} – H^{1}$COSY methods[21].

Figure 10,

Figure *11*.

Another method Proton (^{1}H) NMR of degradation products (N – mono oxide and dioxide) are determined and the results are found as per the study conducted in previous papers. The results were recorded as per the procedure followed for conducting that study.**Table *6***

Mass Spectroscopy - LC-MS (ESI- QTOP) analysis *- Electron spray ionization source, Quadrupole mass analyzer. Separation of DP's and Posaconazole by SB –column of – C_{18}, with amovable phase of incline elution in the ratio of 40:60 and acetonitrile: methanol (1:1 v/v) – buffer is 10 mM ammonium formate (3.5 pH), flow rate 1ml/min. The flow rate of MS is 0.2ml/min, at 260nm wavelength. Drying / Nebulization with purified Nitrogen at (300 ^oC, 35 psi).A study by LC-MS/MS – sciexwith electron source ionization at a voltage of 5500V* ***Error! Reference source not found.****, operated at 550 ^oC results are retrieved by AB Sciex LC-MS (ESI- QTOP)analyst software (V: 1.5.2) for control of the system and document acquirements, data display - protonated DPI and DP II ions at m/z 717, 700, 685, 530, 426, 425, 413, 412, 399, 302, 288, 278,* ***275, 209, 127****. These last three are the characteristic product ions of Posaconazole*[19].**Figure 12**,

Figure *13*, **Figure *14***.

(ESI- QTOP) MS study is conducted of LC- DAD, ESI source (1.2% H_2O_2, 25^{o}C, 24hrs) to identify, and quantify its structural isomers along with epimers of oxidative degradation kinetics along with its degradant products. The protonated Posaconazole [M + H] $^+$at m/z 701.33 and DP I at m/z 717.33 (base peak anextremepower peak) with a molar burden of 700.8 g/mol. Whereas the low-power peak formed at anm/z

value of 733.32. The DP that is not identified by the HPLC isdetected by sophisticated mass spectroscopy. At m/z 723.31 a sodium adduct is detected as the ionization of the molecule happened by sodium ions[23].**Figure *15***

BIOANALYTICAL METHODS

A simple, rapid, simultaneous technique was established by reverse-phase high-performance liquid chromatography to quantify Posaconazole along with itraconazole and voriconazole by taking 2693 serum samples free from the drug initially, triazoles are spiked into separated serum samples of different triazole absorptive readings [0.612, 1.25, 2.5. 5 and 10mg/L]. The reason for introducing this validated method is that LC tandem mass spectroscopy is very accurate and highly specific. It has limitations like - the most expensive, and less available to all diagnostic laboratories. The solvents used are HPLC-grade water, methanol, acetonitrile, and dimethyl sulfoxide, degasification is done with ACN: H_2O (70: 30 v/v), stock solution (1600 mg/L) and sample solution (0.25/ 0.5 to 16 mg/L), isocratic mode of elution,Flow rate 1.0 ml/min, 20 µl is the volume of injection, within a time of the run is given for 12 min. Calibration standards are prepared on Agilent 1290 infinity system equipped above-mentioned solvent system, quadratic pump, C_{18}column at 25^{o}C, rate of flow 1.0 ml/min, and sensor at 255 nm and 262 nm. Then retention time of Posaconazole is found to be 4. 287 $\pm$ 0.1 min, itraconazole of 8.846 $\pm$ 0.1 min along with voriconazole of 3.297 $\pm$ 0.1 min. This method is found to be easy, rapid, has low turnaround time, is less expensive, has low solvent intake, and possible sample recovery to determine antifungal triazoles in a community-related study approach[24].**Figure *16***

Dispersive liquid-liquid microextraction is another novel technique to analyze antifungal triazole (Posaconazole) in human urine samples. In addition with surface-assisted laser desorption ionization/mass spectroscopy (SALDI/MS). The prevailingprocedures for extraction are Solid phase extraction (SPE), and LLE (liquid-liquid extraction). In recent times DLLME (Dispersive liquid-liquid microextraction) had a greater application for extraction from aqueous solutions. SALDI/MS is a mode of high throughput screening (modest, swift, sensitive,and even productive) technique for the quantification of drugs as complex samples. In the optimization of DLLME, many solvents were used with increasing polarity. When chloroform is used then there is a decrease in signal intensities with an increase in its concentration. Mass spectra are produced using SALDI (Colloidal Au) which has 4.0 and 2.2 times greater signal-to-noise ratios in comparison with SALDI (Colloidal Ag/Pd)[25]

This study draws a comparative conclusion on the interaction of posaconazole and dasatinib upon developing a bioanalytical study. A U – HPLC method of gradient elution, with a solvent system of Formic acid: ACN(90:10) v/v, Standards are prepared in concentrations of 5, 50, 100, 500, 1000, and, 5000 ng/ml, whereas samples are supernatant aliquot of 100µl after centrifugation (3500 rpm) of 4^{o}C for 10 min, collected in the Eppendorf tubes and stored in – 80^{o}C, along with 0.3 ml/minrate of flow,1.0 µlvolume of sample injection, Temperature in the autosampler is 10^{o}C, and a probable column temperature is 40 ^{o}C, developed by taking serum samples from rats (Adult male Sprague Dawley) of 267$\pm$ 18g, along with IAEC approval, Pharmacokinetic study is performed by taking 8 rats/ group made 12 hrs to have fasted later drugs are dissolved in sodium CMC to administer orally, followed by sample collection from the tail vein from 0 to 72 hrs after administration. Along with

it a bioanalytical UPLC – MS/MS (ESI / Triple quadrupole analyzer) is equipped to quantify drug interactions in levels of plasma in comparison with their pharmacokinetic profiles. Results of posaconazole and dasatinib were found to be m/z 701.3 to 683.4 and 488.2 to 401.1 respectively. These are interpreted by DAS software with Non-compartmental MWU – test, 8.0 version of GraphPad prism / SPSS software 23.0 version for analysis the found P value < 0.05 (statistically significant)[26].Posaconazole injections of single doses (100, 200, and 300 mg) tested for safety, and tolerability in 36 healthy adults. The results were found to be an increase in dose and a gradual increase in the AUC but decreased distribution and clearance rate. It is tolerated at all doses. Whereas the plasma concentrations of the sample are determined by (Liquid chromatography- Mass spectroscopy/ Mass spectroscopy) method[27].Synchronized estimation of Posacanazole, itraconazole, voriconazole, and hydroxy–itraconazole. A simple, robust LCMS/MS method is developed[28].

DEGRADATION IMPURITIES ANALYSIS IN DRUG

The necessity of degradation studies is to identify the degradant impurities present In the Posaconazole which in case help in therapeutic drug monitoring Posaconazole is made to undergo various forced degradation condition (acidic, oxidation, basic, temperature, hydrolysis). Various methodologies like HPLC, NMR, FTIR, and MS/MS are used in degradation analysis. Majorly HPLC is in use for degradation impurity analysis due to its high sensitivity, selectivity, and reproducibility.[23]

According to the literature the degradation impurities identification is done through a forced degradation pattern, most commonly acidic, basic oxidative thermal, and photolytic degradations are seen. Posaconazole was degraded by oxidative degradation and the drug is estimated by using HPLC-MS by the usage of mobile phase like MeOH:water (75:25)in isocratic elution mode protonated Posaconazole [M+H]+ at m/z 701.33 was identified. Another study uses HPLC by equipping a PDA detector for degradation impurity analysis where they used API and subjected to degradation by acid, base hydrolysis, thermal and photolytic stress, as stated by ICH guidelines and compared with the criterion using water and methanol through gradient elution technique by setting time (min)/% solution B: 0.01/55, 15/70, 20/70, 30/90,35/90, 37/55 and 45/55 and calculated LOD, LOQ, robustness, linearity accuracy. The impurities which are seen were reported first time in the raw material in this study according to literature, where they used Saccharomyces cerevisiae ATCC MYA 1942 microorganism HPLC-DAD (diode array detector) with the isocratic method utilized. First-order kinetics is used for photolytic degradation of product They established a linear, precise, accurate, and robust validated method. Preparative HPLC is made used for impurity determinationforced degradation studies are conducted according to ICH guidelines. The use of water and acetone as mobile phases leads to poor peaks shape, it has been overcome by the addition of tetrahydrofuran which gives better peak shapes.

Table *7*

OTHER METHODS

Extraction of antifungal agents from Mentha piperita and Mentha spicata by hydro-distillation – obtained components are Menthol, Menthone, Iso menthone, carvone, limonene. Method of Disc diffusion andMethod of microdilution broth is performed to check anti-fungal activity, as per the results obtained with a greater zone of inhibition against Fusarium solani (19 – 20 mm: MIC values 120.3 and 115.4 µg/ ml) and aspergillus flavus (28 – 30 mm: MIC values 65.4 and 50.6 µg/ ml) against other strains. These extracted oils were estimated by Gas chromatographyaccomplished by flame ionization detector and Gas chromatography in consort withMass spectroscopy[14]. The novel analytical technique with the help of electro membrane extraction (EME) via MALDI analyzer and results are quantified with mass - spectroscopy. The motto aims to find trace amounts of analytical components in a complex matrixed sample mainly of peptides. Being advantageous, it has spread its wings. EME is mostly coupled with LC and CE since both the acceptor and donor of aqueous, also can be equipped with GC by SPME (solid phase liquid extraction) and LLME (liquid-liquid microextraction). Also, pH and elemental prospective analysis are done by Voltammetry and AAS (Atomic absorption spectroscopy) along with EME where α – cyano- 4- hydroxycinnamic acid plays a key role here[33]

Figure *17*

CONCLUSION

Qualitative and quantitative analytical techniques were compiled into a mini–note, for an antifungal drug like Posaconazole. A comparative study is drawn at a point with itraconazole also various formulations of Posaconazole were compared qualitatively. As per the finding, the triazole broad-spectrum of the second-generation antifungal drugclinched its existence in prescription gives an effective therapy in mucormycosis.

TABLES AND FIGURES

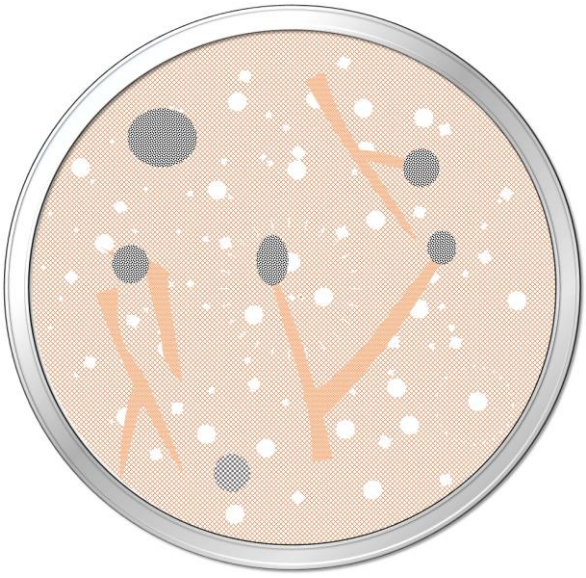


Figure 1: Classical representation of depicted mucormycosis (black fungus) with hyphae of host cell tissue in COVID-19.

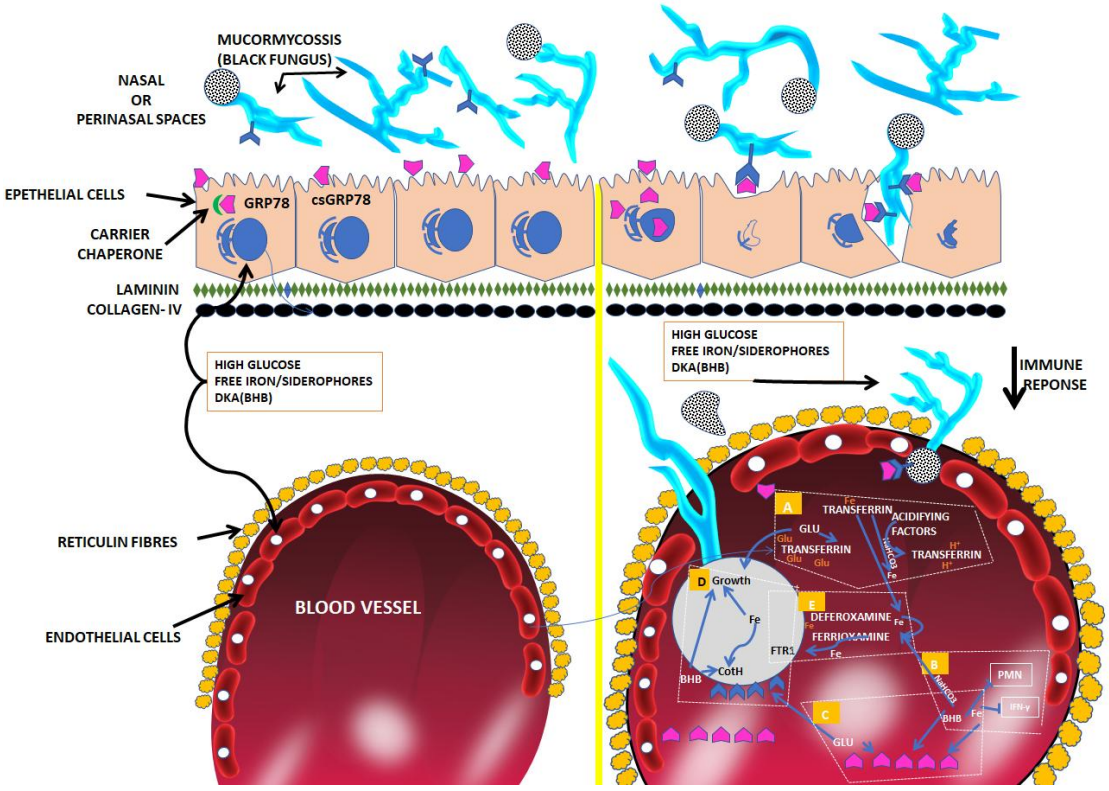


Figure 2

Left: Spores of black fungus drive into the epithelial cells of nasal space by angioinvasion thereby inducing a decline in oxygen and nourishment, that leads to cell death; also drive into the host cell to destruct mainly epithelial cells of the respiratory tract by cooperating with carriers' chaperones (GRP78, csGRP78).

Right: Furthermore, immune-compromised patients with elevated blood glucose levels were having a greater occurrence of mucormycosis attacks because of loss of attachment between Fe^{+2} carriers and transferrin.

to the impairment of the binding of siderophores (iron carriers) to transferrin (Deferoxamine and ferrioxomine imbalance) due to high glucose, immune response failure, and diabetic ketoacidosis.

Figure3: Endothermic peak by DSC.

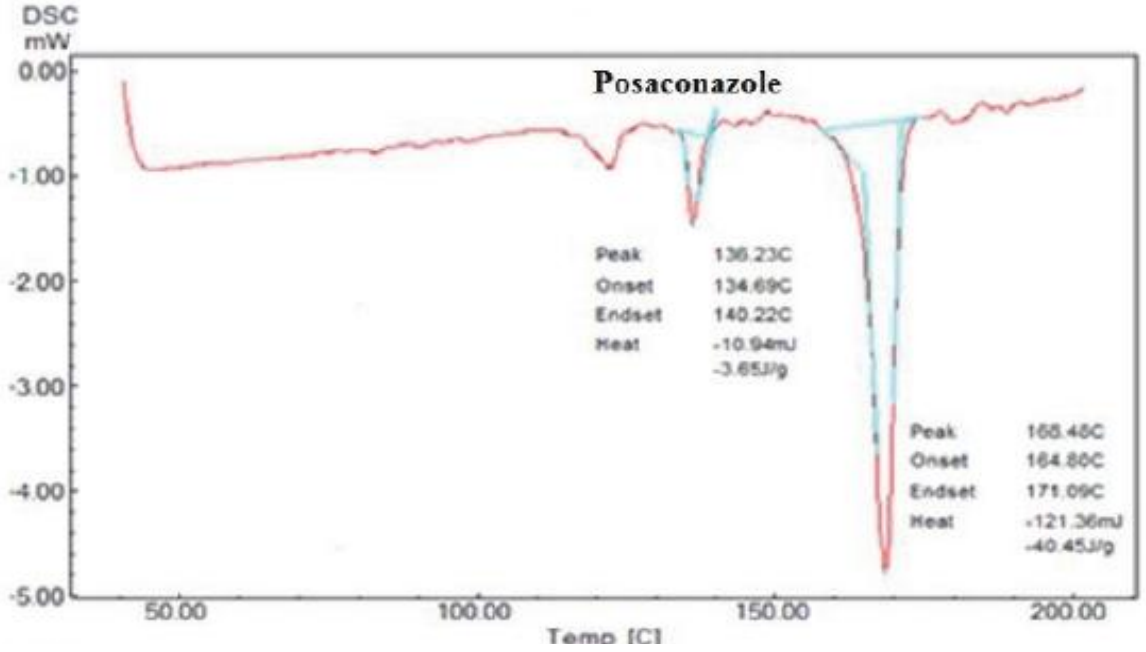
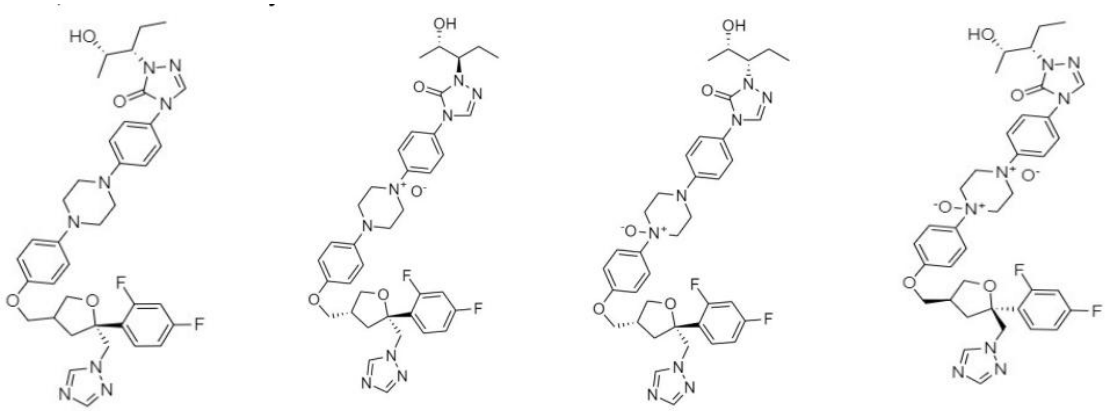


Figure 4: Chemical structures of Posaconazole and its N – Oxide derivatives (N – Oxide I, II, and N- dioxide) [20].



Posaconazole
$C_{37}H_{42}F_2N_8O_4$
700.8 g/mol

N-Mono Oxide; F-1
$C_{37}H_{42}F_2N_8FO_5$
716.79 g/mol

N-Mono Oxide; F-2
$C_{37}H_{42}F_2N_8FO_5$
716.79 g/mol

N, N-Dioxide
$C_{37}H_{42}F_2N_8FO_6$
732.79 g/mol

Figure 5: These are the obtained chromatograms of degradation products formed in oxidative degradation under investigation of delayed-release tablets **20**Error! Reference source not found.

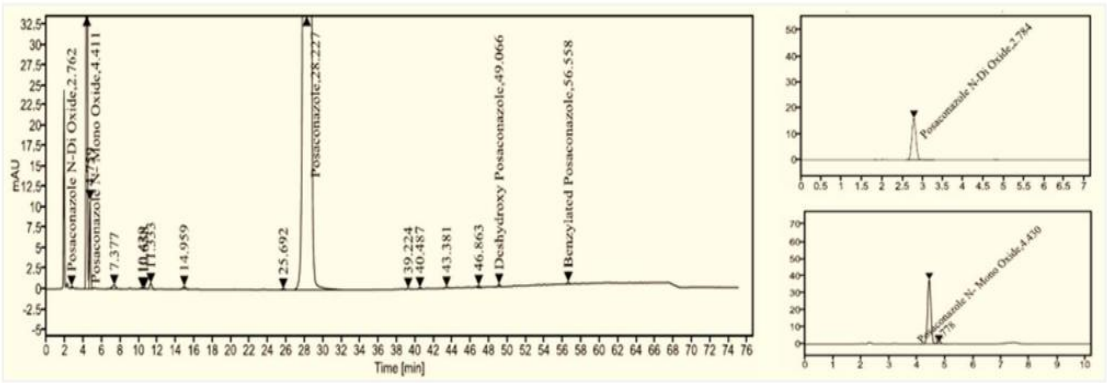


Figure 6: Overlay of the chromatogram of stress study samples[20].

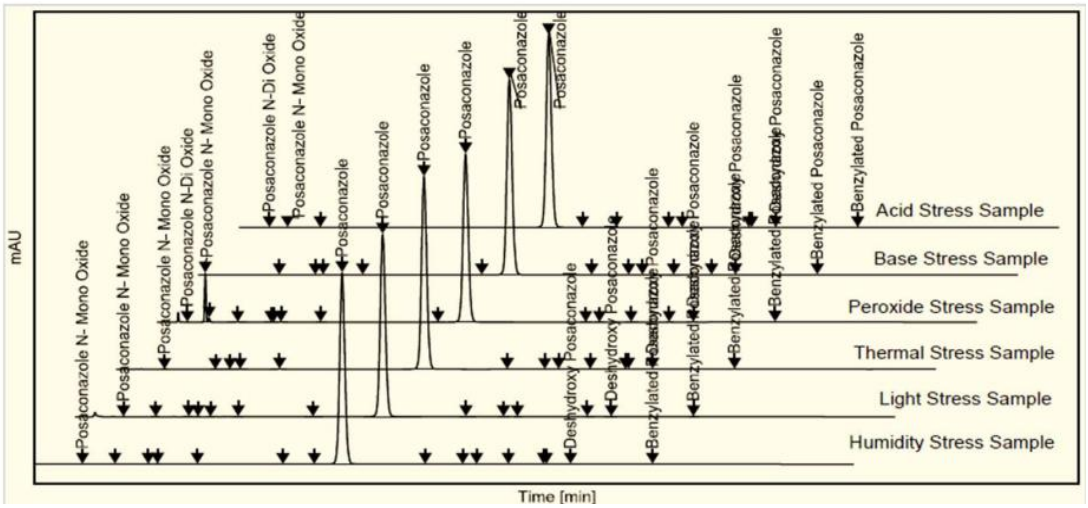


Figure7:The chromatogram spectra oxidative degradation kinetics of Posaconazole, DP, and Mass balance at 0, 9, 24, 35, 48, 57, 72, and 81h of oxidative degradation in the presence of 3% (w/w) H_2O_2 [22]

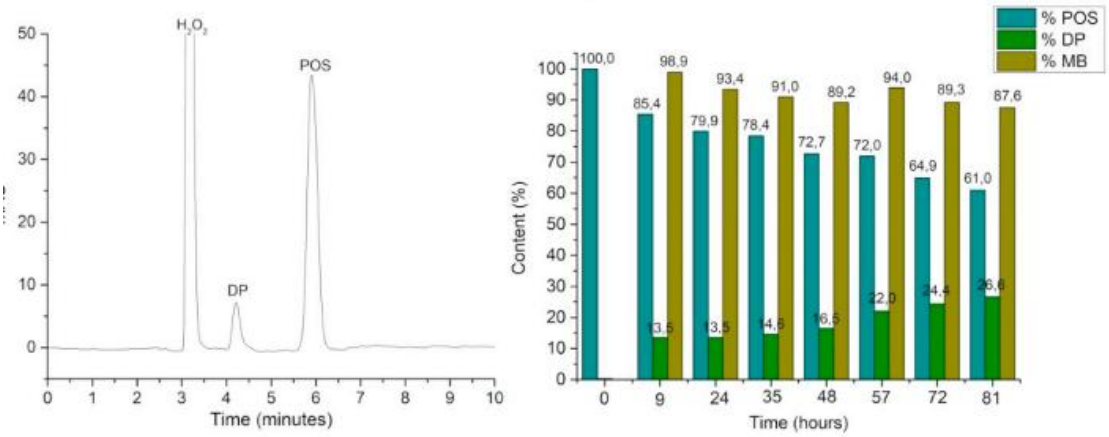


Figure 8: Spectra of Infrared of degradation products (N – mono oxide) formed in oxidative degradation under investigation of delayed-release tablets[21].

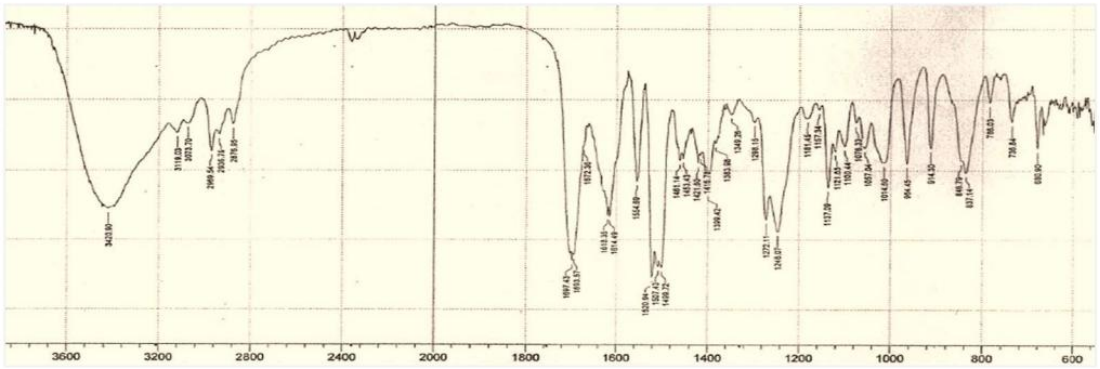


Figure 9: IR spectra of degradation products (N – dioxide) formed in oxidative degradation under investigation of delayed-release tablets[21].

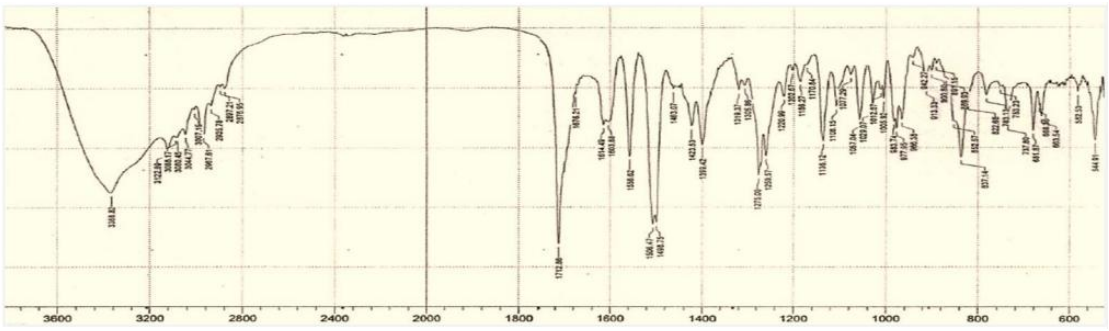
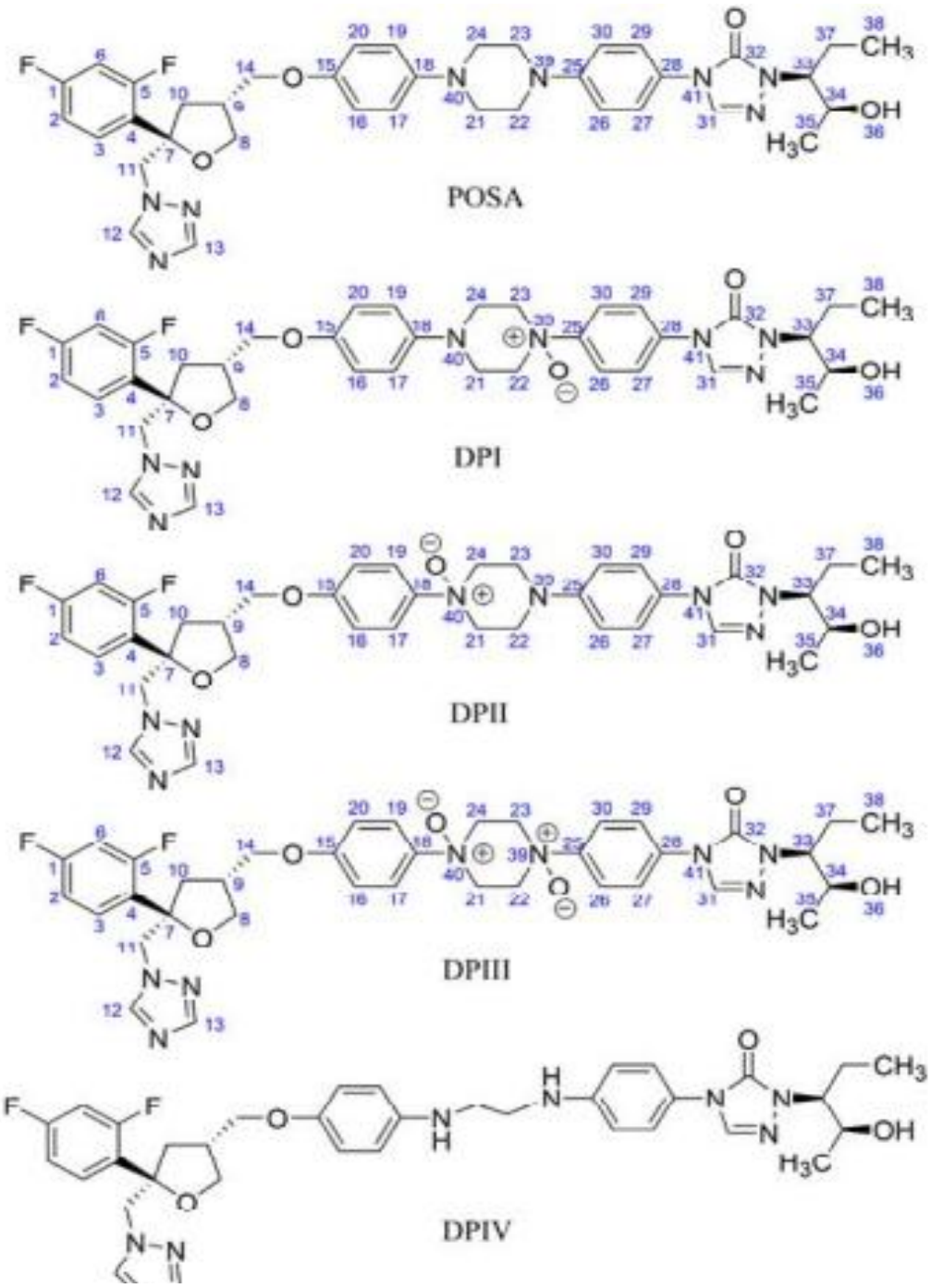


Figure 10: These are the chemical structures by NMR spectroscopy of Posaconazole, DP I, DP II, DP III, and DP IV[23]



POSA

DPI

DPII

DPIII

DPIV

Figure 11: These are the comparative Proton (^{1}H) NMR forms of Posaconazole and DP I, DP II, and DP III ***Error! Reference source not found.*****.**

Position[a]	Posaconazole		DPI		DPII		DPIII	
	δ_H (ppm), Multiplicity	J (Hz)[b]	δ_H (ppm), Multiplicity	J (Hz)	δ_H (ppm), Multiplicity	J (Hz)	δ_H (ppm), Multiplicity	J (Hz)
2	6.98 (td, 1H)	8.4, 2.2	6.98-7.02 (m, 1H)		6.99 (td, 1H)	8.8, 2.4	7.00 (td, 1H)	8.4, 2.4
3	7.28-7.33 (m, 1H)		7.29-7.34 (m, 1H)		7.30-7.34 (m, 1H)		7.30-7.34 (m, 1H)	
6	7.23-7.28 (m, 1H)		7.25-7.28 (m, 1H)		7.24-7.29 (m, 1H)		7.26-7.30 (m, 1H)	
8	4.03, 3.75 (dd, dd, 2H)	8.2, 7.8; 8.5, 7.7	4.04, 3.73-3.76 (dd, m, 2H)	8.8, 7.6	4.04, 3.75-3.78 (dd, m, 2H)	8.4, 7.2	4.05, 3.76-3.80 (dd, m, 2H)	8.8, 7.2
9	2.50-2.56 (m, 1H)		2.55-2.58 (m, 1H)		2.55-2.62 (m, 1H)		2.56-2.64 (m, 1H)	
10	2.39-2.43, 2.14 (m, dd, 2H)	13.1, 8.1	2.38-2.44, 2.15 (m, dd, 2H)	12.8, 8.0	2.40-2.46, 2.17 (m, dd, 2H)	13.2, 8.0	2.41-2.46, 2.19 (m, dd, 2H)	13.2, 8.0
11	4.59 (dd, 2H)	23.9, 14.5	4.59 (dd, 2H)	21.6, 14.4	4.59 (dd, 2H)	22.4, 14.4	4.60 (dd, 2H)	22.8, 14.4
12	8.34 (s, 1H)		8.35 (s, 1H)		8.34 (s, 1H)		8.36 (s, 1H)	
13	7.78 (s, 1H)		7.78 (s, 1H)		7.77 (s, 1H)		7.79 (s, 1H)	
14	3.77, 3.69 (dd, dd, 2H)	9.3, 6.0; 9.2, 7.8	3.77-3.79, 3.67-3.71 (m, m, 2H)		3.88, 3.80-3.82 (dd, m, 2H)	9.6, 6.4	3.87-3.91, 3.81-3.83 (m, m, 2H)	
16/20	6.81 (d, 2H)	9.0	6.83 (d, 2H)	8.8	6.96 (d, 2H)	9.2	7.03 (d, 2H)	9.2
17/19	6.94 (d, 2H)	9.0	6.99 (d, 2H)	8.8	8.10 (d, 2H)	9.2	8.07 (d, 2H)	9.2
21a/24a	3.15-3.17 (m, 4H)		3.60-3.65 (m, 2H)		4.08-4.12 (m, 2H)		4.80-4.85 (m, 2H)	
21b/24b			3.45 (d, 2H)	11.6	2.97 (d, 2H)	10.0	3.13 (d, 2H)	8.0
22a/23a	3.31-3.32 (m, 4H)		4.12-4.17 (m, 2H)		3.64-3.72 (m, 4H)		4.80-4.85 (m, 2H)	
22b/23b			3.00 (d, 2H)	10.0			3.13 (d, 2H)	8.0
26/30	7.10 (d, 2H)	9.0	8.36 (d, 2H)	8.0	7.15 (d, 2H)	8.8	8.33 (d, 2H)	9.2
27/29	7.53 (d, 2H)	9.0	7.87 (d, 2H)	9.2	7.55 (d, 2H)	9.2	7.93 (d, 2H)	8.8
31	8.32 (s, 1H)		8.55 (s, 1H)		8.34 (s, 1H)		8.58 (s, 1H)	
33	3.82 (m, 1H)		3.80-3.82 (m, 1H)		3.78-3.82 (m, 1H)		3.79-3.83 (m, 1H)	
34	3.84 (m, 1H)		3.82-3.84 (m, 1H)		3.80-3.82 (m, 1H)		3.80-3.87 (m, 1H)	
35	1.13 (d, 3H)	5.8	1.13 (d, 3H)	5.6	1.12 (d, 3H)	5.6	1.14 (d, 3H)	6.0
36	4.64 (d, OH)	4.8	4.74 (d, OH)	4.4	4.70 (s, OH)		4.87 (s , OH)	
37	1.69-1.72 (m, 2H)		1.68-1.75 (m, 2H)		1.68-1.75 (m, 2H)		1.69-1.76 (m, 2H)	
38	0.76 (t, 3H)	7.3	0.75 (t, 3H)	7.2	0.75 (t, 3H)	7.2	0.76 (t, 3H)	7.2

These are comparative Carbon (^{13}C) NMR forms of Posaconazole and DP I, DP II, and DP III **Error! Reference source not found.**.

Position[a]	Posaconazole		DPI		DPII		DPIII	
	δc (ppm)	DEPT	δc (ppm)	DEPT	δc (ppm)	DEPT	δc (ppm)	DEPT
1	161.8	C	160.9	C	162.4	C	164.2	C
2	110.9	CH	111.5	CH	111.5	CH	111.5	CH
3	128.4	CH	129.0	CH	129.0	CH	129.0	CH
4	126.1	C	126.7	C	126.6	C	126.7	C
5	158.6	C	158.2	C	160.1	C	160.8	C
6	104.4	CH	105.0	CH	104.7	CH	105.0	CH
7	83.3	C	83.8	C	83.9	C	83.9	C
8	69.8	CH_2	70.4	CH_2	70.3	CH_2	70.2	CH_2
9	38.4	CH	39.0	CH	38.8	CH	38.8	CH
10	37.5	CH_2	38.1	CH_2	38.0	CH_2	38.0	CH_2
11	55.2	CH_2	55.7	CH_2	55.8	CH_2	55.7	CH_2
12	144.9	CH	145.5	CH	145.5	CH	145.6	CH
13	150.4	CH	151.0	CH	151.0	CH	151.0	CH
14	68.7	CH_2	69.2	CH_2	69.2	CH_2	69.3	CH_2
15	152.2	C	152.6	C	158.5	C	158.9	C
16/20	115.0	CH	115.6	CH	114.5	CH	114.9	CH
17/19	117.5	CH	118.0	CH	122.6	CH	122.4	CH
18	145.4	C	145.3	C	149.3	C	147.8	C
21/24	49.5	CH_2	45.4	CH_2	66.9	CH_2	63.7	CH_2
22/23	48.2	CH_2	67.2	CH_2	44.1	CH_2	63.7	CH_2
25	149.6	C	154.4	C	149.5	C	153.0	C
26/30	115.7	CH	122.6	CH	116.3	CH	122.4	CH
27/29	122.6	CH	121.5	CH	123.2	CH	121.8	CH
28	125.6	C	134.7	C	126.4	C	135.1	C
31	134.7	CH	134.8	CH	135.2	CH	134.8	CH
32	152.4	C	152.8	C	152.9	C	152.6	C
33	62.5	CH	63.2	CH	63.0	CH	63.3	CH
34	67.1	CH	67.6	CH	67.6	CH	67.6	CH
35	19.8	CH_3	20.4	CH_3	20.4	CH_3	20.4	CH_3
37	21.2	CH_2	21.8	CH_2	21.8	CH_2	21.8	CH_2
38	10.5	CH_3	11.0	CH_3	11.0	CH_3	11.0	CH_3

Figure 12: Protonated destruction pathways of DP I and DP II.

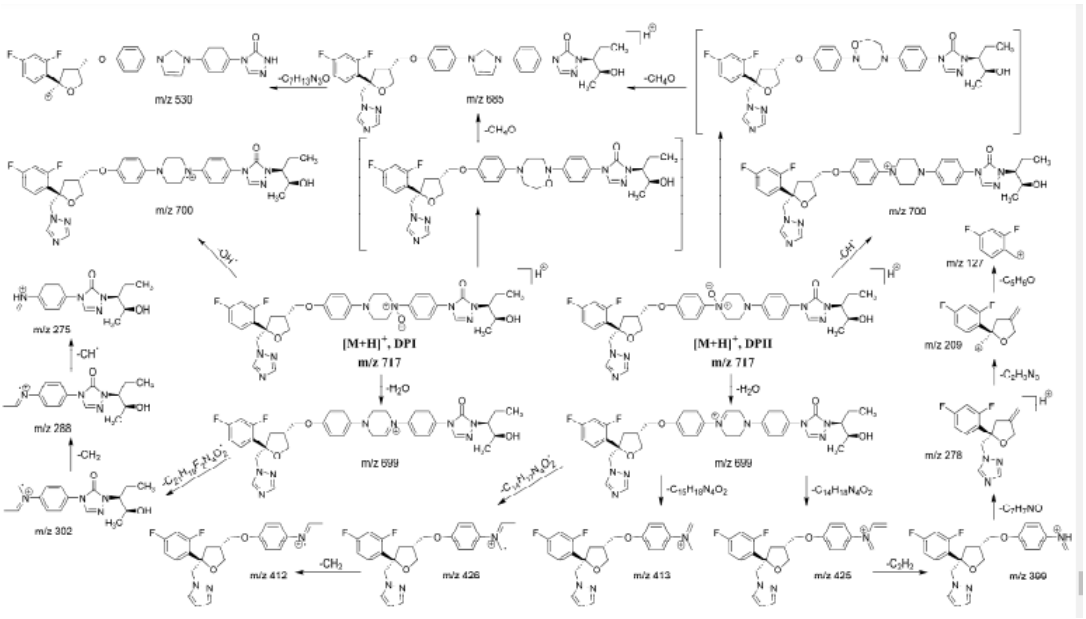


Figure 13:Protonated destruction pathway of DP III.

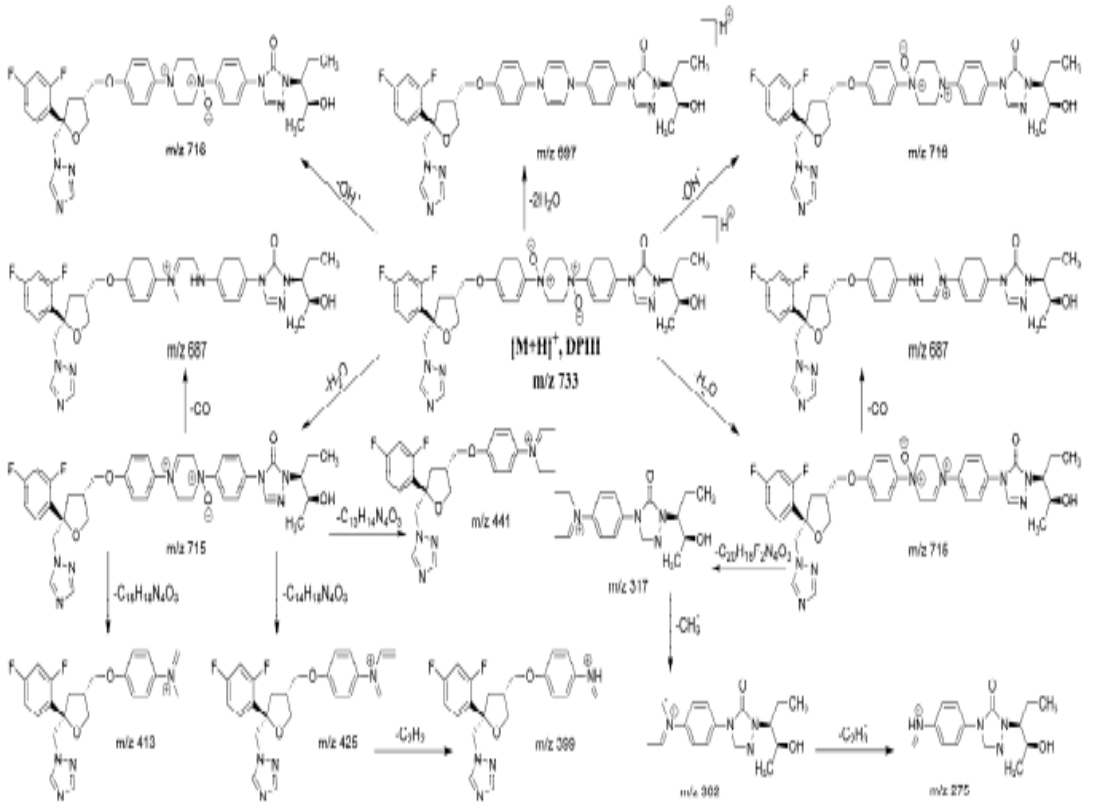


Figure 14: Possible mechanisms for the groundwork of DP I, DP II, DP III

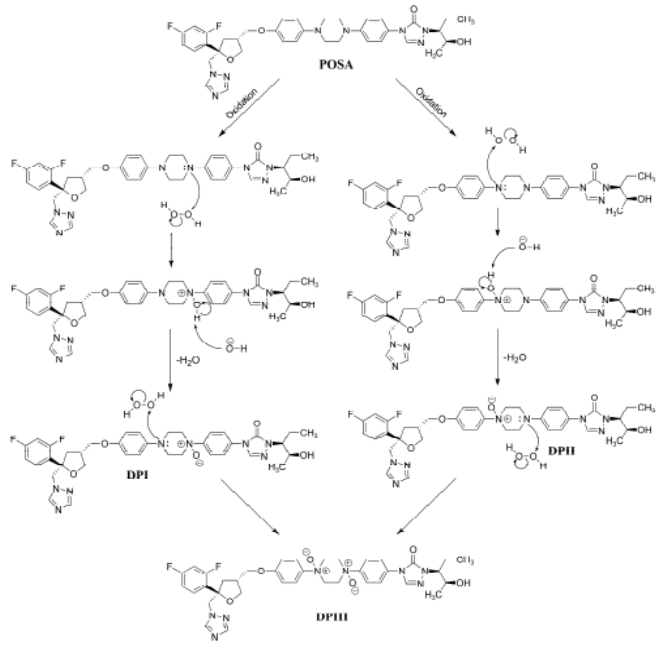


Figure 15: Mass spectra with ESI source analysis of Posaconazole after oxidative degradation kinetics along with its degradant products.

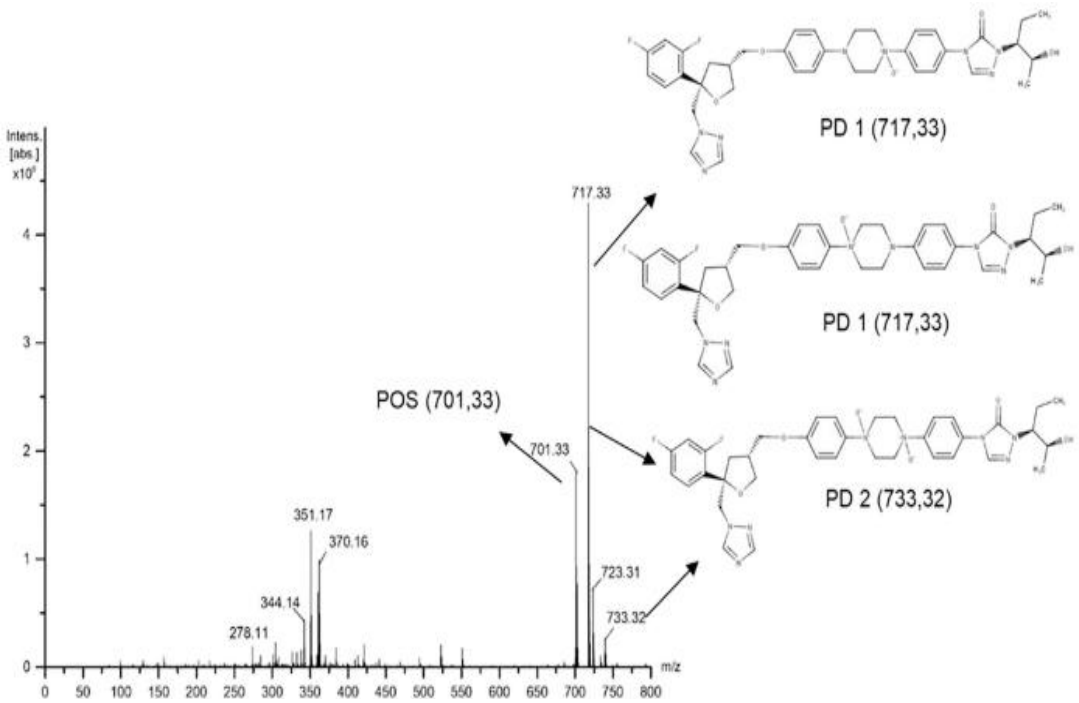


Figure 16: A RP – HPLC chromatogram for a pool of 2693 serum samples is spiked with all three triazole drugs: a comparative study

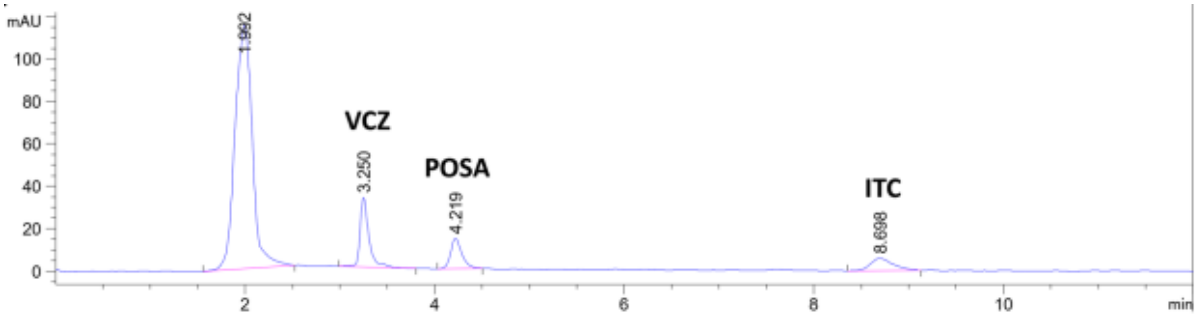


Figure 17: Achiral and chiral separation of antifungals by electrophoretic chromatography i.e., capillary electrophoretic assisted high-performance liquid chromatography[34]

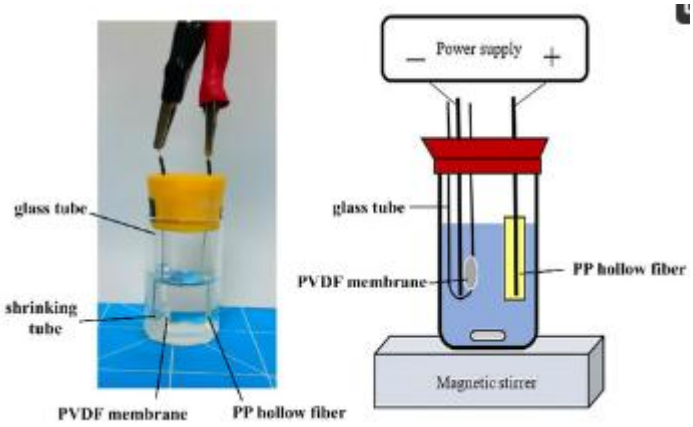


Table 1 Physical properties of Posaconazole [79]

S. No	Name of Property	Value	Units
1.	Solubility	1 g/mL	(g/mL)
2.	Crystalline solubility at acidic (1.2) pH	719 (µg/ml)	(µg/ml)
3.	Crystalline solubility basic (6.5) pH	0.20 (µg/ml)	(µg/ml)
4.	Log P (K o/w) at 25^{o}C	4.4 – 4.7	NA
5.	Melting point	168 - 172	(^{o}C)
6.	pKa	2.27	NA
7.	Molecular weight	700.8	g/mol.

Table 2: Flow properties of Posaconazole

S. No	Name of Property	Value
1.	Bulk density	0.15 $\pm$ 0.005
2.	Tapped density	0.25 $\pm$0.01
3.	Carr's index	35 $\pm$ 0.003
4.	Hausner's ratio	1.54 $\pm$ 0.03
5.	Property	Very poor

Table 3:pharmacokinetic properties of Posaconazole

Properties	Drug
Bioavailability [%]	98% when delivered in two 12-hourly split doses 220% when given in four split doses six hours apart, in the fasted condition
Protein binding [%]	>98
Apparent volume of distribution/F [L/kg]	18± 7
Maximum time to achieve greater concentration in plasma[h]	6 ± 3
Process of breakdown	Glucuronidation to inactive metabolites in the liver
Rate of clearance [L/h/kg]	0.3 ± 2
Elimination half-life [h]	15–35
Elimination route	NMT 1% excreted unaffected in the urine;66% excreted unaffected in feces.

Table 4:HPLC method analysis of Posaconazole.

Method	**Detector**	**Formulation/ Matrix**	**Column**	**Eluent**	**Injection volume**	**Reference**
HPLC	DAD	Oral suspension	Zorbox-C18((4.6 × 250 mm, 5 μm)	ACN/15 mM KH_2PO_4(30:70 to 80:20, linear over 7 minutes)	1.5ml/min	[11]
UHPLC	UV	Oral suspension	Kinetex-C18 (2.1 × 50 mm, 1.3 μm)	Acetonitrile/15 mM KH_2PO_4 (45:55)	0.4ml/min	[11]
HPLC	PDA	Bulk	Shim-pack C8 (250 x 4.6 mm; 5 µm)	MeoH/ H_2O (75:25, v/v)	1ml/min	[12]
HPTLC		Suspension	Aluminum plate (20x10 cm) with pre-	Acetone/chloroform (2:1v/v)	-	[13]

			coated silica gel			
HPLC	UV	Human serum	Sunfire C_{18} 51m (4.6 · 150 mm)	Acetonitrile/ultrapure water (60:40v/v)	0.8ml/min	[14]
UPLC	UV MS/MS	Human plasma	ReproSil-Pur Basic C18 (150mm ×2.0mm ID; 5 ——— m)	Aq. $NH_4H_2PO_4$ /ACN (50:50, v/v)	0.8ml/min	[15]
HPLC	fluorometric	Human serum	Nucleodur 100-5 C18ec (125mm ×4.0mm ID; 5 ——— m)	Formic acid (H_2O: ACN - 55:45, v/v)	1ml/min	[16]
HPLC	MS/MS	Dried blood	(Phenomenex Kinetex C18 (50 mm × 2.1 mm, 2.6 m)	acetonitrile/water (90:10, v/v)	0.8ml/min	[17]
HPLC	UV, MS/MS	Human urine, feces and blood	Zorbax Rx-C8 (250mm ×4.6mm ID; 5 ——— m) and C18	Water/methanol/formic acid and methanol/ammonium acetate (85:15, v/v)		[18]

Table 5: Data of the Forced degradation study in a method validation of preparative HPLC ***Error! Reference source not found.*****.**

Type of degradation	Assay (%)	Degradation (%)	Mass balance	Purity factor	Purity check
Acidity	94.7	1.3	96	1.000	Pass
Alkalinity	98.6	0.2	99	1.000	Pass
Oxidation	60.9	35.9	96	1.000	Pass
Photolytic	93.1	3.3	96	1.000	Pass

Table 6: Proton (^{1}H) NMR chemical shifts of degradation products (N – mono oxide and dioxide) formed in oxidative degradation as per functional groups under investigation of delayed-release tablets***Error! Reference source not found.*****.**

S.NO	Degrant products	Chemical shift value "δ"	No. of Protons	Multiplicity
1.	N – mono Oxide	A. 0.724 – 0.762 B. 1.116 – 1.128 C. 1.693 – 1.726 D. 2.143 – 2.601 E. 3.073 – 3.096 F. 3.317 G. 3.598 – 3.887 H. 4.017 – 4.137 I. 4.543 – 4.679 J. 6.820 – 7.567 K. 7.868 – 7.890 L. 8.049 – 8.071 M. 8.558	a. 3 b. 3 c. 4 d. 2 e. 5 f. 4 g. 2 and OH h. 3 i. 2 j. 10 k. 1 l. 1 m. 1	Triplet Doublet Triplet Multiplet Triplet Singlet Multiplet Triplet Multiplet Multiplet Doublet Doublet Singlet
2.	N – dioxide	A. 0.740 – 0.776 B. 1.132 – 1.144 C. 1.74 – 1.739 D. 2.159 – 2.618 E. 3.020 – 3.070 F. 3.334 G. 3.759 – 3.917 H. 4.033 – 4.072 I. 4.551 – 4.926 J. 7.001 – 7.316 K. 7.909 – 7.931 L. 8.082 – 8.104 M. 8.569	a. 3 b. 3 c. 4 d. 2 e. 5 f. 4 g. 2 and OH h. 3 i. 2 j. 10 k. 1 l. 1	Triplet Doublet Triplet Multiplet Triplet Singlet Multiplet Triplet Multiplet Multiplet Doublet Doublet Singlet

			m. 1	

Table 7: degradation studies of Posaconazole by HPLC

Detector	Column	Eluent	Flow rate	
DAD/ESI TOF	Discovery® C8 column (250×4.6 mm, 5 µm)	Methanol/water (75:25) (isocratic)	1.0ml/min	[23]
PDA	InertsilODS-3V C18 (150x 4.6mm with	Water/methanol (Gradient)	1.0ml/min	[29]

	5µm)			
DAD/QTOF	C8 Shim-pack column (250x4.6mm, 5µ m)	Methanol/water (75:25) (isocratic)	1.0ml/min	[30]
ESI/TOF	Agilent ZORBAX SB-Phenyl column (75 mm×4.6mm, 3.5 µm)	Eluent A Water/tetrahydrofuran / Phosphoricacid (770:230:1, v/v/v) Eluent B water/acetonitrile/ Phosphoricacid (200:800:1, v/v/v).	1.5ml/min	[31]
PDA MS/MS	X-Bridge C18 250 mm X 4.6 mm, 5 µm	25mM dipotassium hydrogen phosphate/ methanol/water (gradient)	1.3ml/min	[32]

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.