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QUANTIFICATION OF OTESECONAZOLE IN RAT PLASMA USING LC-MS/MS AND ITS APPLICATION TO PHARMACOKINETIC STUDY

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ABSTRACT
Background: Oteseconazole is a new molecule launched for human treatment. However, the information is not available in the public domain for analyzing blood samples. This study describes the method development and validation using LC-MS/MS to measure the concentration of Oteseconazole in rat plasma. **Methodology:** The analysis method was developed using a phenyl column, which was utilized to accomplish separation. Furthermore, the mobile phase was a combination of acetonitrile and 0.1% formic acid in water, with a ratio of 30:70 (v/v). The sample was introduced into the system at a 1.0 mL/min flow rate, and the injection volume was 10 µLand analyzed for five minutes using mass spectrometer +ESI mode. **Results and discussion:** MRM is used to quantify Oteseconazole and Posaconazole by analyzing the transitions of their respective m/z values. The concentration ranges of Oteseconazole were 5-100 ng/mL. The correlation coefficient of Oteseconazole was found to be 0. 999. HQC, MQC, LQC, and LLQC precision and accuracy were determined to be 98.60%, 98.69%, 96.11%, and 94.48%, respectively. Respectively, the accuracy recovery of Oteseconazole was determined to be 97.48%. In pharmacokinetic studies, it was observed that Oteseconazole exhibited an average AUC0-t value of 1386 ng-hr/ml and a Cmax value of 44.864 ng/ml in rats. **Conclusion**: The validated approach has effectively demonstrated the determination of pharmacokinetic parameters after the oral administration of Oteseconazole in Wister rats.

INTRODUCTION

Bioanalysis is the study of analytes found in biological samples, including biomarkers, medications, and metabolites, which is an integral part of the pharmacokinetic (PK)/pharmacodynamic (PD) evaluation of a novel new chemical molecule from the time of its discovery and during numerous stages of drug and drug product development till its market authorization [1-2]. This procedure encompasses several stages and entails the acquisition of samples derived from clinical or preclinical research subjects to the results through different critical processes [3-4]. Each step is crucial for ensuring accurate and reliable results [5-6]. To obtain accurate results, it is crucial to storage and sample preparation methods as the degradation and extraction of drug molecules from the mixture of blood components should be robust, reliable, and stable [7-8]. Another aspect is eliminating impurities in the sample matrix and optimizing the performance

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of the analytical system.[9-11]. Oteseconazole, a newly developed orally accessible and particular inhibitor of the fungus cytochrome P450 enzyme 51 (CYP51), has demonstrated considerable effectiveness in managing the condition of recurrent vulvovaginal candidiasis (RVVC) among affected individuals [12, 13]. Developed by Mycovia Pharmaceuticals and granted approval for medicinal use by the US FDA in April 2022, this medication is marketed as Vivjoa [14-15]. However, the information is not available in the public domain to analyze blood samples, although it has been performed for drug approval, and generic drug development needs the information for easy development. Analysis of the drug molecules in blood requires significant information for the generic drug approval and conductance of bioequivalence study in human volunteers. So, we have attempted this study to provide the ready information for the generic drug development organization, which can employed in the human blood sample analysis with less effort and costs. Additionally, pharmacokinetic data from the rat plasma could be used to predict human clinical data, which provides guidance. Its structure is shown in **Figure 1.** The Molecular formula of Oteseconazole is $C_{23}H_{16}F_7N_5O_2$, and its molar mass is 527.403 g·mol−1[16].

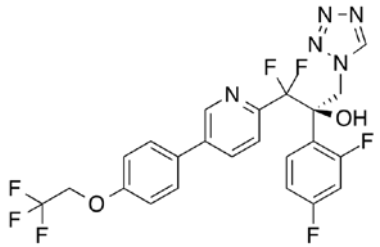

Figure 1: Chemical Structure of Oteseconazole

Oteseconazole is a popular choice of medicine for various indications currently being studied. However, it has not been studied in animals such as rats for the bioanalysis and its pharmacokinetic approach in rats. This study aims to provide a validated analytical protocol for assessing oteseconazole in Rat plasma matrices under the criteria set out by regulatory agencies, such as the FDA, by utilizing LC-MS/MS. The devised methodology was successfully employed in a pharmacokinetic study of Oteseconazole using plasma samples from rats.

MATERIALS AND METHODS

Materials

Oteseconazole and Posaconazole (Internal Standard) were Purchased from Zydus Cadila Health Care Ltd, Secunderabad, India. The Merck Chemical Division in Mumbai provided the LCMS-grade methanol, acetonitrile, and all other chemicals. HPLC-grade water obtained from the Milli-Q water purification system was employed during the experiment. The chromatographic apparatus was a Waters 2695 HPLC equipped with a high-speed Autosampler, column evaporator, degasser, and a SCIEX QTRAP 5500 Mass Spectrometer using Empower-2 software.

Methods

Stock and working solutions

Oteseconazole Stock Solution (200ng/ml)

To create the stock solution, first, weigh out 5mg of Oteseconazole working standard and add it to a 100 ml volumetric flask. The solution was diluted using a diluent as acetonitrile (20): purified water (80) to the mark. To prepare an intermediate stock solution, pipette out 0.4 mL of the solution and dilute using diluent as acetonitrile (20): purified water (80) to a final volume of 10 mL. To prepare the working standard solution, transfer 1 mL of the solution to a 10 mL volumetric vial, then add diluent as acetonitrile (20): purified water (80) to the mark.

Preparation of Internal Standard Stock Solution (240ng/ml)

An accurately weighed 6 mg of Posaconazole was added in a 100 ml volumetric vial. The volume was adjusted with diluent as acetonitrile (20): purified water (80) to the mark to prepare the Posaconazole working standard. Further, the solution of volume 0.4 ml was pipette out in a 10 ml volumetric flask and diluted with diluent as acetonitrile (20): purified water (80). In the end, the 1ml solution was further pipette out and transferred to a volumetric flask with a capacity of 10 ml and diluted to the designated mark by employing a diluent as acetonitrile (20): purified water (80) [17-18].

Oteseconazole Preparation of Standard Solution

The precisely 500µl of the standard stock solution was transferred into a 2 ml centrifuged tube. This mixture was supplemented with 500 µl of internal standard stock, 300 µl of acetonitrile, 500 µl of diluent as a combination of acetonitrile (30), purified water (70), and 200 µl of plasma. After a 20-minute spin, the liquid supernatant was filtered and transferred into an HPLC vial.

Conditions of liquid chromatography & mass spectrometry

The method, reagents, and columns required for the analysis are selected based on the experience. A phenyl analytical column,

150mm x 4.6mm, 3.5µm, operating at room temperature, was utilized to separate the oteseconazole molecule. The mobile phase used in this study consisted of a mixture of acetonitrile and 0.1% formic acid and a purified ratio of 30:70 (v/v) selected based on the number of trials. The mobile phase was subjected for sonication to degas and filtered through the 0.45µm membrane filter using the filtration assembly. The substance was delivered into the system at a 1.0 mL/min flow rate, with an injection volume of 10 µL. The differences in hydrophobicity have been employed to partition a polar stationary phase and a polar mobile phase. The liquid chromatography (LC) process was conducted for five minutes in +ESI mode. The mass spectrometer was in operation [19-21]. The determination of the mass-to-charge ratio transitions for Oteseconazole and Posaconazole (m/z 528.1205→383.614 & 701.3308→629.2165, respectively) was accomplished through the utilization of multiple reactions monitoring (MRM), as depicted in **Figure 2** and **Figure 3**.

Table 1: Test Parameter for +Electrospray Ionization [ESI]mode mass spectrometer

Drug Name	Posaconazole	Oteseconazole
Molecular Weight (g/mol)	700.8	527.4
Vaporizer temperature (°C)	350	350
Capillary temperature (°C)	350	350
Spray voltage positive ionization (V)	3500	3500
Spray voltage negative ionization (V)	2800	2800
Sheath gas (AU)	42	42
Sweep gas (AU)	1	1
Auxiliary gas (AU)	12	12
Retention Time	4.54	4.35
Precursor Ion (m/z)	701.3308	528.1205
Product Ion (m/z)	629.2165	383.6143
Fragmentation voltage (V)	135	170
Collision Energy (V)	17	23

Preparations of Linearity solutions

The linearity of the solution is a critical evaluation parameter for a method to assess the range of concentrations for which the process is being used. At concentrations of 5.00, 12.50, 25.00, 37.50, 50.00, 62.50, 75.00, and 100.00 ng/mL, standards for calibration curves were produced and centrifuged at 4000 RPM for 15–20 minutes. The supernatant solution was gathered and introduced into the chromatograph using an HPLC container**.** The QC samples were prepared using the procedure mentioned above and contained Oteseconazole concentrations of LLOQQC 5.00 ng/mL, LQC 25.00 ng/mL, MQC 50.00 ng/mL, and HQC 75.00ng/mL [22-24].

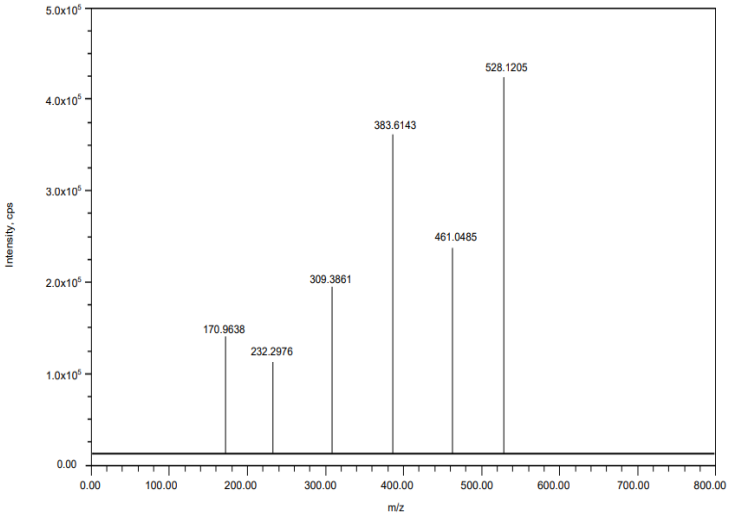

Figure 2: Mass spectrum of Oteseconazole

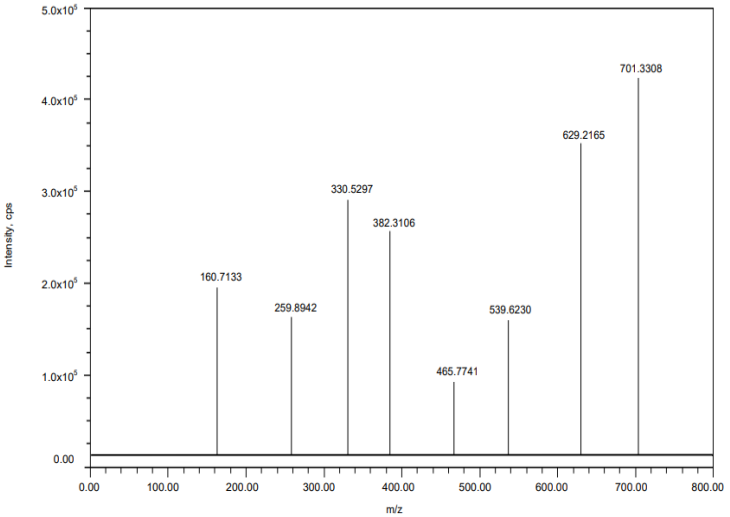

Figure 3: Mass spectrum of Posaconazole

Extraction protocol

Extraction of the drug molecule from the plasma is the most critical method, and the method selected is liquid-liquid extraction to isolate Oteseconazole from rat plasma. Furthermore, centrifugation was employed to separate layers containing the active moiety. The plasma samples of volume 200 µL were centrifuged and treated with phosphoric acid 0.1% v/v, followed by vortexed for 2 min, and then labeled based on the corresponding time intervals. 300 µL of diluent composed of 30% acetonitrile and 70% of 0.1% formic acid in purified water and and mixed thoroughly with the plasma sample. The vortex

cyclo mixture was supplemented with 500 µL of acetonitrile to precipitate all the proteins. The resulting solution was centrifuged at 4000 RPM for 15–20 minutes. The supernatant solution was collected and injected into the chromatograph using a container designed for LC/MS [25-30].

Bio-analytical method validation

According to the FDA's bioanalytical method guidelines [31-32], the devised method underwent comprehensive validation by computing all validation parameters, as detailed below. The acceptance criteria are recorded in Table 2.

Table 2: Recommendations and Acceptance Criteria for Bioanalytical Method Validation

Parameters	Chromatographic Assays (CCs)	Ligand Binding Assays (LBAs)
Calibration Curve	• Non-zero calibrators should be ± 15% of nominal (theoretical) concentrations, except at LLOQ, where the calibrator should be ± 20% of the nominal concentrations in each validation run. • 75% and at least six non-zero calibrator levels should meet the above criteria in each validation run.	Acceptance Criteria: • Non-zero calibrators should be ± 20% of nominal (theoretical) concentrations, except at LLOQ and ULOQ, where the calibrator should be ± 25% of the nominal concentrations in each validation run. • 75% and at least six non-zero calibrator levels should meet the above criteria in each validation run. • Anchor points should not be included in the curve fit
Selectivity	• Blank and zero calibrators should be interference-free at the analyte(s) retention times and the IS. • Spiked samples should be ± 20% LLOQ. • The IS response in the blank should not exceed 5% of the average IS responses of the calibrators and QCs	• For ≥ 80% of sources, the unspiked matrix should be BQL and spiked samples should be ± 25% at LLOQ and ± 20% at H QC.
Specificity	• Blank and zero calibrators should be interference-free at the analyte(s) retention times and the IS. • Spiked samples should be ± 20% LLOQ. • The IS response in the blank should not exceed 5% of the average IS responses of the calibrators and QCs	QCs should meet ± 20%, or 25% at the LLOQ and ULOQ.
Sensitivity	• The analyte response at the LLOQ should be ≥ five times the analyte response of the zero calibrator. • The accuracy should be± 20% of nominal concentration (from ≥ five replicates in at least three runs). • The precision should be ± 20% CV (from ≥ five replicates in at least three runs).	• The accuracy should be ± 25% of the nominal concentration (from ≥ three replicates in at least six runs). • The precision should be ± 25% CV (from ≥ three replicates in at least six runs). • The total error should be ≤ 40%.
Accuracy	The run should meet the calibration curve acceptance criteria and include the LLOQ calibrator. • This run has no QC acceptance criteria Within-run and between runs: • ± 15% of nominal concentrations; except ± 20% at LLOQ.	The run should meet the calibration acceptance criteria and include the LLOQ calibrator. • This run has no QC acceptance criteria Within-run and between runs: • ± 20% of nominal concentrations; except ±25% at LLOQ, ULOQ
Precision	The run should meet the calibration curve acceptance criteria and include the LLOQ calibrator. • This run has no QC acceptance criteria Within-run and between runs: • ± 15% CV, except ± 20% CV at LLOQ	The run should meet the calibration acceptance criteria and include the LLOQ calibrator. • This run has no QC acceptance criteria Within-run and between runs: • ± 20% CV, except ± 25% at LLOQ, ULOQ

Selectivity

Selectivity refers to the ability of an analytical procedure to accurately distinguish and measure analytes even when interfering substances are present in the biological matrix. We use blank samples from at least six independent sources/lots to determine selectivity. These blank samples are processed without including an analyte or internal standard (IS) [33-34].

Linearity

A calibration curve represents the relationship between the response and the known concentration of the analyte. It must be prepared using the same biological matrix as the samples. Additionally, separate calibration curves are needed for each analyte that will be measured. A method's range refers to the concentration interval within which accuracy, precision, and linearity have been demonstrated [35-36].

Accuracy and precision

During method development, it is crucial to verify whether the technique is suitable for validation by studying replicate QCs at numerous concentrations across the assay range. This comprises examining replicate QCs at different concentrations across the assay range. Method validation studies should include at least six independent runs, each with a calibration curve and several sample concentrations measured in repeats, to calculate accuracy and precision [37-38].

Recovery

Six quality control samples were thawed or made fresh from the deep freezer (LQC, MQC, and HQC). The internal standard was added to the quality control samples (extracted samples) before they were injected. A 100 percent extraction of the analyte was achieved by processing blank matrix samples screened from a single lot. These samples were injected with six sets of each quality control dilution at low, middle, and high concentrations. Additionally, an internal standard was included in the process. At each QC level and for ISTD, the percent CV of recovery should be less than 15.00 percent. For all QC levels, the total mean recovery percent CV should be less than 20.00 percent [39-40].

Matrix effects

A variation in analyte reactivity induced by intervening and sometimes undetectable components in the sample matrix is known as the matrix effect. The matrix effect was measured eight times for each analyte and internal standard at LQC and HQC concentration levels. Eight different screened plasma batches were used to make two replicates of blank plasma samples. The LQC concentration was spiked with ISTD using one set of eight independent blank matrices, whereas the HQC concentration was spiked with ISTD using another set. The analysis used spiked analyte(s) and ISTD to reconstitute the solution to obtain one set of aqueous samples comparable to final LQC and HQC concentrations [40-41].

Stability experiments

Stability tests are essential to ensure that the concentration of the analyte remains unchanged throughout sample preparation, processing, analysis, and storage conditions. Evaluating the analyte's stability within the matrix is under investigation by utilizing quality controls for stability, including low and high concentrations. Once the storage conditions have been implemented at time zero, we analyze aliquots of the quality controls with low and high stability. Conducting and assessing at least three stability tests for every concentration level, storage condition, and time point is imperative. The FDA has advised the following stability measures for biological investigations. Changing the analyte in any way can impact chromatographic behavior, making the method development process more complex[42-43]. The above parameters can make the development and validation processes much more accessible to implement. The approach is unacceptable for its intended purpose if selectivity cannot be demonstrated. It will be challenging to develop the approach if recovery is uneven and the analytes are fractionated after being adjusted. Accuracy, precision, range, and other qualities would be considerably altered under such circumstances [44-45]. The samples were subjected to multiple stability parameters, such as benchtop, wet extract, and dry extract, for 18 hours and long-term stability at 28 days, and the spiked concentration was 25 and 75 ng/ml. The samples were analyzed for the content analysis of otseconazole with a previously standardized and validated method [46-47].

Application of the bio-analytical method to pharmacokinetics study

A cohort of six male Wistar rats weighing between 180 and 220 g was utilized to conduct the pharmacokinetic experiments. The animals were accommodated in ventilated enclosures that were adequately supplied with food and water for seven days before the initiation of the experiments [48-50]. The rats were fasted

overnight before being given a dose. A pharmacokinetic study was conducted on Oteseconazole in a group of six rats. The animal study protocol has been approved by the Institute of Animal Ethics Committee (Registration Number: 1250/PO/RcBi/s/18/CPCSEA). The rats were given a single dose of 150 mg Oteseconazole capsules. Samples were collected at various time intervals: 1, 2, 5, 10, 15, 20, 25, and 30 hours after administering the dose. At each time point, 5 ml of blood was collected as an aliquot in K2 EDTA vacutainer containers [51-52]. Furthermore, a pre-dose sample was obtained to assess the potential for plasma interference. The collected samples were centrifuged to obtain plasma, which was subsequently stored at a temperature of -70 °C. The spiked plasma samples were analyzed alongside quality control (QC) samples at four concentrations. The software application WinNonlin (Version 5.2) was used to determine the pharmacokinetic parameters of Oteseconazole [52-54].

RESULTS

Selectivity

When the peak response of blank samples was compared to the reaction of spiked LLOQ samples containing IS mixtures, the selectivity of the method was shown to reveal the lack of interferences at Oteseconazole of both the analyte against the guidance document criteria shown in Table 2 and depicted in **Figure 4** and **Figure 5**. So, it concludes that the IS posaconazole is compatible with Oteseconazole and can freely be used as an internal standard for any bioanalytical methods in the future.

Linearity

The standard curves were linear within the concentration range of 5-100 ng/mL of Oteseconazole. The observed average correlation coefficient was 0.999. The determination of sample quantity was accomplished through the computation of the ratio between the peak areas of the analyte and the internal standard (IS). The peak area ratios of the plasma concentrations were graphically shown, as presented in **Table 3** and **Figure 6**. The response of the multiple concentrations is linear and can be used to analyze Oteseconazole in bioanalytical samples.

Precision and accuracy

Six duplicates containing Oteseconazole were analyzed at three distinct quality control (QC) levels to assess the intra-assay precision and accuracy. Analyzing the three levels of QC samples on independent runs determined the inter-assay precision. The suggested method's percent mean accuracy varied from 94.48 percent to 98.69 percent, and the precision (percent CV) for LQC, MQC, and HQC was 0.22 to 2.03 percent. **Table 4** summarises the findings. Acceptable precision and accuracy results exhibit that the developed method is capable of analysing the analyte accurately and precisely.

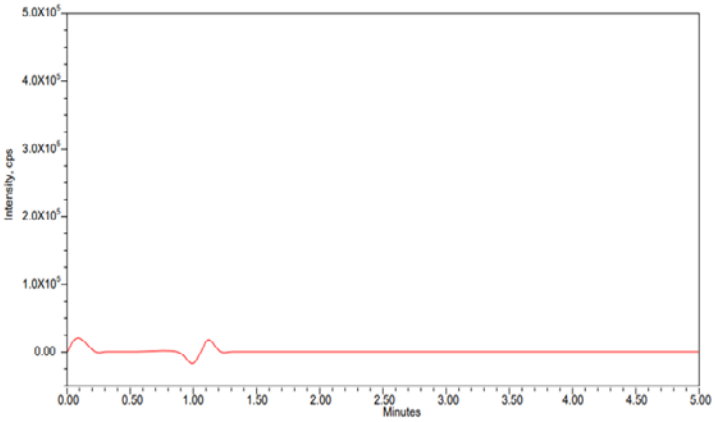

Figure 4: Blank chromatogram of Oteseconazole

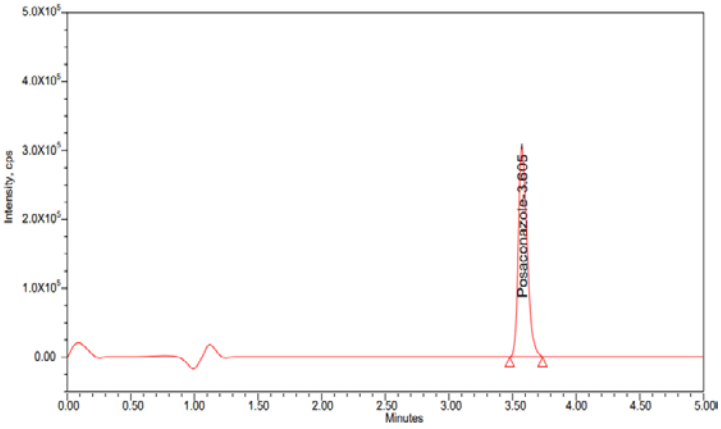

Figure 5: Blank chromatogram+ IS

Table 3: Linearity Results of Oteseconazole

Final conc. in ng/ml	RES	Area response ratio
5.00	0.248	0.081
12.50	0.611	0.201
25.00	1.219	0.400
37.50	1.835	0.599
50.00	2.435	0.797
62.50	3.042	0.997
75.00	3.661	1.203
100.00	4.878	1.590
Slope	**0.0159**	
Intercept	**0.00158**	
R^2 Value	**0.99999**	

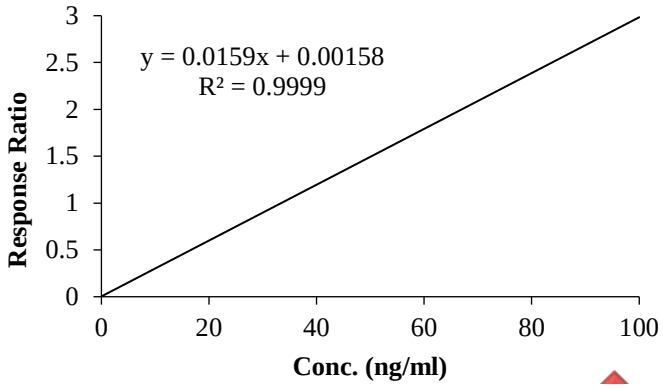

Figure 6: Calibration Curve concentration v/s Area ratio of Oteseconazole

Recovery and Matrix Effect

The recoveries of Oteseconazole and IS were determined by calculating their values at the three quality control (QC) levels, with each level having six duplicates. The recovery of analytes from samples is a measure of the effectiveness of their separation, as represented in **Table 5**. The coefficient of variation (% CV) for LQC, MQC, and HQC were 0.83%, 0.22%, and 0.16%, respectively, as indicated in **Table 6** for Oteseconazole. The recovery of the sample is acceptable in the developed method and can be adapted directly for use in upcoming bioanalysis.

Table 4: Precision and accuracy Results of Oteseconazole

Injections	**HQC**	**MQC**	**LQC**	**LLQC**
1	$3.625x10^5$	$2.407x10^5$	$1.175x10^5$	$0.229x10^5$
2	$3.617x10^5$	$2.403x10^5$	$1.166x10^5$	$0.226x10^5$
3	$3.611x10^5$	$2.415x10^5$	$1.184x10^5$	$0.227x10^5$
4	$3.623x10^5$	$2.426x10^5$	$1.179x10^5$	$0.235x10^5$
5	$3.604x10^5$	$2.418x10^5$	$1.162x10^5$	$0.238x10^5$
6	$3.613x10^5$	$2.409x10^5$	$1.181x10^5$	$0.231x10^5$
Mean	$3.616x10^5$	$2.413x10^5$	$1.175x10^5$	$0.231x10^5$
SD	0.00784	0. 00837	0.00873	0.00469
% CV	0.22	0.35	0.74	2.03
% Mean Accuracy	98.60%	98.69%	96.11%	94.48%

Table 5: Recovery of Oteseconazole

	LQC		**MQC**		**HQC**	
Parameter	**Extracted**	**Unextracted**	**Extracted**	**Unextracted**	**Extracted**	**Unextracted**
Mean	$1.152x10^5$	$1.176x10^5$	$2.393x10^5$	$2.411x10^5$	$3.603x10^5$	$3.615x10^5$
SD	0.00796	0.00933	0.00889	0.00800	0.01010	0.00788
% CV	0.69	0.79	0.37	0.33	0.28	0.22
%Mean recovery	94.23%	96.20%	97.87%	98.61%	98.24%	98.57%
Overall Recovery	97.28 %					

Table 6: Matrix Effect of Oteseconazole

	LQC	**MQC**	**HQC**
Mean	0.9799	0.9927	0.9966
SD	0.00817	0.00216	0.00159
%CV	0.83	0.22	0.16

Stability

Six replicates of quality control (QC) samples at low and high concentrations were evaluated to determine the stability of Oteseconazole in plasma. Oteseconazole standard solutions were added in the appropriate volumes to generate drug-free plasma samples.

The findings in **Table 7** were determined to be within the acceptable range, suggesting that Oteseconazole exhibits favorable stability [55-56]. Hence, the method is suitable for adoption in the commercial analysis of the sample without investing much time and cost for the industries.

Table 7: Oteseconazole QC sample stability results using LC-MS/MS

Stability Parameters	Spiked concentration	Mean ±SD	% RSD	% Accuracy
Bench Top	25(ng/ml) 75(ng/ml)	$1.163x10^5$ ±0.00930 $3.610x10^5$ ±0.00611	0.80 0.17	95.13% 98.43 %
Freeze-Thaw		$1.160x10^5$ ± 0.00700 $3.610x10^5$ ±0.00556	0.60 0.15	94.89 % 98.43 %
Wet Extract (18 hrs.)		$1.152x10^5$±0.00649 $3.585x10^5$ ± 0.00903	0.56 0.25	94.23% 97.75%
Dry Extract (18 Hrs.)		$1.152x10^5$ ±0.00665 $3.588x10^5$ ±0.01108	0.58 0.31	94.23 % 97.87 %
Long Term (28 Days)		$1.024x10^5$ ± 0.00684 $3.167x10^5$ ± 0.00888	0.84 0.28	83.76 % 86.35 %

Pharmacokinetic application

The pharmacokinetic characteristics of Oteseconazole have been determined by calculation. The values for the highest plasma concentration (C_{max}) and the time taken to achieve maximum plasma concentration (T_{max}) were directly extracted from the plasma time profile curve depicted in **Figure 7**. The pharmacokinetic characteristics of Oteseconazole were determined using the WinNonlin software tool (Version 5.2). The stability of the research samples was determined using incurred sample reanalysis (ISR). The linear trapezoidal method was employed to estimate several pharmacokinetic parameters, such as AUC 0-t, AUC 0-∞, t1/2, C_{max}, T_{max}, and clearance (CL). The estimations above have been consolidated and shown in **Table 8**. The time required to achieve the highest concentration, 44.864 ng/mg, is about 10 hours. So, these parameters can be used as a reference for pursuing the studies in humans because a survey of VIVJOA™ (oteseconazole) capsules in human subjects also mentions the time to peak plasma concentrations of oteseconazole was approximately 5 to 10 hours [57]. Hence, the study performed using a suitable validated method in rat plasma can also be co-related to understanding the essential pharmacokinetic study in humans.

Table 8: Mean plasma concentration-time curves of Oteseconazole

Pharmacokinetic parameters	Oteseconazole
AUC_{0-t}	1386 ng-hr/ml
C_{max}	44.864 ng/ml
$AUC_{0-\infty}$	1386 ng-hr/ml
T_{max}	10 Hrs
$T_{1/2}$	25 Hrs

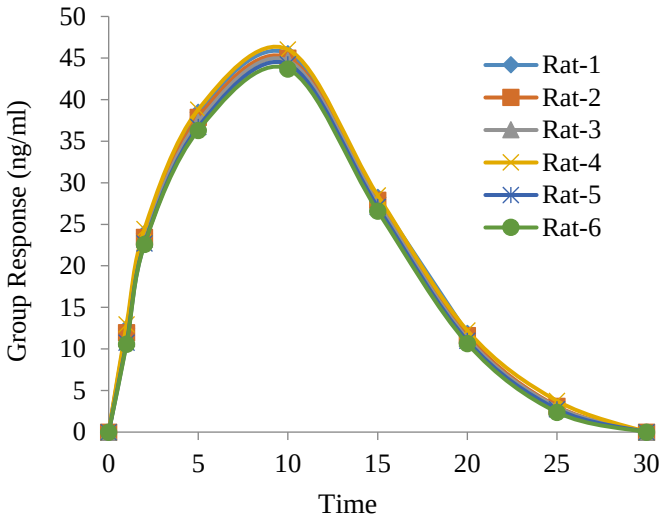

Figure 7: Mean plasma concentration-time profile Oteseconazole in Rat Plasma

Study Limitations

The study has been carried out in rat plasma; it should be carefully used to extract Oteseconazole in human plasma, and the method should be employed after careful validation for human plasma.

DISCUSSION

The internal standard posaconazole is highly useful to verify the analysis of otseconazole during analysis without interference. The linear response was obtained after analyzing multiple concentrations of drug molecules, which is used for further standard calculations. The precision and accuracy results show that the developed method can analyze the analyte accurately and precisely as the results are within acceptable limits. The adequate recovery from the samples indicates that the process is

feasible. Oteseconazole exhibits favorable stability at executed stability conditions. Thus, the method is more suitable for the analysis of blood samples. The time required to achieve the highest concentration is about 10 hours, which matches the data reported in the studies performed on human subjects. Hence, the study conducted using a suitable validated method in rat plasma can also be co-related to understanding the basic pharmacokinetic study in humans. The developed method significantly helps the generic industry to implement in human subjects with little or no modifications.

CONCLUSION

In LC-MS/MS, a technique for correctly and precisely identifying Oteseconazole has been established for quick analysis and maintaining sensitivity. Internal standards Posaconazole helps ensure the accuracy of the analysis of Oteseconazole in Rat Plasma. Oteseconazole has been verified throughout a dynamic linear range of 5-100 ng/mL. The method is suitable with a correlation coefficient (r^2) of 0. 999, which meets USFDA requirements, and validation parameters were all confirmed to be within the permitted range. The method successfully quantified Oteseconazole in pharmacokinetics studies involving male Wistar rats. Stability studies were conducted on the technique, and the results consistently fell within the assay variability limits at every stage.

The pharmacokinetic study of Oteseconazole in rat plasma was successfully conducted using this optimized method, comparable with the human study data from clinical trials. Plasma concentrations of Oteseconazole were measured, and the primary pharmacokinetic parameters were determined, similar to the reported human study data. Hence, the developed method can be employed to create new novel formulations of Oteseconazole and calculate the pharmacokinetic parameters in rat plasma. Also, the parameters obtained can be used to predict pharmacokinetic parameters in humans.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed equally. Krishna has crafted the concept, conducted the experiments, evaluated the data, and drafted, and revised the manuscript. Om Shelke has helped with the literature search, evaluation of data, and drafting, revising, and submission of the manuscript. Neetu Shorgar has supervised the entire process of crafting the concept, conducting the experiments, evaluating of data, and revising the manuscript.

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