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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF ANTIBIOTIC DRUG IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

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Abstract:

The present research focuses on the development and validation of a stability-indicating Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the estimation of Ozenoxacin (OZE), a recently introduced antibiotic drug, in bulk and its semisolid pharmaceutical dosage form. Various chromatographic conditions were optimized using an Inertsil C18 column (4.6 × 250 mm) with a mobile phase consisting of Methanol: Phosphate Buffer (60:40, pH 3.5), and detection was carried out at 309 nm. The method demonstrated excellent resolution, specificity, and reproducibility with a retention time of approximately 4.92 minutes. Validation was carried out in accordance with ICH guidelines for parameters including accuracy, precision, specificity, linearity, robustness, ruggedness, LOD, and LOQ. The method showed linearity in the range of 80% to 120% of test concentration with R^2 value close to 0.9998. Forced degradation studies under acid, base, oxidative, thermal, and UV conditions confirmed the stability-indicating nature of the method. The results revealed that there was no interference from excipients or degradation products. Thus, the proposed RP-HPLC method is accurate, precise, and suitable for routine analysis of Ozenoxacin in pharmaceutical formulations.

Keywords: *Ozenoxacin; RP-HPLC; Method Validation; Semisolid Formulation; Stability-Indicating; Forced Degradation; ICH Guidelines; Antibiotic Analysis; Quantitative Estimation.*

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INTRODUCTION:

Analytical method development and validation play a pivotal role in ensuring the quality, efficacy, and safety of pharmaceutical products. Among the various analytical techniques, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) has gained widespread application due to its high resolution, accuracy, and reproducibility in the separation and quantification of drug compounds. The development of a reliable RP-HPLC method is essential for the routine analysis of active pharmaceutical ingredients (APIs), particularly for newly introduced or less extensively studied drugs.

Ozenoxacin is a novel, non-fluorinated quinolone antibiotic that exhibits broad-spectrum antibacterial activity, especially against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). It is primarily used for the topical treatment of impetigo and other superficial skin infections. As a relatively new antibiotic in clinical practice, the availability of a robust, validated analytical method for its estimation in bulk and pharmaceutical dosage forms is essential to ensure consistent therapeutic performance and regulatory compliance.

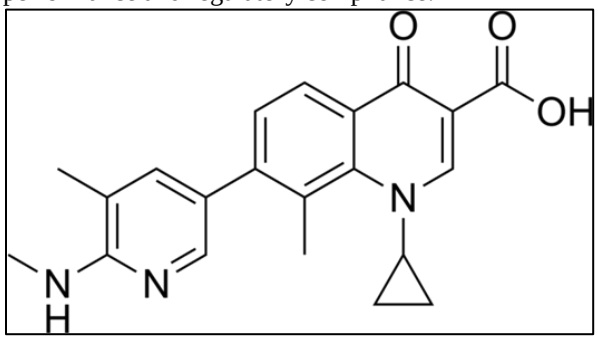

Figure 1: Structure of Ozenoxacin

Despite its increasing use, limited literature is available regarding the analytical estimation of Ozenoxacin by RP-HPLC, especially methods that are optimized and validated as per International Council for Harmonisation (ICH) guidelines. Hence, there is a need to develop a simple, sensitive, specific, and cost-effective RP-HPLC method that can accurately quantify Ozenoxacin in bulk drug and marketed formulations. Such a method should undergo rigorous validation to confirm its reliability in terms of linearity, precision, accuracy, robustness, specificity, and system suitability.

The present research aims to address this gap by developing and validating a novel RP-HPLC method for the estimation of Ozenoxacin. The study will include method optimization involving critical parameters such as mobile phase composition, flow rate, column selection, and detection wavelength. Furthermore, the developed method will be subjected to validation as per ICH Q2(R1) guidelines, ensuring its suitability for routine quality control and stability testing in pharmaceutical industries. Ultimately, this work will contribute to strengthening the analytical tools available for the quality assurance of Ozenoxacin-containing formulations and support regulatory submissions with scientifically justified data.

MATERIALS AND METHODS:

MATERIALS:

The drug selected for the present investigation, Ozenoxacin, was procured as a gift sample from Swapnroop Drugs and Pharmaceuticals, India. For the purpose of method development and validation, a marketed pharmaceutical dosage form was also selected. The commercial formulation used was OZX Cream, manufactured by Abbott Healthcare Private Limited, which contains Ozenoxacin at a concentration of 1%. This formulation was utilized for comparative and validation studies.

All reagents and chemicals used in the study were of analytical or HPLC grade to ensure accuracy and reproducibility of the results. The solvents used included acetonitrile, methanol, ortho-phosphoric acid, and HPLC-grade water, all of which were obtained from Merck Ltd., India. These high-purity chemicals and solvents were chosen to avoid interference in the chromatographic analysis and to maintain the integrity of the RP-HPLC method.

METHODS & PROCEDURE

Identification and Characterization of Drug

Before initiating the experimental work, it is crucial to identify and characterize the selected drug compound. Understanding the physical and chemical properties of the drug, such as appearance, solubility, melting point, and spectral behavior, provides essential information regarding its purity and helps in selecting appropriate solvents and chromatographic conditions. These fundamental evaluations aid in the development of a robust and stable analytical method.

Selection and Procurement of Drug

Ozenoxacin (OZE), a non-fluorinated quinolone antibiotic, was selected as the model drug for the development and validation of an RP-HPLC method. The pure drug was generously gifted by a pharmaceutical industry located in India. After procurement, the drug was analyzed for its physical properties, including color, odor, and melting point, to confirm its identity and quality.

Physico-Chemical Characterization

The characterization of the drug molecule is essential for confirming its identity and ensuring its purity prior to method development. Physico-chemical studies involved determining parameters such as

melting point, UV-visible spectrophotometric properties, and solubility profile. These tests play a pivotal role in establishing a baseline for further analytical assessments.

Solubility Studies

As an initial step in method development, the solubility of Ozenoxacin was tested in a variety of solvents to identify a suitable solvent system for standard solution preparation and mobile phase selection. The goal was to find a common solvent that ensures complete solubility, chemical stability, and compatibility with chromatographic systems for accurate quantification.

Melting Point Determination

The melting point of Ozenoxacin was determined using a standard capillary tube method. A small quantity of the sample was placed in a closed-end capillary tube, and the tube was inserted into a melting point apparatus. The temperature at which the drug melted was noted, confirming its identity and purity, and the results were tabulated.

FT-IR Spectral Analysis

Fourier Transform Infrared Spectroscopy (FTIR) was employed to analyze the functional groups and chemical structure of Ozenoxacin. The IR spectrum was recorded using a Shimadzu FTIR-8400S spectrometer in the range of 4000–400 cm^{-1}. FTIR is a reliable technique based on the absorption of specific frequencies of infrared light by different functional groups in a molecule. These vibrational frequencies are dependent on molecular structure and bonding, making FTIR an effective tool for confirming the identity of the drug substance.

UV Spectrophotometric Analysis

UV-visible spectrophotometry was carried out using a Shimadzu-1800 spectrophotometer with 1 cm quartz cells. The absorption spectrum of Ozenoxacin was recorded over the range of 200–400 nm to determine the maximum wavelength of absorption (λmax). This parameter was used to support both identity confirmation and the selection of detection wavelength in HPLC analysis.

Selection of Mobile Phase and Preparation of Standard Solutions

Preparation of Standard Solution

A standard stock solution of Ozenoxacin was prepared by accurately weighing 5 mg of the drug and dissolving it in methanol, followed by volume adjustment to 25 mL to obtain a concentration of 200 µg/mL. From this stock, 1 mL was diluted further to 20 mL with methanol to achieve a final working concentration of 10 µg/mL. This solution was used for HPLC injection and method optimization trials.

Mobile Phase Optimization

Various mobile phase combinations were evaluated for achieving sharp, symmetrical peaks with consistent retention times. Initially, methanol and water in different ratios were tested, followed by combinations of methanol with phosphate buffer at different pH values. The mobile phases tested included:

- Methanol
- Methanol: Water (90:10)
- Methanol: Water (80:20)
- Methanol: Phosphate Buffer (80:20), pH 5
- Methanol: Phosphate Buffer (70:30), pH 4.5
- Methanol: Phosphate Buffer (60:40), pH 4
- Methanol: Phosphate Buffer (60:40), pH 3.5

Among these, the mobile phase consisting of Methanol: Phosphate Buffer (60:40), pH 3.5 was selected as optimal, providing well-resolved, sharp, and reproducible peaks for Ozenoxacin.

Chromatographic Conditions

The final chromatographic conditions for RP-HPLC analysis were established after multiple trials to ensure system suitability and method reproducibility. The analysis was carried out using an Inertsil C18 column (4.6 mm × 250 mm, 5 µm particle size). The optimized mobile phase consisted of Methanol and Phosphate Buffer (60:40) with a pH adjusted to 3.5. The flow rate was maintained at 1.0 mL/min, and the detection was carried out at a wavelength of 309 nm. The injection volume was set at 20 µL, and the column was operated at ambient temperature. These conditions were consistently used for all further validation and analytical runs.

Preparation of Calibration Curve

Preparation of Standard Solutions

A standard stock solution of Ozenoxacin (OZE) was prepared by accurately weighing 10 mg of the drug and dissolving it in methanol. The final volume was adjusted to 100 mL to achieve a concentration of 100 µg/mL. This stock solution was further diluted with the mobile phase to obtain a series of standard solutions with concentrations ranging from 2 to 20 µg/mL.

Procedure for Calibration Curve

The mobile phase was first allowed to equilibrate with the stationary phase until a stable baseline was achieved. Each prepared concentration was injected into the HPLC system, and the corresponding peak areas were recorded. A calibration curve was then constructed by plotting the peak area against drug concentration, which is depicted in Figure No. 12. This curve served as the reference for quantifying OZE in samples.

System Suitability Test

System suitability testing is a critical component of method validation, ensuring that the chromatographic system performs consistently and is suitable for analysis. This test was performed by injecting five replicates of a standard solution of Ozenoxacin. The

standard solution was prepared by dissolving 10 mg of OZE in the mobile phase and diluting to a concentration of 10 µg/mL. After equilibrating the system with filtered mobile phase, 20 µL of the solution was injected, and chromatographic parameters such as retention time, theoretical plates, tailing factor, and peak symmetry were evaluated. The results confirmed the adequacy and reliability of the system for further analysis, as presented in Table No. 10.

Application of Proposed Method for Estimation of OZE in Laboratory Sample

Preparation of Laboratory Standard and Sample Mixture

A laboratory standard solution of Ozenoxacin was prepared by dissolving 10 mg of the drug in 100 mL of mobile phase and sonicating for five minutes. The resulting solution was diluted to achieve a final concentration of 10 µg/mL. Similarly, five different laboratory mixtures were prepared by accurately weighing OZE and diluting with mobile phase to obtain the same concentration. The peak areas of both the standard and sample mixtures were compared to determine the concentration of OZE in the laboratory samples.

Precision

Precision was assessed by conducting replicate analyses of the laboratory-prepared samples. The relative standard deviation (RSD) and standard deviation (SD) were calculated to determine the repeatability and consistency of the method under identical conditions.

Application of Proposed Method for Estimation of OZE in Pharmaceutical Formulation

Standard and Sample Solution Preparation

For standard solution, 10 mg of Ozenoxacin was weighed accurately and dissolved in mobile phase in a 100 mL volumetric flask, sonicated for 10 minutes, and diluted to volume. The cream formulation equivalent to 10 mg of OZE was accurately weighed (from a 200 mg sample), transferred into a 100 mL flask, and treated with 50 mL of mobile phase. The mixture was sonicated, diluted to volume, and filtered through Whatman No. 41 filter paper.

Procedure

An equal volume (20 µL) of both standard and sample solutions was injected separately into the HPLC system after equilibrium. Chromatograms were recorded, and the peak areas were measured. The content of Ozenoxacin in the formulation was determined by comparing the sample peak with the standard peak area.

Validation Parameters

Accuracy

Accuracy was evaluated through recovery studies by the standard addition method. Accurately weighed portions of preanalyzed formulation were mixed with standard OZE solution at different concentration levels (80%, 100%, and 120%). The solutions were diluted appropriately, filtered, and analyzed.

Ruggedness

Ruggedness was examined under varying conditions, including interday, intraday, and by different analysts.

Interday: The analysis was performed over three consecutive days, and the % label claim was calculated.

Intraday: The analysis was repeated at 3-hour intervals within a single day.

Different Analysts: The same procedure was carried out independently by two analysts to confirm reproducibility. The data showed consistent results, confirming the ruggedness of the method.

Specificity

Specificity was determined by evaluating the chromatographic behavior of OZE in the presence of formulation excipients. A single sharp peak with retention time at 4.917 minutes was observed, with no interference from excipients, confirming the specificity of the method.

Linearity and Range

Linearity was tested over the range of 80% to 120% of the test concentration, as per USP guidelines. A series of concentrations was prepared and injected, and a linear relationship was established between concentration and peak area. The method demonstrated excellent linearity, suitable for quantitative analysis.

Robustness

Robustness was evaluated by deliberately varying method parameters such as mobile phase composition, flow rate, and detection wavelength. The method remained unaffected by these minor changes, demonstrating its robustness and reliability.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined based on signal-to-noise ratios. The LOD represents the lowest detectable amount of analyte, while LOQ indicates the lowest quantifiable concentration with acceptable accuracy and precision. These parameters confirm the sensitivity of the method.

Forced Degradation Studies

1. Acidic Degradation

The sample was treated with 10 mL of 0.1N HCl and heated at 60°C for 1 hour. After cooling, it was diluted to 10 µg/mL and analyzed by HPLC. The degradation was evaluated by observing peak purity and percentage degradation.

2. Alkaline Degradation

The test solution was subjected to 1N NaOH under the same heating conditions. It was diluted, filtered,

and analyzed to determine degradation percentage and peak purity.

3. Peroxide Degradation

A 10% hydrogen peroxide solution was used for oxidative stress testing. The sample was heated at 60°C for 1 hour, then prepared and analyzed to assess degradation.

4. Thermal Degradation

The solid and liquid samples were exposed to 100°C for 24 hours in an oven. After treatment, the sample was diluted and analyzed to study degradation behavior.

5. Photodegradation

The solid drug was exposed to direct sunlight for one day, and additional testing under UV light was done for 3 days using concentrations of 30 µg/mL and 50 µg/mL. The prepared solutions were analyzed by HPLC to evaluate any photolytic changes.

RESULTS AND DISCUSSIONS:

The study focused on developing and validating an RP-HPLC method for the estimation of Ozenoxacin (OZE), a recently marketed semisolid antibiotic formulation. The drug's purity, as reported by the supplier, was 99.8% and used as a reference. Initial physicochemical characterization included melting point determination, UV and FT-IR spectroscopy, confirming the identity and purity of OZE.

Melting Point

The melting point of Ozenoxacin was found to be 252°C, which indicates its purity and matches reported literature values.

FT-IR Analysis

The FT-IR spectrum of OZE showed characteristic peaks at 3106.40 cm^{-1} (N–H), 2914.08 cm^{-1} (C–H), 3320.56 cm^{-1} (O–H), 1695.09 cm^{-1} (CO), and 1652.85 cm^{-1} (C=O), confirming the functional groups present in the drug.

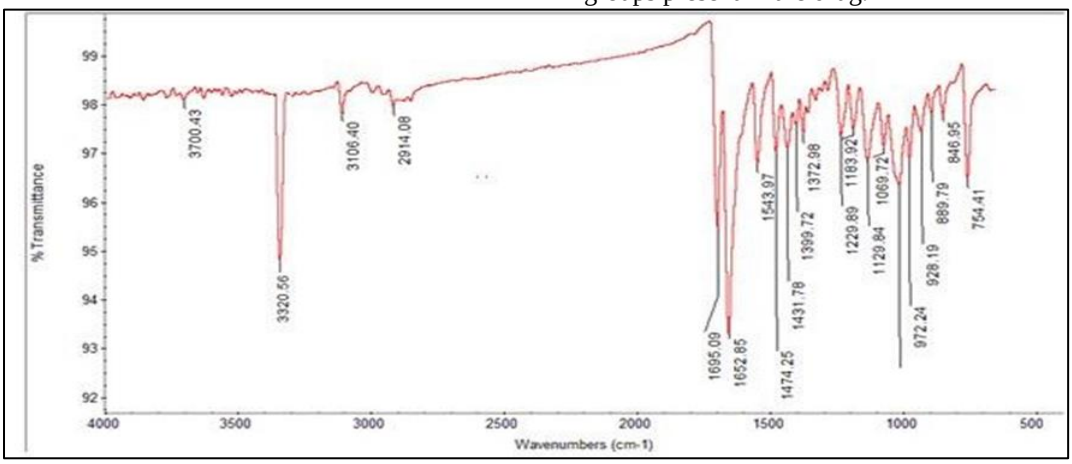

Figure 2: FT-IR Spectra of OZE

UV Analysis

Using UV spectrophotometry, the λmax of Ozenoxacin was found to be 309 nm**,** which was used for HPLC detection.

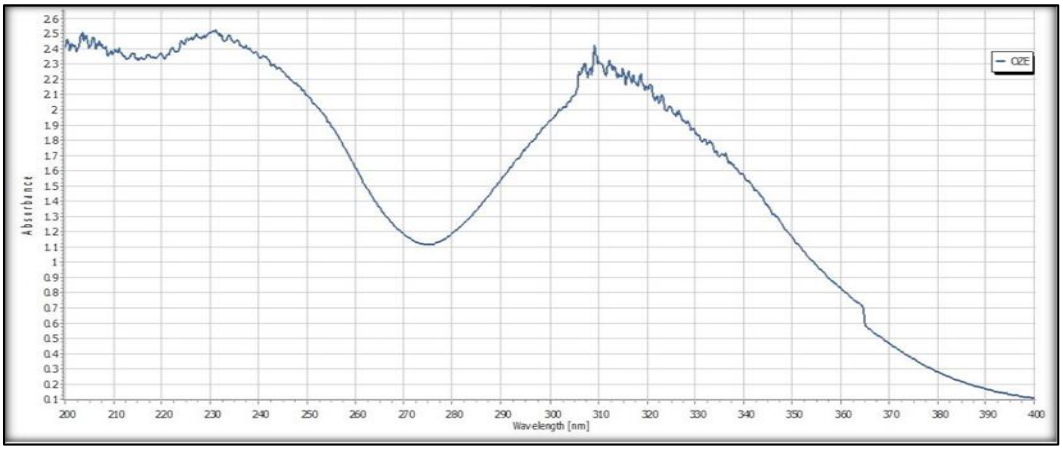

Figure 3: UV Spectra of OZE

Mobile Phase Selection

Various mobile phases were tested, including methanol and methanol-water mixtures. Based on peak shape, retention time, and resolution, methanol: phosphate buffer (60:40, pH 3.5) was selected as the optimized mobile phase for HPLC analysis.

From various mobile phases tried, mobile phase containing Methanol: Phosphate Buffer (60:40) pH 3.5 was selected, since it gives sharp reproducible retention time for OZE.

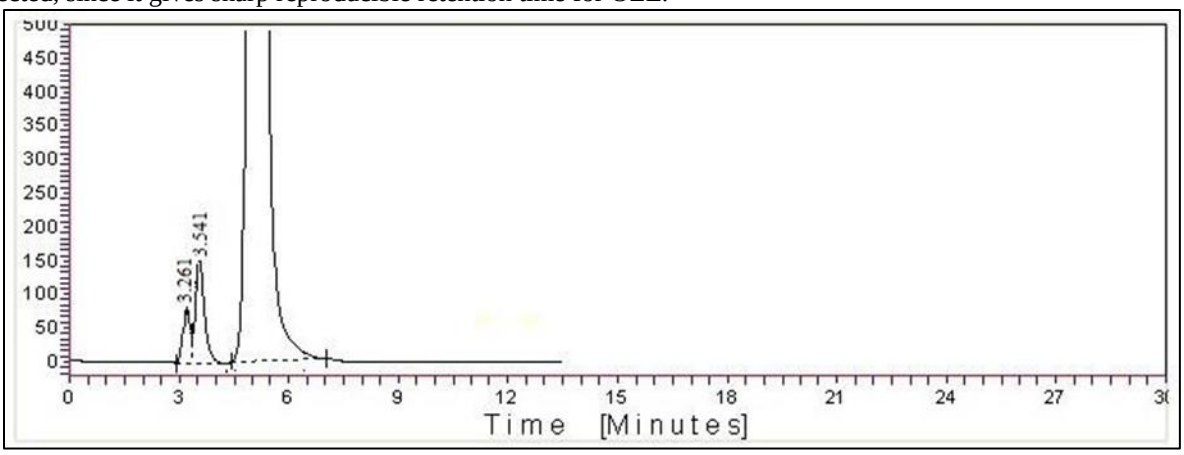

Figure 4: Trial Chromatogram obtained by using Methanol: Water (90:10) as mobile phase.

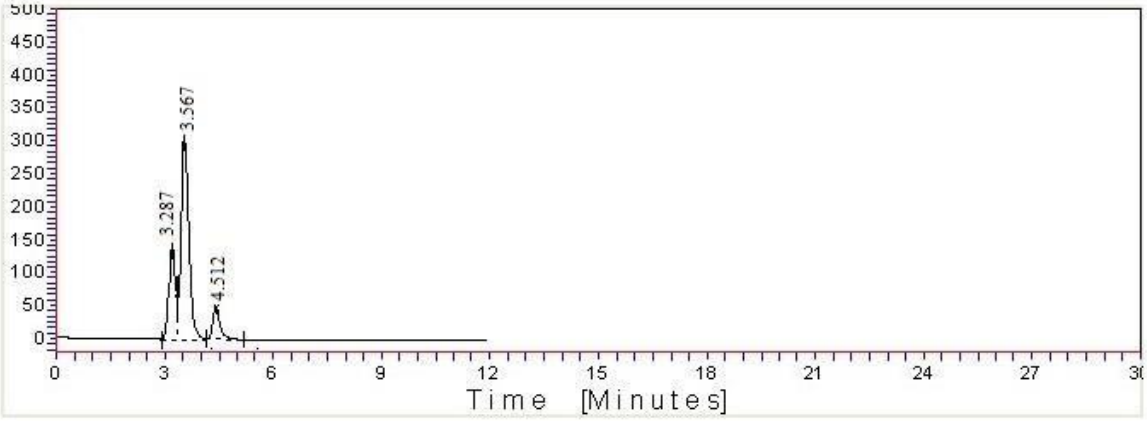

Figure 5: Trial Chromatogram obtained by using Methanol: Phosphate Buffer (80:20) pH 5 as mobile phase.

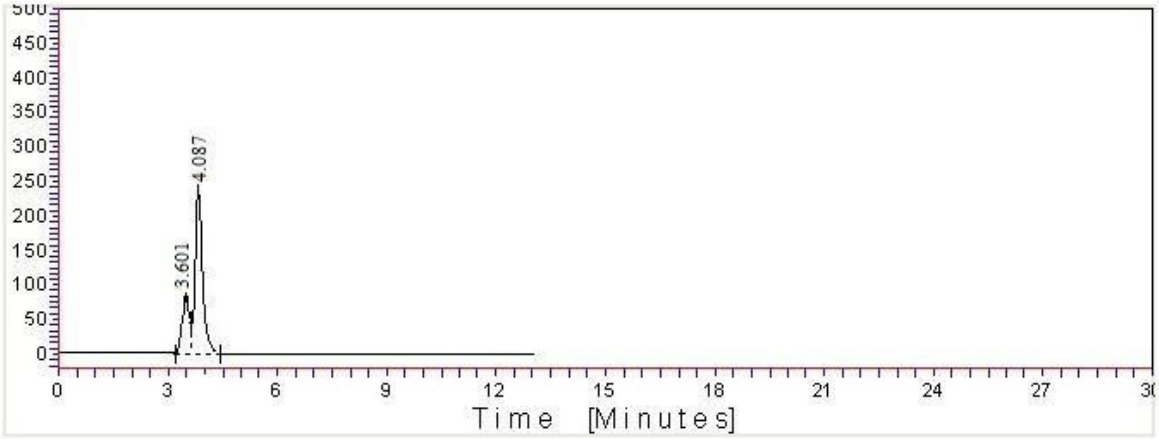

Figure 6: Trial Chromatogram obtained by using Methanol: Phosphate Buffer (70:30) pH 4.5 as mobile phase.

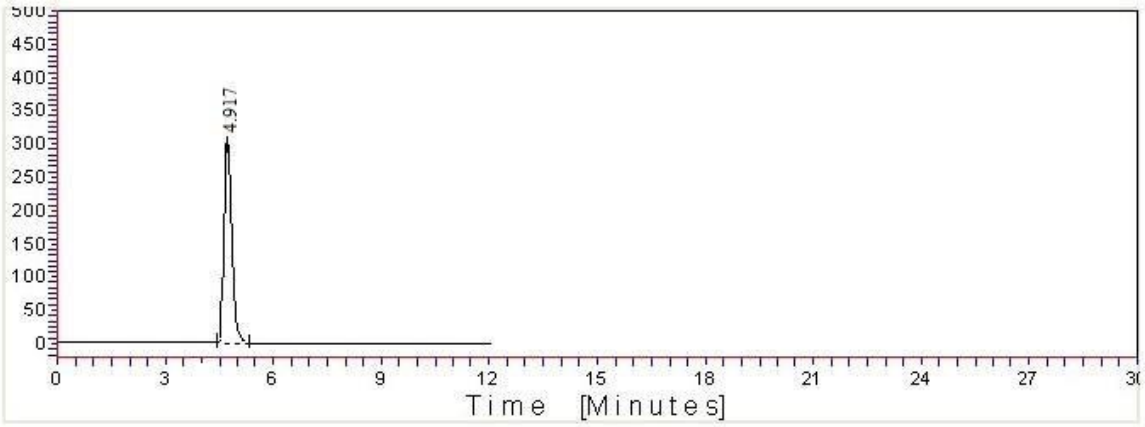

Figure 7: Final Chromatogram obtained by using Methanol: Phosphate Buffer (60:40) pH 3.5 as mobile phase of OZE.

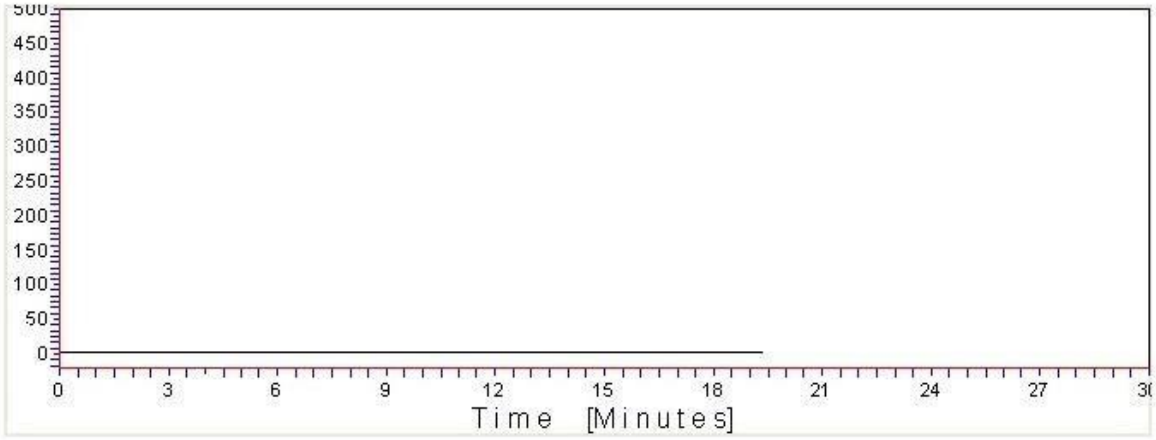

Figure 8: Blank Chromatogram obtained by using Methanol: Phosphate Buffer (60:40) pH 3.5 as mobile phase.

Preparation of calibration curve:

The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The series of concentration from 2-20 µg/ml for OZE drug solutions was injected and peak area was recorded.

Table No.01 : Observation of standard curve of OZE

Sr No	Conc.(g/ml) OZE	Peak Area OZE
1	2	2497.4
2	4	4994.8
3	6	7492.2
4	8	9989.6
5	10	12487
6	12	14984.4
7	14	17481.8
8	16	19979.2
9	18	22976.6
10	20	24974.1

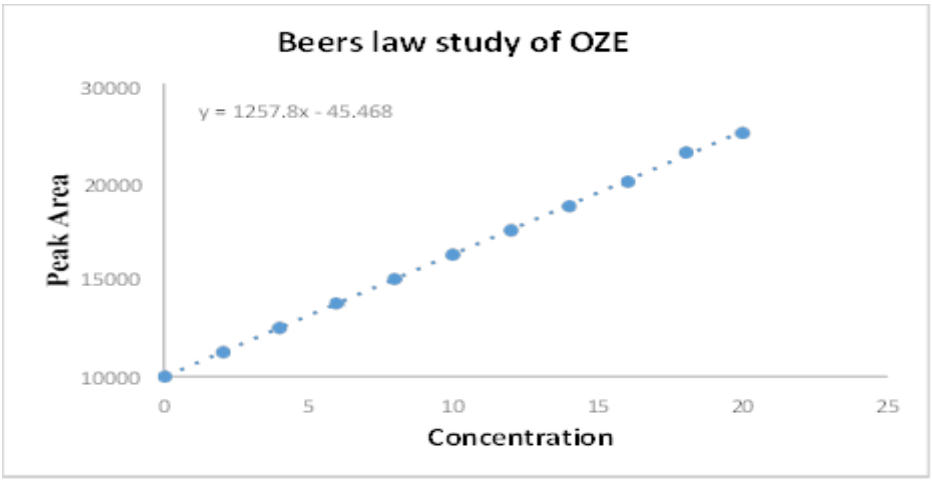

Figure 9: Standard calibration curve for OZE

System suitability test:

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions.

Table No. 02: Result of System Suitability Study

Sr. No	Peak area	Retention Time	Asymmetry	Efficiency
	OZE	OZE	OZE	OZE
1	12487.7	4.917	1.589	32445.4
2	12454.9	4.921	1.523	32456.6
3	12501.1	4.945	1.501	32444.2
4	12489	4.919	1.533	32481.1
5	12433.5	4.920	1.556	32432.8
Mean	12473.24	4.9244	1.5404	32452.02
± S.D	28.05918744	0.01161034	0.033597619	18.30988804
C.V	0.002249551	0.002357717	0.021810971	0.000564214

Application of proposed method for estimation of OZE Laboratory Sample: The standard and Sample solution of OZE was prepared and inject. The peak area of standard and sample laboratory was compared to obtain the concentration.

Table No. 03: Results and statistical data for estimation of OZE in lab. Sample

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Standard	Peak Area of Sample	% Drug Estimation
	OZE	OZE	OZE	OZE	OZE
1	10	10	12487	12536.9	100.4
2		10		12412.1	99.4
3		10		12462.0	99.8
				Mean	99.87
				±S.D.	0.503
				C.V.	0.005

*Results are mean of three replicates

Application of proposed method for estimation of OZE in formulation:

Equal volume (20µL) of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content OZE was calculated by comparing a sample peak with that of standard.

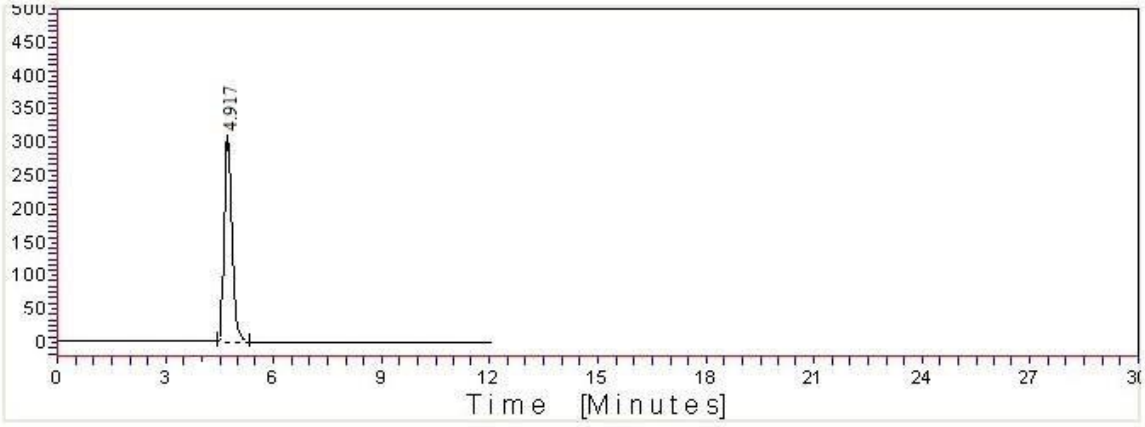

Figure 10: Chromatogram obtained by formulation of OZE

Validation parameters:

Accuracy:

It was ascertained on the basis of recovery studies performed by standard addition method. The results of recovery studies and statistical data are recorded.

Table No.04: Results and statistical data for Recovery study of OZE

Sr. No	wt. of formulation (mg)	Amount of Drug Added in (µg/ml)	Peak Area of stand.	Peak Area of sample	% Recovery
	OZE	OZE	OZE	OZE	OZE
1	200	1	12487	12437.1	99.6
2		1		12586.9	100.8
3		1		12599.4	100.9
4		2		12624.4	101.1
5		2		12674.3	101.5
6		2		12512.0	100.2
7		3		12437.1	99.6
8		3		12524.5	100.3
9		3		12536.9	100.4
				Mean	100.49
				S.D.	0.649
				C.V	0.006

*Results are mean of three replicates

Precision:

Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method. The Results and statistical data of Precision Study of OZE is recorded and shown in following table.

Table No.05: Results and statistical data of Precision Study Brand Name: OZX

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Label claim
	OZE	OZE	OZE	OZE	OZE
1	10	200	12487	12536.9	100.4
2		200		12412.1	99.4
3		199.9		12462.0	99.8
				Mean	99.87
				±S.D.	0.503
				C.V.	0.005

*Results are mean of three replicates

Ruggedness:

The studies of ruggedness were carried out under two different conditions-

- Days
- Analyst.

Interday (Different days):

Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated. Data obtained for day 1, day 2, and day 3 is shown in Table 06.

Table No.06: Results and statistical data of Interday Study Brand Name: OZX

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Label claim
	OZE	OZE	OZE	OZE	OZE
1	10	200	12487	12499.5	100.1
2		200		12437.1	99.6
3		200		12524.5	100.3
				Mean	100.00
				±S.D.	0.361
				C.V.	0.004

*Results are mean of three replicates

Intraday:

It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. Interval within a day. The percent label claim was calculated using formula & Result and statistical data are shown in Table No. 07

Table No.07: Results and statistical data of Intraday Study Brand Name: OZX

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Label claim
	OZE	OZE	OZE	OZE	OZE
1	10	200	12487	12586.9	100.8
2		200.1		12599.4	100.9
3		200		12624.4	101.1
				Mean	100.93
				±S.D.	0.153
				C.V.	0.002

*Results are mean of three replicates

Different analyst:

The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

Table No.08: Result and statistical data of Different analyst study

Sr. No	% Label claim	
	ANALYST I	ANALYST II
	OZE	OZE
1	100.3	99.9
2	100.4	100.1
3	100.5	99.6
4	101	100.3
5	101.1	100.4
Mean	100.66	100.06
S.D	0.364691651	0.320936131
C.V	0.003623005	0.003207437

*Results are mean of three replicates.

Specificity:

Specificity was measured as ability of the proposed method to obtain well separated peak for OZE without any interference from component of matrix. Mean retention time for – OZE – 4.917. The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix.

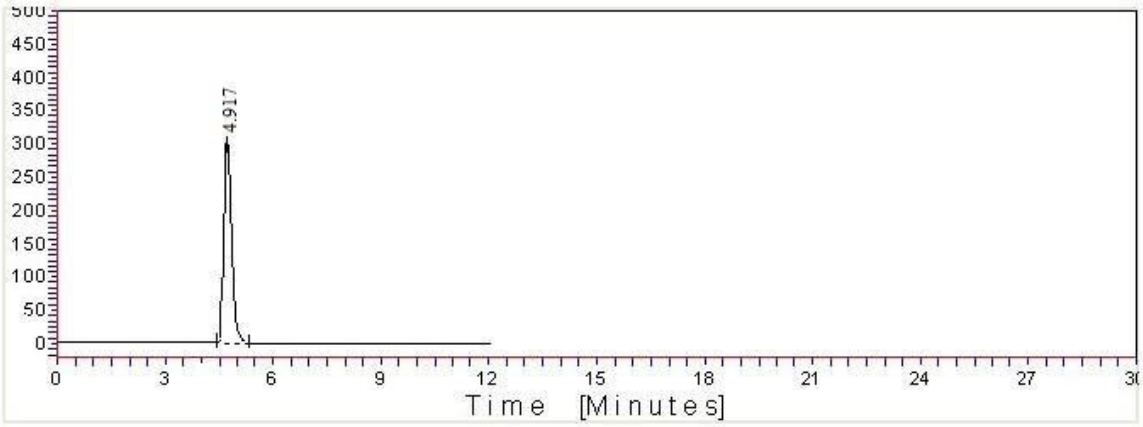

Figure 11: Chromatogram obtained by formulation of OZE

Linearity and range:

According to USP formulation equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions was recorded. OZE marketed formulation was found to be linear in the range ± 20% of the test concentration of the respective drug.

Table No.09: Observations of Linearity and range study for OZE.

Sr. No.	%Label claim	Peak area OZE
1	80	9989.6
2	90	11238.3
3	100	12487
4	110	13735.7
5	120	15184.4

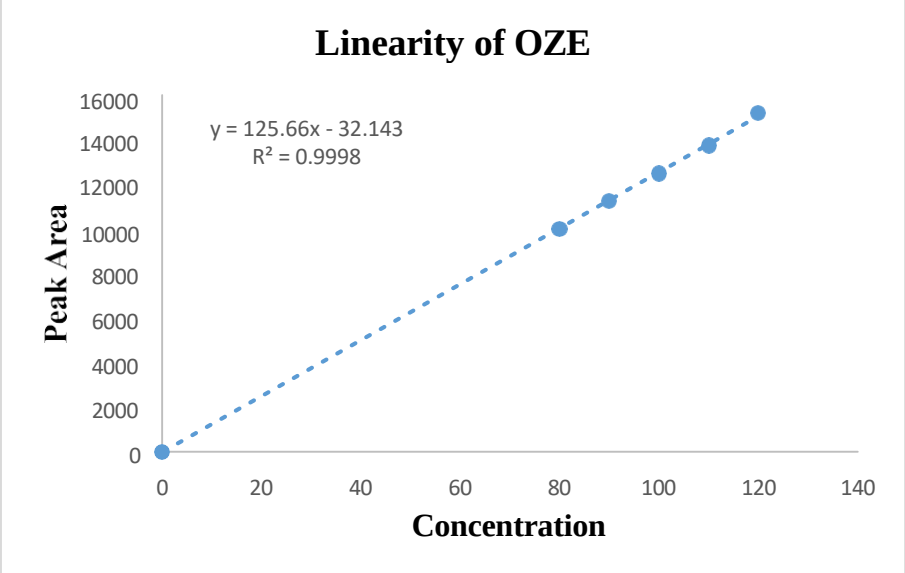

Figure 12: Plot of linearity and range study for OZE

The robustness study indicated that the factors selected remained unaffected by small variation of organic composition of mobile phase, wavelength and the flow rate. The system suitability results should lie within the limit. Hence the method was robust.

Table No.10: Result of Robustness study of OZE

Sr. No.	Condition	Parameter	Peak Area	RT
01	Change of wavelength	307 nm	12401.8	4.921
02		309 nm	12491.2	4.918
03		311 nm	12487	4.917
04	Change in Temperature	30 ºC	12456	4.912
05		25 ºC	12487	4.917
06		20 ºC	12432	4.920
07	Change in Flow rate	0.8 ml/min	12501	4.987
08		1ml/min	12487	4.917
09		1.2 ml/min	12431	4.899
10	Change in Mobile Phase	55:45	12478	4.901
11		60:40	12487	4.917
12		65:35	12491	4.945

f) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision accuracy.

Table 11: LOD & LOQ of OZE

Sr. No.	Drug Name	LOD (g/ml)	LOQ (g/ml)
1	OZE	0.072	0.219

Forced degradation study

Acid stress degradation:

Forced degradation in acidic media was performed, the result of OZE with acid degradation shown in bellow table 12.

Table No. 12: Acid Stressed Degradation of OZE

OZE			
Sr. No.	**Parameter**	**Unstressed Test**	**Stressed Test**
01	Purity-1 angle	0.056	0.056
02	Purity-1 threshold	0.177	0.177
03	Purity Flag	No	No
04	Area	12487	11213.33
05	% Degradation	10.2	

Base stress degradation:

Forced degradation in alkali media ie NAOH was performed, the result of OZE with Base degradation shown in bellow table 13.

Table No. 13: Alkali Stressed Degradation of OZE

OZE			
Sr. No	**Parameter**	**Unstressed Test**	**Stressed Test**
01	Purity-1 angle	1.076	1.076
02	Purity-1 threshold	0.782	0.782
03	Purity Flag	No	No
04	Area	12487.1	10976.07
05	% Degradation	12.1	

Peroxide stress degradation:

Forced degradation in peroxide media ie H_2O_2 was performed, the result of OZE with peroxide degradation shown in bellow table 14.

Table No. 14: Peroxide Stressed Degradation of OZE

OZE			
Sr. No	**Parameter**	**Unstressed Test**	**Stressed Test**
01	Purity-1 angle	0.132	0.132
02	Purity-1 threshold	0.365	0.365
03	Purity Flag	No	No
04	Area	12487	11013.53
05	% Degradation	11	.8

Thermal stress degradation:

Forced degradation in Thermal stress degradation by heating the sample in oven was performed, the result of OZE with Thermal degradation shown in bellow table.

Table No. 15: Thermal Stressed Degradation of OZE

OZE			
Sr. No	**Parameter**	**Unstressed Test**	**Stressed Test**
01	Purity-1 angle	0.056	0.056
02	Purity-1 threshold	0.187	0.187
03	Purity Flag	No	No
04	Area	12487	12212.29
05	% Degradation	2.	2

Photo degradation

Forced degradation in Photo stress degradation by heating the sample by exposing the 30 and 50 microgram per ml to UV light was performed, the result of OZE with Photo degradation shown in bellow table 16.

Table No. 16: Photo Stressed Degradation of OZE

OZE			
Sr. No	**Parameter**	**Unstressed Test**	**Stressed Test**
01	Purity-1 angle	0.072	0.072
02	Purity-1 threshold	0.132	0.132
03	Purity Flag	No	No
04	Area	12487	12424.57
05	% Degradation	0.	5

The stress conditions applied for degradation study involved acid, base, neutral, sunlight, thermal, UV photolysis, and oxidative degradation for find out the stability nature of the drug. The degradation samples were prepared by taking suitable aliquots of the drug and drug product solution and then undertaking the respective stress testing procedures for each solution.

Table No 17: Stressed Degradation studies of OZE

Sr. No.	Injection	% Degradation	Purity Angle	Purity Threshold	Purity Flag
1	Acid Degradation	10.2	0.056	0.177	No
2	Base Degradation	12.1	1.076	0.782	No
3	Peroxide Degradation	11.8	0.132	0.365	No
4	Thermal Degradation	2.2	0.056	0.187	No
5	UV Degradation	0.5	0.072	0.132	No

CONCLUSIONS:

From the studies it can be concluded that RP-HPLC technique can be successfully used for the estimation of the Ozenoxacin (OZE) in their pharmaceutical formulations. The method shows good reproducibility, the RP-HPLC method is accurate, precise, specific, reproducible and sensitive. The analysis of semisolid dosage formulation of Ozenoxacin (OZE) can also be successfully performed. No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies. The forced degradation studies were carried out as per ICH guidelines and the results revealed suitability of the method to study stability of OZE under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations. it was also concluded that the method is simple, sensitive and ability to separate the drug from degradation products and excipients found in the

dosage form. No interference of additives, matrix etc. is encountered in these methods.

REFERENCES:

1. Dictionary.com Unabridged (v 1.1), Random House, Inc., 2007.
2. Merriam-Webster's Medical Dictionary, Merriam-Webster, Inc., 2007
3. The American Heritage Science Dictionary, Houghton Mifflin Company, 2007.
4. Satoskar RS, Bhandarkar SD, Rege NN. Pharmacology and Pharmacotherapeutics, 21st edition, Popular Prakashan, Mumbai, India. p:1, 2009.
5. Anthony GC. Foundations of Osteopathic Medicine, 3rd edition, American Osteopathic Association, Lippincott Williams & Wilkins. p: 12, 2010.
6. Durand VM, David HB. Essentials of Abnormal Psychology, 5th edition, Wadsworth Publishing Company. p: 13, 2009.
7. Pizzi R. Salving with Science: The roaring twenties and the great depression, The Pharmaceutical Century: Ten decades of drug discovery supplements to American Chemical Society. p: 34, 2000.
8. Frey R, Lesney MS. Anodynes and Estrogens: The pharmaceutical decade, The Pharmaceutical Century: Ten decades of Drug Discovery Supplement to American Chemical Society. p: 92-109, 2000.
9. Henderson R. Managing innovation in the information age, Harvard Business Review. p: 100-105, 1994.
10. Sedlacek HH, Sapienza AA, Volker A. Ways to successful strategies in drug research and development, VCH, Wienheim and New York.
11. Tsinopoulos C, Mc Carthy I. The evolution of strategies for drug discovery: From accidental discovery to rational drug design, Proceedings of R&D management conference, Dublin. p: 467-474, 2001.
12. Watari N, Sugiyama Y, Kaneniwa N, Hiura M. Prediction of hepatic first pass metabolism and plasma levels following intravenous and oral administration of barbiturates in the rabbit based on quantitative structure pharmacokinetic relationships. Journal of Pharmacokinetics and Biopharmaceutics, 1998, 16, 279-301.
13. Wrighton AS, Branden MV, Ring JB. The human drug metabolizing cytochromes P450. Journal of Pharmacokinetics and Biopharmaceutics, 1996, 24 (5), 461-473.
14. Mc Ginnity FD, Soars GM, Urbanowicz AR, Riley JR. Evaluation of fresh and cryopreserved hepatocytes as in vitro drug metabolism tools for the prediction of metabolic clearance. Drug Metabolism and Disposition, 2004, 32 (11), 1247-1253.
15. Shen SC, Chang WC, Chang CL. An extract from wax apple (Syzygium samarangense (Blume) Merrill and Perry) effects glycogenesis and glycolysis pathways in tumor necrosis factor-α-treated FL83B mouse hepatocytes. Nutrients, 2013, 5 (2), 455-467.
16. Kern A, Bader A, Pichlmayr R, Sewing KF. Drug metabolism in hepatocyte sandwich cultures of rats and humans. Biochemical Pharmacology, 1997, 54 (7), 761-772.
17. Hengstler JG, Utesch D, Steinberg P, Platt KL, Diener B, Ringel M, Swales N, Fischer T, Biefang K, Gerl M, Bottger T, Oesch F. Cryopreserved primary hepatocytes as a constantly available in vitro model for the evaluation of human and animal drug metabolism and enzyme induction. Drug Metabolism Reviews, 2000, 32 (1), 81-118.
18. Atherden LM, Edr. Bentley and Drivers Text Book of Pharmaceutical Chemistry, 8th Edition, Oxford University Press, 1966, 4th Impression.
19. Deorge RF, Edt, Wilson. Gisvold's Text Book of Organic Medicinal and Pharmaceutical Chemistry, 8th edition, Lippincott Company, 1982
20. Topliss JG. Quantitative Structure – Activity Relationships of Drugs, Vol 19, Academic Press, London, 1983.
21. Korolkovas A. Essentials of Medicinal Chemistry, 2nd edition, Wiley Interscience, New York 1988.
22. Definition and classification of Drug or Pharmaceutical Regulatory aspects of drug approval, 2013.
23. Bazaj ME. Edition, Indian Pharmaceutical Guide, Pamposh Publications, New Delhi, 1987.
24. Lachman L, Lieberman HA, Kaning JL. The Theory and Practice of Industrial Pharmacy, 3rd Edition, Varghese Publishing House, Bombay, 1990.
25. Mithal BM. Text Book of Pharmaceutical formulations, 4th Edition, Vallabh Prakashan, New Delhi, 1991.
26. CIMS, Current Index of Medicinal Specialties, (Jan- Mar) 2010.
27. Rappoport Z, Liebman JF. The chemistry of hydroxylamines, oximes and hydroxamic acids, Part-1. John Wiley & Sons Ltd. p: 516, 2009.
28. Paul K, Nagarajan JSK, Chandan RS. Standardization an ARP-HPLC method for the estimation of a ceclofenac in dosage form. International Journal of Research in Pharmacy and Chemistry, 2011, 1 (4), 853-859.

29. Day RA, Underwood AL. Quantitative Analysis, 5th Edition, Prentice Hall, New Delhi, 1986.
30. Willard HH, Merrit LL Jr, Dean JA, Settle FA Jr. Instrumental Methods of Analysis, 6th Edition, CBS Publishers, New Delhi, 1986.
31. Gore AB, Munde MK, Rukhe NB, Kulkarni NS. A Review on HPLC Method Development and Validation for Gliptin Class: New Oral Antidiabetic Agents. Research Journal of Pharmaceutical Dosage Forms and Technology. 2022;14(1):79- 86.
32. Stojanović J, Krmar J, Protić A, Svrkota B, Djajić N, Otašević B. DoE Experimental design in HPLC separation of pharmaceuticals; a review. Archives of Pharmacy. 2021 Aug 27;71(Notebook 4):279-301.
33. Nalini CN, Kumar V. A Review of Different Analytical Techniques for Fexofenadine Hydrochloride and Montelukast Sodium in Different Matrices. Critical reviews in analytical chemistry. 2020;2:1-4
34. Vyas AJ, Nathwani GP, Patel AI, Patel NK, Patel AB. Validated stability indicating RP-HPLC DAD method for simultaneous determination of amitriptyline hydrochloride and pregabalin in presence of stress degradation products in tablet dosage form. Chemical Methodologies. 2019 Jul 1;3(4):494-08.
35. Vhanmane A, Tamboli A, More S. RP-HPLC Method Development and Validation for the Simultaneous Estimation of Gabapentin and Amitriptyline Hydrochloride in Pharmaceutical Dosage Forms. Journal of Drug Delivery and Therapeutics. 2019;9(3- s):62-8.
36. Jagadeesh K, Annapurna N. Stability Indicating Method Development and Validation of Metformin and Ertugliflozin by High-Performance Liquid Chromatography with PDA Detection and its application to tablet dosage form. Asian Journal of Pharmaceutical and Clinical Research. 2019;12(3): 353-58.
37. Gedawy A, Al-Salami H, Dass CR. Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide. Journal of food and drug analysis. 2019;27(1):315-22.
38. Kant R, Bodla RB, Kapoor G, Bhutani R. Optimization of a single HPLC-PDA method for quantifying metformin, gliclazide, pioglitazone, dapagliflozin, empagliflozin, saxagliptin, linagliptin and teneligliptin using central composite design. Bioorganic chemistry. 2019;91:103-11.
39. Gundala A, Prasad KV, Koganti B. Application of quality by design approach in RP- HPLC method development for simultaneous estimation of saxagliptin and dapagliflozin in tablet dosage form. Brazilian Journal of Pharmaceutical Sciences. 2019; 24:55.
40. Donepudi S, Achanta S. Simultaneous estimation of saxagliptin and dapagliflozin in human plasma by validated high performance liquid chromatographyUltraViolet method. Turkish Journal of Pharmaceutical Sciences. 2019;16(2):227.
41. Padmaja BR, Sivagami B, Chandrasekar R, Babu MN. A highly validated RPHPLC method development for the simultaneous estimation of dapagliflozin and saxagliptin in tablet dosage forms. Int J Pharm Sci Drug Res. 2018;10(5):372-8.
42. Deepan T, Dhanaraju MD. Stability indicating HPLC method for the simultaneous determination of dapagliflozin and saxagliptin in bulk and tablet dosage form. Current Issues in Pharmacy and Medical Sciences. 2018;31(1):39-43.
43. Singh N, Bansal P, Maithani M, Chauhan Y. Development and validation of a stability-indicating RP-HPLC method for simultaneous determination of dapagliflozin and saxagliptin in fixed-dose combination. New Journal of chemistry. 2018;42(4):2459-66