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Stability Indicating Validated Novel RP-HPLC Method for Dexlansoprazole and LC-MS/MS Study of Degradation Product

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ABSTRACT

This work effectively developed and validated a stability-indicating RP-HPLC technique for precisely measuring dexlansoprazole in bulk samples. An Acetonitrile and 0.5 mmol Ammonium Acetate (pH 4.5) gradient mobile phase, a 1 ml/min flow rate, and detection at 283 nm were all part of the method's unique chromatographic conditions. A Kromasil C18 column was also used. Dexlansoprazole had a 5.14-minute retention period, and the technique showed a linear range of 5–30 mg/ml with a strong correlation value of 0.997. The technique showed high sensitivity with a limit of detection (LOD) of 1.2 mg/ml and a limit of quantification (LOQ) of 3.64 mg/ml. The accuracy of the approach was shown by the percentage recovery of dexlansoprazole, which varied from 98.6% to 102%. The International Council for Harmonisation (ICH) gave standards for validating the created approach, which were followed. It covered several variables: linearity, LOD, LOQ, accuracy, precision, robustness, and solution stability. The technique demonstrated stability and the capacity to successfully separate the degradation products from the analyte peaks, establishing its validity as a stability-indicating technique. LC-MS/MS spectra were also used to establish the degradants' structures.The quantitative measurement of dexlansoprazole in bulk form may be accomplished effectively using this established RP-HPLC technique. It is a useful instrument for quality control and stability evaluation of dexlansoprazole in pharmaceutical formulations because of its accuracy, precision, and stability-indicating capabilities.

Keywords: Dexlansoprazole, HPLC, Validation, Stability Indicating, LC-MS/MS.

INTRODUCTION

Proton pump inhibitors (PPIs), such as dexlansoprazole, are frequently used to treat gastroesophageal reflux disease (GERD) and other gastrointestinal problems. It is a lansoprazole derivative with better pharmacokinetic characteristics, such as increased acid suppression and a longer duration of action. The therapeutic effectiveness of dexlansoprazole (Figure1), which comes in delayed-release capsule form, depends on the exact and accurate measurement of the active pharmaceutical ingredient (API) in pharmaceutical formulations [1-3].

The creation of accurate and effective analytical techniques for the determination of dexlansoprazole is of utmost relevance in the field of pharmaceutical analysis. These techniques are intended to enhance pharmacokinetic investigations and therapeutic drug monitoring and to guarantee the quality, safety, and efficacy of pharmaceutical goods containing dexlansoprazole. High-performance liquid chromatography (HPLC), one of the several analytical methods available, is frequently used for the examination of dexlansoprazole due to its sensitivity, selectivity, and adaptability [4-5].

Determining an adequate stationary phase, optimizing the mobile phase's composition, choosing an appropriate detection wavelength, and validating the technique's performance parameters are all crucial steps in developing an analytical method for dexlansoprazole. For the analytical technique to comply with regulatory criteria and guarantee the accurate quantification of dexlansoprazole, it must have excellent sensitivity, precision, accuracy, linearity, and robustness. Determining the degradation products is also essential for evaluating the stability and quality of formulations containing dexlansoprazole.

Advanced methods, such as liquid chromatography-mass spectrometry (LC-MS/MS), which offers excellent selectivity and sensitivity, are often used to identify and quantify degradation products [6].

In this study, we describe the creation and approval of an HPLC technique for dexlansoprazole analysis and identify its degradation products by LC-MS/MS. The technique intends to offer precise and reliable dexlansoprazole measurement in pharmaceutical formulations and evaluate its stability under various circumstances. To guarantee the method's dependability and usefulness in regular analysis, the validation is carried out by specified rules [7].

The findings of this study will advance the field of developing analytical methods for dexlansoprazole and offer insightful information to regulatory authorities, researchers, and pharmaceutical analysts involved in the development, production, and quality assurance of pharmaceutical products containing dexlansoprazole [8-9].

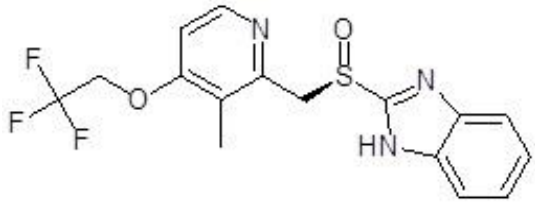


Figure (1): Chemical Structure of Dexlansoprazole.

Drug substance analysis must be accurate and exact in order to guarantee the quality and safety of drugs. Like any pharmaceutical substance, dexlansoprazole is subject to degradation in various storage and environmental situations. The identification and measurement of degradation products are essential for determining the stability of the drugs and ensuring that they keep their intended therapeutic qualities. This work helps maintain the drug's quality and safety profile by developing and verifying an HPLC technique combined with LC-MS/MS to analyze dexlansoprazole and its breakdown products. Establishing validated analytical methods is a fundamental requirement to meet these regulatory standards. By thoroughly developing and validating the proposed HPLC method for the analysis of dexlansoprazole, this study provides an essential contribution to complying with regulatory requirements and facilitates the approval process for the drug's manufacturing and commercialization.

This study addresses key aspects such as drug quality and safety, regulatory compliance, method selection and optimization, and scientific knowledge enhancement. The findings of this research will contribute significantly to the field and provide valuable insights for pharmaceutical analysts, researchers, and regulatory authorities involved in the development, production, and quality control of dexlansoprazole-containing pharmaceutical products [1-5].

There are some work carried out by some of the researchers as follows,

The study conducted by Sahu et al. focused on developing and validating a stability-indicating HPLC method for determining dexlansoprazole in pharmaceutical dosage forms. The researchers aimed to establish a reliable analytical method to accurately quantify dexlansoprazole and differentiate it from its potential degradation products. [10]. Sharma et al. developed and validated an RP-HPLC method for simultaneously estimating

dexlansoprazole and domperidone in capsule dosage forms. The researchers utilized a liquid chromatography system with a reversed-phase column and UV detection. The method demonstrated good linearity, accuracy, and precision, making it suitable for analyzing these drugs in pharmaceutical formulations. [11]. Lu et al. developed a reversed-phase high-performance liquid chromatography (RP-HPLC) method for determining dexlansoprazole in delayed-release capsules. The study utilized a liquid chromatography system with a reversed-phase column and UV detection, demonstrating the method's suitability for the quantitative analysis of dexlansoprazole in pharmaceutical formulations.[12]. Qian and Yin (2019) developed and validated an HPLC method for determining dexlansoprazole in human plasma, enabling pharmacokinetic studies. The researchers utilized a liquid chromatography system with a suitable column and UV detection. The validated method demonstrated good linearity, sensitivity, precision, and accuracy, making it suitable for analyzing dexlansoprazole levels in plasma samples for pharmacokinetic investigations.[13]. Xu et al. developed an LC-MS/MS method to quantify dexlansoprazole simultaneously, its two main metabolites, and lansoprazole in human plasma. The study employed liquid chromatography and tandem mass spectrometry for accurate and sensitive analysis. The method demonstrated good linearity, precision, accuracy, and selectivity, making it suitable for pharmacokinetic studies and therapeutic drug monitoring of these compounds in clinical settings [14].

Through some of the HPLC and few LC-MS methods, the detailed analysis of dedredation products is not reported to date. Hence, the study aims to develop and validate an HPLC method for the analysis of dexlansoprazole, a drug used to treat gastric acid-related disorders. Additionally, the study aims to detect and characterize the degradation products of dexlansoprazole using LC-MS/MS. The method development and validation will ensure accurate quantification of dexlansoprazole and provide valuable insights into its stability and potential degradation pathways, contributing to understanding its quality and safety in pharmaceutical formulations.

METHODS

Chemicals

Dexlansoprazole was obtained as a gift sample from ZIM Laboratory Pvt Ltd, Nagpur, Maharashtra, India. HPLC grade acetonitrile and Analytical reagent (AR) grade Ammonium acetate, Sodium hydroxide, hydrogen peroxide, and hydrochloric acid were procured from Merck Laboratory Pvt. Ltd., Mumbai, Maharashtra, India. High purity HPLC grade water obtained from MiliporeM-lli Q plus system (Milford, MA, USA)

Instrument and Equipment

Table (1): List of Instrument and Equipment.

Sr. No.	Particulars	Manufacturers	Model
1	HPLC	Perkin Elmer	Series 200
2	UV-Vis spectrophotometer	Perkin Elmer	Lambda 25
3	FT-IR Spectrophotometer	Shimadzu	IR Affinity-1S

Sr. No.	Particulars	Manufacturers	Model
4	LC-MS/MS	Shimadzu	LC/MS/MS 8040
5	Weighing balance	Shimadzu	AUX220
6	Digital pH meter	LABINDIA	Pico^{+}
7	Micropipette (100-1000µl)	Biosystem	-
8	Micropore filter	Elix	-
9	Filtration assembly	-	-

Characterization

Color: A small quantity of DXLwas injected into the watch glass to inspect the color visually.

Odor: The drug was placed in the watch glass and was smelled.

Melting Point: The melting point of DXL was determined using Thill's tube apparatus and is uncorrected. The results of characterization are shown in (Table 2).

Solubility: In flask 1, 1 ml of water was added after accurately weighted DXL (10 mg) was transferred to a 10 ml volumetric flask (VF). For one minute, the solution was combined. The drug was discovered to be soluble.

In flask 2, 1 ml of methanol was added after accurately weighing 10 mg of DXL was transferred to 10 ml of VF. For one minute, the solution was combined. The drug was discovered to be easily soluble.

In flask 3, 1 ml of acetonitrile was added after accurately weighing 10 mg of DXL was put to 10 ml of VF. For one minute, the solution was combined. The drug was discovered to be easily soluble.

In flask 4, 1 ml of acetone was added after accurately weighing 10 mg of DXL was transferred to 10 ml of VF. For one minute, the solution was combined. The drug was discovered to be easily soluble. The results of Solubility are shown in (Table 3.)

Identification

FT-IR Spectrum analysis by ATR method

The IR spectrum was obtained by using ATR. The characteristic infrared absorptions of a functional group of DXL are summarized in (Table 4). Based on the FTIR spectrum (Figure 2), the identification of DXL was established. [10,11,12]

Determination of Wavelength of maximum absorbance of DXL

A UV-VIS spectrophotometer was used to calculate the wavelength from the UV spectrum of the DXL. To make the stock solution, 10 mg of DXL were precisely weighed, transferred to a 10 ml volumetric flask, dissolved in acetonitrile, and then brought up to 10 ml with acetonitrile. This yielded a drug stock solution with 1000 g/ml concentration. DXL's working standard solution was diluted to 100 g/ml. From a stock solution of 100 g/ml, an additional 10 g/ml was made in order to determine the wavelength.

The absorption spectra of the reference standard (acetonitrile) used as a blank were compared to the standard solution of DXL at 10 g/ml in the wavelength range of 200-400 nm (Figure 3) [13,14].

Chromatographic condition

A filtered and degassed combination of ACN (HPLC grade) and 0.5 mmol ammonium acetate (pH 4.5) was employed as the diluent to accomplish chromatographic separation in gradient mode. Other factors include the detection wavelength of 283 nm and a flow velocity of 1 ml/min.Dexlansoprazole separation was optimized using a runtime of 11 min, an injection volume of 20 l, and a column temperature of 300 c. The solution stability and the stress-degraded samples Samples were examined using a UV detector with a 200–400 nm wavelength range (Table 5). [15,16]

Preparation of 0.5 mmol Ammonium acetate (pH 4.5) buffer

Ammonium acetate accurately weighed at 19.64 mg, was transferred to a 500 ml volumetric flask, where it was diluted with HPLC-grade water and adjusted to a pH of 4.5 using acetic acid. Before use, the resulting solution was degassed in an ultrasonic bath and filtered through a 0.45-m membrane filter [17,18].

Preparation of standard solution

10 mg of DXL were accurately weighed and then transferred to a 10 ml volumetric flask. Acetonitrile and 1 ml of 0.5 mmol Ammonium acetate (pH 4.5) were added to make up the volume. The resultant solution contained around 1000 ppm. Before use, the resulting solution was degassed in an ultrasonic bath and filtered through a 0.45 mm membrane filter [19,20].

Initialization of Instrument

The HPLC was prewashed using mili Q water/methanol for 45 mins. The HPLC instrument was switched on. The column was washed with either HPLC grade water (mili Q Water) or methanol for 45 minutes. A filled HPLC syringe with the mobile phase was injected into the injector holder. The mobile phase was run to see if any peaks were observed [21,22].

Running Standard Solution of DXL

A 10 ml volumetric flask was constructed with a 20 g/ml stock solution. Acetonitrile and 0.5 mmol ammonium acetate (90:10) were employed as the diluent to bring the volume up to the required level. Before use, the solution was degassed in an ultrasonic bath and filtered through a 0.45 m membrane filter. The solution was injected into the HPLC system's injector following filtration. The chromatogram was subsequently obtained (Figure 4). [23,24,25]

Validation of Developed RP-HPLC Method

System Suitability Parameter

A system suitability test was performed with 100 µg/ml freshly prepared standard solution of DXL. After being injected in the HPLC system under optimized conditions, the system was run for drug solution. Parameters to be evaluated for the system's suitability and results obtained are given (Table 6).

Specificity

Preparation and Running of Placebo Solution

Magnesium stearate (3 mg) and starch powder (1.5 mg) were transferred to a 100 ml volumetric flask, and diluents [acetonitrile and 0.5mmol ammonium acetate (90:10)] was added for dissolution of the drug (10 mg).

This mixture was sonicated for 20 minutes to dissolve the content, and the volume was made up to the mark with diluents. After filtration, the solution was injected into the injector of the HPLC system [26,27,28].

Standard Solution of DXL

10 mg of the drug was accurately weighed and transferred to 10 ml of volumetric flask and dissolved in 10 ml of diluent [acetonitrile and 0.5mmol ammonium acetate (90:10)] to get the resultant solution contained 1000µg/ ml DXL [29,30].

Linearity

Linearity was assessed by creating standard solutions with varied DXL concentration levels ranging from 5 to 30 g/ml. The response curve was plotted between conc Vs.Area in (Table 7), and the calibration curve was generated as concentration *Vs*— area (Figure 5).

Precision

Repeatability

20µg/ml of the standard stock solution of DXL was prepared by taking 0.2ml from the stock solution of 1000 µg/ml in a 10ml volumetric flask, and the volume was made up to the mark with diluent. The resultant solution was filtered through a 0.45 µm membrane filter and degassed under the optimized condition. The peak area of the resultant solution was assessed using six determination test concentrations, i.e.20µg/ml of DXL. (Table 8) shows the data for repeatability analysis for the precision of the method [31,32,33].

Intra and Inter Day

Intraday and inter-day precision were carried out with 10µg/ml,15 µg/ml, and 20 µg/ ml of standard stock solution of DXL. The dilution was prepared with acetonitrile and 0.5 mmol ammonium acetate (90:10). The resultant solution was filtered through a 0.45 µm membrane filter and degassed under the ultrasonic bath prior to use and in triplicate and was injected into the HPLC system. This solution was analyzed within one day (intraday). Data forthe Interday precision of the method at 283 nm are shown in (Table 9).

Accuracy

The standard addition technique was used to assess the method's accuracy. For this purpose, a concentration of 10µg/ml was selected for DXL to prepare the sample matrix of the bulk drugs. 0.1ml of the stock solution of DXL was taken in three10 ml volumetric flasks. To these three flasks, 0.08, 0.1, and 0.12 ml of the standard stock solution of DXL were added, respectively, and the volumes were made up to the mark with diluent. To the sample matrix, aliquots of standard solution were added such that the sum of total concentration was in the 80, 100, and 120%of the target concentration range. The peak areas of the sample matrix and after standard addition were measured in triplicate, and concentration was found for peak areas. The results are shown in (Table 10) [34,35].

Limit of Detection/ Limit of Quantitation

LOD and LOQ are determined per the standard reported procedure and the ICH Q2 guidelines.

Robustness

To guarantee that the analytical procedure's validity is upheld everytime it is utilized, a set of system appropriateness parameters (such as the Resolution test) should be defined as part of the robustness evaluation. To determine the method's robustness, the

change in pH, flow rate, and wavelength was done at 10 µg/ml in triplicate analysis; from this, the standard deviation and relative standard deviation were calculated. The values of SD and RSD are given in (Table 11).[36,37]

Forced Degradation Study

Preparation of Standard Solution of DXL

Accurately weighed 10 mg of DXL was transferred to a 10 ml volumetric flask and added acetonitrile and 1 ml of 0.5mmol Ammonium acetate (pH 4.5) make up the volume with Acetonitrile (Resultant solution conc. 1000 ppm). From the stock solution, 10 µg/ml Resultant solution was prepared, filtered through a 0.45µm membrane filter, and degassed under an ultrasonic bath before use [38,39].

Hydrolytic Degradation of DXL

The acidic degradation of DXL was investigated using a standard stock solution with a concentration of 1000 g/ml. 1 ml of the stock solution was transferred to a volumetric flask with a 10 ml mark containing clean water. After a 30-minute break, transfer 1 ml of the resulting solution to a 10-ml volumetric flask and dilute it with 90:10 (acetonitrile:ammonium acetate) to the proper volume. The finished mixture had a concentration of 10 g/ml—the dilution injection into an HPLC apparatus. In (Figure 6), the obtained chromatogram is displayed [40,41].

Acidic Degradation of DXL

The acidic degradation of DXL was investigated using a standard stock solution with a concentration of 1000 g/ml. 1 ml of the stock solution was added to a 10 ml volumetric flask that had previously held 0.1N HCl, and the volume was adjusted to the proper level. After a 30-minute break, transfer 1 ml of the resulting solution to a 10-ml volumetric flask and dilute it with 90:10 (acetonitrile:ammonium acetate) to the proper volume. The finished mixture had a concentration of 10 g/ml. (Figure 7) depicts the chromatogram and the dilution that was fed into the HPLC apparatus [42,43].

Alkaline Degradation of DXL

The acidic degradation of DXL was investigated using a standard stock solution with a concentration of 1000 g/ml. 1 ml of the stock solution was added to a 10 ml volumetric flask that had previously contained 0.1N NaOH and had been filled to the appropriate level. Following a 30-minute interval from the resulting solution, 1 ml of the solution is removed, transferred to a 10-ml volumetric flask, and the volume is adjusted to the proper concentration using the diluents acetonitrile and 0.5 mmol ammonium acetate (90:10). The finished mixture had a concentration of 10 g/ml. (Figure 8) depicts the chromatogram and the dilution that was fed into the HPLC apparatus [44,45].

Oxidative Degradation of DXL

The acidic degradation of DXL was investigated using a standard stock solution with a concentration of 1000 g/ml. 10 ml of the volumetric flask, which had previously held 3% H2O2, was filled to the proper level with 1 ml of the stock solution. After a 30-minute break, transfer 1 ml of the resulting solution to a 10-ml volumetric flask and dilute it with 90:10 (acetonitrile:ammonium acetate) to the proper volume. The finished mixture had a concentration of 10 g/ml. (Figure 9) depicts the chromatogram and the dilution that was introduced into the HPLC apparatus [46].

Thermal Degradation of DXL

The drug was maintained in a heated oven at 110°C for varying lengths. A precise transfer of 10 mg of DXL into a volumetric flask of 10 ml was accomplished, and the final solution was prepared in the diluent to achieve a concentration of 10 g/ml. (Figure 10) displays the HPLC system's chromatograms and dilution injectors [47].

Photolytic Degradation of DXL

To create a solution that contained 1000 g/ml of photolytic solution at room temperature, 10 mg of the medication was carefully weighed, transferred to a volumetric flask, and then dissolved in 10 ml of diluent solution. The photolytic degradation of the medication was investigated by placing the stock solution in the UV chamber for an hour. A portion of the sample was taken out. The withdrawn sample was diluted with a diluent solution to get the 20 g/ ml DXL. The HPLC system received the injection of the dilution. The chromatogram is displayed in (Figure11) [48].

LC-MS Study

All the LC-MS studies were performed in positive and negative modesto determine the probable mass of degradants obtained when the drug was subjected to acidic degradation using 0.1N HCl and oxidative degradation using 3% w/v hydrogen peroxide. A

run of degradation sample was performed in the HPLC system with the same mobile phase, which was optimized during method development. It was then introduced into the mass spectrometer, and the number of fragments was observed in the form of mass spectra, which are given in (Figures 12(a),12(b),12(c),13(a)and 13(b)). The number of degradants, probable structure, and their mass are given in (Tables 12 and 13) [49,50,51].

RESULT

Identification and Characterization of DXL

Characterization

Table (2): Organoleptic Parameter of DXL.

Sr. No.	Test	Observation
1	Color	White coloured powder
2	Odor	Odorless
3	Melting point	138-142°C

Table (3): Determination of Solubility of DXL.

Sr. No.	Solvents	Solubility	
		Observed	Reported
1	Water	Insoluble	Insoluble
2	Methanol	Freely soluble	Freely soluble
3	Acetone	Freely soluble	Freely soluble
4	Acetonitrile	Freely soluble	Freely soluble

Table (4): Characterization of infrared absorption of a functional group of DXL.

Sr. No.	Functional Group	Wavenumber(cm^{-1})
1	N-H & C=C	1585.49
2	O-H	1438.9
3	S=O	1303.88
4	C-F	1265.30
5	C-O	1161.15
6	C=C	975.95
7	C-H	740.67

FT-IR Spectrum analysis by ATR method

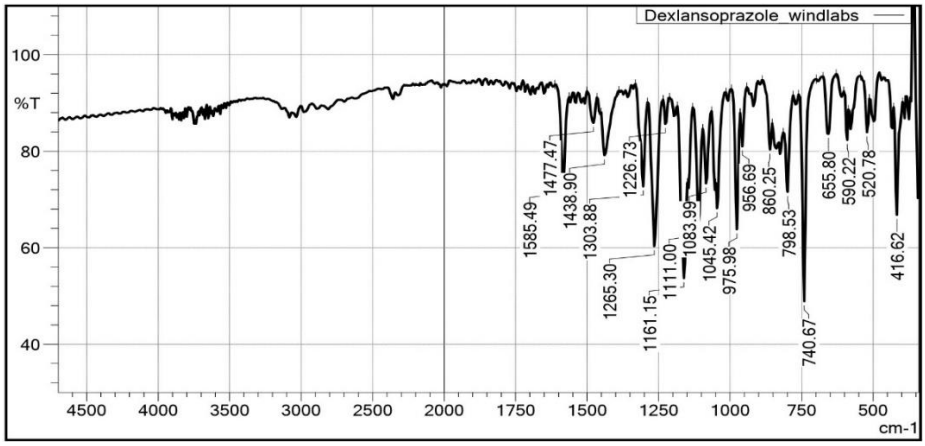


Figure (2): FT-IR Spectrum of Dexlansoprazole.

Determination of Wavelength of Maximum Absorption λmax of DXL

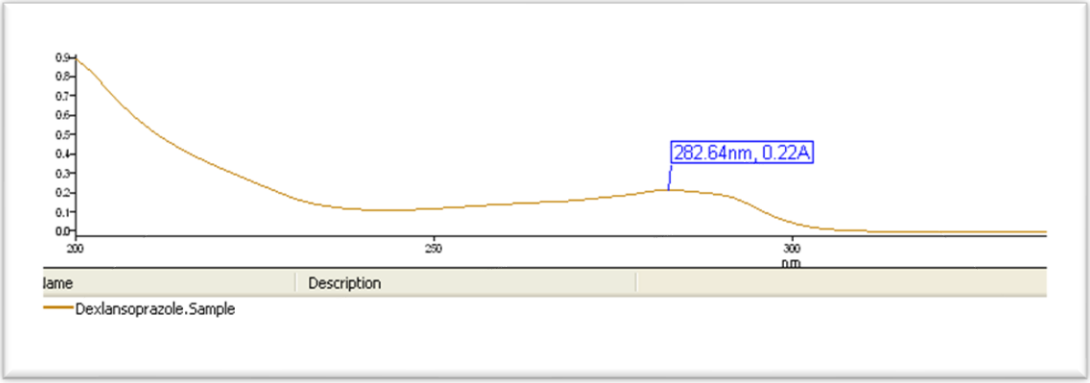


Figure (3): Absorption spectra of 10 µg/ml of standard DXL solution in reference solvent.

Table (5): Chromatographic Condition.

Sr. No.	Parameters/ condition	Description/ values
1	Column name	Kromasil C18 (100mm*4.6mm*5µ)
2	Detector wavelength	283nm
3	Flow rate	1 ml/min
4	Injection volume	20 ml
5	Mobile phase	0.5 mmol Ammonium acetate(pH 4.5): Acetonitrile Steps Time Ratio 0 0.5 75.25 1 4.0 25.75 2 4.0 75.25 3 3.0 75.25
6	Run time	11 min

IDENTIFICATION

Validation of Developed RP-HPLC Method

System Suitability Parameter

Table (6): System suitability parameter.

Sr. No.	Parameter	Limit	Result
1	Tailing factor	T < 2	1.5
2	Theoretical plate	N > 2000	13718.19
3	Retention time	-	5.14

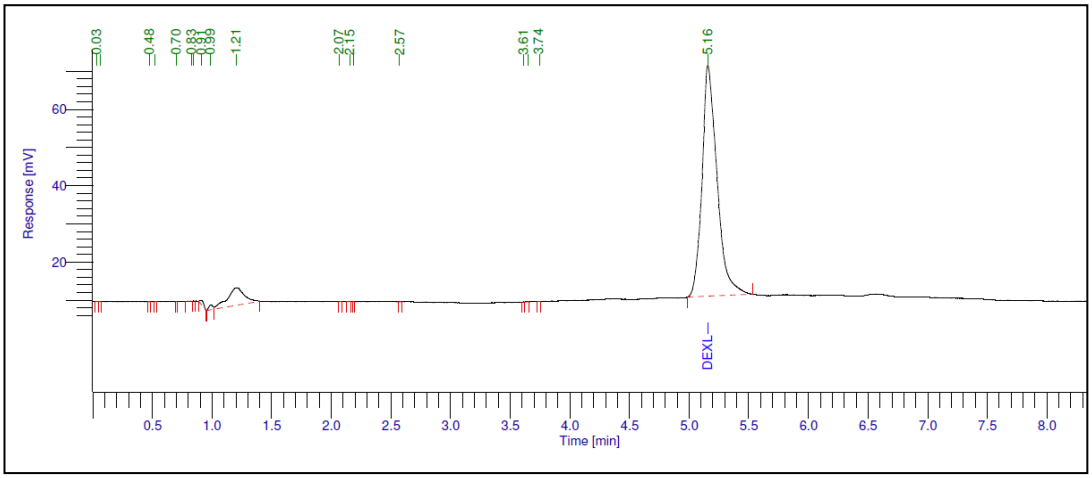


Figure (4): Chromatogram of Excipients and DXL.

Linearity

Table (7): Linearity response for DXL.

Sr. No.	Concentration(µg/ml)	Area(mV*sec)
1	0	0
2	5	131838
3	10	323389
4	15	513730
5	20	693819
6	25	922493
7	30	1086046

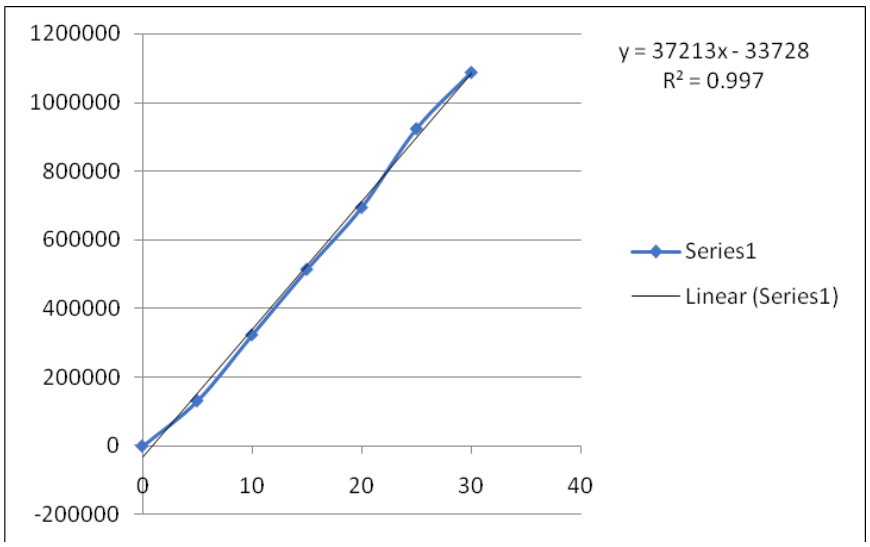


Figure (5): Calibration curve of dexlansoprazole.

Precision

Repeatability

Table (8): Data Showing Repeatability Analysis.

Sr. No.	Conc. (mg/ml)	Peak Area (mV*sec)	Mean ± SD	%RSD
1	20	710519	715326 ±4859.04	0.67
2		710928		
3		714632		
4		717101		
5		723803		
6		714974		

Intraday and Interday Precision

Table (9): Intraday and Interday Precision data of DXL.

Intraday

Sr. No.	Conc. (mg/ml)	Peak Area(µV*sec)			Mean ± SD	%RSD
		0 hr	2hr	4 hr		
1	10	321881	318814	324154	321616.3± 2679.82	0.83
2	15	513730	521341	518665	517912± 3860.96	0.74
3	20	681819	686256	690145	686073.3±4166	0.60

Interday

Sr. No.	Conc. (mg/ml)	Peak Area(µV*sec)			Mean ± SD	%RSD
		Day 1	Day 2	Day 3		
1	10	321881	328814	319019	323238± 5036.52	1.55
2	15	513730	519582	515856	516389.3± 2962.23	0.57
3	20	681819	678916	688577	683097.3± 4945.99	0.72

Limit of Detection

The slope and standard deviation of the response were used to calculate the detection limit, which was determined to be 1.2 g/mL.

Limit of Quantification

The response's standard deviation and slope were used to quantify the limit, which was discovered to be 3.64 g/ml.

Accuracy

Table (10): Accuracy study based on recovery data.

Sr. No.	Recovery study (% Drug)	Standard conc. (µg/ml)	Spiked Conc. (µg/ml)	Conc. After spiking (µg/ml)	Amount recovered (µg/ml)	% Amount recovered
1	0%	10	0	10	9.89	98.9
2	80%	10	8	18	18.2	101.1
3	100%	10	10	20	20.4	102
4	120%	10	12	22	21.7	98.63

Robustness

Table (11): Robustness study of DXL.

Conc. (µg/ml)	Area at flow rate 0.8ml/min	The area at a flow rate of 1.2ml/min	Area at pH 4.0 (buffer)	Area at pH 5.0 (buffer)	Area at wavelength 278nm	An area at wavelength 288nm
15	525730	505644	512365	518956	520189	510652
15	523935	509155	515845	514875	517856	508859

Conc. (µg/ml)	Area at flow rate 0.8ml/min	The area at a flow rate of 1.2ml/min	Area at pH 4.0 (buffer)	Area at pH 5.0 (buffer)	Area at wavelength 278nm	An area at wavelength 288nm
15	519536	504581	518956	512658	520059	506945
Average	523067	506460	515722	515496.3	519368	508818.6
SD	3186.92	2393.69	3297.22	3194.64	1311.04	1853.82
%RSD	0.6	0.47	0.63	0.61	0.25	0.36

The results show that the method is robust in the given concentration range and within the acceptable limit per ICH guidelines.

Forced Degradation Study

Hydrolytic Degradation of DXL

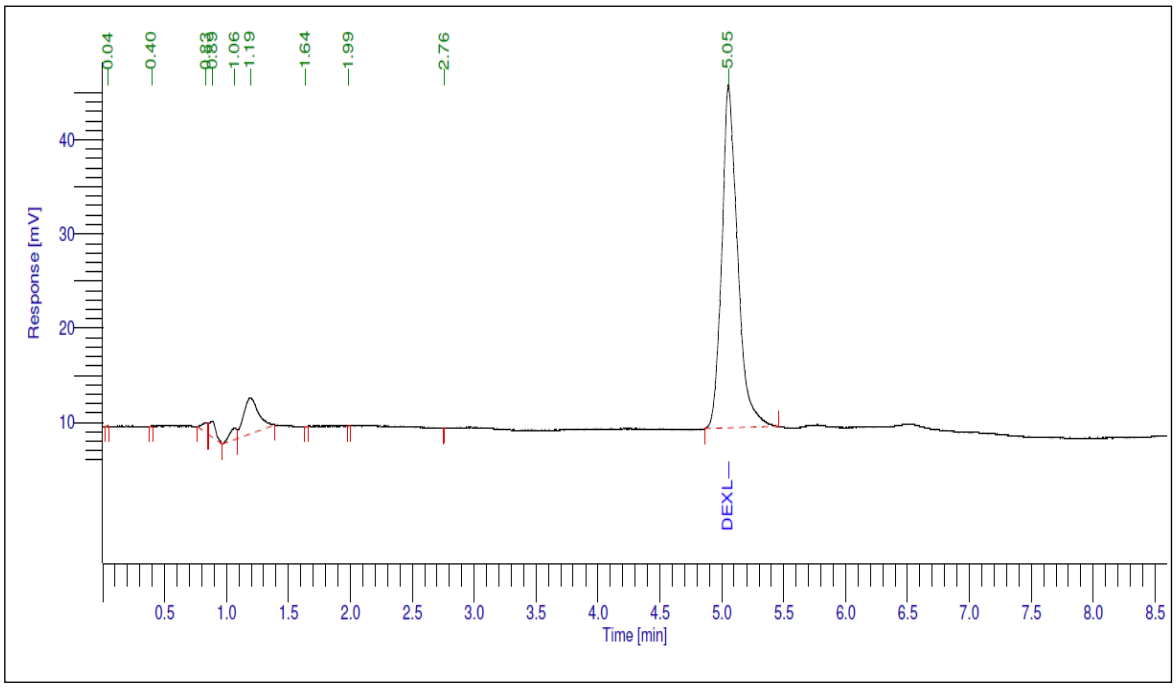


Figure (6): Chromatogram of DXL in Hydrolytic condition of 30 min at room temperature.

Acidic Degradation of DXL

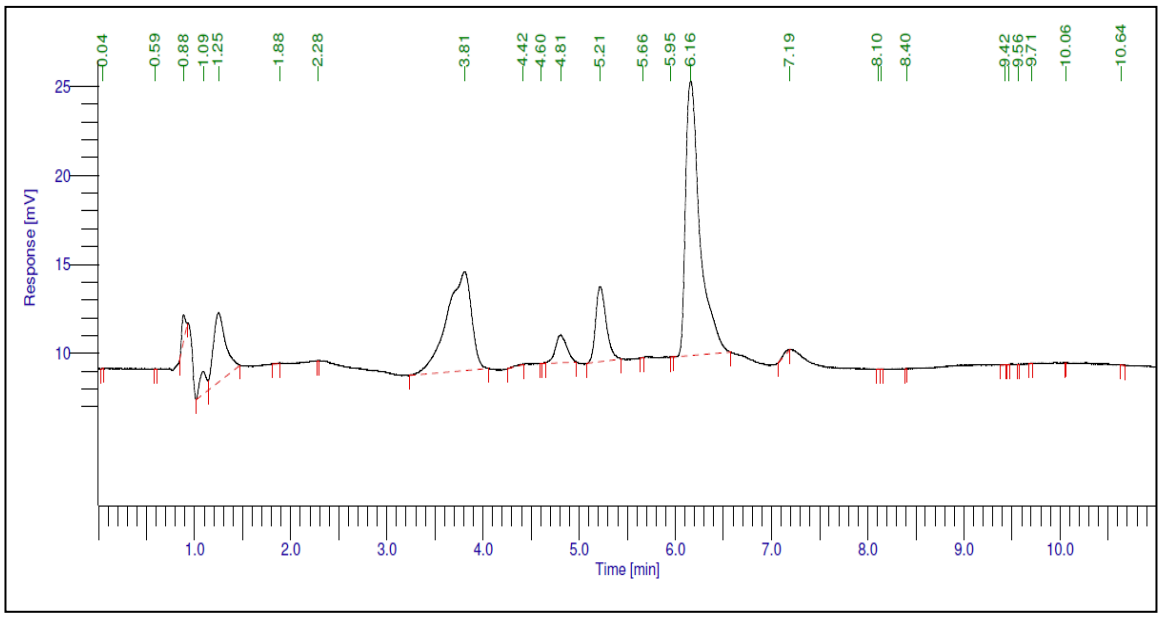


Figure (7): Chromatogram of DXL in Acidic Condition of 30 min at room temperature.

Alkaline Degradation of DXL

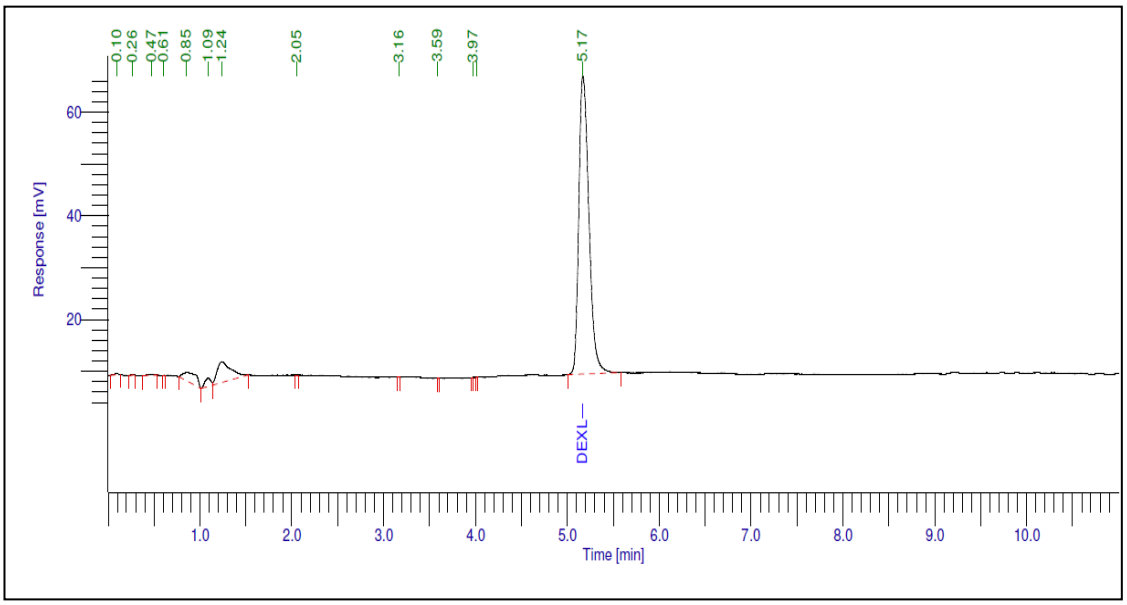


Figure (8): Chromatogram of DXL in Alkaline Condition of 3 hours at room temperature.

Oxidative Degradation of DXL

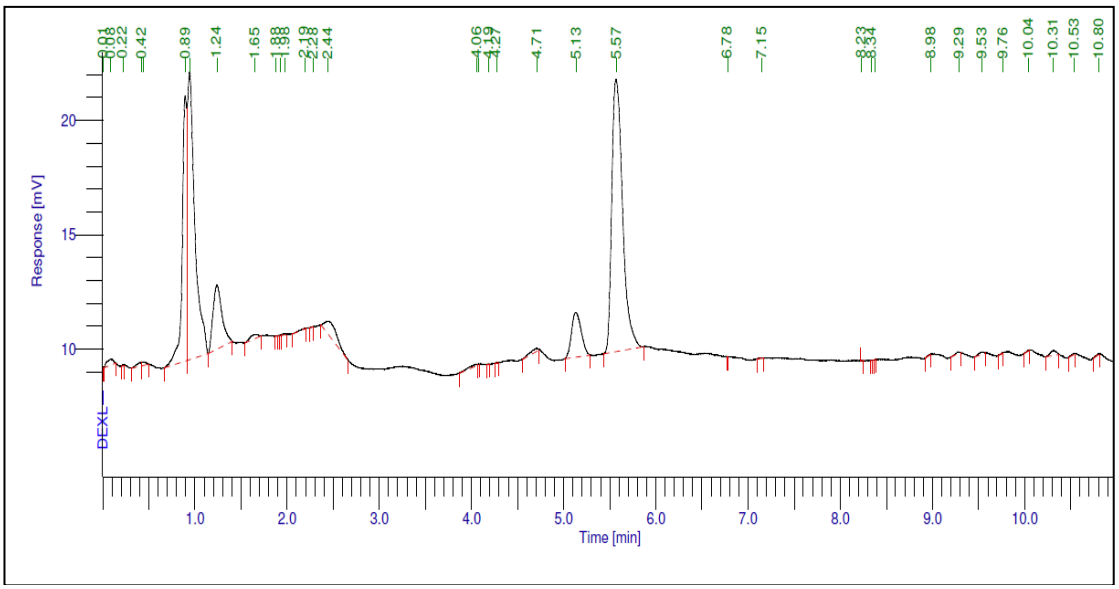


Figure (9): Chromatogram of DXL in Oxidative Condition of 30 min at room temperature.

Thermal Degradation of DXL

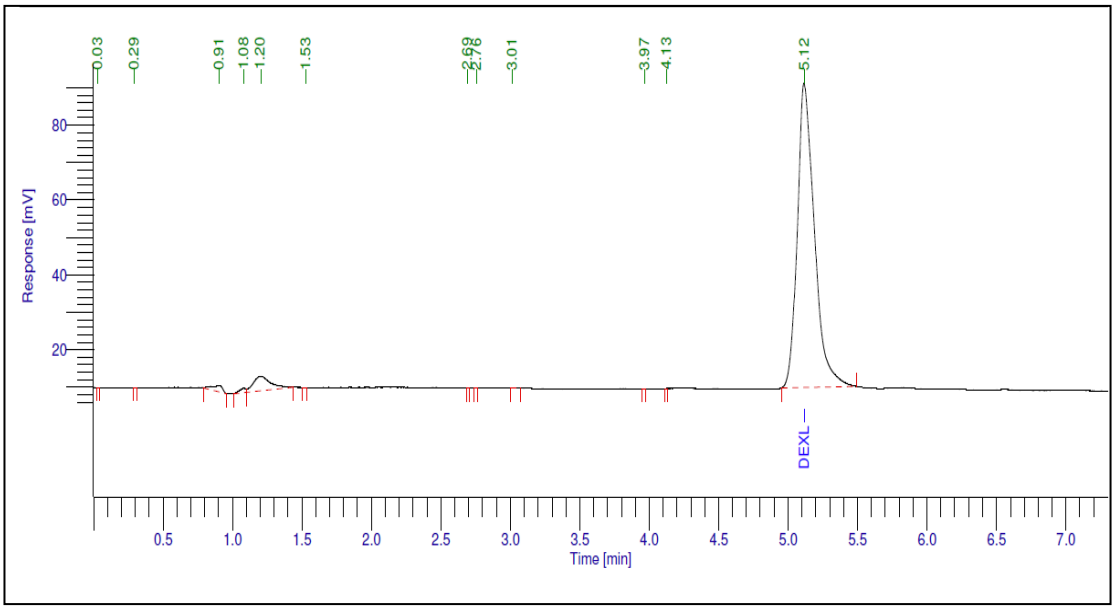


Figure (10): Chromatogram of DXL in Thermal Condition of 1 hour at 110 ^{0}C temperature.

Photolytic Degradation of DXL

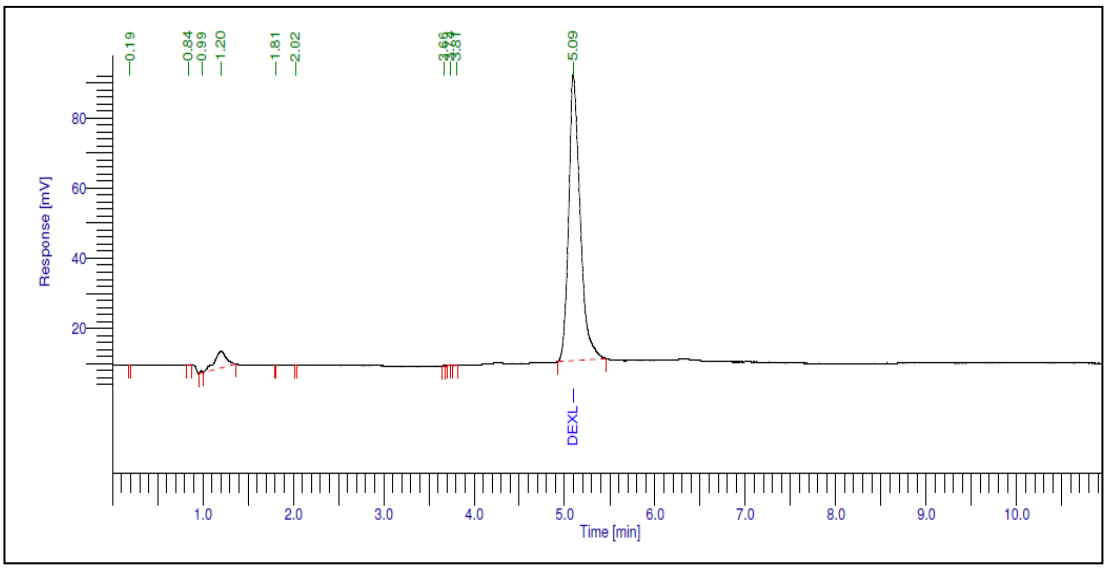


Figure (11): Chromatogram of DXL in Photolytic Condition.

LC-MS Study

LC-MS Spectra of DXL in Acidic Condition

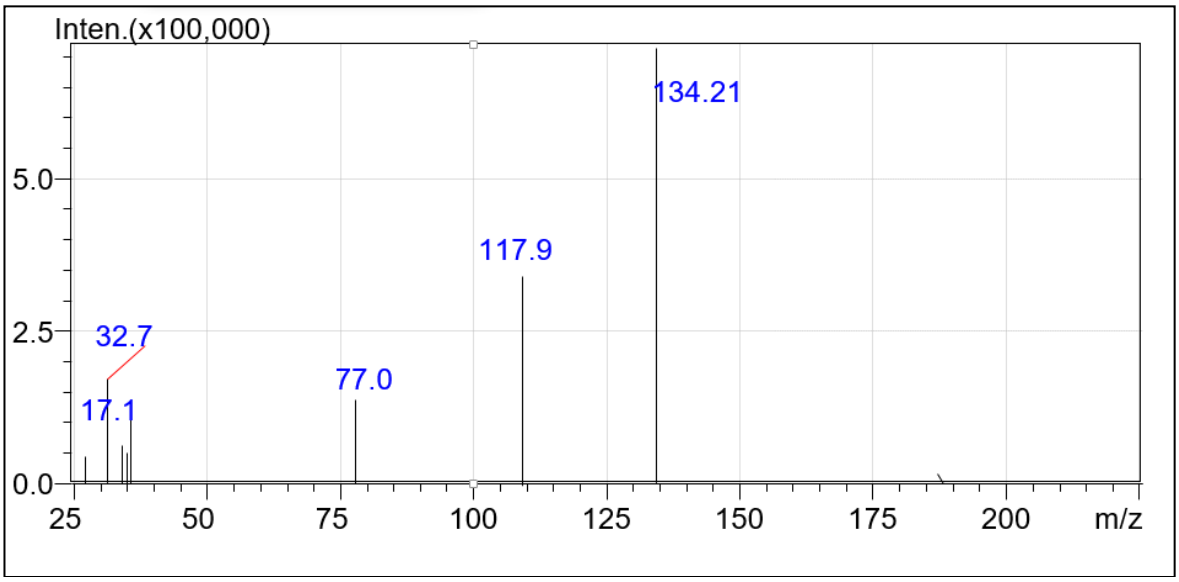


Figure (12 a): LC-MS Spectra of DXL in Acidic Condition.

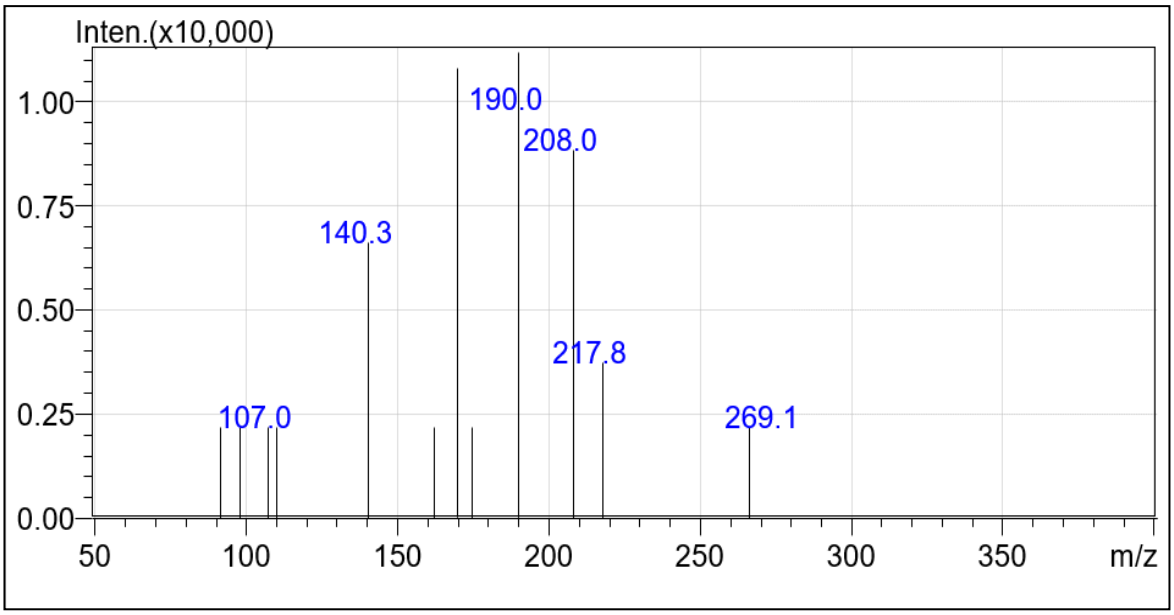


Figure (12 b): LC-MS Spectra of DXL in Acidic Condition.

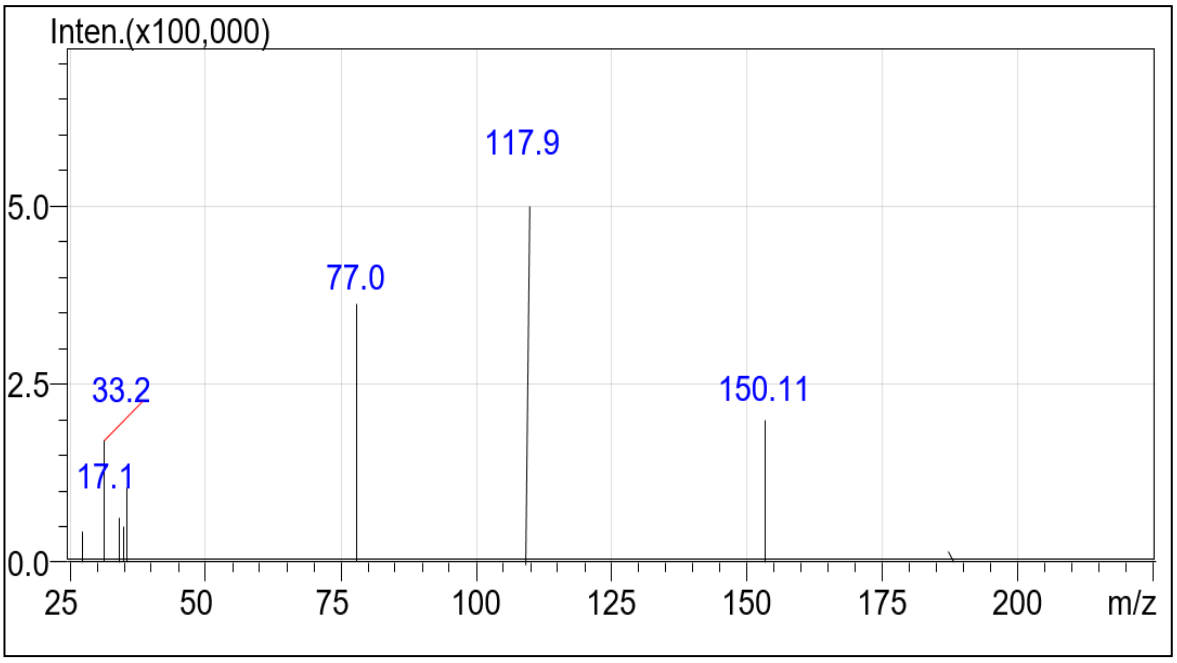


Figure (12 c): LC-MS Spectra of DXL in Acidic Condition.

LC-MS Spectra of DXL in Oxidative Condition

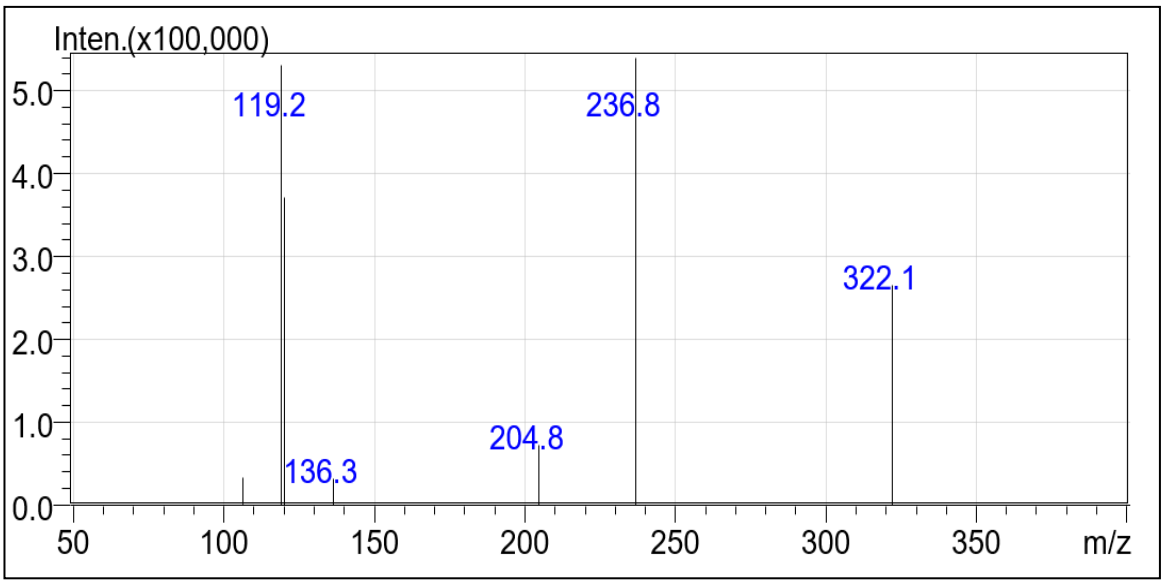


Figure (13 a): LC-MS Spectra of DXL in Oxidative Condition.

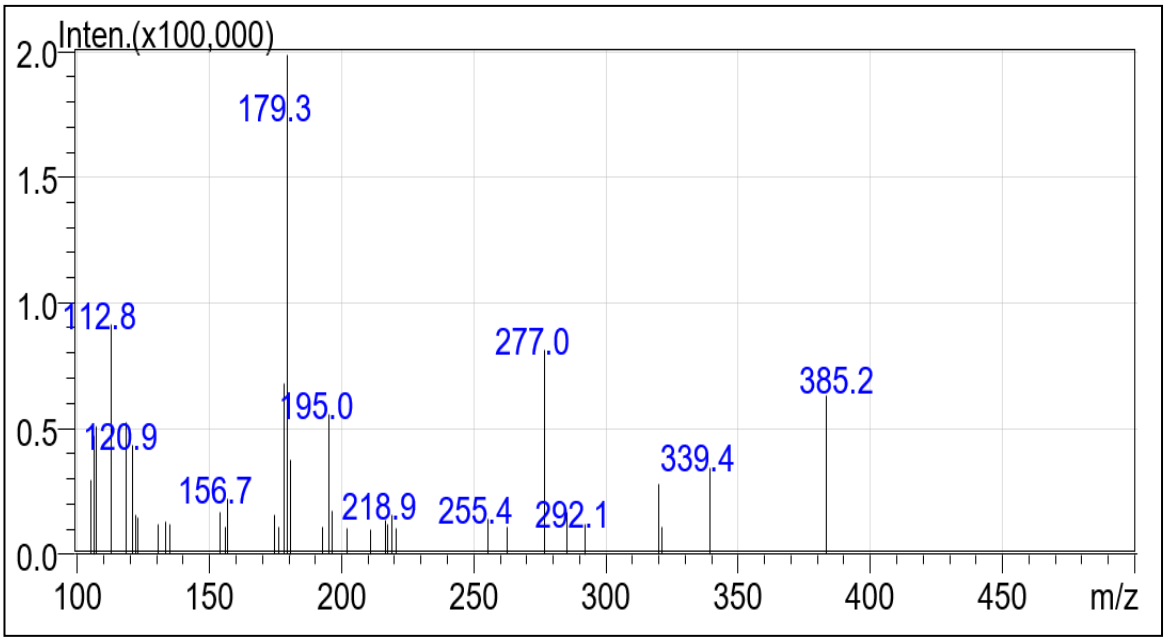


Figure (13 b): LC-MS Spectra of DXL in Oxidative Condition.

Table (12): Interpretation of MS spectra of DXL in acidic degradation condition.

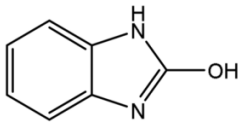
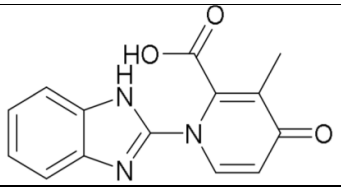
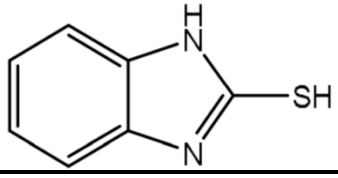
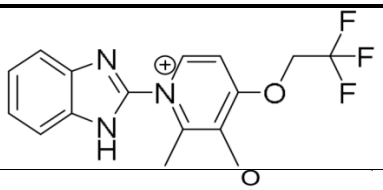
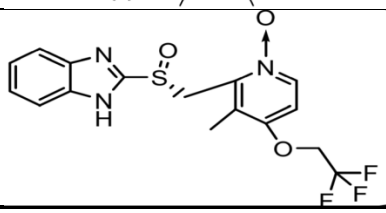
Sr. No.	m/z Ratio	Probable molecular formula	Probable molecular structure	Exact mass	Error
1	134.21	$C_7H_6N_2O$		134.06	0.15
2	269.1	$C_{14}H_{11}N_3O_3$		269.11	0.01
3	150.11	$C_7H_6N_2S$		150.13	0.02

Table (13): Interpretation of MS spectra of DXL in oxidative degradation condition.

Sr. No.	m/z Ratio	Probable molecular formula	Probable molecular structure	Exact mass	Error
1	322.1	$C_{16}H_{15}N_3F_3O$		322.12	0.02
2	385.2	$C_{16}H_{14}N_3F_3O_3S$		385.16	0.04

In acidic degradation, 3 and 2 oxidative degradants were recognized by LC-MS scan.

DISCUSSION

The infrared (IR) spectrum of dexlansoprazole (delaxaprazole) provides information about the functional groups present in the compound. The characterization of the IR absorption peaks for specific functional groups is as follows:

N-H & C=C (Aromatic Ring): The absorption peak at 1585.49 cm-1 indicates the

presence of the N-H stretching vibration and the C=C stretching vibration in the aromatic ring. O-H (Alcohol): The absorption peak at 1438.9 cm-1 corresponds to the O-H stretching vibration, suggesting the presence of an alcohol functional group. S=O (Sulfoxide): The absorption peak at 1303.88 cm-1 represents the S=O stretching vibration, indicating the presence of a sulfoxide functional group. C-F (Fluorine): The absorption peak at 1265.30 cm-1 indicates the presence of a C-F bond, likely from a fluorine-containing functional group. C-O (Ether or Alcohol): The absorption peak at 1161.15 cm-1 suggests the presence of a C-O bond, which can be attributed to an ether or alcohol functional group. C=C (Alkene): The absorption peak at 975.95 cm-1 corresponds to the C=C stretching vibration, indicating the presence of an alkene functional group. C-H (Alkyl): The absorption peak at 740.67 cm-1 represents the C-H stretching vibration, indicating the presence of alkyl groups in the molecule. [52-54]. The UV abortion spectra are obtained at 282.64 nm.

The system suitability parameters, namely tailing factor, theoretical plate count, and retention time, were evaluated to assess the performance and suitability of the HPLC system for the analysis of dexlansoprazole.

Tailing factor: The tailing factor measures the peak symmetry, with a value of less than 2 indicating a well-shaped peak. In this study, the obtained tailing factor was 1.5, which is within the acceptable limit, indicating good peak symmetry and efficient separation.

Theoretical plate count: The theoretical plate count represents the efficiency of the column in separating the analyte. A value above 2000 is generally considered satisfactory for chromatographic separations. The obtained theoretical plate count was 13718.19, indicating excellent column efficiency and the ability to resolve closely eluting peaks.

Retention time: The retention time is when the analyte passes through the chromatographic system. In this study, the retention time for dexlansoprazole was 5.14 units. The specific limit for retention time was not provided in the results. However, the obtained retention time can be a reference for future experiments or comparisons [54-56].

The linearity data points indicate a clear linear relationship between the concentration of dexlansoprazole and the corresponding peak area. As the concentration increases, the peak area also increases consistently and proportionally. This suggests that the response of the HPLC method is linear within the studied concentration range. The mean and % RSD were found within the acceptable limits.

The repeatability of the assay technique was assessed, and the assay's % RSD (intraday) was calculated. The method's intermediate accuracy was examined by having a different person carry out the same operation under identical experimental conditions on a different day (interday). The results are reported in Table 9. The mean standard deviation and % RSD were found within the acceptable limits. % recovery studies were found in a range of 98.63 % to 102 %. This is within the acceptable limits.

The behavior of a drug's degradation under various situations was researched according to the results of force degradation experiments. The medication was found to be unstable under alkaline, thermal, and photolytic

settings, although it was found vulnerable to acidic and oxidative degradation conditions.

The LCMS studies were carried out to get insights into the degradation behavior, and the results obtained are shown in Table 12 and Table 13.

The mass spectrometry (MS) analysis of DXL (Dexlansoprazole) under two different degradation conditions, acidic and oxidative, provides valuable insights into the degradation products formed during these processes.

Three peaks were observed in the acidic degradation condition (Table No. 12). Peak 1, with an m/z ratio of 134.21, corresponds to a probable molecular formula of C7H6N2O, and its exact mass and error values indicate a relatively accurate measurement. Peak 2 (m/z 269.1) has a probable molecular formula of C14H11N3O3, again with a precise mass measurement and a small error. Peak 3 (m/z 150.11) is associated with a probable molecular formula of C7H6N2S, and the exact mass is close to the expected value with a minor error. Identifying these peaks and their structural significance would require further analysis and comparison with known degradation pathways.

Two peaks were detected in the oxidative degradation condition (Table No. 13). Peak 1 (m/z 322.1) corresponds to a probable molecular formula of C16H15N3F3O, with a precise mass measurement and a small error. Peak 2 (m/z 385.2) has a probable molecular formula of C16H14N3F3O3S, and its exact mass is in good agreement with the expected value, although there is a slightly larger error compared to the peaks in Table No. 12.

Overall, these results provide initial information about the degradation products formed under acidic and oxidative conditions for DXL. However, further investigations, including structural elucidation and comparison with known degradation pathways, are necessary to confirm the identities of these peaks and their relevance to the degradation of DXL in pharmaceutical formulations or other contexts. Understanding the degradation pathways is crucial for ensuring the stability and efficacy of DXL in various applications.

CONCLUSION

In conclusion, we have created an effective RP-HPLC technique for assessing DXL as a bulk medication that is trustworthy and robust in demonstrating stability. A Kromasil C18 column and a UV detector set to 283 nm are used in the procedure, and the mobile phase is made up of acetonitrile and 0.5 mmol ammonium acetate at a pH of 4.5. The medication had a retention duration of 5.1 minutes, and all system suitability parameters fell within desirable ranges, demonstrating the method's appropriateness and dependability.

Investigating DXL's degradation behavior under various circumstances showed it was vulnerable to oxidative and acidic degradation. However,the medication remained stable under alkaline, thermal, and photolytic conditions. Following LC-MS analysis of materials under oxidative stress, it was possible to identify potential structures and chemical formulae based on the MS spectra.

The created stability-indicating RP-HPLC technique was verified by ICH Q2 recommendations. The validation parameters' precision, accuracy, specificity, and robustness were determined to meet the standards. This demonstrates the validity of the approach and supports its use for routine DXL

analysis, resulting in precise and accurate quantification.

The established approach is useful for evaluating DXL's stability and quality control in bulk medication form. It provides a quick, precise, linear, and reliable method to measure the drug's efficacy and degradation under various circumstances. The results of this study help the creation of top-notch pharmaceutical formulations incorporating DXL and aid in understanding the stability profile of DXL.

Consent for publication: The authors give the Publisher the Author's permission to publish the work.

Data Availability: All data generated for this study are included in the article.

Author Contributions: **Ritesh Bhole:** Conceptualization, Data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualization, and writing review editing. **Chandrakant Bonde**: Conceptualization, writing original draft Writing-original draft, data formal analysis, investigation, writing review & editing, **Ghansham Girase**: project administration, resources, software, supervision, validation, **Shailendra Gurav:** software, supervision, validation, visualization, and writing review & editing.

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