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## Research Paper

# Development and Validation of RP-HPLC Method for The Estimation of Proton Pump Inhibitor in Bulk and Pharmaceutical Dosage Form

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## ABSTRACT

A simple, precise, and accurate reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of a proton pump inhibitor (PPI) in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved using a C18 column with a mobile phase consisting of acetonitrile and phosphate buffer (pH adjusted to optimal conditions), delivered at a specific flow rate and monitored at an appropriate wavelength using a UV detector. The method was validated according to ICH guidelines for parameters including linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantitation (LOQ), and robustness. The results demonstrated excellent linearity over a specified concentration range, with high recovery and minimal interference from excipients. The proposed method is suitable for routine quality control analysis of PPIs in both bulk drugs and formulated products.

## INTRODUCTION

Drugs are physiological compounds used for disease treatment, alleviation, or prevention. Originating from botanical substances, they have evolved through scientific advancements, enhancing the quality of pharmaceutical compounds and synthetic processes. [1-2]

### Significance of specificity of analytical methods in drug analysis

The pharmaceutical sector uses liquid chromatography technology, notably LC-UV, to assess drug material purity, identifying UV-active analytes with accuracy and precision.

### Importance of impurity quantification in pharmaceutical industry

The pharmaceutical sector preserves purity and health advantages by limiting impurities depending on nature, toxicity, and dosage, with

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limited commercialization necessary to manage contaminants.

### Pharmaceutical Analysis

Pharmaceutical analysis is a vital part of analytical chemistry, involving the separation, identification, and determination of components in matter samples. It ensures drug quality, quality control, and uses physical and physicochemical methods. [3-5]

### High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is an advanced method with high resolution, small diameter columns, and controlled flow of the mobile phase. It is highly automated and sensitive, and is often used in the pharmaceutical sector to qualify medications before release. Reversed phase HPLC (RP-HPLC) is also used.

### Normal phase HPLC

Although it is classified as "normal", it is not the most widely utilised kind of HPLC. The column is filled with microscopic silica particles, and the solvent is non-polar. A typical column has an internal diameter of 4.6 mm (and may be less than that), and a length of 150 to 250 mm. Polar chemicals in the mixture being transported through the column will adhere longer to the polar silica than non-polar ones. The non-polar chemicals will therefore travel more swiftly through the column.

### Reverse phase HPLC

Reverse phase HPLC involves altering silica to non-polar by attaching hydrocarbon chains, using a polar solvent, and observing the attraction between the solvent and polar molecules. Non-polar chemicals are less soluble and slow down passage through the column.

### Functional description of the instrument

The HPLC comprises of the following component (Figure 1): Mobile phase reservoir Pump Injector Column Detector and Data system.

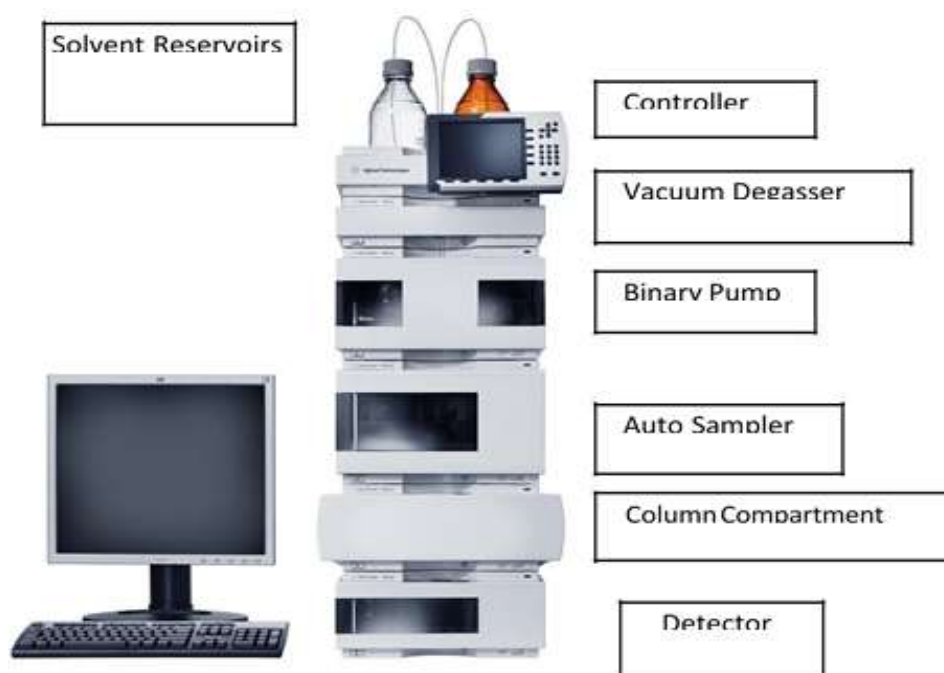


Figure 1: HPLC Instrumentation

## Mobile phase reservoir

Glass bottles serve as prevalent solvent reservoirs, equipped with lids, Teflon tubing, and filters for interfacing with the pump inlet and purge gas (helium) for the elimination of air.

## Pump

High-pressure pumps propel solvents through stationary phase beds, ensuring flow rate stability and incorporating cost-effective attributes such as electronic feedback and multi-headed

combinations. Their flow rate ranges from 0.01 to 5 mL/min, with a maximum pressure of 300 MPa, and includes integrated degassing systems.

## Isocratic flow and gradient elution

Isocratic separation preserves a stable mobile phase composition, whereas gradient elution alters it. Reverse-phase chromatography employs solvent A as water and solvent B as an organic solvent.

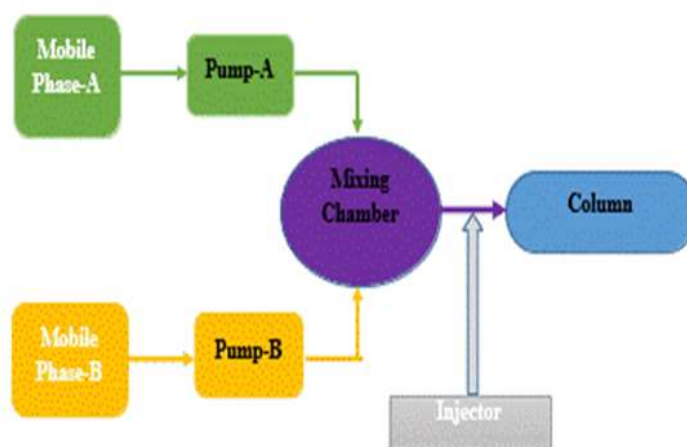


Figure-02 : High pressure Gradient Flow Chart

## Injector

The introduction of LC systems includes injection valves, auto samplers, microprocessors, liquid chromatography, and solvent dissolution for liquid samples, utilizing straightforward techniques such as injection valves.

## Column

HPLC columns, typically 5-25 cm long, are packed with tiny diameter particles for sample capacity, mobile phase consumption, speed, and resolution. Larger diameter columns may be needed for pure substances. Packing involves experience and specialized equipment; hence

prepacked columns are suggested for most chromatographers.

## Column Efficiency

Column efficiency refers to the performance of the stationary phase to accomplish specified separations. This comprises how well the column is packed and its kinetic performance. The efficiency of a column can be tested by numerous ways which may or may not be affected by chromatographic abnormalities, such as "tailing" or appearance of a "front." For this reason, efficiency might be an intriguing value since manufacturers may utilize different approaches in determining the efficiency of their columns.

## Column-packing materials

Silica ( $\text{SiO}_2 \cdot \text{XH}_2\text{O}$ ) is a commonly used material for packing materials, offering a wide range of commercial products with surface areas ranging from 100 to 800  $\text{m}^2/\text{g}$  and particle sizes from 3 to 50  $\mu\text{m}$ . The silanol groups on the surface provide polar character, which is used in adsorption chromatography utilizing non-polar chemical eluents. The binding of hydrocarbon chains to silica generates a non-polar surface ideal for reversed phase chromatography employing combinations of water and organic solvents. Intermediate surface polarities occur from bonding to silica of other organic compounds. Separation of chemicals in mixtures takes place slowly via differential adsorption on a stationary silica phase, with partition playing an essential role.

## Detector

Optical detectors are often employed in liquid chromatographic systems to monitor light intensity differences produced by UV absorption, fluorescence emission, or refractive index changes. These voltage variations are captured on a strip chart recorder and transmitted into a computer for retention time and peak area data. Other frequent detectors include UV, refractive index, fluorescence, and electrochemical detectors, with UV being the most sensitive.

## Data system

Electronic data systems improve signal analysis by boosting accuracy and precision while lowering operator focus. Preprogrammed computing integrators are suited for regular analysis, while intelligent devices like data stations or minicomputers give higher control levels. Intelligent processors in chromatographs enable automated possibilities, complicated data analysis, and software safe controls to reduce inadvertent usage. [6-12]

## HPLC Method Development and Valiation

HPLC method development involves selecting instruments, defining analyte parameters, and optimizing criteria. Results are reviewed for improvement, with optimization focusing on efficiency and minimizing experimental input.

## The column and flow rate

To ensure irreproducible sample retention during technique development, sturdy and repeatable columns are necessary. A C8 or C18 column made from less acidic silica is recommended for all samples. For low pH and low temperatures, sterically protected bonded phase column packing is preferred. A column with a flow rate of 2  $\text{mL}/\text{min}$  is suited for diverse mobile phases.

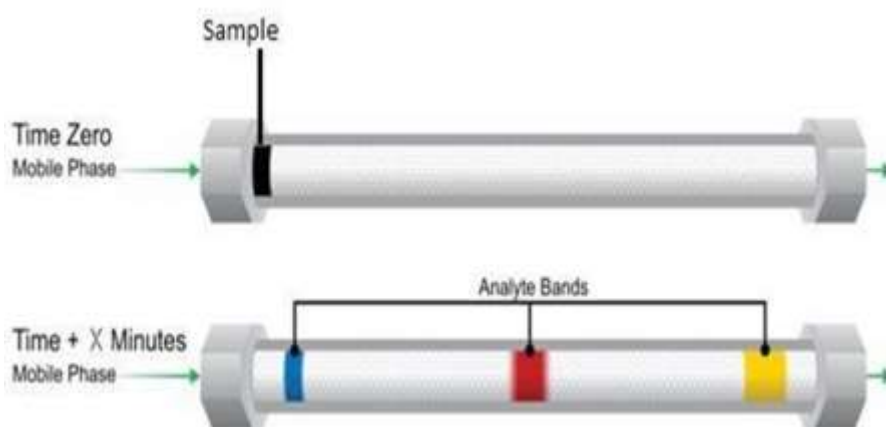


Figure - 03: Separation of components in column

### **The mobile phase**

Acetonitrile is the chosen organic solvent for mobile phase mixes due to UV transmittance and low viscosity. Methanol is a reasonable option. Amine modifiers like tetrahydrofuran are less desirable due to lengthier column equilibration durations and other difficulties. The mobile phase's pH should be determined depending on stability and retention of column silanols. A pH of 2 to 2.5 is recommended for stable columns.

### **Injection volume**

For optimal detection sensitivity, use 25-50  $\mu\text{L}$  injection. For smaller column diameters or smaller particles, use smaller volumes. Dissolve samples in water or acetonitrile, then utilize mobile phase for technique development. Dissolve samples in acetonitrile or methanol.

### **Equilibration of the column with the mobile phase**

The analytical column is equilibrated with the mobile phase before analysis, ensuring precise retention data. Equilibration is important for changes in column, mobile phase, or temperature during technique development. Reliability can be checked by cleaning the column with new mobile phase and reinjecting the sample. Retention times should not change by more than 0.02 minutes between runs.

### **Performance calculations**

The overall system performance was accessed by calculating the following values:

1. Relative retention
2. Theoretical plates
3. Capacity factor
4. Resolution
5. Peak asymmetry
6. Plates per meter [13-18]

### **Validation of Analytical Data**

Method validation seeks to guarantee a method fulfills its intended function, considering aspects including sampling procedure, sample preparation, chromatographic separation, and detection, with consistency in analytical parameters.

#### **Validation Parameters:**

Method validation will be evaluated with different validation parameters are evaluated. Those are listed as below.

- System Suitability
- Specificity
- Precision
- Linearity
- Limit of Detection and Quantitation
- Accuracy
- Stability in Analytical Solution
- Robustness

### **Recovery**

Absolute recovery of a technique is assessed by comparing the response of a processed spiked matrix standard to the pure standard without sample pretreatment. It determines if the method delivers a response for the total amount of analyte in the sample. If an internal standard is utilized, its recovery should be established independently. [19-20]

### **Response function**

Chromatographic analysis employs peak area to construct a calibration model, proving its accuracy by assessing differences between observed and fitted values for six specific concentrations.

### **Sensitivity**

Sensitivity in analytical procedures is determined by calibration line slope and limits of quantitation



(LOQ). Over-range concentrations should be diluted and re-assayed.

### Precision

Precision in analytical methods evaluates random error and agreement between replicate measurements. It contains intra-assay precision, which allows repeating the same methodology with the same analyst, equipment, and chemicals, and inter-assay precision, which allows repeating

under various conditions. Validation should test precision at three unique concentrations.

### Accuracy

The analytical method's accuracy is determined by the percentage bias, which is calculated by comparing the measured value to the true value. Validation assures accuracy within  $\pm 15\%$  at all concentrations.

**Table- 01: Details of Instruments and their applications**

S. No.	Instrument	Principle	Applications
01	UV-Visible spectrophotometer	Absorption of radiation.	Identification and quantification of organic compounds
02	IR Spectrophotometer	Absorption of radiation.	Identity of chemical substance Identity and Quantity of Polymorphic form of chemical substance
03	NMR Spectrophotometer	Absorption of radiation.	Identity of organic chemical substance
04	Mass spectrometer	Mass to charge ratio	Identification, and structural characterization of organic compounds
05	Potentiometer	Electrical potential	Estimation ionic compounds.
06	Polarimeter	Rotation of radiation	To measure the optical rotation and optical purity
07	Thermogravimetry	Thermal Properties	To screen the thermal stability and weight loss.
08	Differential Scanning Calorimetry	Thermal Properties	To screen the melting and crystalline phase formation temperatures
09	HPLC	Liquid Chromatography	Identification, characterization and quantification of chemical compounds
10	GC	Gas Chromatography	Identification, characterization and quantification of volatile chemical compounds
11	HPTLC & TLC	Liquid Chromatography	Identification, characterization and quantification of chemical compounds
12	IC	Ionic Chromatography	Identification, characterization and quantification of ionic compounds

Chromatographic techniques and spectroscopic procedures are vital in pharmaceutical businesses for quality evaluation. HPLC columns offer reproducibility, column longevity, and sensitivity. Reverse phase chromatography is common, and

modern software interacts with statistical techniques for data interpretation. Compliance with regulations is vital for correct analysis. [21-30]



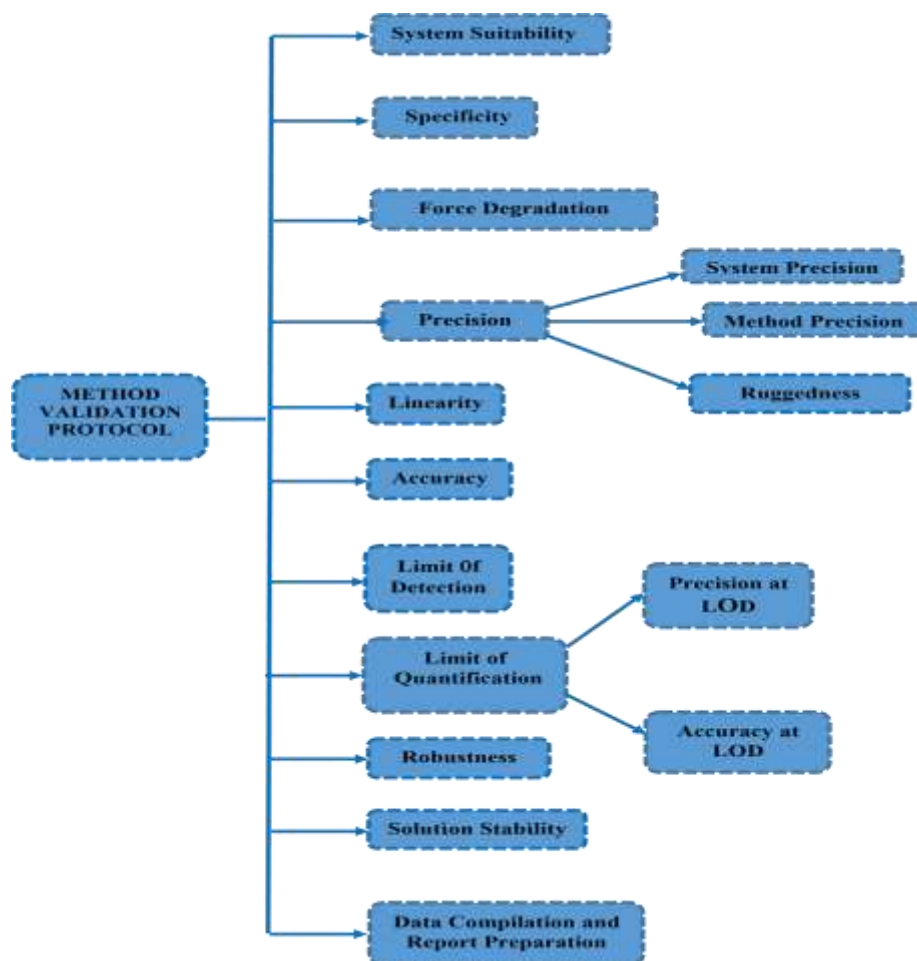
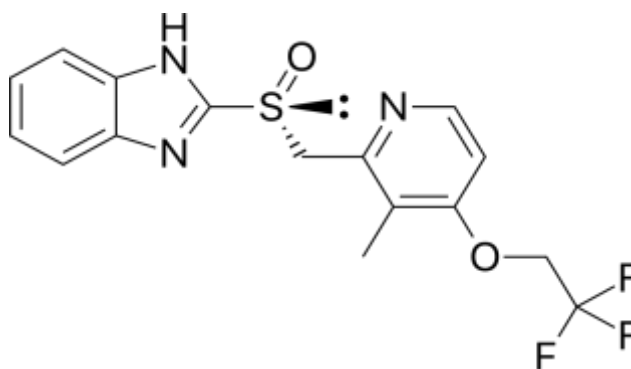


Figure - 04: Method Validation Protocol [30]

## Drug Profile

## Structure

### Dexlansoprazole



**Chemical Name:** (2-([3-methyl-4-(2,2,2-trifluoroethoxy) pyridin-2-yl] methyl sulfinyl) - 1H-benzo[d]imidazole

**Molecular formula:** C<sub>16</sub>H<sub>14</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub> S

**Molecular weight:** 369.36

**Description:** White to off-white powder.

**Solubility:** Insoluble in water, soluble in DMSO, ethanol and free soluble in methanol & organic solvent

**Category:** Dexlansoprazole is in a class of medications called proton pump inhibitors. It works by decreasing the amount of acid made in the stomach

**Mechanism of Action:** Dexlansoprazole blocks the H/K ATPase at the gastric parietal cell's secretory surface, preventing the discharge of hydrochloric acid into the gastric lumen.

**Pharmacodynamics:** Dexlansoprazole, a proton pump inhibitor, lowers gastric acid secretion, but may increase susceptibility to infections, vitamin deficiencies, hypomagnesemia, and false positive results in tests.

**Absorption:** Dexlansoprazole's dual delayed-release formulation includes two unique peaks, with plasma concentration-time profiles. Its mean Cmax and AUC values increase dose-proportionally after oral administration, with meals raising Cmax and AUC.

**Metabolism:** Dexlansoprazole is extensively metabolized in the liver, undergoing oxidation,

reduction, sulfation, glucuronidation, and glutathione conjugation to create inactive metabolites, largely derived from CYP2C19-mediated hydroxylation.

**Route of elimination:** Dexlansoprazole, provided to six healthy male participants, indicated that around 50.7% of the radioactivity was eliminated in urine and 47.6% in feces.

**Adverse effects:** Clinical trials revealed substantial adverse effects such as diarrhea, stomach pain, bloating, nausea, upper respiratory tract infection, vomiting, and flatulence ( $\geq 2\%$ ).

**Uses:** Dexlansoprazole is used for erosive esophagitis and GRD heartburn, lasting longer than lansoprazole and requiring less frequent usage, but lacks clear evidence of improved efficacy.

## MATERIALS & METHODS

### Materials

The drug used for present investigation was obtained from Swapnroop Drugs and Pharmaceuticals Maharashtra.

### Details of Pure Drug

Table No.02: Details of API

Sr. No.	Drug	Supplied by	Quantity	Purity (Assay)
01	Dexlansoprazole	Swapnroop Drugs and Pharmaceuticals	10g	99.8

### Marketed Preparation

Table No.03: Details of Marketed preparation

Sr. No.	Brand name	Mfg by	Content	Quantity
01	Dexlansoprazole 30 Capsule	Cipla Ltd India	Dexlansoprazole	30mg

### Reagents and Chemicals

All reagents and chemicals used were of AR grade and HPLC grade.



**Table No.04: List of Reagent and Chemicals used.**

Sr. No.	Name of chemicals	Manufacturer.
1.	Acetonitrile HPLC Grade	Merck Ltd., India
2.	Methanol HPLC grade.	Merck Ltd., India
3.	Ortho-phosphoric acid.	Merck Ltd., India
4.	Water HPLC grade.	Merck Ltd., India

## Instruments

**Table No.05: List of Instruments used.**

Sr. No.	Instrument	Make	Model
1.	UV-Visible Spectrophotometer	Shimadzu	Double beam carry-UV 1800
2.	HPLC	Waters India	UV Detector
3.	PH Meter	Equip-tronich	Eq-614A
4.	Analytical column	Grace	C18, (4.6 x 150 mm)
5.	Balance	Citizen	CY 104 (Micro-Analytical Balance)
6.	Ultrasonicator	Meta-lab	1.5 L 50

## METHODS & PROCEDURE

### Identification and characterization of drug

Before starting experimental work, it's necessary to examine the drug's physical and chemical properties, enabling the selection of solvents and the development of a rigorous analytical approach.

### Selection and procurement of drug

Dexlansoprazole (DEXL) was chosen as a model drug candidate for method development and validation, gifted by the pharmaceutical sector India, and studied for physical attributes.

### Physico-chemical characterization

The physico-chemical characterisation of drug molecule is crucial with reference to its purity, identification in development and validation of analytical method. The numerous tools utilized for characterisation of medicinal compounds include melting point, UV spectroscopy, solubility research, etc.

### Solubility Studies

The solubility of both drugs was evaluated in several solvents to discover a common solvent for simultaneous drug quantification in a combination.

### Melting point range determination

The melting point of Dexlansoprazole (DEXL) was evaluated by inserting a small sample in a capillary tube and noting the findings in Table 06.

### FT-IR analysis

The FTIR 8400S spectrometer (Shimadzu) was used to record the IR absorbance spectrum of DEXL, which helps comprehend its chemical structure by evaluating the exact frequencies of light absorbed by molecules, based on their molecular surface shape, vibronic coupling, and atom mass.

### UV Spectroscopy Analysis

The Shimadzu 1800-UV visible spectrophotometer and 1cm quartz cells were used to produce the ultraviolet absorption spectrum of DEXL,



examining wavelength maxima as indicated in table no. 07.

### Selection of mobile phase

### Preparation of standard solutions

#### DEXL standard solution

A exact quantity of DEXL was dissolved in methanol, resulting in a final concentration of approximately 30 µg/ml, after further dilution with 20 ml methanol.

### Procedure

Methanol equilibrated, DEXL combination was evaluated, and several solvents were utilized to produce stable separation. Mobile phase compositions were assessed for adequate separation under specific chromatographic settings.

- 1) Methanol: Water (90:10)
- 2) Methanol: Water (80:20)
- 3) Methanol: Water (70:30)
- 4) Acetonitrile: Water (90:10)
- 5) Acetonitrile: Water (80:20)
- 6) Acetonitrile: Phosphate Buffer (90:10) pH 5.5
- 7) Acetonitrile: Phosphate Buffer (80:20) pH 5.5
- 8) Acetonitrile: Phosphate Buffer (70:30) pH 5

The mobile phase comprising Acetonitrile: Phosphate Buffer (70:30) pH 5 was chosen because to its sharp repeatable retention time for DEXL.

### Chromatographic conditions

The following chromatographic conditions were established by trial and error and were kept constant throughout method.

**Column:** Intersil 4.6 (id) x 250 mm

**Particle size packing :** 5 µm

**Stationary phases :** C18 Intersil

**Mobile phase :** Acetonitrile: Phosphate Buffer (70:30) pH 5

**Detection wavelength :** 275 nm

**Flow rate :** 1 ml/min.

**Temperature :** Ambient

**Sample size :** 20 µL

### Preparation of calibration curve:

### Preparation of standard solutions:

#### DEXL standard stock solution

10 mg of DEXL was dissolved in methanol to 100 ml mark, then diluted with mobile phase to get varied concentrations.

### Procedure

The mobile phase equilibrated with the stationary phase, and DEXL concentrations from 3-30 µg/ml were injected, with peak area recorded and graph plotted in Fig. No.12.

### System suitability test

System suitability is a pharmacopoeial requirement ensuring adequate resolution and reproducibility of chromatographic systems for analysis, tested using data from five replicate injections of standard solutions.

### Preparation of standard drug solution.

#### DEXL standard solution

A precise weighed quantity of 10 mg of DEXL was dissolved in mobile phase, diluted to a final concentration of 30 µg/ml.

### Procedure

The mobile phase was equilibrated with the stationary phase until a steady baseline was achieved, and a standard drug solution was injected in five replicates.



### Application of proposed method for estimation of DEXL Laboratory Sample Preparation of laboratory mixture (standard)

A 10 mg DEXL was weighed, shaken, and diluted to obtain a 30 µg/ml laboratory sample.

### Preparation of laboratory mixture (sample)

Five DEXL laboratory mixtures were prepared by weighing drug samples, comparing peak area, and estimating drug amounts using specific methods.

$$\% \text{ Estimation} = \frac{A_t}{A_s} \times \frac{D_s}{D_t} \times \frac{W_s}{W_t} \times 100$$

Where-

At	= Area count for sample solution.
As	= Area count for standard solution.
Ds	= Dilution factor for standard.
Dt	= Dilution factor for sample.
Ws	= Weight of standard (mg)
Wt	= Weight of sample (mg)

### Application of proposed method for estimation of DEXL in formulation

#### Standard stock solution

A 10 mg DEXL solution was weighed, shaken, and mixed with mobile phase to create laboratory mixtures with a concentration of 30 µg/ml.

#### Sample solution preparation

The tablets were weighed, averaged, ground, and powdered to 10 mg of DEXL. The powder was

$$\text{Assay} \left( \frac{mg}{ml} \right) = \frac{A_t}{A_s} + \frac{D_s}{D_t} + \frac{W_s}{W_t} + \frac{p}{100} \times wt \frac{mg}{ml} \text{ of test sample}$$

$$\% \text{ Label claim} = \frac{\text{Assay (mg/ml)} \times 100}{\text{Label claim in mg/ml}}$$

Where -

At = Area count for sample solution.

As = Area count for standard solution.

Ds = Dilution factor for standard.

Dt = Dilution factor for sample

P = Potency of drug.

### Validation parameters

diluted, filtered, and sonicated before being added to a flask.

#### Procedure

Standard and sample solution were injected separately, chromatograms recorded, and DEXL content calculated by comparing sample peak with standard. Tablet drug amount calculated using formula.

#### 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 in Table No. 13

#### Preparation of standard solution

Pre-analysed formulation was mixed with standard DEXL solution, adjusted, and filtered. Drug contribution was determined from total drugs, and drug content calculated using marketed formula.

The % Recovery was then calculated by using formula

$$\%Recovery = \frac{A}{B + C} \times 100$$

Where,

A	= Total amount of drug estimated.
B	= Amount of drug found on preanalysed basis.
C	= Amount of pure drug added.

Results are shown in the Table No. 13

### Precision

The precision of an analytical method, expressed as S.D or R.S.D of series of measurements, was confirmed through replicate estimation of drugs using the proposed method.

### Ruggedness

Ruggedness refers to an analytical method's ability to withstand minor changes in experimental conditions, studied under two conditions: days and the analyzer.

### Interday (Different days)

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 No. 15

### 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. 16

### 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.

### Specificity

Specificity was measured as ability of the proposed method to obtain well separated peak for DEXL without any interference from component of matrix.

### Mean retention time for –

### DEXL – 4.573

The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix.

Typical chromatogram is shown in the Fig. No. 14

### Linearity and range

USP tablet powder was diluted to 80%-120% of test concentration, revealing DEXL's linearity within  $\pm 20\%$  of the test concentration, as shown in Fig. No.15.

### Robustness

The robustness study confirmed that the method's reliability remains unaffected by small variations in parameters and environmental factors, indicating its suitability for normal usage.

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

Limit of detection (LOD) and limit of quantitation (LOQ) are the lowest analyte in a sample that can be detected but not quantitated accurately.

$$LOD = \frac{3.3 \sigma}{S}$$

$$LOQ = \frac{10 \sigma}{S}$$

Where,

$\sigma$  = The standard deviation of the response S = The slope of the calibration curve

The results of LOD and LOQ are shown in table 20

## RESULT & DISCUSSION

Dexlansoprazole (DEXL) is a commonly used clinical drug, with a purity of 99.8% reported by the supplier. The study analyzed its physico-chemical characterization using melting point, UV spectroscopy, and solubility studies.

### Melting point range determination

The drug's melting point was determined by placing a small sample in a capillary tube and holding it on a melting point apparatus, as shown in Table 06.

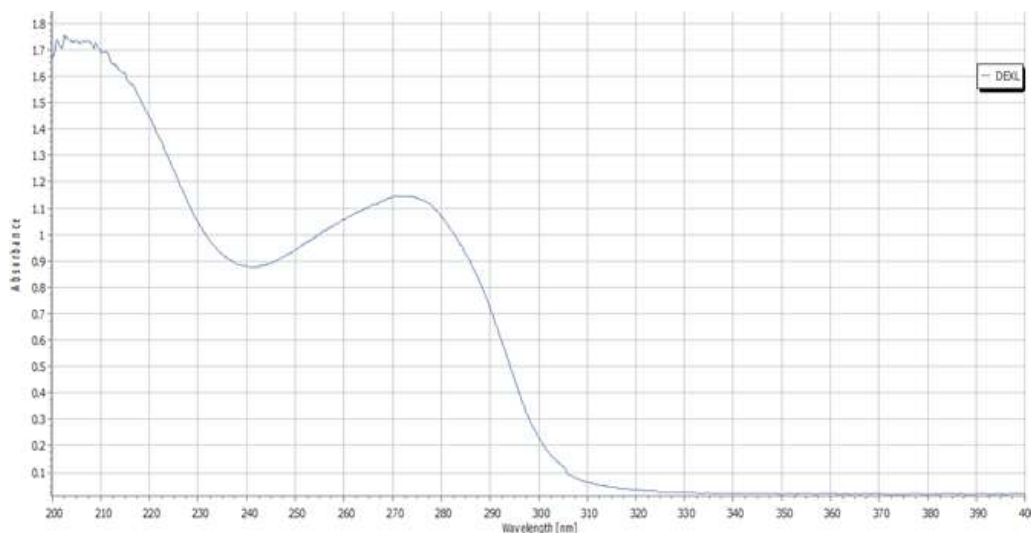
**Table No 06: Melting point range analysis result**

Sr. No.	Name of Drug	Melting point
1	DEXL	139 °C

## FT-IR analysis

### FT-IR of Dexlansoprazole

The FTIR 8400S spectrometer (Shimadzu) was used to record the IR absorbance spectrum of Dexlansoprazole (DEXL) within the 4000 to 400 cm-1 range.



**Fig No 05: FT-IR Spectra of DEXL**

IR spectroscopy theory explains how molecules absorb specific light frequencies, providing information about their structure and functional

groups. The FTIR spectra of DEXL confirmed its drug's properties.

### UV Spectroscopy Analysis



The Shimadzu 1800-UV visible spectrophotometer and 1cm quartz cells were used to obtain the ultraviolet absorption spectrum of DEXL, analyzing wavelength maxima as shown in table no. 07

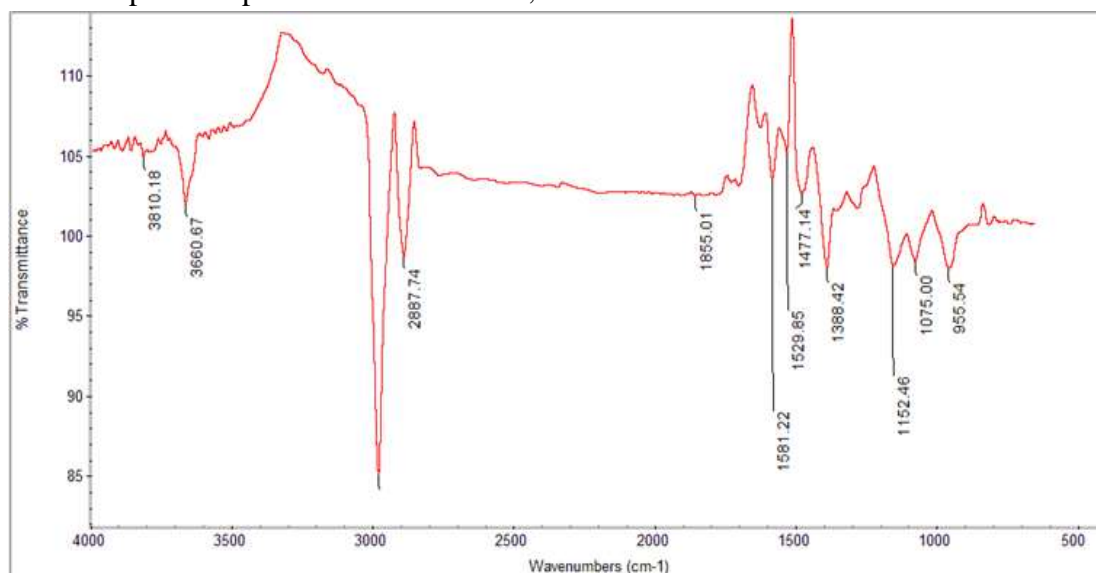


Fig. No. 06: - UV Spectra of DEXL

Table no 07: Drug wavelength maxima ( $\lambda$  max)

Sr. No.	Name of Drug	Observed value ( $\lambda$ max) nm
1	DEXL	275

From the spectra the wavelengths selected for estimation of drug was 275 nm.

#### Selection of mobile phase

#### Preparation of standard solutions

#### DEXL standard solution

DEXL was dissolved in acetonitrile, diluted to 30  $\mu$ g/ml, and filtered through Whatman filter paper. Different mobile phase compositions were evaluated for acceptable separation.

Table no 08: List of mobile phase tried

Sr. No.	List of mobile phase tried
1	Methanol: Water (90:10)
2	Methanol: Water (80:20)
3	Methanol: Water (70:30)
4	Acetonitrile: Water (90:10)
5	Acetonitrile: Water (80:20)
6	Acetonitrile: Phosphate Buffer (90:10) pH5.5
7	Acetonitrile: Phosphate Buffer (80:20) pH5.5
8	Acetonitrile: Phosphate Buffer (70:30) pH 5

From various mobile phases tried, mobile phase containing Acetonitrile: Phosphate Buffer (70:30)

pH 5 was selected, since it gives sharp reproducible retention time for DEXL.

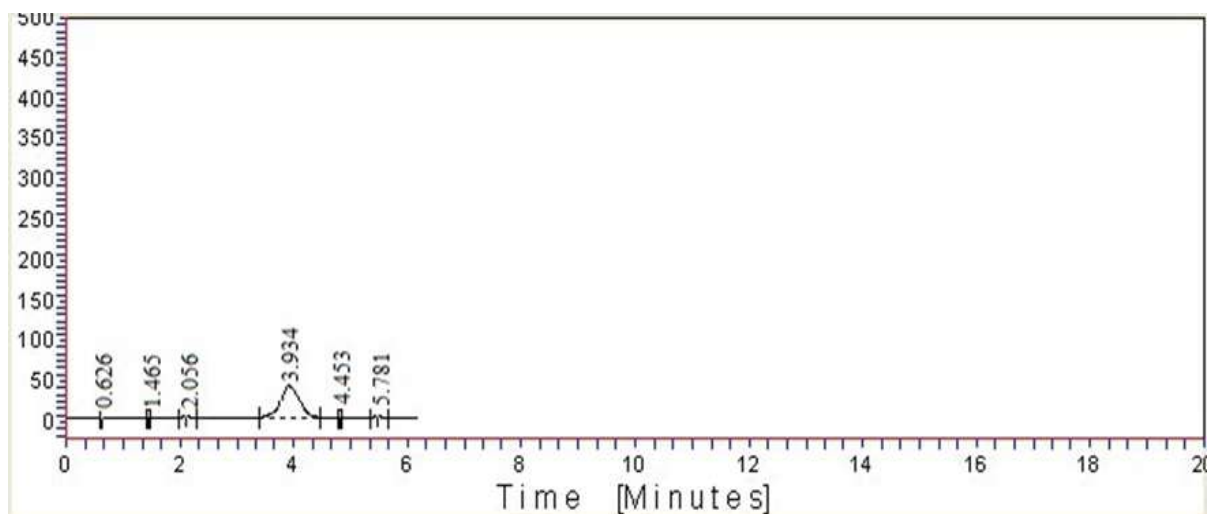


Fig. No.07: Trial Chromatogram obtained by using Methanol: Water (90:10) as mobile phase.

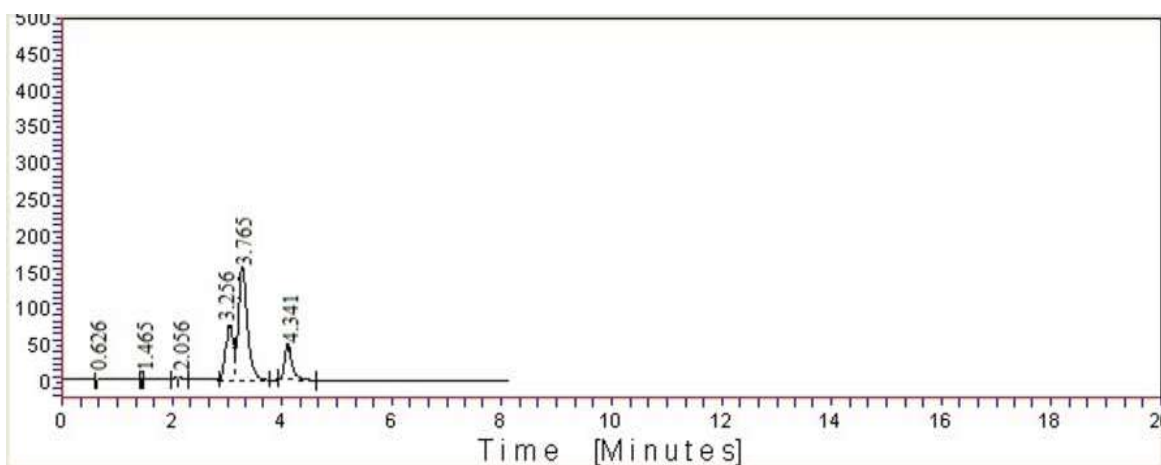


Fig. No.08: Trial Chromatogram obtained by using Acetonitrile: water (80:20) as mobile phase

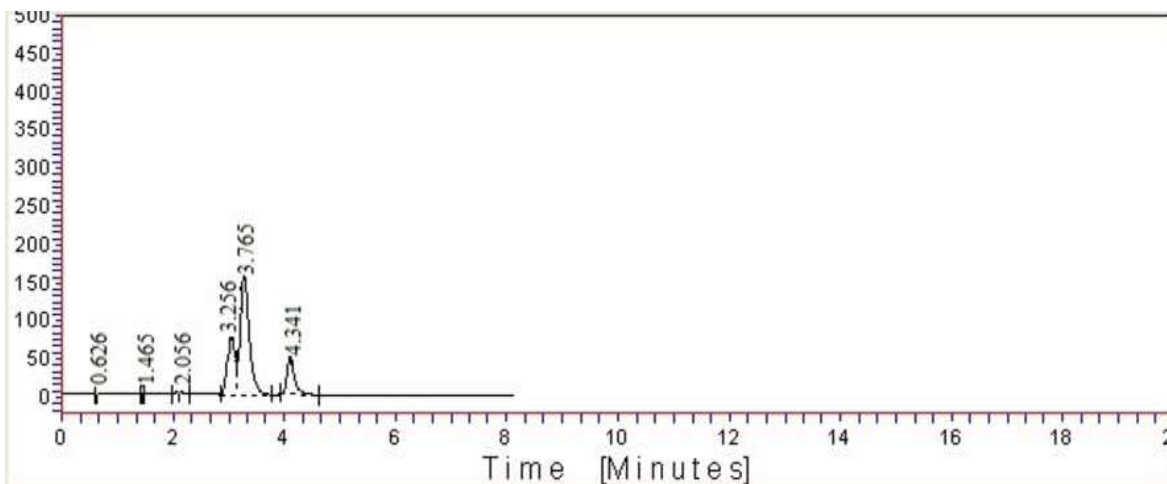


Fig. No.09: Trial Chromatogram obtained by using Acetonitrile: Phosphate Buffer (90:10) pH 5.5 as mobile phase.

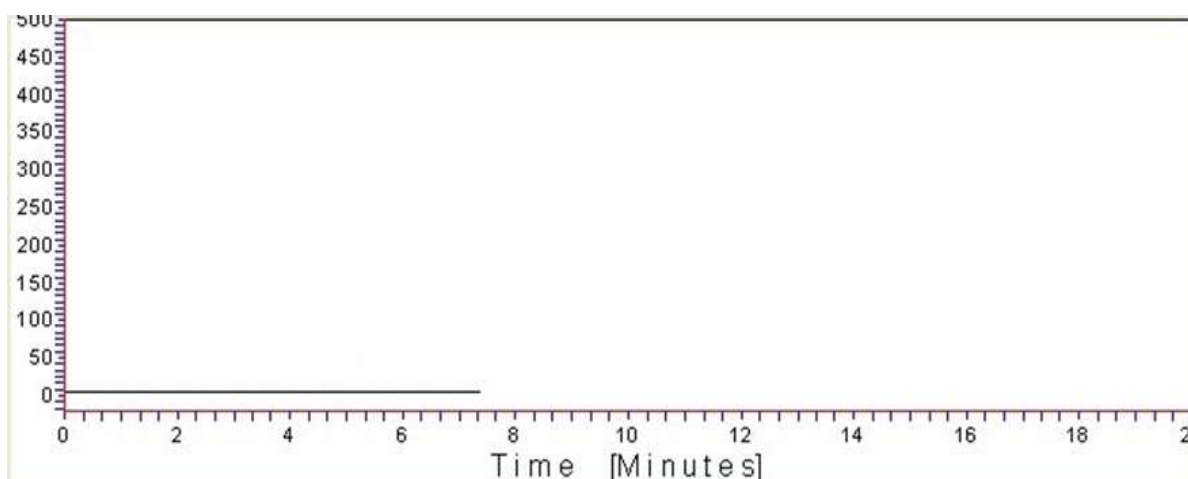


Fig. No.10: Final Chromatogram obtained by using Acetonitrile: Phosphate Buffer (70:30) pH 5 as mobile phase of DEXL.

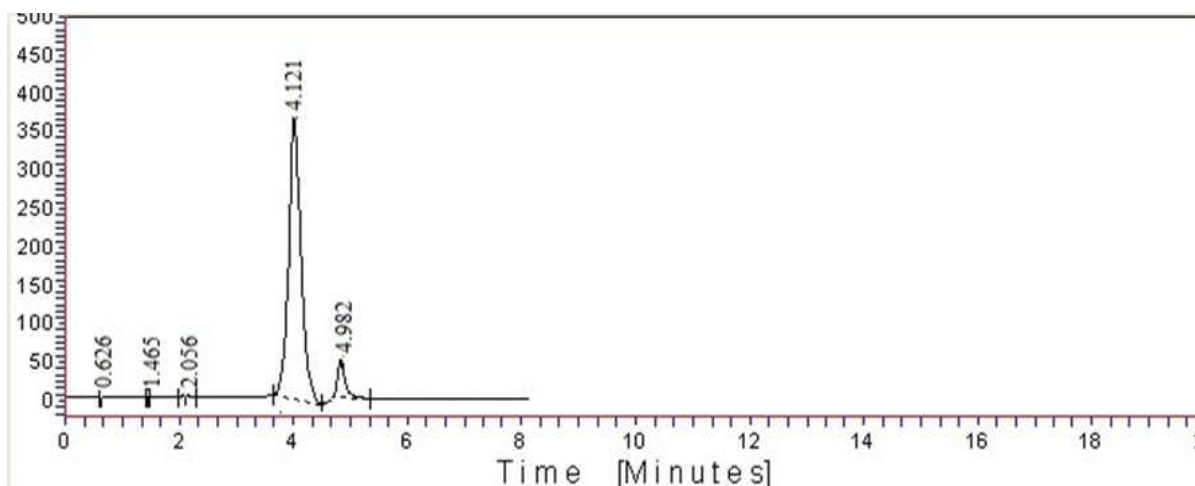


Fig. No.11: Blank Chromatogram obtained by using Acetonitrile: Phosphate Buffer (70:30) pH 5 as mobile phase.

**Chromatographic conditions:**

The following chromatographic conditions were established by trial and error and were kept constant throughout method.

**Column:** Intersil 4.6 (id) x 250 mm

**Particle size packing :** 5  $\mu$ m

**Stationary phases :** C18 Intersil

**Mobile phase :** Acetonitrile: Phosphate Buffer (70:30) pH 5

**Detection wavelength :** 275 nm

**Flow rate :** 1 ml/min.

**Temperature :** Ambient

**Sample size :** 20  $\mu$ L

**Preparation of calibration curve**

The mobile phase equilibrated with the stationary phase, and DEXL drug solutions were injected at concentrations ranging from 3-30  $\mu$ g/ml, with a graph plotted.

**Table No. 09: Observation of standard curve of DEXL**

Sr. No.	Conc.( $\mu$ g/ml) DEXL	Peak Area DEXL
1	3	37865.7
2	6	75731.3

3	9	113597.0
4	12	151462.6
5	15	189328.3
6	18	227193.9
7	21	265059.6
8	24	302925.2
9	27	345790.9
10	30	378656.5

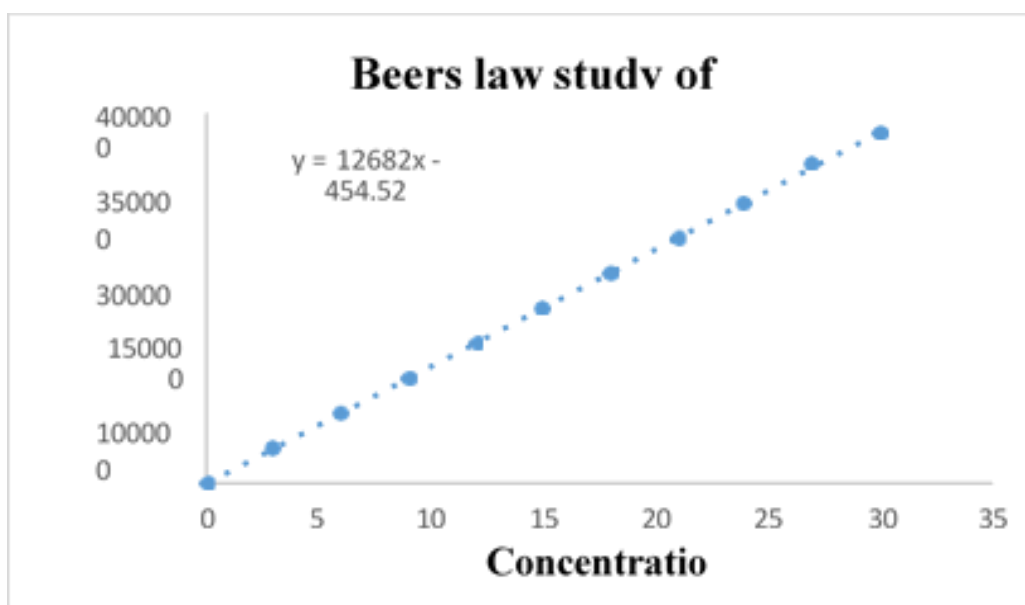


Fig. No. 12: Standard calibration curve for DEXL

**System suitability test**

System suitability is a pharmacopoeial requirement ensuring adequate resolution and

reproducibility of chromatographic systems for analysis, tested using data from five replicate injections of standard solutions.

**Table No. 10: Result of System Suitability Study**

Sr. No	Peak area	Retention Time	Asymmetry	Efficiency
	DEXL	DEXL	DEXL	DEXL
1	378656.5	4.573	1.188	4225564.2
2	378618.6	4.581	1.181	4225145.8
3	378202.1	4.579	1.197	4225871.5
4	378391.4	4.557	1.123	4225623.9
5	378652.7	4.575	1.188	4225564.2
Mean	378504.26	4.573	1.1754	4225553.92
+ S. D	201.446725	0.009486833	0.029837895	261.0208555
C.V	0.000532218	0.002074532	0.025385312	0.000061772

**Application of proposed method for estimation of DEXL Laboratory Sample**

The standard and Sample solution of DEXL was prepared and inject. The peak area of standard and

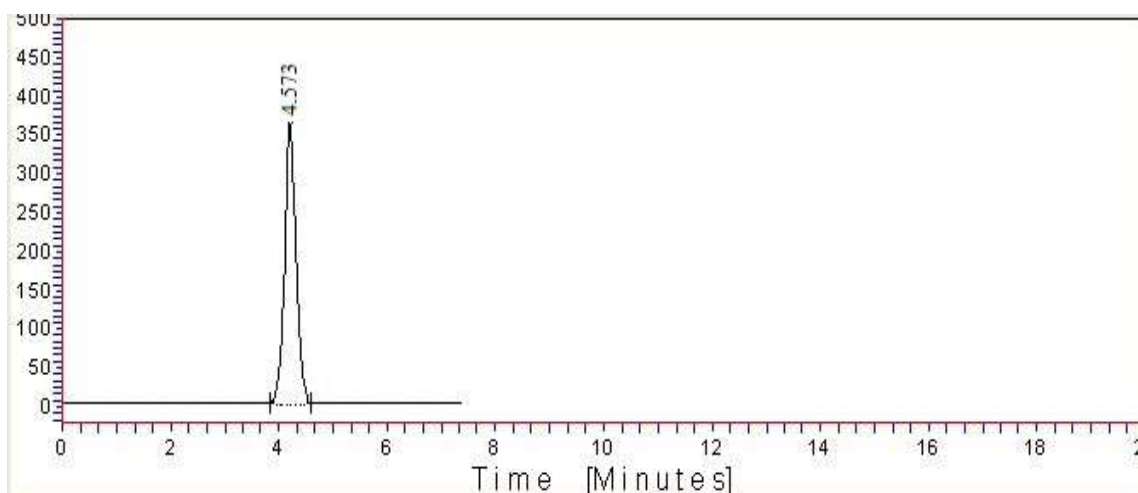
sample laboratory was compared to obtain the concentration.

**Table No 11.: Results and statistical data for estimation of DEXL in lab. Sample**

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Standard	Peak Area of Sample	% Drug Estimation
	DEXL	DEXL	DEXL	DEXL	DEXL
1	10	10	378656.5	377520.5	99.7
2		10		377141.9	99.6
3		10		379035.2	100.1
				<b>Mean</b>	99.80
				<b>±S.D.</b>	0.265
				<b>C.V.</b>	0.003

### Application of proposed method for estimation of DEXL in formulation

Standard and sample solutions were injected separately, chromatograms recorded, and major peaks measured. DEXL content was calculated by comparing sample peak with standard.



**Fig. No.13: Chromatogram obtained by formulation of DEXL**

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Drug Estimation
	DEXL	DEXL	DEXL	DEXL	DEXL
1	10	50	378656.5	378277.8	99.9
2		50		379792.5	100.3
3		49.9		380171.1	100.4
				<b>Mean</b>	100.20
				<b>±S.D.</b>	0.265
				<b>C.V.</b>	0.003

### 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 in Table No. 13

**Table No.13: Results and statistical data for Recovery study of DEXL**

Sr. No	wt. of formulation	Amount of Drug Added in ( $\mu\text{g/ml}$ )	Peak Area of stand.	Peak Area of sample	% Recovery
	DEXL	DEXL	DEXL	DEXL	DEXL
1	50	1	378656.5	377520.5	99.7
2		1		377141.9	99.6
3		1		379035.2	100.1
4		2		379792.5	100.3
5		2		380171.1	100.4
6		2		380549.8	100.5
7		3		382443.1	101
8		3		382821.7	101.1
9		3		382064.4	100.9
				<b>Mean</b>	100.40
				<b>S.D.</b>	0.541
				<b>C.V</b>	0.005

### Precision

The precision of an analytical method, expressed as S.D or R.S.D, was determined through replicate

estimation of drugs using the proposed method, as shown in the table.

**Table No.14: Results and statistical data of Precision Study**

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Label claim
	DEXL	DEXL	DEXL	DEXL	DEXL
1	10	50	378656.5	379792.5	100.3
2		50.1		380171.1	100.4
3		49.9		380549.8	100.5
				<b>Mean</b>	100.40
				<b>±S.D.</b>	0.100
				<b>C.V.</b>	0.001

**Brand Name:** Dexlanzol 30

**Interday (Different days)**

### Ruggedness

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

The same procedure was performed on different days for marketed formulation analysis, and the % label claim was calculated, as shown in Table No. 15.

- 1) Days
- 2) Analyst.

**Table No.15: Results and statistical data of Interday Study**

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Label claim
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	DEXL	DEXL	DEXL	DEXL	DEXL
1	10	50	378656.5	378277.8	99.9
2		50		379792.5	100.3
3		50		380171.1	100.4
				<b>Mean</b>	100.20
				<b>±S.D.</b>	0.265
				<b>C.V.</b>	0.003

**Brand Name:** Dexlanzol 30

**Intraday**

The study used the same procedure as the marketed formulation analysis, recording absorbance at 3 hours intervals within a day, and calculated the percent label claim using a formula.

**Table No.16: Results and statistical data of Intraday Study**

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Label claim
	DEXL	DEXL	DEXL	DEXL	DEXL
1	10	50	378656.5	377141.9	99.6
2		50		379035.2	100.1
3		50.1		379413.8	100.2
				<b>Mean</b>	99.97
				<b>±S.D.</b>	0.321
				<b>C.V.</b>	0.003

**Brand Name:** Dexlanzol 30

**Different analyst**

Two different analysts prepared the sample solution using the same procedure as before, and the % label claim was calculated as per the marketed formulation estimation.

**Table No.17: Result and statistical data of Different analyst study**

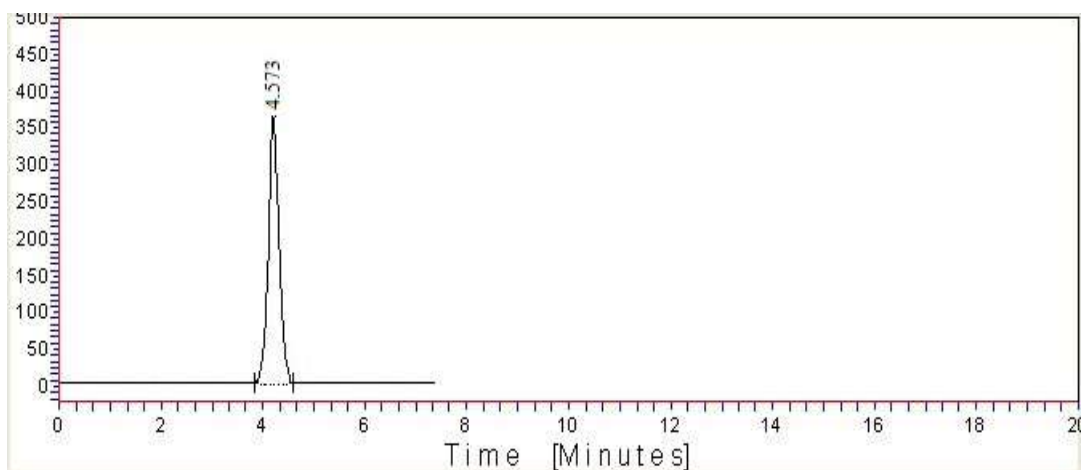
Sr. No	% Label claim	
	Analyst I	Analyst Ii
	DEXL	DEXL
1	99.6	99.9
2	100.3	100.1
3	100.4	100.8
4	99.4	100.9
5	99.8	101.1
<b>Mean</b>	99.9	100.56
<b>□ S. D</b>	0.435889894	0.527257053
<b>C.V</b>	0.004363262	0.005243209

**Specificity**

The proposed method demonstrated specificity by obtaining a well-separated peak for DEXL without

matrix interference, with a mean retention time of 4.573, closely resembling standard laboratory mixture.





**Fig. No.14: Chromatogram obtained by formulation of DEXL**

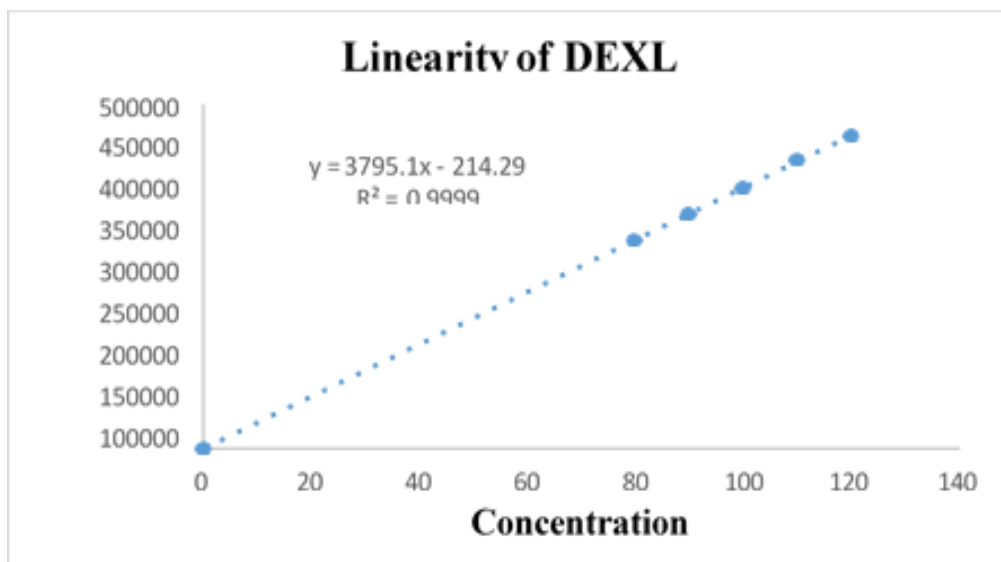
### Linearity and range

within  $\pm 20\%$  of the test concentration, as shown in Fig. No. 15.

USP tablet powder was diluted to 80%-120% of test concentration, revealing DEXL's linearity

**Table No.18: Observations of Linearity and range study for DEXL.**

Sr. No.	%Label claim	Peak area DEXL
1	80	302925.2
2	90	340790.85
3	100	378656.5
4	110	419522.15
5	120	454387.8



**Fig. No.15: -Plot of linearity and range study for DEXL**

### Robustness

The robustness study found that factors selected remained unaffected by small variations in mobile

phase, wavelength, and flow rate, indicating the method's suitability remains within the limit.

**Table No.19: Result of Robustness study of DEXL**

Sr. No.	Condition	Parameter	Peak Area	RT
01	Change of wavelength	273 nm	378618.6	4.575
02		275 nm	378694.4	4.573
03		277 nm	378656.5	4.572
04	Change in Temperature	30 °C	378505	4.578
05		25 °C	378618.6	4.573
06		20 °C	378202.1	4.579
07	Change in Flow rate	0.8 ml/min	378391.4	4.588
08		1ml/min	378652.7	4.573
09		1.2 ml/min	378467.2	4.528
10	Change in Mobile Phase	65:35	379035.2	4.581
11		70:30	378656.5	4.573
12		75:25	378652.7	4.548

### Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of detection refers to the minimum amount of analyte in a sample that can be detected but not quantitated accurately.

**Table 20: LOD & LOQ of DEXL**

Sr. No.	Drug Name	LOD $\mu$ g/ml	LOQ $\mu$ g/ml
1	DEXL	1.02	2.18

### CONCLUSION

The developed and validated RP-HPLC method for the estimation of Dexlansoprazole (DEXL) in bulk and tablet dosage forms has proven to be accurate, precise, specific, robust, and reproducible. Utilizing a mobile phase of Acetonitrile: Phosphate Buffer (70:30) at pH 5 and a detection wavelength of 275 nm, the method demonstrated excellent chromatographic performance with optimal retention time, peak symmetry, and system suitability parameters. Validation studies as per ICH guidelines confirmed the method's reliability across accuracy, precision, specificity, ruggedness, robustness, and linearity. The results indicate no interference from excipients or matrix components, confirming the method's suitability for routine quality control

analysis. This RP-HPLC method can be confidently applied for the routine estimation of Dexlansoprazole in pharmaceutical dosage forms, and it may be extended to other formulations in future research.

### CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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