ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DEXKETOPROFEN TABLETS BY RP-HPLC

Dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY,

CHENNAI

In partial fulfillment for the requirement for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

Submitted by

[Reg. No. 261330352]

Under the guidance of

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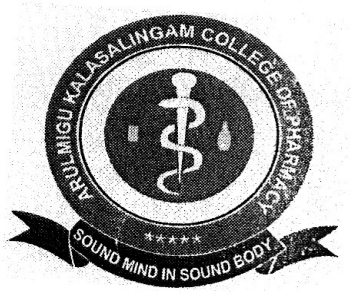

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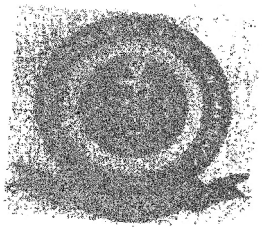

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This is to certify that the investigation described in the dissertation entitled "**ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DEXKETOPROFEN IN TABLET DOSAGE FORM BY RP-HPLC**" submitted by Reg.No:261330352 was carried out in the Department of Pharmaceutical Analysis, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil. which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under my supervision and guidance for the partial fulfillment of degree of MASTER OF PHARMACY in PHARMACEUTICAL ANALYSIS.

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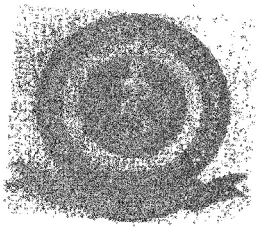

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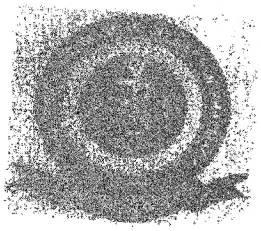

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EXAMINERS:

1.

2.

Affectionately dedicated to

My beloved

Parents

Acknowledgement

"Success is how high you bounce when you hit bottom"

"If you can dream it, you can do it"

The completion of my M. Pharmacy is not only fulfillment of my dreams but also the dreams of my parents who have taken lots of pain for me in completion of my higher studies. I solicit my deep sense of appreciation and love to my wonderful parents and consider my self-privilege to have seen an entity of almighty in them.

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I express my Deepest Affection & thanks to my supporters.

Yes! My thesis is the sensible team effort of all these people, mentioned or non-mentioned here, still, it is too less to express my deep sense of thanks to them.

Last… but not the least, I wish to express my gratitude towards "God" and my parents, who gave me the strength and courage to fulfill my dream and has showered upon me his choicest blessings.

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LIST OF ABBREVATIONS

A	Absorbance
API	Active Pharmaceutical Ingredient
A_s	Asymmetry Factor
AUC	Area Under Curve
C_p	Viscosity
F	Flow Rate
GLC	Gas Liquid Chromatography
GPC	Gel Permeation Chromatography
GSC	Gas Solid Chromatography
h	Peak Height
HETP	Height Equivalent to a Theoretical Plate
HPLC	High Performance Liquid Chromatography
i.d.	Internal Diameter
IUPAC	International Union of Pure and Applied Chemistry
k	Retention Factor or Capacity Factor
L	Length of Column
LOD	Limit of Detection
LOQ	Limit of Quantitation
N	Plate Number
n	Noise
ODS	Octadecylsilane
PDA	Photo Diode Array
Rs	Resolution
RP-HPLC	Reverse phase high performance liquid chromatography
S	Signal
T_f	Tailing Factor
TLC	Thin Layer Chromatography
t_M	Void Time
t_R	Retention Time
USP	United States Pharmacopeia

UV	Ultra Violet
V_0	Intraparticle Volume
V_C	Empty Column Volume
V_E	Interstitial Volume
V_M	Void Volume
V_R	Retention Volume
W_b	Peak Width
α	Separation Factor or Selectivity

INDEX

1.0 INTRODUCTION [1-5]

Analytical chemistry, like other areas of chemistry and science, has gone through drastic changes and growth. Analytical chemistry may defined as the "Science and art of determining the composition of materials in terms of the elements or compounds contained". In analytical chemistry it is of prime importance to gain information about the qualitative and quantitative composition of substances and chemical species, that is to find out what a substance is composed of and exactly how much .

Analytical method is a specific application of a technique to solve an analytical problem. The use of instrumentation is an exciting and fascinating part of chemical analysis that interacts with all areas of chemistry and with many other areas of pure and applied science. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs and water. The manufacture of materials, whose composition must be known precisely such as substances used in integrated circuit chips, is monitored by analytical instruments.

Instrumental or physicochemical methods are based on the theory of relations between the content and the corresponding physicochemical and physical properties of the chemical system being analyzed. Changes in the system properties are either detected or recorded through the measurement of current, electrode potential, electrical conductivity, optical density, refractive index etc. with suitable and sensitive instruments. In instrumental analysis physical property of substance is measured to determine its chemical composition.

Measurements of physical properties of analyte such as conductivity, electrode potential, light absorption or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of variety of inorganic and biochemical analyses. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction, and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination.

1.1 PRINCIPAL TYPES OF CHEMICAL INSTRUMENTATION

- **Spectroscopic Techniques**
  1) Ultraviolet & visible Spectrophotometry
  2) Fluorescence & Phosphorescence Spectrophotometry
  3) Atomic Spectrometry (Emission & absorption)
  4) Infrared Spectrophotometry
  5) Raman Spectroscopy
  6) Radiochemical techniques including activation analysis
  7) Nuclear Magnetic Resonance Spectroscopy
  8) Electron Spin Resonance Spectroscopy

- **Electrochemical Techniques**
  1) Potentiometry
  2) Volta metric techniques
  3) Amperometric techniques
  4) Coulometry
  5) Electrogravimetry
  6) Conductance techniques

- **Chromatographic Techniques**
  1) High Performance Liquid Chromatography
  2) Gas chromatography

- **Miscellaneous Techniques**
  1) Thermal analysis
  2) Mass Spectrometry
  3) Kinetic techniques

- **Hyphenated Techniques**
  1) GC-MS (Gas Chromatography – Mass Spectrometry)
  2) ICP-MS (Inductively Coupled Plasma - Mass Spectrometry)
  3) GC-IR (Gas Chromatography – Infrared Spectroscopy)
  4) MS-MS (Mass Spectrometry – Mass Spectrometry)

1.2 INTORDUCTION TO CHROMATOGRAPHY [5-7]

Chromatography was first invented by Michael Tswett, a Russian botanist in 1906 in Warsaw for the separation of colored substance into individual component.

Chromatography is defined as a procedure by which solutes are separated by a dynamic differential migration process in a system consisting of two or more phases, one of which moves continuously in a given direction and in which the individual substances exhibit mobilities by reason of differences in adsorption, partition, solubility, vapour pressure, molecular size or ionic charge density. The individual substances thus obtained can be identified or determined by analytical methods.

Chromatography was invented nearly 100 years ago, but it is only in the past few years that the development of the technique and associated instrumentation has reached a level that might be called the '*steady state*'.

Separation Process:

The Chromatographic method of separation, in general, involves the following steps:

- Adsorption or retention of substance or substances on the stationary phase.
- Separation of the adsorbed substances by the mobile phase.
- Recovery of the separated substances by the continuous flow of the mobile phase.
- Qualitative and quantitative analysis of the eluted substances.

1.2.1 DIFFERENT FORMS OF CHROMATOGRAPHY

The different forms of chromatography are shown in Fig. 1.

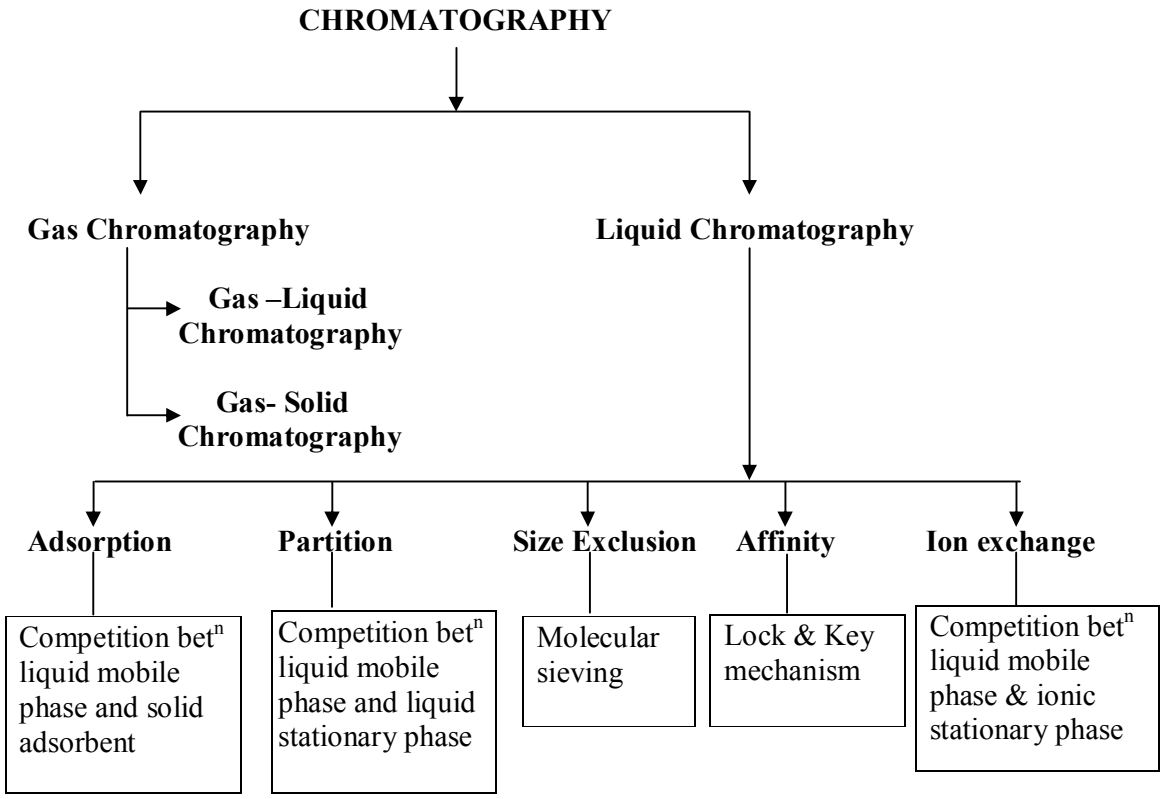


Fig 1: Different Forms Of Chromatography

1.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY [8]

High performance Liquid Chromatography (HPLC), also known as high pressure liquid chromatography, is essentially a form of column Chromatography in which the stationary phase consists of small particle (3-50 μm) packing contained in a column with a small bore (2-5mm), one end of which is attached to source of pressurized liquid eluent (Mobile phase).

In High Performance Liquid Chromatography, the mobile as well as the stationary phase compete for the distribution of the sample components. In case of HPLC, separation is based on:

- **Partition** **:** Separation due to differences in solubility characteristics between two phases.

- **Adsorption** : Separation due to difference in interaction with the surface of the stationary phase.
- **Ion Exchange**: Due to differences in the affinity of ions for the ion exchanger.
- **Size Exclusion***:* Due to differences in molecular weight and size of the molecules to be separated.

Today, HPLC is the most widely used analytical separation method. The method is popular because it is non-destructive and may be applied to thermally labile compounds (unlike GC); it is also very sensitive technique since it incorporates a wide choice of detection methods. With the use of post-column derivatization methods to improve selectivity and detection limits, HPLC can easily be extended to trace determination of compounds that do not usually provide adequate detector response. The wide applicability of HPLC as separation method makes it a valuable separation tool in scientific fields.

1.3.1 MODES OF SEPARATION IN HPLC

There are different modes of separation in HPLC. They are

- Normal Phase Chromatography (NPC);
- Reversed Phase Chromatography (RPC);
- Ion Exchange Chromatography;
- Ion Pair Chromatography;
- Size Exclusion Chromatography; and
- Affinity Chromatography

1.3.2 METHODS USED IN HPLC

1) Normal phase chromatography

For a polar stationary bed like silica we need to choose a relatively non polar mobile phase. This mode of operation is termed as *Normal phase chromatography*. Here the least polar component elutes first, and increasing the mobile phase polarity leads to decrease in elution time. Isopropanol, diisopropyl ether and acetic acid are used as modifiers or tail reducers. Nonpolar solvents like Pentane, Hexane, Iso Octane, Cyclohexane, etc. are more popular. It is extremely important to control the water content of the stationary phase while using silica columns. Variation in moisture content of silica leads to variation in retention times. Therefore, it is a common

practice to saturate the mobile phase with water before using it as eluent in Normal phase chromatography. It is mainly used for separation nonionic, nonpolar to medium polar substances.

2) Reverse phase chromatography

For hydrocarbon type or nonpolar stationary phase, we need to choose a polar mobile phase. This mode of partition chromatography is called as *Reverse phase chromatography*. Here the most polar component elutes first. Increasing mobile phase polarity leads to decrease in elution time. Common solvents used in this mode include Methanol/Acetonitrile/Isopropanol etc. Control of pH is another way to control resolution. In the case of an aromatic acid, a low pH buffer will suppress the ionization, and will increase the capacity factor, while with a high pH buffer, the capacity factor will decrease. Small amounts of modifiers like sodium phosphate/ sodium acetate reduce peak tailing, and increase the separation efficiencies. Mostly used for separation of ionic and polar substances**.**

3) Ion- Pair Chromatography

Ion-pair and RP- HPLC have several similar features. The column and mobile phase used for both these separation techniques are similar, differing mainly in the addition of an ion-pair reagent to the mobile phase for ion-pair chromatography (IPC). If RPC method development is unable to provide an adequate separation due to poor band spacing, IPC provides selectivity option.

1.3.3 Elution Techniques

(a) Isocratic elution:

A separation in which the mobile phase composition remains constant throughout the procedure is termed **isocratic** (meaning constant composition).

(b) Gradient elution:

The mobile phase composition does not have to remain constant. A separation in which the mobile phase composition is changed during the separation process is described as a gradient elution. One example is a gradient starting at 10% methanol and ending at 90% methanol after 20 minutes. The two components of the mobile phase are typically termed "A" and "B"; A is the "weak" solvent which allows the solute to elute only slowly, while B is the "strong" solvent which rapidly elutes the solutes from the column. Solvent A is often water, while B is an organic solvent miscible with water, such as acetonitrile, methanol, THF or isopropanol.

In isocratic elution, peak width increases with retention time linearly according to the equation for N, the number of theoretical plates. This leads to the disadvantage that late-eluting peaks get very flat and broad. Their shape and width may keep them from being recognized as peaks.

Gradient elution decreases the retention of the later-eluting components so that they elute faster, giving narrower (and taller) peaks for most components. This also improves the peak shape for tailed peaks, as the increasing concentration of the organic eluent pushes the tailing part of a peak forward. This also increases the peak height (the peak looks "sharper"), which is important in trace analysis. The gradient program may include sudden "step" increases in the percentage of the organic component, or different slopes at different times - all according to the desire for optimum separation in minimum time.

In isocratic elution, the selectivity does not change if the column dimensions (length and inner diameter) change - that is, the peaks elute in the same order. In gradient elution, the elution order may change as the dimensions or flow rate change.

The driving force is originated in reversed phase chromatography in the high order of the water structure. The role of the organic mobile phase is to reduce this high order by reducing the retarding strength of the aqueous component.

1.3.4 HPLC Instrumentation

A liquid chromatography consists of a reservoir containing the mobile phase, a pump to force the mobile phase through the system at high pressure, an injector to introduce the sample into the mobile phase, a chromatographic column, a detector, and a data collection device such as a computer, integrator, or recorder. Short, small bore columns containing densely packed particles of stationary phase provide for the rapid exchange of compounds between the mobile and stationary phases. In addition to receiving and reporting detector output, computers are used to control chromatographic settings and operations, thus providing for long periods of unattended operations.[9-10]

The different parts of HPLC system are schematically shown in the Fig. 2.

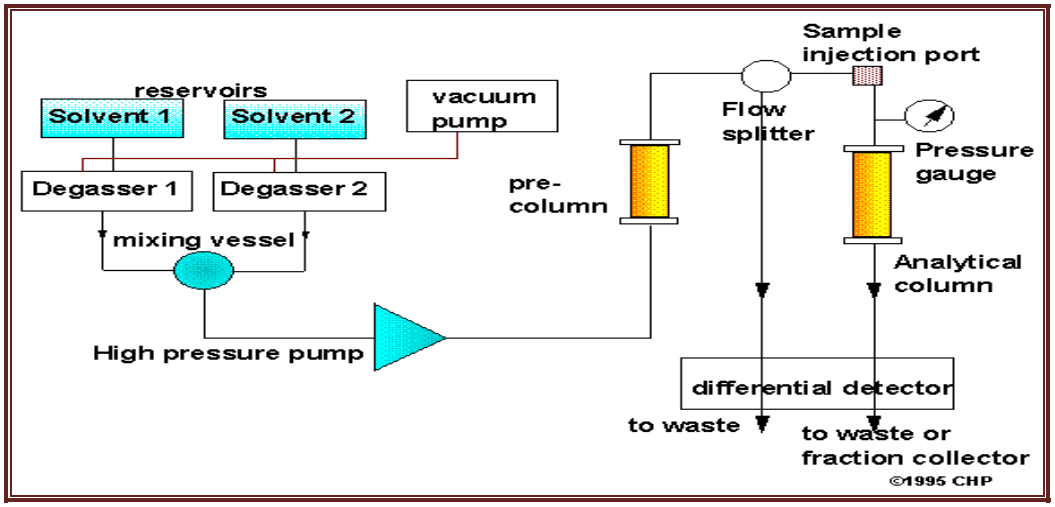


Fig. 2: High Performance Liquid Chromatography (Schematic).

1.4 HPLC METHOD DEVELOPMENT

1.3.1 METHOD DEVELOPMENT[9-10]

Today the development of a method of analysis is usually based on prior art or existing literature, using the same or quite similar instrumentation. It is rare today that an HPLC based method is developed that does not in some way relate or compare to existing, literature based approaches. The development of any new or improved method usually tailors existing approaches and instrumentation to the current analyte, as well as to the final needs or requirements of the method.

Method development usually requires selecting the method requirements and deciding on what type of instrumentation to utilize and why. In the development stage, decisions regarding choice of column, mobile phase, detectors, and method of quantitation must be addressed. In this way, development considers all the parameters pertaining to any method.

There are several valid reasons for developing new method of analysis:

- There may not be suitable method for a particular analyte in the specific sample matrix.
- Existing method may be inaccurate, artifact , and /or contamination prone, or they may be unreliable (have poor accuracy or precision)
- Existing methods may be too expensive, time consuming or energy intensive, or they may not be easily automated.

- Existing methods may not provide adequate sensitivity or analyte selectivity in samples of interest.
- Newer instrumentation and techniques may have evolved that provide opportunities for improved methods, including improved analyte identification or detection limits, greater accuracy or precision , or better return on investment.
- There may be a need for an alternative method to confirm for legal or scientific reasons analytical data originally obtained by existing methods.

Separation Goals And Steps Involved In HPLC Method Development

The major steps involved in the methods development are shown in the Fig. 3 and the separation goals in HPLC method development are shown in Table 1.

Goal	**Comment**
Resolution	Precise and rugged quantitative analysis requires that *Rs* be greater than 1.5.
Separation time	<5-10 min is desirable for routine procedures.
Quantitation	≤2% (1 SD) for assays; ≤5% for less-demanding analyses; ≤15% for trace analyses.
Pressure	<150 bar is desirable, <200 bar is usually essential (new column assumed).
Peak height	Narrow peaks are desirable for large signal/ noise ratios.
Solvent consumption	Minimum mobile-phase use per run is desirable.

Table 1: Separation Goals In Hplc Method Development

HPLC method development is based on few basic steps includes: [11-12]

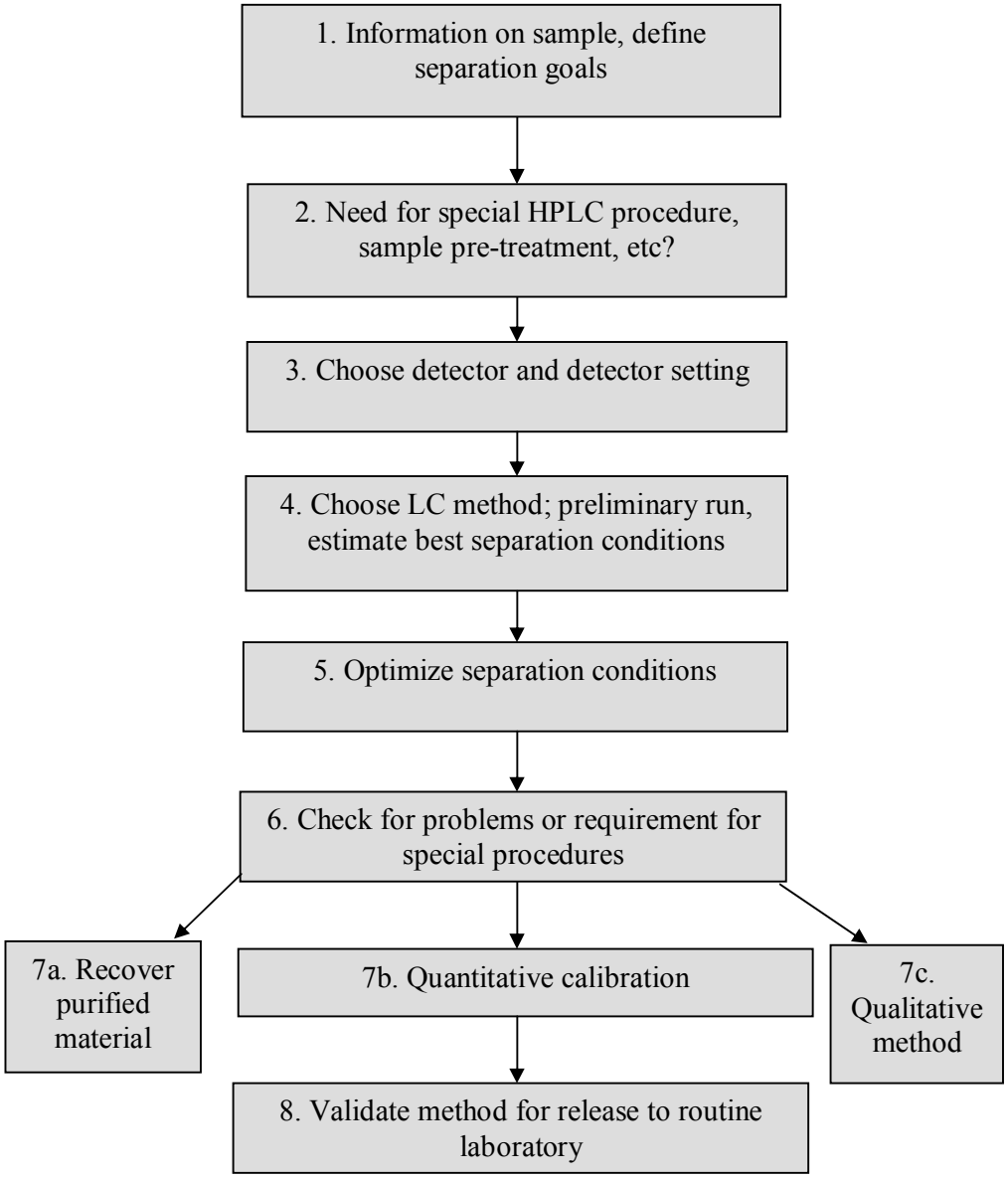


Fig 3: Steps in HPLC Method Development

Once the instrumentation has been selected, based on the criteria suggested above, it is important to determine "Analyte Parameters" of interest. To develop a method it is necessary to consider the properties of analytes of interest that may be advantageous to establish optimal ranges of analyte parameter values. It is important that method development may be performed using only analytical standards that have

been well identified and characterized, and whose purity is already known. Such precautions will prevent problems in the future and will remove variables when one is trying to optimize or improve initial conditions during method development.

1.3.2 Step-by-Step HPLC method development[13-16]

Documentation starts at the very beginning of the method development process, a system for full documentation of the development studies must be established. All data relating to these studies must be established. All data relating to these studies must be recorded in laboratory notebooks or an electronic database.

Different steps used in method development are:

1. Literature collection

- Search the literature (USP, EP, JP, IP, Chromatography journals, patents, internet etc.) for available/ existing HPLC methods for the given molecule or molecules having similar structures.
- Check the suitability of reported literature methods and see if the requirements are met. If required, modify the method to meet the requirements such as resolution of possible impurities as per the synthetic process.
- Specify the components to be evaluated in the HPLC method considering all the possible impurities that can be present in drug substance which could be intermediates, raw materials, process related impurities and degradants.
- Collect samples, standards and all possible impurities of each stage, if available.

2. Chemical structure and other information

- Obtain information on analyte solubility, the pKa values (if available) and functional groups since it is a prerequisite for effective method development.
- Based on the structure and the functional group present it can be determined whether the compound is basic, acidic or neutral.

3. Polarity

- Depending on the solubility and nature (acid/base/ionic/non-ionic) of the molecule select the appropriate mode given below.
- Reversed phase chromatography - acids, bases and non-ionic sample
- Ion-pair chromatography - ionic samples.
- Ion exchange chromatography - ionic samples.

- ❖ Normal phase chromatography - isomers, non-polar/non-ionic samples, chiral molecules.
- ❖ Size exclusion chromatography - high molecular weight samples such as proteins.

4. pH/pka Value of compounds

- ❖ Based on pH/pKa values, the nature of the compound (acidic, basic or neutral in nature) and polarity of the compound can be assumed.

Note: if the compound is acidic, acidic mobile phase is preferable. For a basic compound, low pH and basic mobile phase is preferable. For a neutral compound neutral mobile phase is suitable.

5. Solubility

- ❖ Check the solubility of the sample in solutions like mobile phase, mobile phase organic mixtures, water-organic mixtures and mixtures of acids like perchloric acid, phosphoric acid etc.

Note: If the analyte is soluble in polar solvents like water, methanol, chloroform, acetonitrile or tetrahydrofuran, the method development should be initiated in reversed phase chromatography. If the analyte is soluble in non-polar solvents like ethyl acetate, n-hexane, dioxane etc. then the method development can be initiated in normal phase chromatography.

6. Column selection

- ❖ **Reversed phase**

Column containing C_4, C_8, C_{18}, Cyano and phenyl, amino stationary phases comes under reversed phase category and can be used against a more polar mobile phase. The retention of non-polar compounds increases as the length of the alkyl ligand (C_4, C_8, C_{18} etc.) increases.

- ❖ **Normal phase**

Columns containing cyano, phenyl and silica stationary phases come under normal phase category and are used against non-polar mobile phases.

The impact of the bonded phase, internal diameter, particle surface area, pore volume and size of theoretical plates, peak symmetry, selectivity and resolution should be evaluated.

7. Selection of detector

- Select a suitable detection technique based on the structure and nature of the molecule.
- UV detectors :compounds having chromophores
- Fluorescence Detector : Compounds having Fluorescence properties
- Electrochemical Detector : Easily oxidizable compounds
- RI Detector: Universal Detector: but cannot be used with gradient elution. This detector is used for compounds which do not contain chromophores.
- ELSD: Universal Detector, but can be used with gradient analysis with higher sensitivity.

8. Mobile phase selection

- **Choice of solvents for reversed phase chromatography**

The solvents like Water, Methanol, Acetonitrile, Tetrahydrofuran, Isopropyl alcohol, Ethanol etc.

Note: when THF is used as mobile phase component, the content should not be more than 20%**.**

- **Organic modifiers**

Triethylamine, Diethyl amine, Trifluoroacetic acid, Phosphates, Perchlorates, Chlorides, Bromides, Nitrates etc.

- **Choice of buffering agent**

Phosphates of sodium, potassium, ammonium etc, perchlorates of ammonium, sodium, potassium etc., ammonium acetate etc. Buffering agent should be selected appropriately in their buffering range to maintain a constant pH of the mobile phase.

- **Choice of solvents for normal phase chromatography**

Solvents like Isopropyl alcohol, Ethanol, n-hexane, Dioxin, Chloroform etc. Organic modifiers used with normal phase chromatography: Triethylamine, Diethyl amine, Trifluoroacetic acid, Formic acid and Acetic acid.

- **Ionic compounds**

Compounds which are highly polar or ionic in nature do not retain on reversed phase columns and have poor peak symmetry. For such compounds the method should be developed either in ion exchange chromatography mode or ion

pair chromatography is preferred because it can be run on the conventional reversed phase columns such as $C_{8,}$ C_{18} etc. with mobile phase additives.

Ion pair reagents (sodium lauryl Sulphate, heptane Sulphonic acid, Tetra butyl ammonium hydroxide, etc.) of concentrations 0.001%-0.5% can be added to the mobile phase to get optimum retention, peak symmetry and separations.

Note: Concentrations more than specified range should be justified for acidic compounds cationic ion pair reagents like tetra butyl ammonium hydrogen sulphate, tetra methyl ammonium bromide, sodium per chlorate, can be used as additives in the mobile phase at suitable pH.

For basic compounds, anionic ion pair reagents like sodium salt of butane, pentane, hexane or octane sulphonic acid, per chlorates etc. can be used as additives in the mobile phase at suitable pH.

9. Instrument set up and Initial Studies:

a) Set up the required instrumentation. Verify installation and operational and performance qualifications of instrumentation using laboratory standard operating procedures (SOP's).

b) Always use new consumables (e.g. solvents filters and gases). For example never start method development on an HPLC column that has been used before.

c) Prepare the analyte standard in a suitable injection / introduction solution and in known concentration and solvents. It is important to start with an authentic, known standards rather than with a complex sample matrix. If the sample is extremely close to the standard (eg. Bulk drug), then it is possible to start work with actual sample.

d) Begin the analysis using the analytical conditions described in the existing literature.

e) Evaluate feasibility of method with regard to the analytical figures of merit obtained

10) Optimization

During optimization change one parameter at a time, and isolate set of conditions, rather than use a trial and error approach. . Work from an organized, methodical plan, and document every step (Keep a lab notebook) in case of dead ends.

11) Demonstration of analytical figures of merit

Document the originally determined analytical figures of merit [limit of quantitation (LOQ), limit of detection (LOD), linearity, time per analysis, cost, sample preparation etc]

12) Evaluation of method development with actual samples

Ensure that the sample solution leads to unequivocal, absolute identification of the analyte peak of interest apart from all other matrix components.

13) Determination of percent recovery of actual sample and demonstration of quantitative sample analysis

Determine percent recovery of spiked, authentic standard analyte into sample matrix that is shown to contain no analyte. Show reproducibility of recovery (average ± standard deviation) from sample to sample and whether recovery has been optimized. It is not necessary to obtain 100% recovery as long as the results are reproducible and known with a high degree of certainty.

1.3.4 Chromatographic Parameters :

A) RETENTION TIME
B) RETANTION VOLUME
C) SEPERATION FACTOR
D) CAPACITY FACTOR
E) ASYMMETRIC FACTOR
F) THEORETICAL PLATES.
G) RESOLUTION

A) RETENTION TIME (Rt): It is the difference in time between the point of injection and the appearance of peak maxima. Retention time is the time required for 50 % of a component to be eluted from a column. It is measured in minutes or seconds.

B) RETENTION VOLUME (Vr): It is the volume of carrier gas required to elute 50 % of the component from the column.

$$R.V = R.T \times \text{Flow rate.}$$

C) SEPERATION FACTOR: Separation factor is the ratio of partition - co-efficient of the two components to be separated.

$$S = \frac{Kb}{Ka} = \frac{Ka}{Kb} = \frac{(tb - t\,o)}{(ta - t\,o)}$$

Where, to = Retention time to unretained substance

Kb, Ka = Partition Coefficient of b and a

tb, ta = Retention time of substance b and a

S = depends on liquid phase, column temperature.

D) CAPACITY FACTOR: It is generally required to be calculated by the difference in the retention time of main peak and the retention time of uracil injected to the retention time of the main peak.

$$K' = \frac{Rt\text{-}Ru}{Rt}$$

Where**, Rt** : is the retention time of main peak,

Ru: is the retention time of uracil.

K': is the capacity factor

It is one of the important factor need to be determined while performing method development of multi formulation, it should be always less then 2.

E) ASSYMETRIC FACTOR: A chromatographic peak should be symmetrical about its center and said to follow Gaussian distribution. But due to some factors, the peak is not symmetrical and shows tailing and fronting.

- **FRONTING**: It is due to the saturation of stationary phase and can be avoided by using less quantity of sample.
- **TAILING:** It is due to more active adsorption sites and can be eliminated by support pretreatment, more polar mobile phase, increasing the amount of liquid phase.

F) THEORETICAL PLATES (PLATE THEORY): A theoretical plate is an imaginary or hypothetical unit of a column where distribution of solute between stationary and mobile phase has attained equilibrium. A theoretical plate can also be called as a functional unit of the column.

G) RESOLUTION (Rs) : Resolution is measure of the extent of separation of the two components and the base line separation achieved.

1.4 METHOD VALIDATION [16-18]

Validation is defined as follows by different agencies

Food and Drug administration (FDA): Establishing documentation evidence, which provides a high degree of assurance that specific process, will consistently produce a product meeting its predetermined specification and quality attributes.

World Health Organization (WHO): Action of providing that any procedure, process, equipment, material, activity, or system actually leads to the expected results.

European Committee (EC): Action of providing in accordance with the principles of good manufacturing practice, that any procedure, process, equipment material, activity or system actually lead to the expected results. In brief validation is a key process for effective Quality Assurance.

Analytical method validation

Analytical monitoring of a pharmaceutical product or of specific ingredients within the product is necessary to ensure its safety, efficacy throughout all phases of its shelf life. Such monitoring is in accordance with the specifications elaborated during product development.

Analytical method validation is the corner stone of process validation without a proven measurement system it is impossible to confirm whether the manufacturing process has done what it purports to do. All new analytical methods developed are validated.

Steps followed for validation procedures

1. Proposed protocols or parameters for validations are established
2. Experimental studies are conducted
3. Analytical results are evaluated
4. Statistical evaluation is carried out
5. Report is prepared documenting all the results

1.4.1 VALIDATION PARAMETERS

Analytical methods are required for the identification, batch analysis and storage stability data for active constituents of Pharmaceutical product, and for post-registration compliance purposes. Analytical method development as a first step is carried out to ensure that the API used and the dosage forms that are developed and manufactured for human consumption are meeting the regulated quality norms. Every newly developed method must be validated prior to sample analysis. Validation must also be repeated if a parameter has been modified or if the validation was strongly performed in another laboratory, to ensure that the methods are transferable. A verification is necessary if the analyst or instrument have been changed, or if the sample type has been modified.

The objective of validation of an analytical method is to demonstrate that the procedure, when correctly applied, produces results that are fit for purpose. Method validation is a practical process designed and experimentally carried out to ensure that an analytical methodology is accurate, specific, reproducible and rugged over the specified range of analysis. Validation provides both assurance and reliability during normal use and documented evidence that the method is 'fit for purpose'.

Method validation ensures the validity of a measurement before it is carried out and is essential part of quality assurance procedures. The process of method validation provides information on the critical factors affecting the method output, thus enabling suitable quality control procedures to be implemented to ensure data quality. The extent of method validation will vary with applications, sector and regulatory compliance.

Typical validation characteristics which should be considered are listed below

- Accuracy
- Precision
- Specificity
- Detection limit
- Quantitation limit
- Linearity
- Range
- Robustness etc.
- Ruggedness

1.4.2 ICH VALIDATION PARAMETERS [19,20]

Type of analytical procedure	Identification	Testing for impurities		Assay-dissolution (measurement only) - content/potency
Characteristics		Quantitation	Limit	
Accuracy	-	+	-	+
Precision Repeatability Interm. Precision	 - -	 + (1)	 - -	 + (1)
Specificity (2)	+	+	+	+
Detection Limit	-	(3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

Table 2 : Data Elements Required For Validation According To ICH

- \- signifies that this characteristic is not normally evaluated
- \+ signifies that this characteristic is normally evaluated
- (1) In cases where reproducibility has been performed, intermediate precision is not needed
- (2) Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)
- (3) May be needed in some cases

1.5.3USP VALIDATION PARAMETERS [19,20]

Analytical Performance Characteristics	Category I	Category II		Category III	Category IV
		Quantitative	Limit Tests		
Accuracy	Yes	Yes	*	*	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	*	Yes
Detection Limit	No	No	Yes	*	No
Quantitation Limit	No	Yes	No	*	No
Linearity	Yes	Yes	No	*	No
Range	Yes	Yes	*	*	No
* May be required, depending on the nature of the specific test.					

Table 3: Data Elements Required for Validation According to USP

1) Accuracy

The accuracy of an analytical method is defined as the degree to which the determined value of analyte in a sample corresponds to the true value. Accuracy may be measured in different ways and the method should be appropriate to the matrix. The accuracy of an analytical method may be determined by any of the following ways:

- Analyzing a sample of known concentration and comparing the measured value to the 'true' value. However, a well characterized sample (e.g. reference standard) must be used.
- Spiked-placebo (product matrix) recovery method. In the spiked-placebo recovery method, a known amount of pure active constituent is added to formulation blank (sample that contains all other ingredients except the active ingredient), the resulting mixture is assayed, and the results obtained are compared with the expected result.
- Standard addition method. In the standard addition method, a sample is assayed, a known amount of pure active constituent is added, and the sample is again assayed. The difference between the results of the two assays is compared with the expected answer.

In both methods (spiked-placebo recovery and standard addition method), recovery is defined as the ratio of the observed result to the expected result expressed as a percentage.

The accuracy of a method may vary across the range of possible assay values and therefore must be determined at several different fortification levels. The accuracy should cover at least 3 concentrations (50%, 100% and 150%) in the expected range.

Accuracy may also be determined by comparing test results with those obtained using another validated test method.

Acceptance criteria:

The expected recovery depends on the sample matrix, the sample processing procedure and on the analyte concentration. The mean % recovery should be within the following ranges: For assay: 98%-102%

2) Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Precision may be considered at three levels:

- System Precision (Repeatability)
- Method Precision (Reproducibility)
- Intermediate precision (Ruggedness)

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. A minimum of 6 replicate sample determinations should be made together with a simple statistical assessment of the results, including the percent relative standard deviation. The following levels of precision are recommended.

Acceptance criteria: For an assay the %RSD should be NMT 2%

3) Specificity [21-23]

It signifies the instruments, ability to measure or identify the analyte without any interference from sample matrix, impurities, precursors or degradation product.

Forced degradation

Forced degradation provides useful information about the degradation pathways and degradation products during the product storage. The information thus obtained will facilitate pharmaceutical development in areas such as formulation development, manufacturing, and packaging, where knowledge of chemical behavior can be used to improve the quality of drug product.

HPLC methods should be able to separate, detect, and quantify the various drug-related degradants that can form on storage or manufacturing, plus detect and quantify any drug-related impurities that may be introduced during synthesis. Forced degradation studies (chemical and physical stress testing) of new chemical entities and drug products are essential to help develop and demonstrate the specificity of such stability-indicating methods. In addition to demonstrating specificity, forced degradation studies can be used to determine the degradation pathways and degradation products of the APIs that could form during storage, and facilitate formulation development, manufacturing, and packaging. Procedures for the preparation of specific degradation products needed for method validation often emerge from these studies.

For marketing applications, current FDA and ICH guidance recommends inclusion of the results, including chromatograms of stressed samples, demonstration of the stability indicating nature of the analytical procedures, and the degradation pathways of the API in solid state, solution, and drug product. The chemical structures

of significant degradation products and the associated procedures for their isolation and/or characterization are also expected to be included in the filing. The experimental protocol for performing forced degradation studies will depend on the active ingredients and formulation involved because the chemistry of each compound is different. In general, a target of approximately 10-30% degradation of the API during forced degradation, or exposure to energy in slight excess of what is typically used in accelerated storage is recommended. In this way, the "worst-case" degradation products can be studied. The following will provide some suggestions for performing forced degradation studies based upon available guidance from the ICH and FDA.

Stress testing is likely to be carried out on a single batch of the drug substance. The testing should include the effect of temperatures [in 10°C increments (i.e., 50°C, 60°C) above that for accelerated testing], humidity (i.e., 75% relative humidity or greater) where appropriate, oxidation, and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension.

The stress studies should assess the stability of the drug substance in different pH solutions, in the presence of oxygen and light, and at elevated temperatures and humidity levels. The guidance does not specify pH, temperature ranges, specific oxidizing agents, or conditions to use, the number of freeze-thaw cycles, and so on. In general, values anywhere between 5% to 20% degradation of the drug substance have been considered as reasonable and acceptable for validation of chromatographic assays.

Different forced degradation conditions to be used for drug substances & products [16]

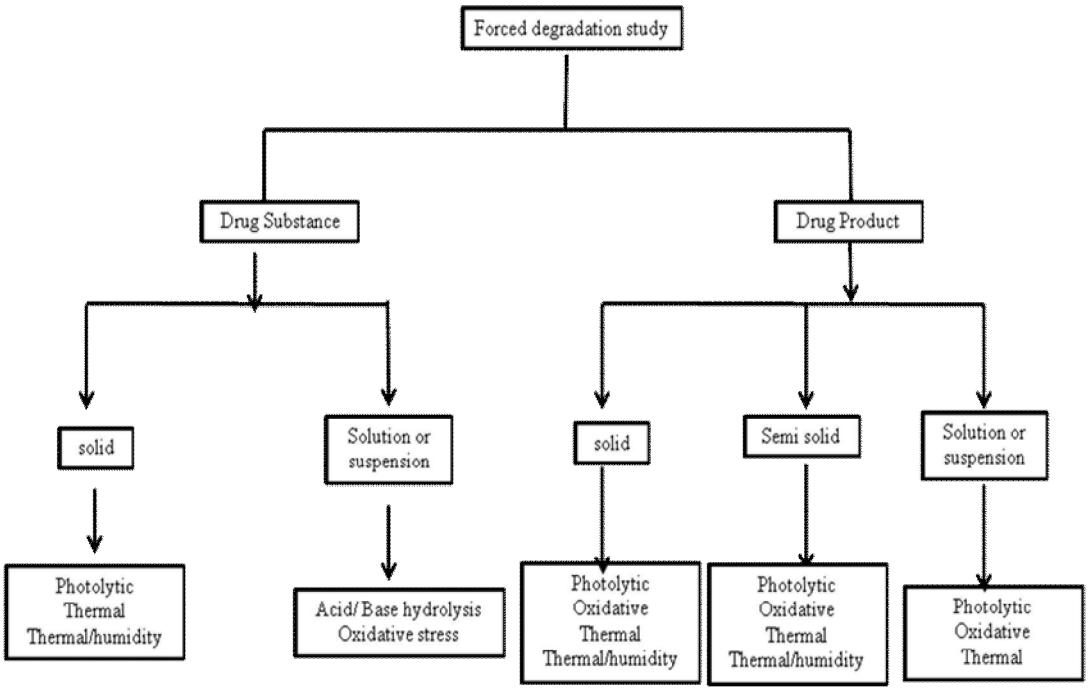


Fig 4: Different Forced Degradation Conditions To Be Used For Drug Substances & Products

However, for small pharmaceutical molecules for which acceptable stability limits of 90% of label claim is common, pharmaceutical scientists have agreed that approximately 10% degradation is optimal for use in analytical validation. In designing forced degradation studies, it must be remembered that more strenuous conditions than those used for accelerated studies (25°C/60% RH or 40°C/75% RH) should be used.

It must be noted that a forced degradation study is a "living process" and should be done along the developmental time line as long as changes in the stability-indicating methods, manufacturing processes, or formulation changes are ongoing. Forced degradation is only considered complete after the manufacturing process is finalized, formulations established, and test procedures developed and qualified.

At a minimum, the following conditions should be investigated:

1. Acid and base hydrolysis
2. Hydrolysis at various pH
3. Thermal degradation
4. Photolysis degradation

4) Detection limit

The detection limit of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated as an exact value.

The LOD may be determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level (lowest calibration standard) at which the analyte can be reliably detected. The lowest calibration standard which produces a peak response corresponding to the analyte should be measured 'n' times (normally 6-10). The average response (X) and the standard deviation (SD) calculated.

The LOD = 3.3 * SD/ Slope of Calibration curve

SD = Standard Deviation

5) Quantitation limit

The limit of quantitation is the lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the stated experimental conditions. The limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products or low levels of active constituent in a product. The LOQ may be determined by preparing standard solutions at estimated LOQ concentration (based on preliminary studies). The solution should be injected and analyzed 'n' times (normally 6-10). The average response and the standard deviation (SD) of the n results should be calculated and the SD should be less than 20%. If the SD exceeds 20%, a new standard solution of higher concentration should be prepared and the above procedure repeated.

The LOQ = 10 * SD/ Slope of Calibration curve

SD = Standard Deviation

6) Linearity

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) and/or by using separate weighing of synthetic mixtures of the test product components, using the proposed procedure.

Linearity is determined by series injections of whose concentrations span 50-150 percent of the expected concentration range. The response should be directly proportional to the concentrations of the analytes or proportional by means of a well-defined mathematical calculation. A linear regression equation applied to the results should have an intercept not significantly different from zero (0). If a significant nonzero intercept is obtained, it should be demonstrated that this has no effect on the accuracy of the method.

7) Range

The range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. The range is normally expressed in the same units as the test results (e.g. percentage, parts per million) obtained by the analytical method.

For assay tests, the ICH requires the minimum specified range to be 80 to 120 percent of the test concentration, and for the determination of an impurity, the range to extend from the limit of quantitation, or from 50 percent of the specification of each impurity, whichever is greater, to 120 percent of the specification.

8) Robustness

Robustness tests examine the effect that operational parameters have on the analysis results. For the determination of a method's robustness, a number of method parameters, for example, pH, flow rate, column temperature, injection volume, detection wavelength or mobile phase composition, are varied within a realistic range, and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range.

Obtaining data on these effects helps to assess whether a method needs to be revalidated when one or more parameters are changed, for example, to compensate for column performance over time. In the ICH document, it is recommended to consider the evaluation of methods robustness during the development phase, and any results that are critical for the method should be documented. This is not however, required as part of a registration.

9) Ruggedness

Ruggedness tests examine the effect that operational parameters have on the analysis results. The ruggedness was determined by changing the column same manufacture but different lot numbers and Analyst variability and system to system variability. In all the ruggedness conditions the method parameters shall meet the system suitability criteria.

10) Solution stability

Solution stability was determined by Preparing the samples and standard solutions at room temperature and inject at initially and after 1 day, 2day, 3day if possible Similarly Prepare the mobile phase and keep it at room temperature in well closed condition and inject into HPLC system. Evaluate the all the system suitability parameters.

Proposed Acceptance Criteria for the Different Characteristics of Validation by ICH [18 ,20]

Characteristics	Proposed Use Acceptance Criteria
Linearity	$r^2 \geq 0.99$, similar response ratios
Precision-System	RSD < 2 %
Precision-Method	RSD < 2 %
Precision Repeatability/Reproducibility	RSD ≤ 2 %
Accuracy	FDA 98-102 %, EPA 50-150 %
Specificity	No interference
Detection Limit	>2 times base line
Quantitative Limit	Signal to Noise ratio = 10:1
Range	Concentration where data can be reliably detected

Table 4: Proposed ICH Acceptance Criteria For The Different Characteristics Of Validation

CHAPTER 3

REVIEW OF LITERATURE

The following methods have been reported for the estimation of Dexketoprofen individually and in combination with other drugs.

1) M.T.Harde et al [23]

A simple, fast, accurate and precise method has been developed for the simultaneous determination of thiocolchicoside and dexketoprofen from pharmaceutical formulation by reversed-phase high performance liquid chromatography. The separation was carried out on C18 column using mobile phase consisting of a mixture of methanol: phosphate buffer and pH adjusted to 4.5 with orthophosphoric acid inthe ratio (65:35 v/v). The flow rate was maintained at 1.0 ml/min. The UV detection was carried out at a wavelength of 260 nm. The retention time for thiocolchicoside and dexketoprofen was found to be 3.02 min and 8.91 min respectively. Linear response obtained for thiocolchicoside was in the concentration range 4-24 µg/ml (r2 = 0.9998) and dexketoprofen in the range 5-30 µg/ml (r2 = 0.9990). The relative standard deviation in the tablets was found less than 2% for six replicates. The method was validated according to theICH guidelines with respect to linearity, precision, accuracy, limit of detection, limit of quantification and robustness. Thus, proposed method can be successfully applicable to the pharmaceutical preparationcontaining the above mentioned drugs without any interference of excipients.

2) Rao J R et al [24]

An accurate, simple, sensitive and precise reverse phase high performance liquid chromatographic (RP-HPLC) method for the determination of paracetamol and dexketoprofen trometamol was developed and validated in the bulk drug and in tablet dosage form. Thermo Hypersil ODS–C18 (250 mm × 4.6 mm, 5.0 µ) used as stationary phase and methanol: ammonium acetate buffer (65: 35 v/v) as mobile phase at a flow rate of 1.0 ml/min and the detection wavelength was 256 nm. The

retention time for paracetamol and dexketoprofen trometamol was found to be 3.20 and 5.94 min, respectively. Proposed method was validated for precision, accuracy, linearity range and robustness.

3) Dhaneshwar et al [25]

In the present study, comprehensive stress testing of Dexketoprofen trometamol was carried out according to ICH guideline Q1A (R2). Dexketoprofen trometamol is subjected to stress conditions of hydrolysis, oxidation, photolysis and neutral decomposition. Additionally, the solid drug is subjected to 50 °C for 60 days in dry-bath, and to the combined effect of temperature and humidity, with and without light, at 40°C/75% RH. The drug was found to degrade significantly in oxidative and photo condition whereas it is found to be stable in acidic, alkaline and neutral condition. The drug is relatively stable in the solid-state, except formation of minor products under accelerated conditions. Successful separation of drug from degradation products formed under stress conditions is achieved on a Thermo Hypersil BDS–C_{18} (250 mm × 4.6 mm, 5.0 µ) from Germany with isocratic conditions and simple mobile phase containing methanol: acetate buffer pH adjusted to 4 with glacial acetic acid (65: 35) at flow rate of 1 mL/min using UV detection at 255 nm. The method is validated according to ICH guidelines. The developed method is found to be precise, accurate, specific and selective.

4) D. V. Pokharkar et al [26]

A simple, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of Dexketoprofen trometamol and Paracetamol from tablet dosage form using a Hypersil BDS, C18 column (5µ, 4.5mm x 250 mm) column and mobile phase composed of 0.01m Potassium Dihydrogen Phosphate:acetonitrile (75:25 v/v) pH 6.0 adjusted with Triethylamine, at flow rate of ml/min. The retention time of Dexketoprofen Trometamol and Paracetamol were found to be 6.732 and 3.256min respectively. Linearity was established for both drugs in the

concentration range of 50-150µg/ml. The percentage recoveries of Dexketoprofen Trometamol and Paracetamol were found to be in the range of 98.12%-101.82% and 98.15%-101.8% respectively. Detection was carried out at wavelength 254nm using photodiode array detector. The separation was carried out at 400^{o}C temperature. Both the drugs were subjected to acid, alkali, neutral hydrolysis, oxidation, dry heat, and UV degradation. The degradation studies indicated Dexketoprofen trometamol and Paracetamol showed degradation in acid and alkali. The degradation products of Dexketoprofen trometamol and Paracetamol in acidic and alkali were well resolved from the pure drug with significant differences in their retention time values. This method can be successfully employed for simultaneous quantitative analysis of Dexketoprofen trometamol and Paracetamol in tablet formulations.

5) Krunal Pandya et al [27]

The study is focused to developed and validate a UV-Spectroscopic method and HPLC Method for simultaneous estimation of Dexketoprofen Trometamol from their dosage form. Equation is applied in spectroscopic method, in which wavelengths, 260nm has been been selected. At this wavelength drug have considerable absorbance. The method was found to be linear in range of 2-12µg/mL for Dexketoprofen Trometamol. The accuracy and precision were determined and validated statistically.

A simple Reverse Phase Liquid Chromatographic method has been developed and validated for determination of Dexketoprofen Trometamol. The separation was carried out using mobile phase consisting of Phosphate buffer: Methanol (30:70). The column was used Luna 5u C18 (2) 100A of size 0.25m *4.6mm with flow rate of 1.0 mL/min using 260nm as detector. The describe method was linear over concentration range of 10-50 ppm for assay of Dexketoprofen Trometamol. The retention time of Dexketoprofen Trometamol was found to be 4.31. Result of analysis was validated statistically. Both the method shows good reproducibility and recovery with less than 1%. All the test above mentioned studies were found to be in acceptance criteria. The method was found to be rapid, specific, precise and

accurate and can be successfully applied for the routine analysis of Dexketoprofen Trometamol bulk and marketed dosage form.

6) Santosh V.Gandhi et al [28]

This paper describes a simple, sensitive, accurate, and validated reverse-phase high-performance liquidchromatographic (RP-HPLC) method for the simultaneous quantification of these compounds as the bulk drug andin tablet dosage forms. Separation was carried out on Jasco HPLC system equipped with HiQ sil C18 HS colum(250 × 4.6 mm i.d.) and PDA detector using Methanol: Sodium acetate buffer (70:30, v/v) with pH adjusted to 5with Glacial acetic acid as the mobile phase and detection was carried out at 265 nm. Results were linear in therange of 5-30 µg mL−1 for Dexketoprofen and 1 –10 µg mL−1 for Thiocolchicoside. The method was successfully applied for the analysis of drugs in pharmaceutical formulation. Results of the analysis were validated statistically and by recovery studies.

7) Chaudhari et al [29]

Two simple spectrophotometric methods have been developed for simultaneous estimation of Thiocolchicoside and Dexketoprofen trometemol from pharmaceutical dosage form. Method-I involved simultaneous equation method and Method-II is the Q-absorbance method. For simultaneous equationmethod, the absorbances of the standard solutions were taken at two wavelengths 368 nm (λ-max of Thiocolchicoside) and 258 nm (λ-max of dexketoprofen trometamol). For Q-absorbance method, the absorbances of the standard solutions were taken at two wavelengths 258 nm (λ-max of dexketoprofen trometamol) and 281 nm (Isoabsorptive point), in methanol. Linearity range was found to be 2-24 µg/ml for dexketoprofen trometamol and Thiocolchicoside in both methods based on the ratio of the two drugs in combined dosage form. The accuracy and precision of the methods were determined and validated statistically. Both methods showed good reproducibility and recovery with RSD less than 2. Proposed methods were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of dexketoprofen trometamol and Thiocolchicoside in

pharmaceutical dosage form

8) Kadam et al [30]

The Literature survey indicates several methods for the determination of Dexketoprofen trometamol. So an attempt was made to develop and validate a simple, precise, accurate, and economical RP-HPLC method as per ICH guidelines for the estimation of Dexketoprofen trometamol in bulk and pharmaceutical dosage forms. A simple reverse phase HPLC method was developed for the determination of Dexketoprofen trometamol present in pharmaceutical dosage forms.C18 (4.6ID x 250mm) in an gradient mode with mobile phase Acetonitrile: Methanol (25:75) was used. The flow rate was 0.9ml/min and effluent was monitored at 262nm. The retention time was 2.9 min for Dexketoprofen trometamol. The linearity ranges were found to be 30-70µg/ml

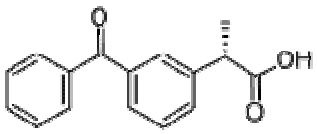
9) Selvadurai Muralidharan et al [31]

Simple, economic, precise, rugged, sensitive and validated RP-HPLC method has been developed to determine dexketoprofen (DKP) tablet. Chromatographic separation was achieved isocratically on Thermo, C18column (250mm × 4.6 mm,5µm and acetonitrile: ammonium acetate buffer (pH 5.0) in the ratio of 400:60 (v/v) using mobile phase, at a flow rate of 1.0 ml/min. Detection was carried out at 225 nm. The retention time for DKP was found to be 4.25 min respectively. The method was validated as per ICH guidelines. The method was linear in the concentration range of 100-600 ng/ml for with correlation coefficient of 0.99 respectively. The mean recoveries obtained for DKP was 98.14 % respectively. The correlation coefficients for all components are close to 1. The present developed and validated method was found to be more accurate, precise, selective, rapid and easily adapted to bio analytical method.

DRUG PROFILE [21, 22, 23]

DEXKETOPROFEN :

Structure :



Chemical name : (2*S*)2 [3(benzoyl) phenyl] propanoic acid

Description : White or almost white crystalline powder.

Molecular formula : $C_{16}H_{14}O_3$

Molecular weight : 254.280g/mol

Melting point : 94°C (201.2°F)

Solubility : Freely soluble in water and sparingly soluble in methanol.

Category : Analgesic

Half life : 1.54±0.63

Pharmacology

Indication:-

Short-term treatment of mild to moderate pain including dysmenorrhoea.

Pharmacodynamics:-

Dexketoprofen is a propionic acid derivative and an isomeric form of ketoprofen. The drug inhibits prostaglandin biosynthesis by inhibition of cyclooxygenase pathway (COX-1 and COX-2)

Mechanism of action:-

Dexketoprofen belongs to a class of medicines called non-steroidal anti-inflammatory drugs (NSAIDs). Prostaglandins are produced in response to injury or certain diseases and would otherwise go on to cause swelling, inflammation and pain. By blocking cyclo-oxygenase, dexketoprofen prevents the production of prostaglandins and therefore reduces inflammation and pain. Along with Peripheral analgesic action it possesses central analgesic action.

Absorption:-

Dexketoprofen is highly lipophilic. Absorption is controlled by diffusion through membranes. After the adminstrartion absorption is rapid.

Metabolism:-

Deketoprofen is metabolized by liver. The major metabolic pathways involves at least two cytochrome p450 enzyme. Dexketoprofen trometamol mostly conjucated to an acyl glucronide.

Route of elimination:-

Deketoprofen was eliminated by renal excretion. After completion of metabolism unchanged drug found in urine. Elimination is rapid, after repeated administration of dosage form no accumulation is found. (25mg Dexketoprofen tablet 3 times a day).

Drug interaction:-

Drug interacted with anti coagulants like warfarin, produce gastrointestinal bleeding increased hemorrhage, interacted with glycosides possible of increased plasma concentration.

Adverse drug reaction:-

Nausea and vomiting, diarrhea, abdominal pain and dyspepsia, common gastritis, constipation. Peptic ulcer, gastrointestinal bleeding are rare.

AIM

To validate the assay method for estimation of Dexketoprofen in Dexketoprofen Tablets by HPLC method, and to demonstrate that the analytical procedure is suitable for its intended purpose.

OBJECTIVES

On literature survey it was found that Dexketoprofen was estimated by HPLC and UV Spectrophotometric methods, in combination with other drugs in tablet and bulk dosage form.

And also no method was available for such estimation in the pharmacopoeia. In view of the need for a suitable method for routine analysis of Dexketoprofen in formulations, attempts are being made to develop simple, precise and accurate analytical methods for estimation of Dexketoprofen and extend it for their determination in formulations.

The utility of the developed methods to determine the content of drug in commercial tablet is also demonstrated. Validation of the method was done in accordance with USP and ICH guidelines for the assay of active ingredients. The methods were validated for parameters like accuracy, linearity, precision, specificity, ruggedness, robustness, and system suitability. This proposed method was suitable for the pharmaceutical analysis in analytical laboratories.

This method validation protocol provides a high degree of assurance that a specific process will consistently produce that the **DEXKETOPROFEN TABLETS 25 mg** meeting its predetermined specification, quality characteristics, and to validate the HPLC method using the working standard, Placebo and drug product.

METHOD DEVELOPMENT

MATERIAL AND INSTRUMENTS
CHEMICALS

Sr. No	Chemical/ Solvent	Make	Grade
1	Potssium dihydrogen orthophosphate	Merck	AR
2	Acetonitrile	Spectrochem	AR
3	O- phosphoric acid	Fisher scientific	AR
4	HPLC water	-	-

Table No. 5: Material and Chemicals

Instruments:

A. Spectrophotometer Double beam UV visible spectrophotometer with 10mm matched quartz cells

Model UV 1700

Make Shimadzu

B. HPLC Gradient System

Pump Agilent 1260 & 1290 series

Detector Variable Wavelength Detector

Software Chemstation

Column Agilent Zorbex C18, (250×4.6 mm 5µ)

C. Analytical Balance Metler Toledo XS105

D. P^H Meter Susima Digital pH meter

E. Sonicator PCI India Ultrasonic Bath Sonicator

F. Filter Nylon & PVDF 0.45µm (Millipore)

All the glassware's used were of borosilicate glass of class A and all the solvents and prepared solutions were filtered through Nylon filter 0.45u.

Drug	Dexketoprofen
Label claim	Dexketoprofen 25 mg
Manufactured by	Caplin point Laboratories, India

Table No. 6 : Details of Marketed formulation

Drug	Procured from	Assay result	Used as
Dexketoprofen Trometamol	Caplin point Laboratories, India	99.20%	Standard

Table no. 7: Details of Procured Drugs

5.2 Study of spectra and selection of wavelength:

Selection of solvent:

Solubility of the drug was checked in solvent like water, methanol, acetone, acetonitrile, dimethylsulphoxide, dimethylforma-mide, dimethyl amine etc. and solubility of dexketoprofen enhanced in Methanol and water that wise it selected as diluent.

Preparation of Diluent:

Methanol: Water-(60:40) v/v.

Dexketoprofen Trometamol standard solution:

An accurately weighed 73.9 mg of Dexketoprofen Trometamol (equivalent to 50mg of deketoprofen) was taken in 250 ml Volumetric flask dissolved and volume was made to mark with diluent. (Concentration of Dexketoprofen 200µg/ml)

The standard solution made further concentration as follows 60µg, 80µg, 100µg, 120 µg, 140µg, these solutions were scanned in the range of 400 to 200 nm in the 1cm cell against blank. The UV absorbance spectrum of Dexketoprofen is shown in Fig.No: 5

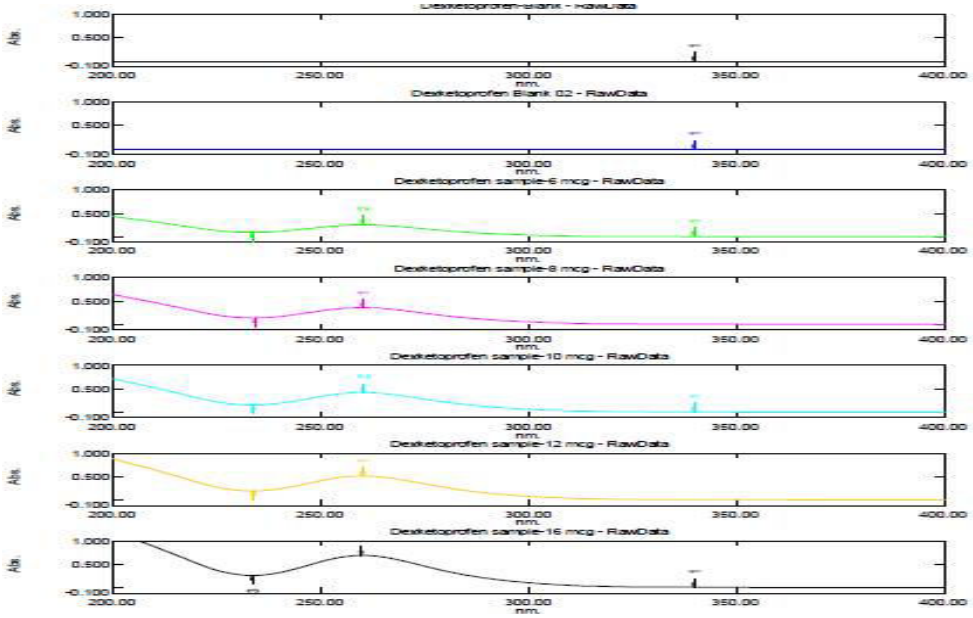


Fig no. 5: Stacked graph of Dexketoprofen

Concentration	Absorbance
Blank	0.000
6mcg	0.255
8mcg	0.359
10mcg	0.421
12mcg	0.512
16mcg	0.689

Table no: 08 Absorbance of uv spectrum in various concentrations

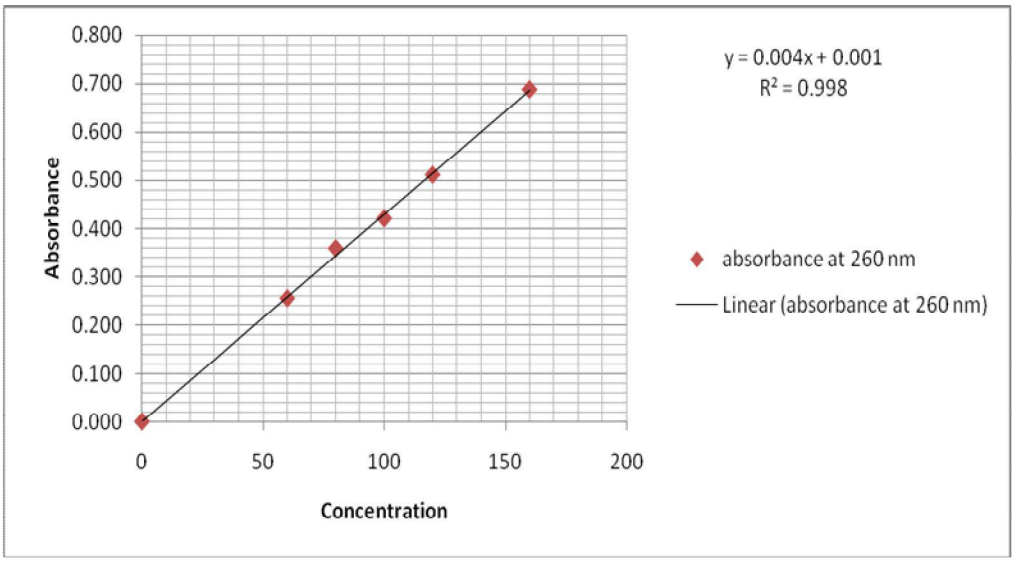


Fig. No. 6 : Linearity of Dexketoprofen

Dexketoprofen obeys Beers law in the range of 0- 160mcg concentrations and shows the

R^2 = 0.998. At 260 nm shows good linearity, Hence this wavelength selected for estimation.

Estimation of Dexketoprofen in Tablet Formulation Form by RP-HPLC

Selection of Mobile Phase

Based on sample solubility, stability and suitability, various mobile phase compositions were tried to get a good resolution and sharp peak.

The standard solution of Dexketoprofen Trometamol was run in different mobil phase. Higher solublitiy of dexketoprofen in water,that wise in mobile phase water used half of amount in preparation of mobile phase.

The following mobile phases were used:

1) Buffer : Methanol:Water [1:52:48]
2) Buffer : Methanol: Water [5:45:50]
3) Buffer : Methanol: Water [2:48:50]

Preparation of Reagent:

Buffer solution :

Weigh accurately about 6.8 g of Potassium dihydrogen orthophosphate and transfer into a 100 mL volumetric flask. Add about 60 mL of purified water to dissolve and dilute up to the volume with water. Adjust the pH to 3.50 ± 0.05 with Orthophosphoric acid.

Given above ratio of mobile phase were prepared. Buffer, Methanol, Water mixed well and it degassed, then it filtered through the 0.45 filter.

TRIAL - 1

Chromatographic conditions:-

Parameter/ Conditions	Description/Values
Column name	C_{18} (250*4.6) mm, 5µ
Detector	VW Detector
Flow rate	1.5 ml/min
Injection volume	20µl
Wavelength	260 nm
Column Temp.	Ambient
Buffer	Phossphate buffer pH 3.5
Mobile phase	Buffer : Methanol:Water (1:52:48)

Table No. 09: Chromatographic condition of trial -1

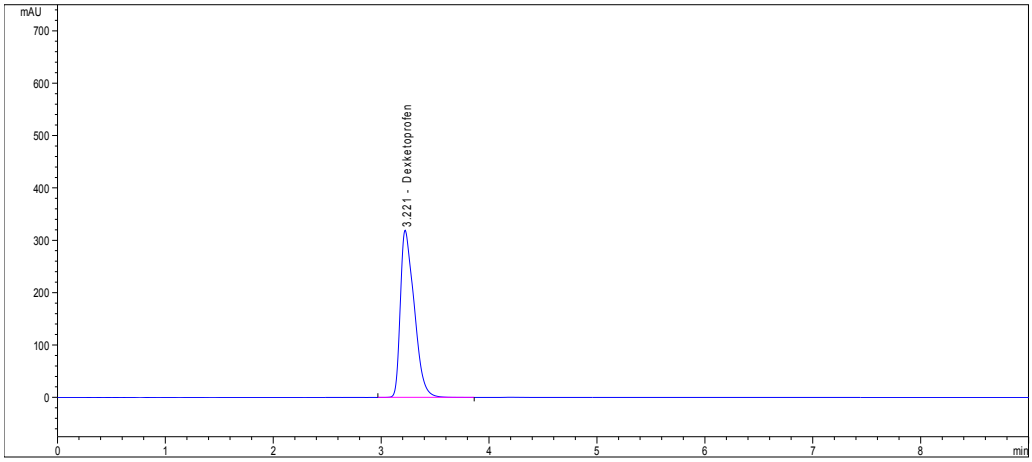


Fig. No. 07 : Chromatogram of trial-1

Observation:-

Peak Name	RT	USP Tailing	USP Plate Count
Dexketoprofen	3.221	0.500	2970

Conclusion:-

USP Plate count of Dexketoprofen is also below the limit. and the retention time of Dexketoprofen is 3 mins.hence it's run in very short run time.so next trial was proposed.

In this method mobile phase's organic solvent ratio is increased,that wise the RT of the dexketoprofen is less efficient,the next trail method, solvent ratio decreased and buffer ratio were increased.

Trial - 2

Chromatographic conditions:-

Parameter/ Conditions	Description/Values
Column name	C_{18} (250*4.6) mm, 5µ
Detector	VW Detector
Flow rate	1.5 ml/min
Injection volume	20µl
Wavelength	260 nm
Column Temp.	Ambient
Buffer	Phossphate buffer pH 3.5
Mobile phase	Buffer : Methanol:Water (5:45:50)

Table no. 10: Chromatographic condition of trial -2

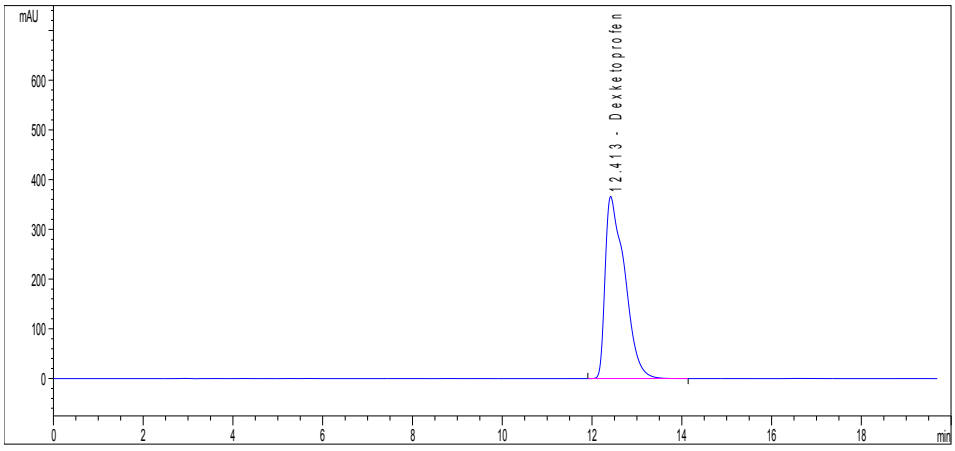


Fig. No. 08 : Chromatogram of trial-2

Observation:-

Peak Name	RT	USP Tailing	USP Plate Count
Dexketoprofen	12.423	0.37	3532

Observation:-

In this trail ratio of buffer increased for increase the retention time of the dexketoprofen, but this trail was showed increased retention time. And run time also 20 minutes, so this trail method was rejected. Next trail method were proposed

Trial - 3

Chromatographic conditions:

Parameter/ Conditions	Description/Values
Column name	C_{18} (250*4.6) mm, 5µ
Detector	VW Detector
Flow rate	1.5 ml/min
Injection volume	20µl
Wavelength	260 nm
Column Temp.	Ambient
Buffer	Phossphate buffer pH 3.5
Mobile phase	Buffer : Methanol:Water (2:48:50)

Table no. 11: Chromatographic condition of trial -3

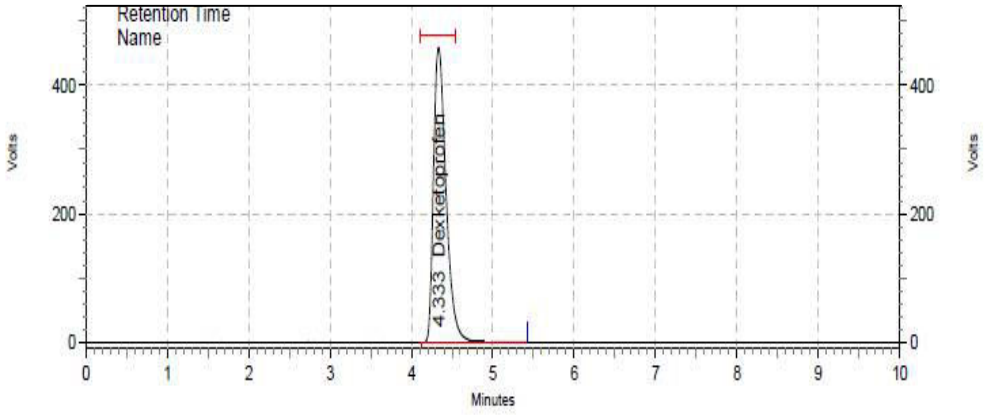


Fig. No. 09 : Chromatogram of trial-3

Observation:-

Peak Name	RT	USP Tailing	USP Plate Count
Dexketoprofen	4.333	1.47	3998

Conclusion:

Tailing factor was <2.0, Plate count was >2000, run time is 10 minutes only, RT was 4mins, so this method is considered as the optimized method.

METHOD VALIDATION

Preparation of Buffer Solution:

Mobile Phase-A:- Weighed accurately 6.8 g of Potassium dihydrogen orthophosphate and transferred into 100 mL volumetric flask. Added 60 mL of purified water. Dissolved and diluted up to the volume with water. Adjusted the pH to 3.50 ± 0.05 with Orthophosphoric acid.

Preparation of Mobile Phase:

Mobile Phase: Prepared a mixture 20 volume of buffer, 480 volume of Acetonitrile, and 500 volume of water, mixed and sonication was done for 10 minutes and filtered the solution through 0.45 µm nylon filter.

Preparation of Diluents: Mobile Phase

Preparation of Standard Solution: (250 mcg/mL of Dexketoprofen)

Weighed accurately 92.3 mg of Dexketoprofen Trometamol WS (Equivalent to 62.5 mg of Dexketoprofen) and transferred into 100 mL volumetric flask. Added 60 mL of diluent and sonication was done for 5 minutes. Dissolved and diluted up to the volume with diluent. Pipette out 10 mL of the above solution and transferred into 25 mL volumetric flask and diluted up to the volume with diluent. Filtered the solution through 0.45 µm nylon filter and collected the solution in an HPLC vial after discarded the first 2 mL of filtrate.

Preparation of Sample Solution: (250 mcg/mL of Dexketoprofen)

Weighed and finely powdered not fewer than 10 tablets. Transferred accurately weighed portion of the powder equivalent to 125 mg of Dexketoprofen into 100 mL volumetric flask. Added 60 mL of diluent and sonication was done for 15 minutes. Dissolved, cooled and diluted up to the volume with diluent. Pipette out 5.0 mL of the above solution and transferred into 25 mL volumetric flask and diluted up to the volume with diluent. Filtered the solution through 0.45 µm nylon filter and collected the solution in an HPLC vial after discarded the first 2 mL of filtrate.

Chromatographic conditions:

Parameter/ conditions	Description/Values
Column name	Agilent Zorbax SB C_{18} (250*4.6) mm,5µm
Flow rate	1.5 ml/min
Injection volume	20 µl
Wavelength	260 nm
Column Temp.	Ambient
Run time	10 min
Buffer	3.50 pH
Mobile phase	Isocratic

Table No. 12: Chromatographic condition of Optimized method

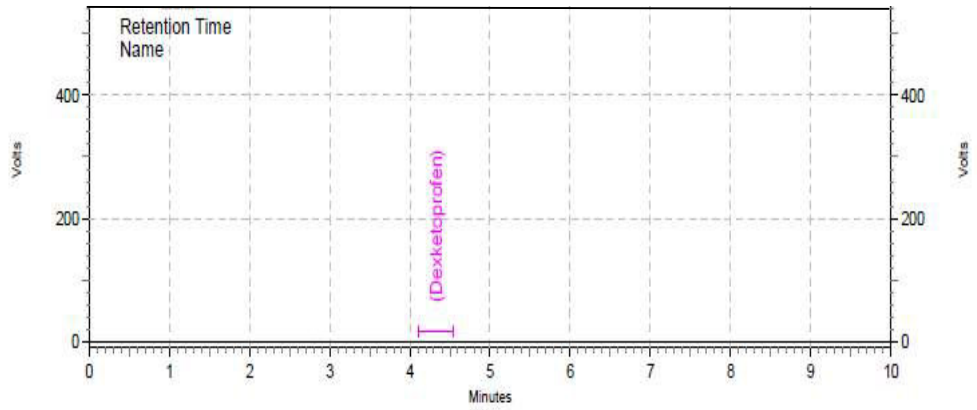
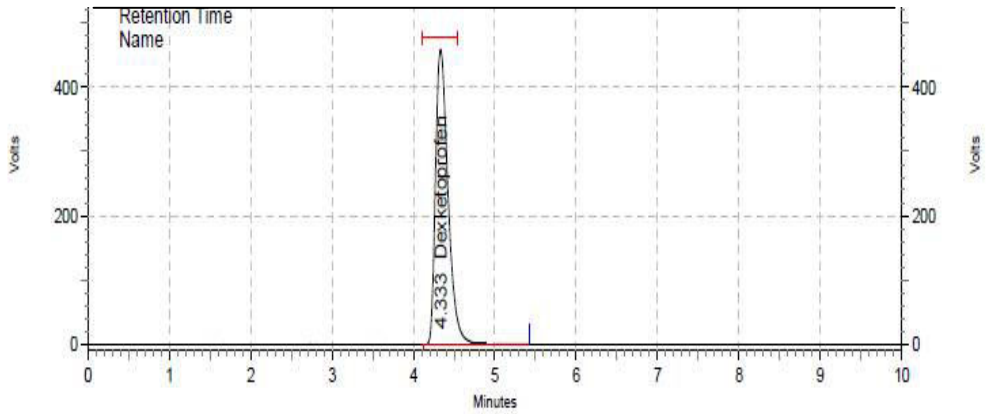


Fig. No. 10 : Chromatogram of Blank



Fig, No. 11 : Chromatogram of Standard

Observation:

Name	RT (min)	Area (µv*sec)	USP Plate Count	USP Tailing
Dexketoprofen	4.333	657221971	3918	1.49

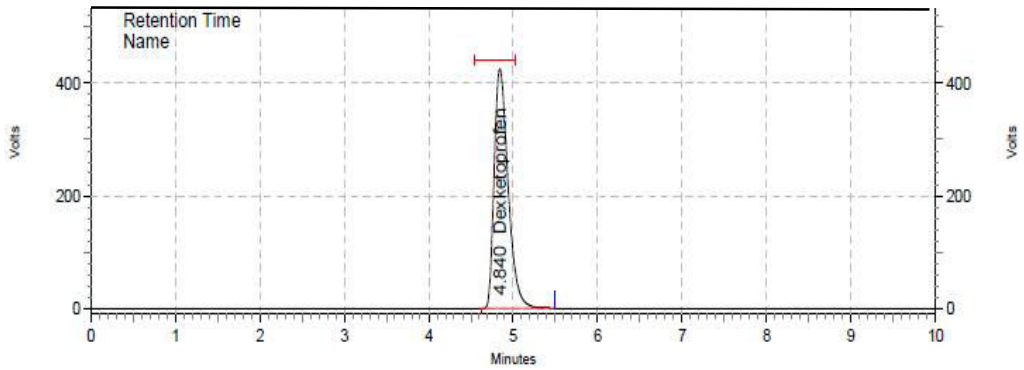


Fig. No. 12 : Chromatogram of Sample

Name	RT (min)	Area (µv*sec)	USP Plate Count	USP Tailing
Dexketoprofen	4.840	657921232	4204	1.48

Calculations:

Content of Dexketoprofen in mg:

SPL Area STD wt 10 100 25 STD Purity in ASB 254.28

= ----------x----------x-------x----------x---------x----------x---------- x Avg. wt

STD Area 100 25 Wt. of SPL 5 100 375.42

= 254.28 is a molecular weight of Dexketoprofen.

= 375.42 is a molecular weight of Dexketoprofen Trometamol.

Content of Dexketoprofen in %:

$$= \frac{\text{Dexketoprofen in mg}}{\text{The Label claim of each Tablet (in mg)}} \times 100$$

Acceptance Criteria:

22.50 mg to 27.50 mg (90.00 % to 110.00 % of label claim)

Laoratory Mixtures	Area Obtained	Amount in mg	Percentage of Drug
Standard	657221971	26.24	104.96
Sample	657921232	26.10	104.40

Table No. 13: Results for Assay

i) System Suitability

Standard solution was prepared as per the proposed test method and injected into the HPLC system in six replicates and chromatograms were recorded. The system is suitable for analysis if

- The USP plate count of Dexketoprofen peak should not be less than 2000.
- The USP tailing for peak should not be more than 2.0
- The % RSD of peak area response for six replicate injections for Dexketoprofen peak not more than 2.0 %.

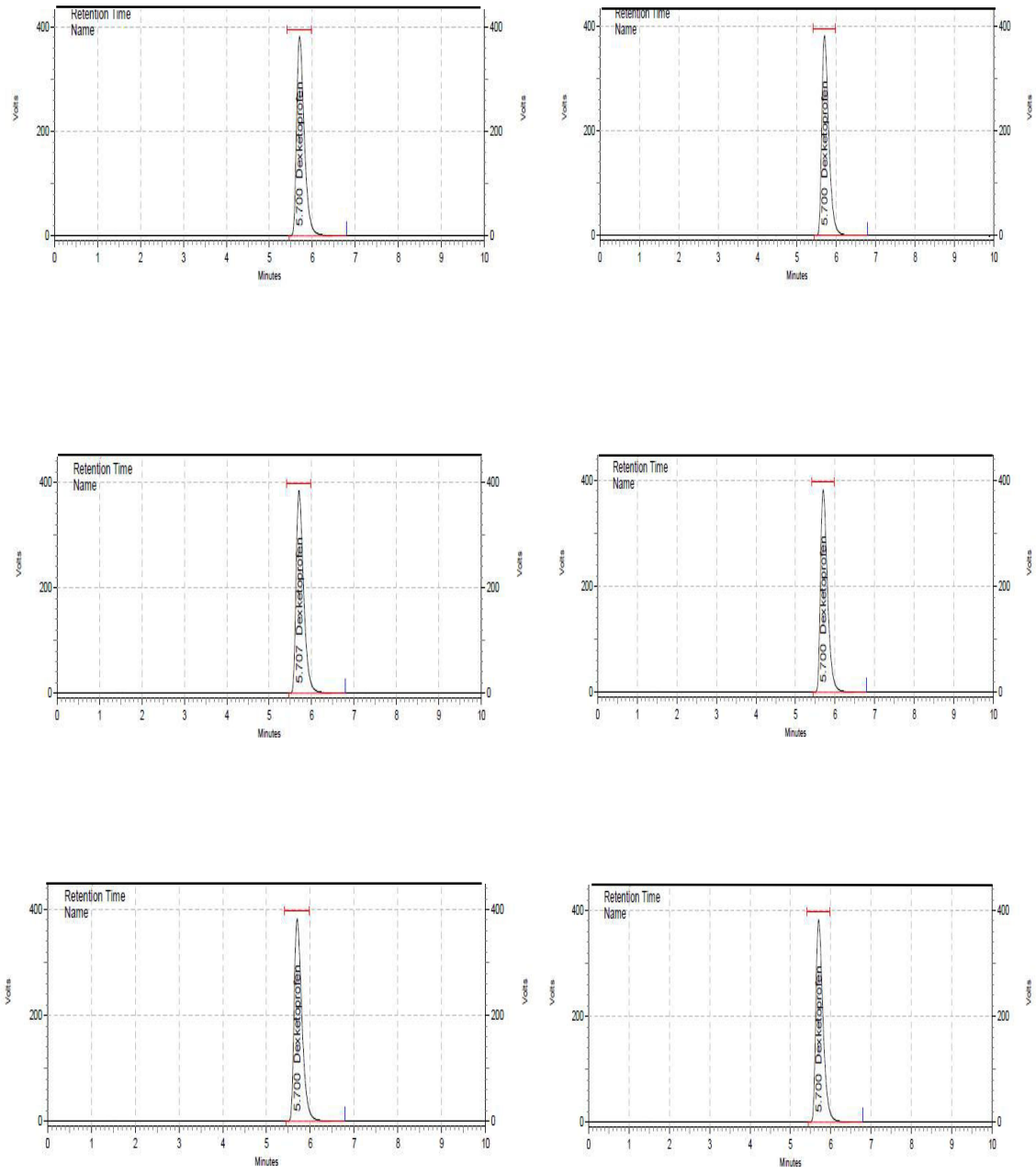


Fig No. 13:Chromatogram for standard solution for system suitability

System suitability results for Dexketoprofen

Name	Inj	RT (min)	Area (µv*sec)	USP Plate Count	USP Tailing
Dexketoprofen	1	5.70	634290937	5051	1.56
Dexketoprofen	2	5.70	634874613	5070	1.54
Dexketoprofen	3	5.71	635331197	5090	1.50
Dexketoprofen	4	5.70	635404281	5073	1.53
Dexketoprofen	5	5.70	635194094	5087	1.54
Dexketoprofen	6	5.70	635491917	5074	1.55
Mean			635097840	5074	1.54
Std Dev.			**450299.0**		
% RSD			**0.071**		

Table No. 14: System suitability results for Dexketoprofen

Observation:

It was observed from the data tabulated above that all the system suitability parameters meet the predetermined acceptance criteria as per the test method and indicates the suitability of the selected system.

ii) Linearity and Range

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. A series of standard concentrations were prepared from 10% to 200% of the targeted concentration of Dexketoprofen. A linearity graph of concentration (µg/ml) versus average area response was plotted for Dexketoprofen peak and the correlation coefficient was calculated.

Preparation of Stock Solution: Accurately weighed and transferred about 92.31 mg of Dexketoprofen Trometamol working standard (Equivalent to 62.5 mg of Dexketoprofen) into a 50 ml volumetric flask. Add about 25 mL of diluent and sonicate for 10 minutes, to dissolve and dilute up to the volume with diluent.

(Concentration of Dexketoprofen is about 1250µg/ml)

Preparation of Linearity Dilutions for Dexketoprofen.

Linearity Dilutions

Linearity Level (%)	Volume of Stock Taken (ml)	Diluted to (ml)	Final Conc. (µg/ml) Dexketoprofen
10	1	50	25
20	1.0	25	50
50	5.0	25	125
100	5.0	25	250
120	6.0	25	300
160	8.0	25	400
200	10.0	25	500

Table No. 15: Linearity Dilutions

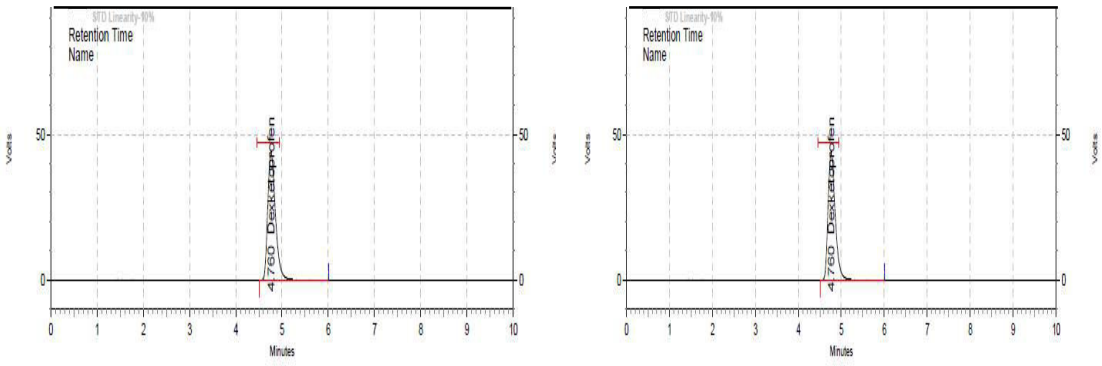


Fig. No. 25: Chromatograms for Linearity at 10%

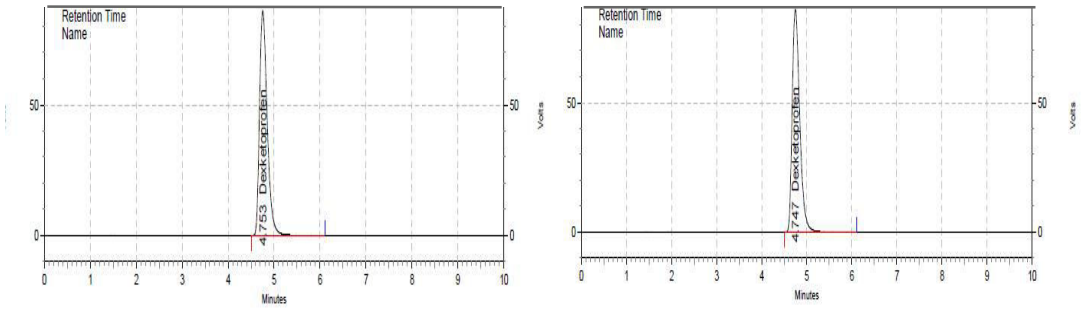


Fig. No. 14 : Chromatograms for Linearity at 20%

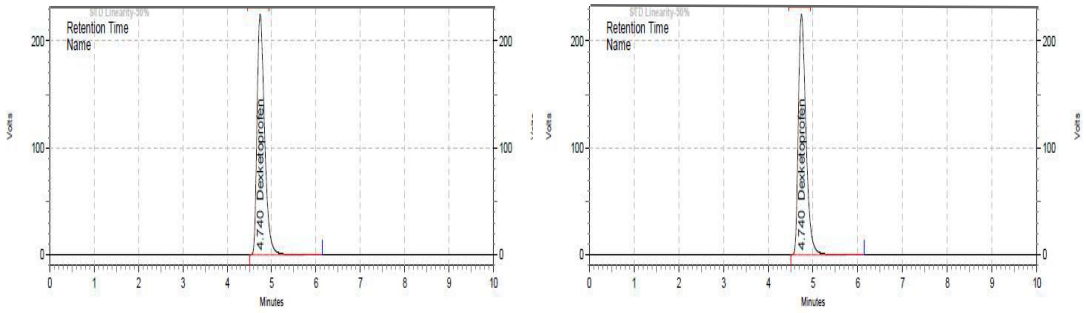


Fig. No.15: Chromatograms for Linearity at 50%

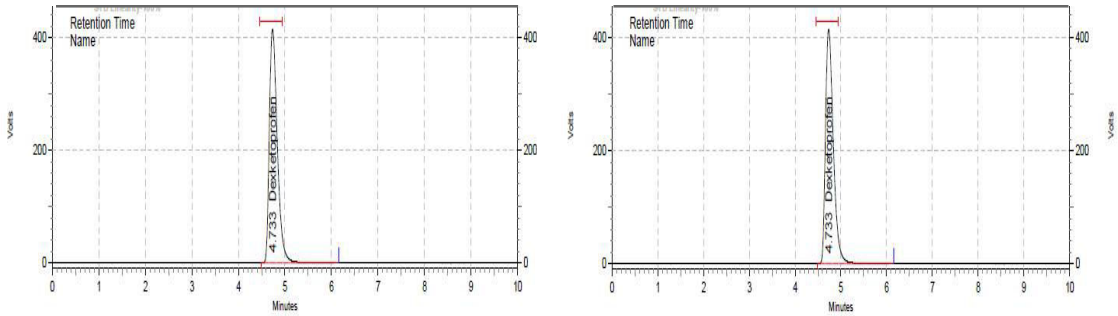


Fig. No. 16 : Chromatograms for Linearity at 100%

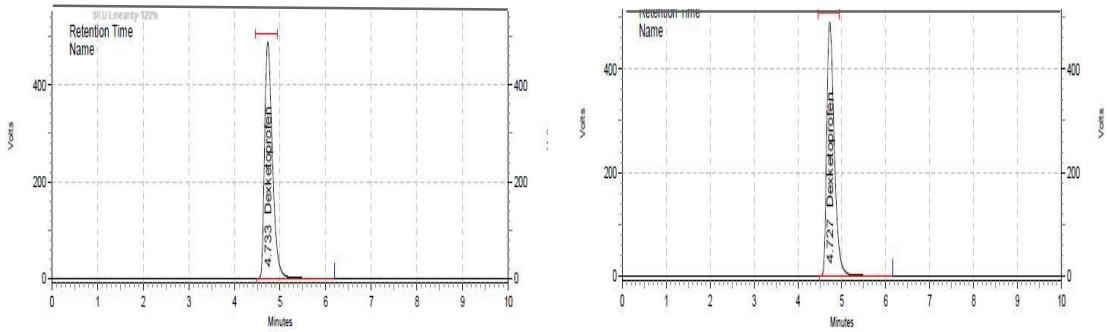


Fig. No. 17 : Chromatograms for Linearity at 120%

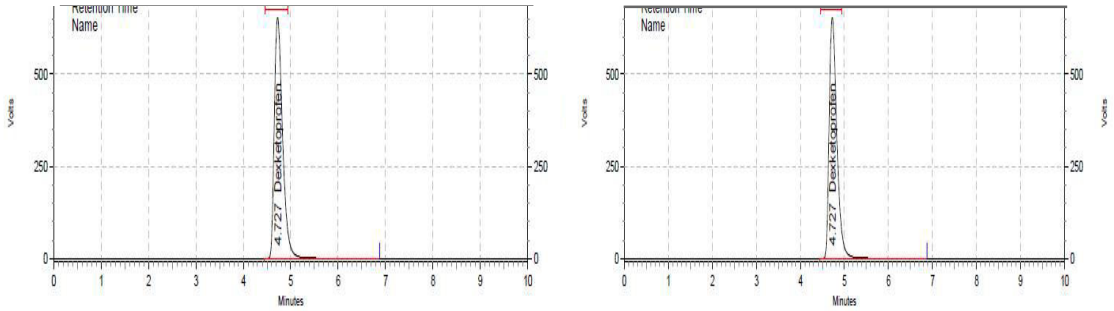


Fig. No. 18 : Chromatograms for Linearity at 160%

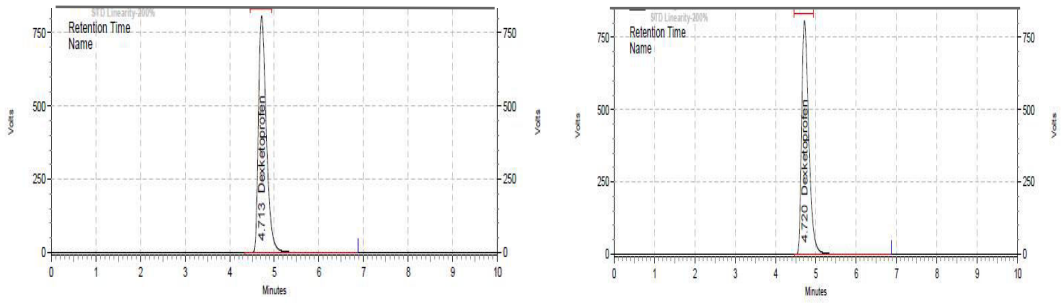


Fig. No.19 : Chromatograms for Linearity at 200%

REPORT:

The linearity response of Dexketoprofen was determined across the range are given in the following tables. The result shown in the table and its graphical representation indicates that the response is linear over the specified range

Linearity data obtained from 10 % to 200 % for Standard:

Linearity level concentration in %	Concentration of Dexketoprofen (µg/mL)	Sample area -1	Sample area-2	Average area
10	25	68651042	68762225	68706634
20	50	134684907	135044793	134864850
50	125	356488901	357047686	356768294
100	250	663177879	663185028	663181454
120	300	783888776	784329728	784109252
160	400	1064539197	1062691472	1063615335
200	500	1333597687	1334595109	1334096398

Table No.16 : Linearity Data

S.No	Parameter	Results obtained	Acceptance criteria
01.	Tailing factor for Dexketoprofen	1.48	NMT 2.0
02.	%RSD of Dexketoprofen area for six standard injections	0.16	NMT 2.0%
03.	Theoretical Plates for the peak due to Dexketoprofen	3774	NLT 2000

Table No.17: System suitability Data

Linearity graph obtained for Standard:

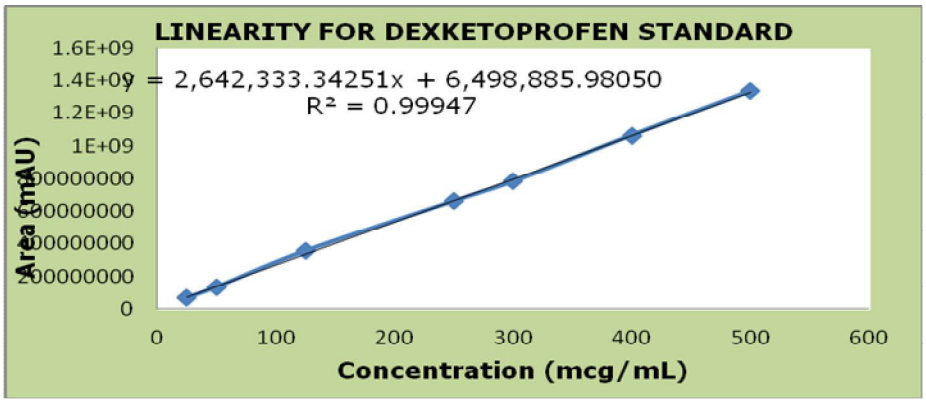


Fig. No. 20 : Graph for Linearity standard

Correlatione co efficient: 0.99947

Slope: 2642333.343

Intercept: 6498885.981

Sample Stock solution (625 mcg/mL of Dexketoprofen):

Weigh and finely powder not fewer than 10 tablets. Transfer accurately weigh portion of the powder equivalent to 125 mg of Dexketoprofen into a 50 mL volumetric flask. Add about 30 mL of diluent and sonicate for 15 minutes to dissolve. Cool and dilute up to the volume with diluent.

Linearity Dilutions

Linearity Level (%)	Volume of Stock Taken (ml)	Diluted to (ml)	Final Conc. (µg/ml) Dexketoprofen
10	1	50	25
20	1.0	25	50
50	5.0	25	125
100	5.0	25	250
120	6.0	25	300
160	8.0	25	400
200	10.0	25	500

Table No. 18: Linearity Dilutions

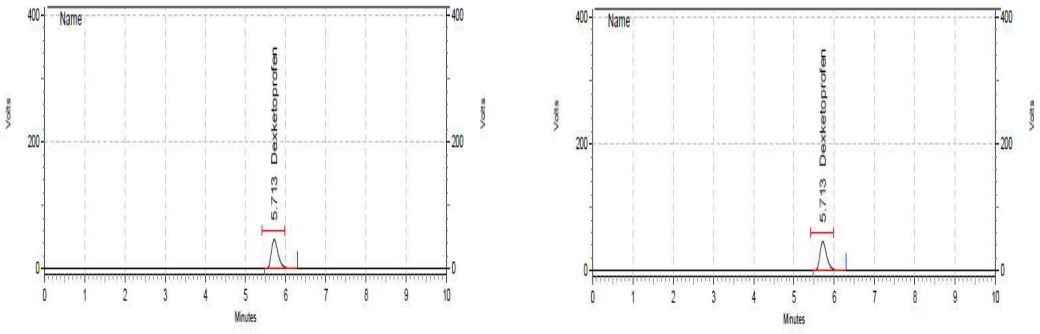


Fig. No. : Chromatograms for Linearity at 10%

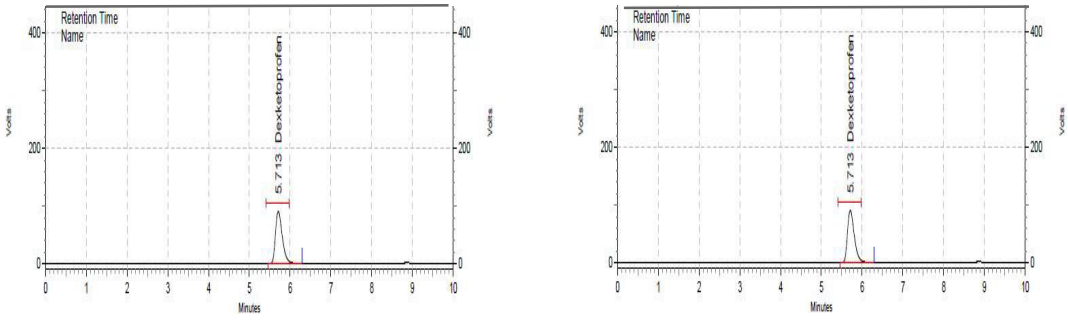


Fig. No.21 : Chromatograms for Linearity at 20%

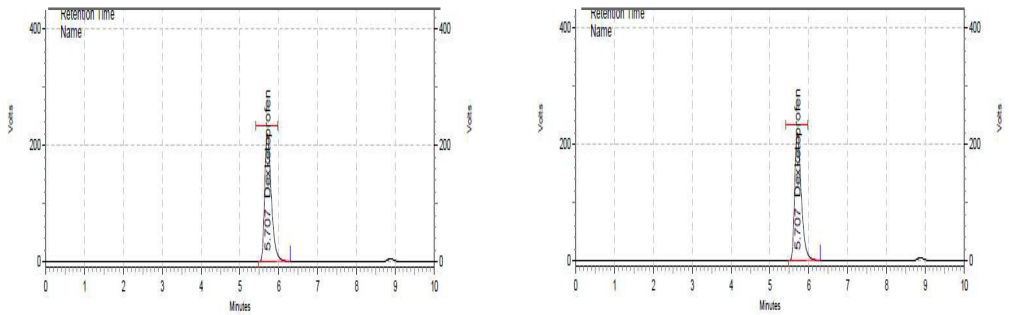


Fig. No.22 : Chromatograms for Linearity at 50%

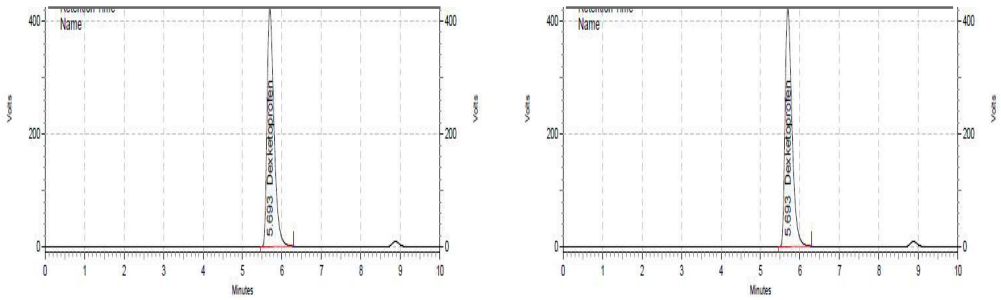


Fig. No.23 : Chromatograms for Linearity at 100%

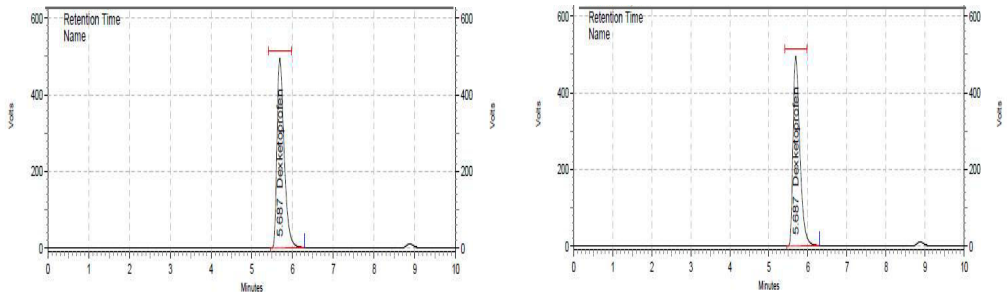


Fig. No.24 : Chromatograms for Linearity at 120%

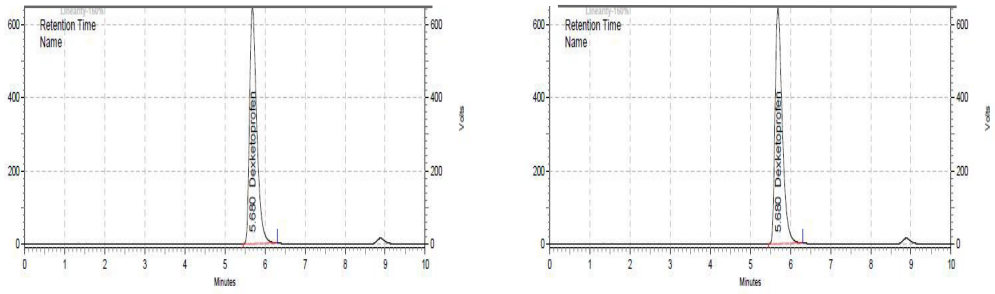


Fig. No. : Chromatograms for Linearity at 160%

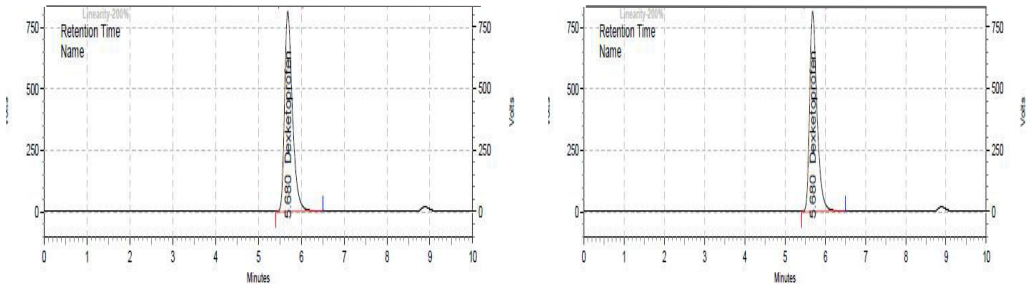


Fig. No.25 : Chromatograms for Linearity at 200%

REPORT:

The linearity response of Dexketoprofen was determined across the range are given in the following tables. The result shown in the below table and its graphical representation indicates that the response is linear over the specified range

Linearity data obtained from 10 % to 200 % for Standard

Linearity level concentration in %	Concentration of Dexketoprofen (µg/mL)	Sample area -1	Sample area	Average area
10	25	72167886	72386578	72277232
20	50	143070269	142038911	142554590
50	125	350084739	350540979	350312859
100	250	687987277	687142472	687564875
120	300	808364399	808727071	808545735
160	400	1096605577	1099591338	1098098458
200	500	1391525072	1391837116	1391681094

Table No. 19: Linearity of Dexketoprofen

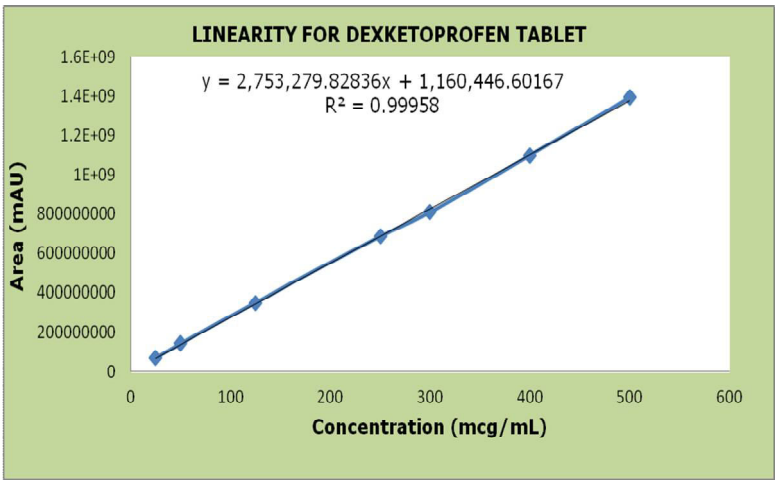


Fig. No. 26 : Linearity Plot of Dexketoprofen

Correlatione co efficient: 0.99947

Slope: 2642333.343

Intercept: 6498885.981

Data Interpretation

The *Correlation coefficient* for Dexketoprofen was found to be **0.9995,** which indicates that the peak responses are linear. This concluded that the method was linear throughout the range selected.

iii) Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurement.

System precision

Standard solution was prepared as per the proposed test method for system precision studies. Six replicate injections were injected into the HPLC system. The % RSD for the peak responses of six replicate injections should be NMT 2.0.

Theoretical plates of the peak obtained for six replicates standard solution injections of Dexketoprofen should be not less than 2000.

The tailing factor of the peak due to Dexketoprofen from six replicates standard solution injections should be not more than 2.0.

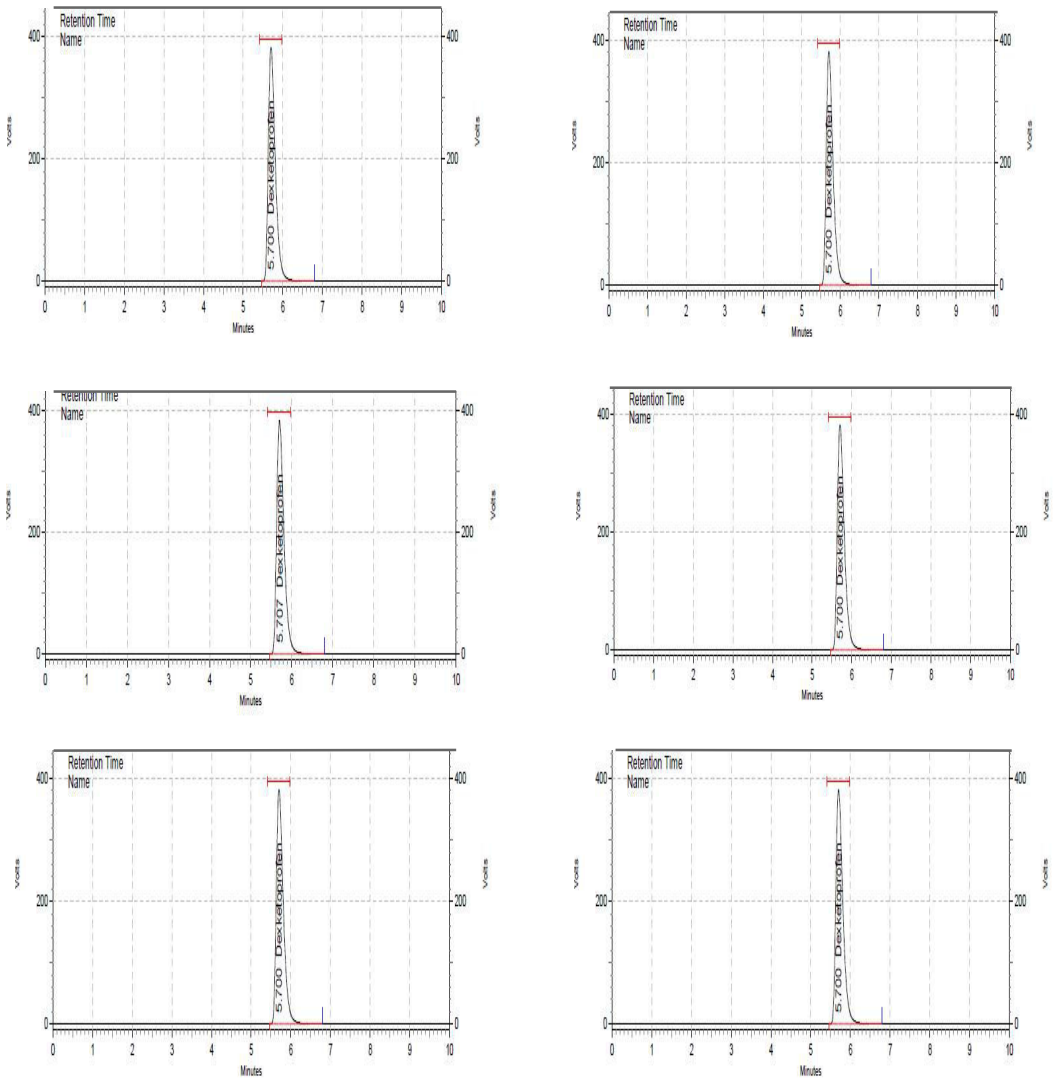


Fig. No. 27 : Chromatograms for system Precision

Peak Results for System Precision

Inj.	RT (min)	Area (µV*sec)	Tailing Factor	Theoretical Plates
1	5.70	634290937	1.56	5051
2	5.70	634874613	1.54	5070
3	5.71	635331197	1.50	5090
4	5.70	635404281	1.53	5073
5	5.70	635194094	1.54	5087
6	5.70	635491917	1.55	5074
Mean		**635097840**	**1.54**	**5074**
% RSD		**0.071**		

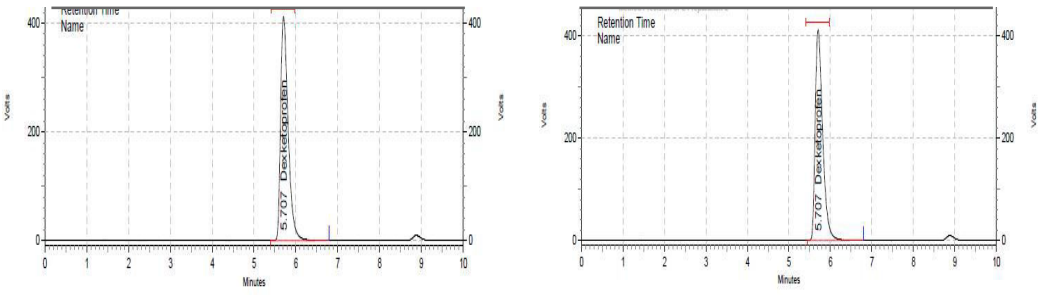
Table No. 20 : Peak Results for System Precision

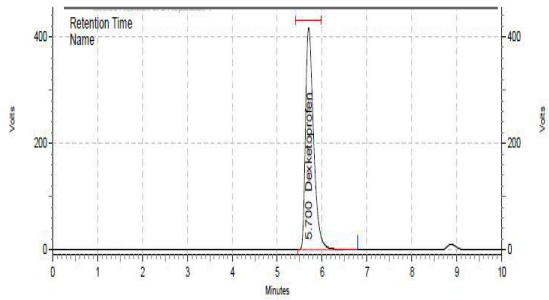
Data Interpretation

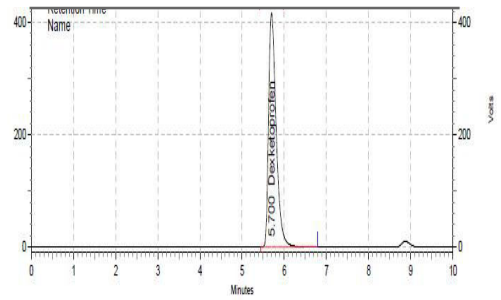
It is observed from the data tabulated above, that the % RSD of the peak responses as peak area was found to be within acceptance criteria indicating an acceptance level of precision for system precision studies.

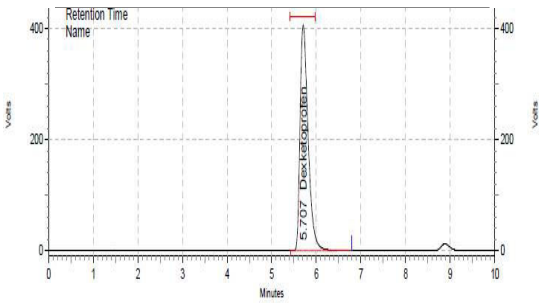
Method Precision

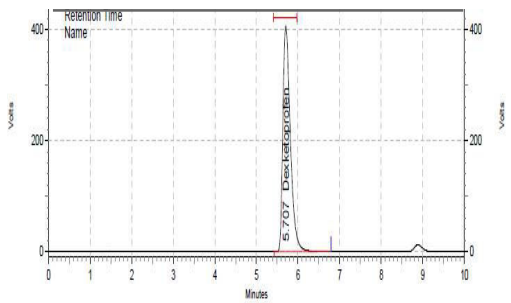
In method precision, a homogenous sample of a single batch should be analysed six times. This indicates whether a method is giving consistent results for a single batch. The % RSD for the six determinations should be *NMT 2.0*

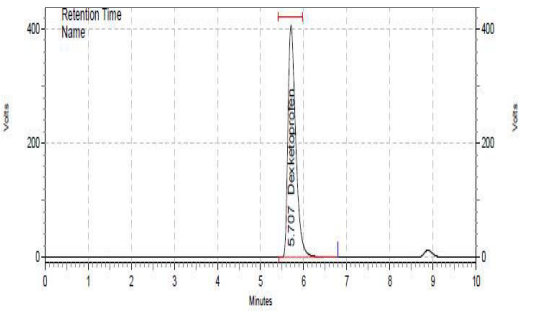


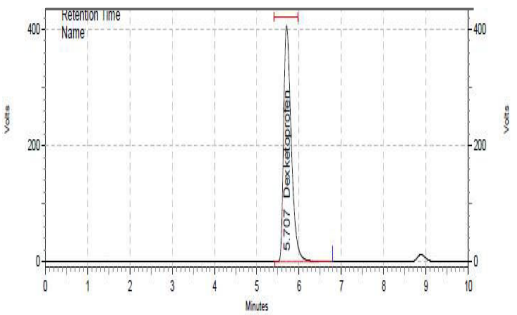

Retention Time
Name
5.700 Dexketoprofen
Volts
Minutes

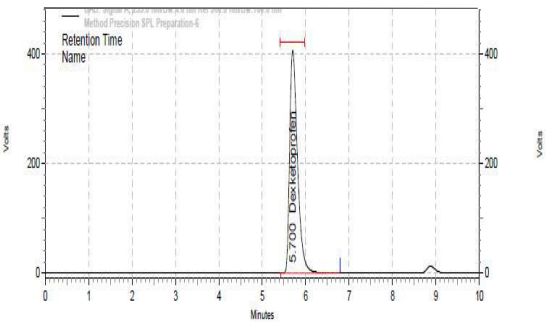

Name
5.700 Dexketoprofen
Volts
Minutes

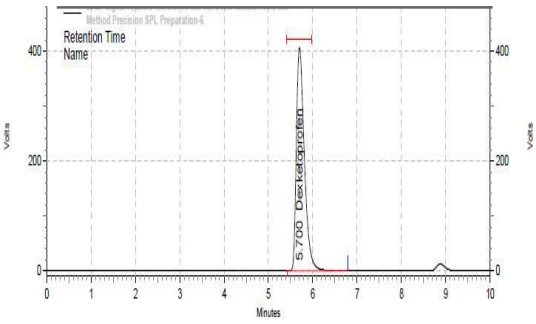

Retention Time
Name
5.707 Dexketoprofen
Volts
Minutes


Retention Time
Name
5.707 Dexketoprofen
Volts
Minutes


Retention Time
Name
5.707 Dexketoprofen
Volts
Minutes


Retention Time
Name
5.707 Dexketoprofen
Volts
Minutes


Retention Time
Name
5.700 Dexketoprofen
Volts
Minutes


Retention Time
Name
5.700 Dexketoprofen
Volts
Minutes

.

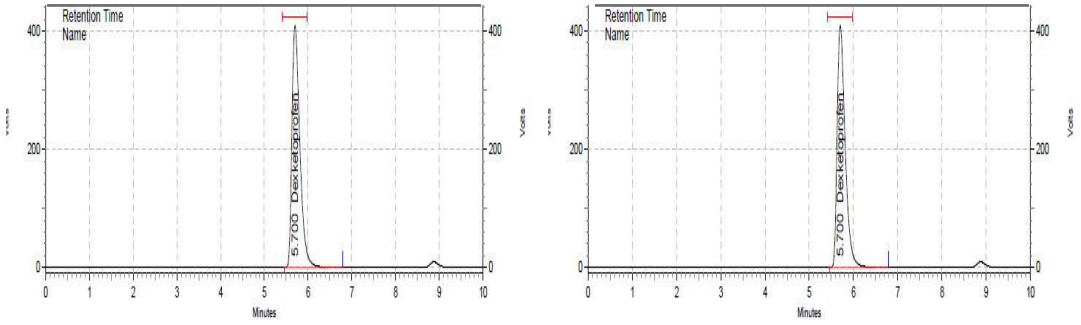


Fig. No. 28 : Chromatograms for Method Precision

Method Precision Results for Dexketoprofen

No. of Sample	Samples Area			Results obtained	
	Sample -1	Sample -2	Average	Amount of drug present in mg	Percentage of drug
01	698152127	698926730	698539429	25.94	103.76
02	692342080	692477262	692409671	25.93	103.72
03	685167327	686704800	685936064	26.21	104.84
04	699465361	698867877	699166619	26.07	104.28
05	691698691	692857520	692278106	26.23	104.92
06	686455597	686143821	686299709	26.12	104.48
				Mean	**104.33**
				Std. Dev	**0.52**
				RSD	**0.494**

Table No. 21: Method Precision Results for Dexketoprofen

Data Interpretation

From the above results, it was concluded that the method is precise.

iv)Accuracy:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy study was conducted by spiking the known amount of active ingredients into the placebo at three different levels (80%, 100% and 120% of target concentration). The samples were analysed as per the proposed test procedure and the % recovery for each spiked level was calculated. ***The % RSD at each spike level should be NMT 2.0. The overall % RSD for % recovery for all spike level should be NMT 2.0. The % recovery at each spike level should be NLT 98.0 and NMT 102.0 of the added amount.***

Procedure for accuracy

Accuracy at 80%:

Accurately weighed 2025 mg of placebo, 147.68 mg of Dexketoprofen trometamol (Equivalent to 50.0 mg of Dexketoprofen) was transferred to 50 ml volumetric flask. To it 30 ml of diluent was added and sonicated for 15 minutes with occasional shaking, cooled at room temperature and diluted to volume with diluent. Further 5 ml is diluted to 25 ml with diluent.

Accuracy at 100%:

Accurately weighed 2025 mg of placebo, 184.60 mg of Dexketoprofen Trometamol (Equivalent to 62.5 mg of Dexketoprofen) was transferred to 50 ml volumetric flask. To it 30 ml of diluent was added and sonicated for 15 minutes with occasional shaking, cooled at room temperature and diluted to volume with diluent. Further 5 ml is diluted to 25 ml with diluent.

Accuracy at 120%:

Accurately weighed 2025 mg of placebo, 221.5 mg of Dexketoprofen Trometamol (Equivalent to 75.0 mg of Dexketoprofen) was transferred to 50 ml volumetric flask To it 50 ml of diluents was added and sonicated for 30 minutes with occasional shaking, cooled at room temperature and diluted to volume with diluent. Further 5 ml is diluted to 25 ml with diluent.

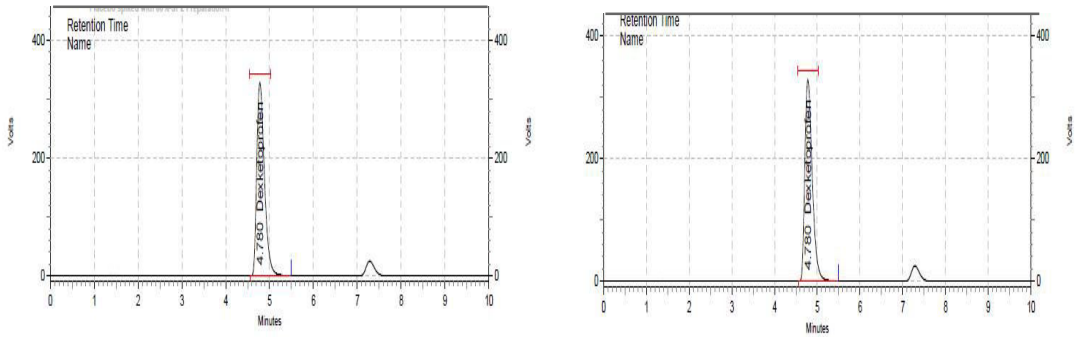


Fig. No. 29 : Chromatograms for Accuracy at 80% level

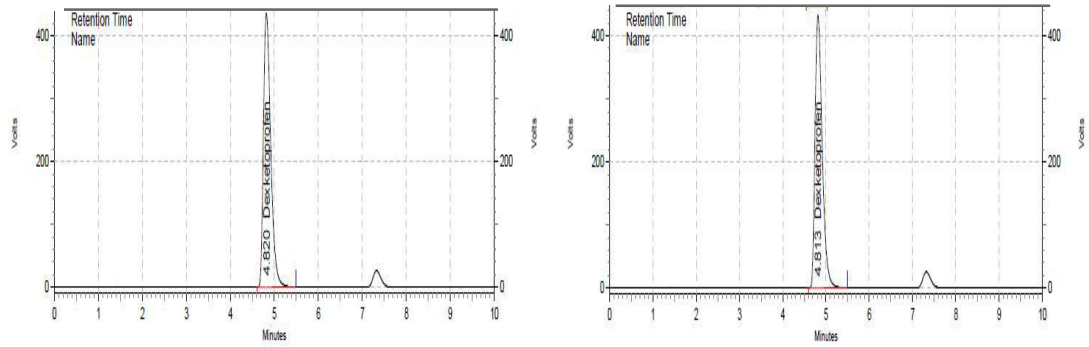


Fig. No.30: Chromatograms for Accuracy at 100% level

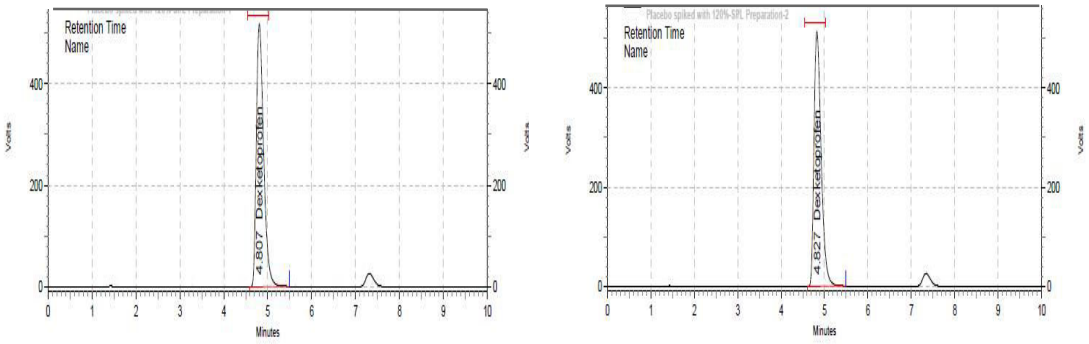


Fig. No. 31: Chromatograms for Accuracy at 120% level

Peak Results for Accuracy of Dexketoprofen

Accuracy Level in %	Dexketoprofen added in mg	Dexketoprofen recovered in mg	% Recovered	Mean of % Recovered
80	0.29774	0.29804	100.10	100.60
	0.29738	0.30031	100.98	
100	0.37216	0.37583	100.47	100.25
	0.37022	0.37339	100.56	
120	0.44464	0.44708	99.70	99.55
	0.44498	0.44305	99.32	

Table No. 22: Peak Results for Accuracy of Dexketoprofen

S.No.	Parameter	Results obtained	Acceptance criteria
01	Tailing factor for Dexketoprofen	1.49	**NMT 2.0**
02	%RSD of Dexketoprofen area for six standard injections	0.371	**NMT 2.0%**
03	Theoretical Plates for the peak due to Dexketoprofen	3968	**NLT 2000**

Table No.23: System suitability Data

Data Interpretation

On the basis of these chromatograms we can say that there is no interference of blank and placebo at the retention time of Dexketoprofen . Hence the method is specific.

v) Specificity

Blank & Placebo Interference: Placebo was injected in triplicate by weighing the equivalent amount present in the finished drug product and analysed for interference due to placebo.

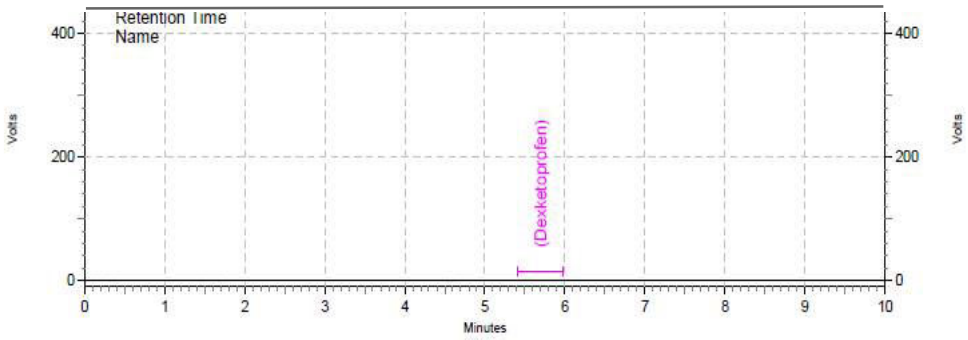


Fig. No. 32 : Chromatogram of Blank (Diluent)

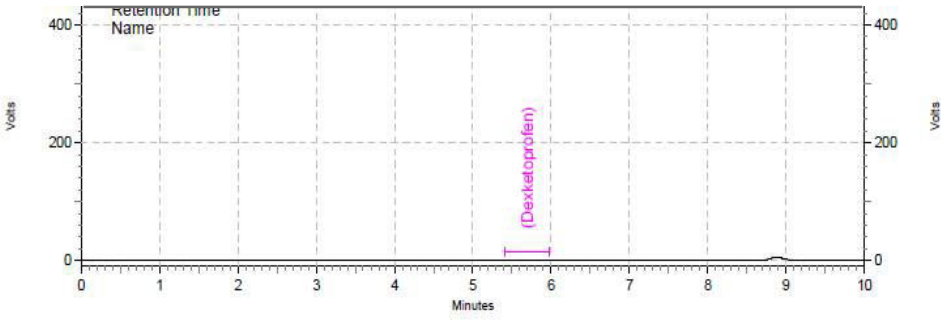


Fig. No. 33 : Chromatogram of Placebo

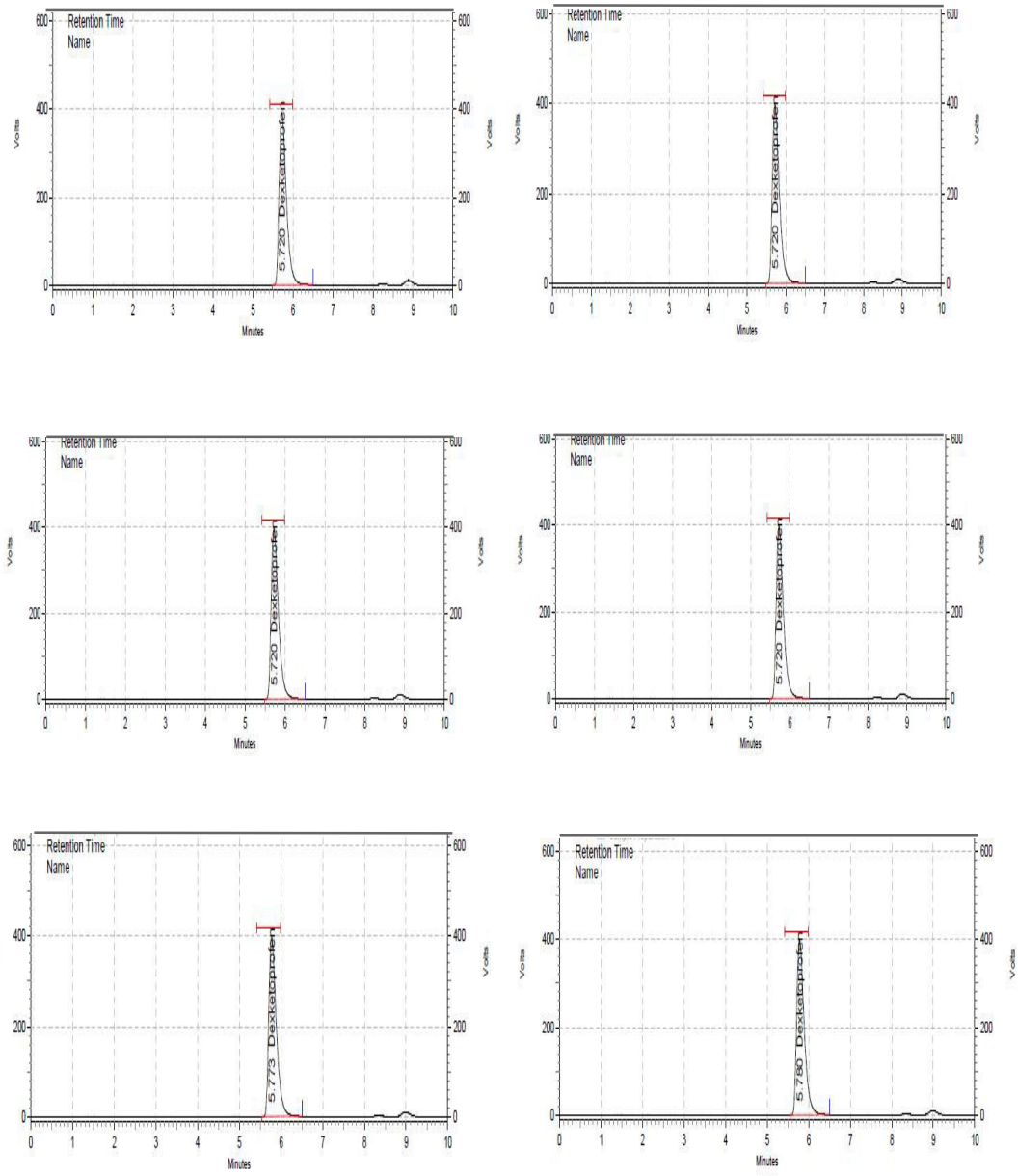
Data Interpretation

On the basis of these chromatograms we can say that there is no interference of blank and placebo at the retention time of Dexkeoprofen .Hence the method is specific.

VI) INTERMEDIATE PRECISION-(RUGGEDNESS):

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

Intermediate precision is to be performed by different analyst coupled with different day.



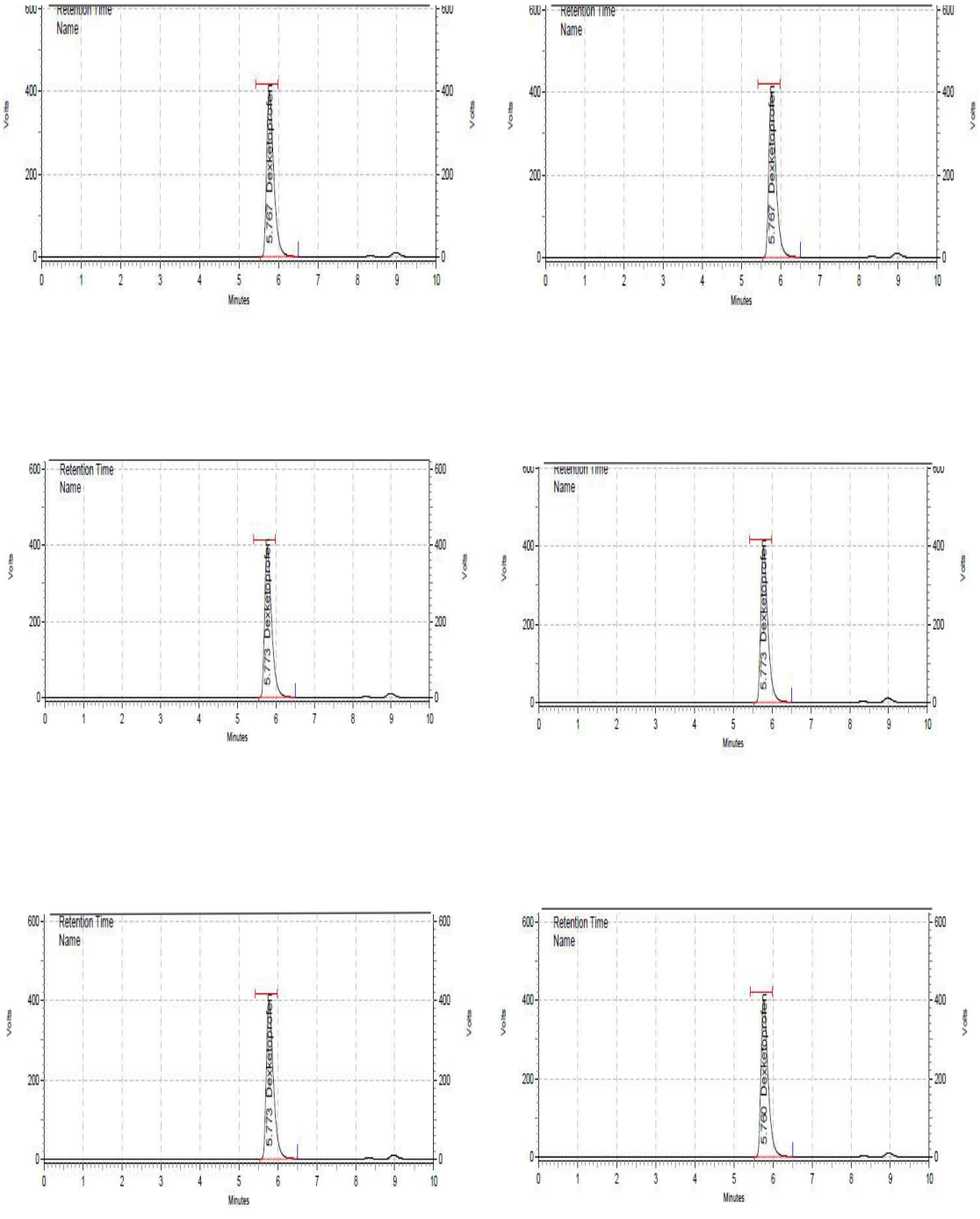


Fig. No.34 : Chromatogram of Intermediate-Precision

Intermediate Precision Results for Dexketoprofen

No. of Sample	Samples Area			Results obtained	
	Sample -1	Sample -2	Average	Amount of drug present in mg	Percentage of drug
01	657221971	657921232	657571602	26.24	104.96
02	671649864	672807248	672228556	26.10	104.40
03	671898722	672457317	672178020	26.22	104.88
04	666650867	667691114	667170991	26.08	104.32
05	670589937	672271795	671430866	26.13	104.52
06	672701057	673997960	673349509	26.09	104.36
				Mean	**104.57**
				Std. Dev	**0.28**
				RSD	**0.266**

Table No.24: Peak Results for intermediate Precision of Dexketoprofen

S.No.	Parameter	Results obtained	Acceptance criteria
01	Tailing factor for Dexketoprofen	1.58	**NMT 2.0**
02	%RSD of Dexketoprofen area for six standard injections	0.146	**NMT 2.0%**
03	Theoretical Plates for the peak due to Dexketoprofen	4893	**NLT 2000**

Table No.25: System suitability Data

REPORT:

System suitability result passes and the results obtained for Intermediate precision are found within the acceptance criteria. Hence, it is concluded that the assay method is capable to generate, repeatable assay results for Dexketoprofen tablets in multiple preparations of a unique batch, besides by a different analyst.

VII) ROBUSTNESS:

The Robustness for the analytical procedure expresses a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during analysis.

Change in flow rate plus (1.7 ml/minute):

For Chromatographic conditions, follow the method of analysis same, except changing the flow to 1.7 ml / minute instead of 1.5 ml / minute.

Change in flow rate minus (1.3 ml/minute):

For Chromatographic conditions, follow the method of analysis same, except by changing the flow to 1.3 ml / minute instead of 1.5 ml / minute.

Change in wavelength plus (262 nm):

For Chromatographic conditions, follow the method of analysis same, except by changing the wavelength to 262 nm instead of 260 nm.

Change in wavelength minus (258nm):

For Chromatographic conditions, follow the method of analysis same, except by changing the wavelength to 258 nm instead of 260 nm.

Change in mobile phase organic content plus (+ 5 %):

Change the least Buffer ratio to 21 instead of 20, and adjust the quantity variation from Acetonitrile concentration to maintain 100%. Maintain other chromatographic condition remains same.

Change in mobile phase organic content minus (- 5 %):

Change the least Buffer ratio to 19 instead of 20, and adjust the quantity variation from Acetonitrile concentration to maintain 100%. Maintain other chromatographic condition remains same.

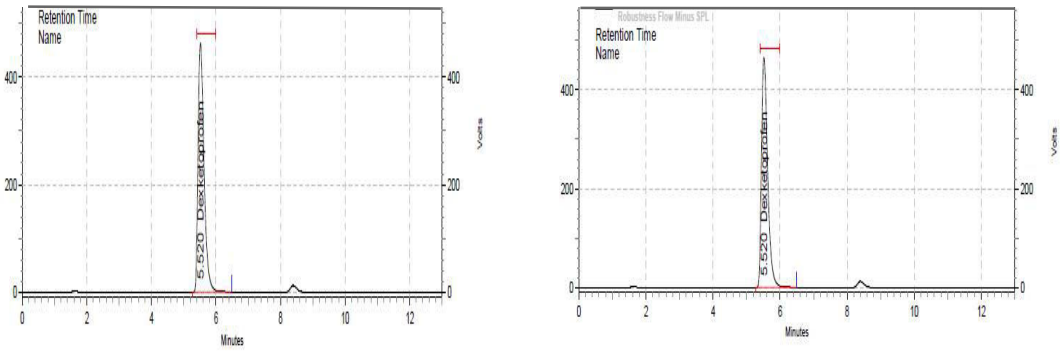


Fig. No. 35 : Chromatogram of Flow rate Minus

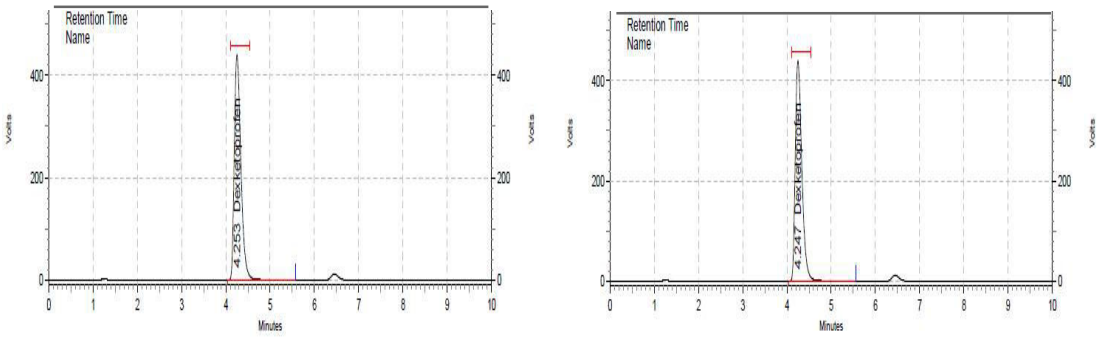


Fig. No. 36 : Chromatogram of Flow rate Plus

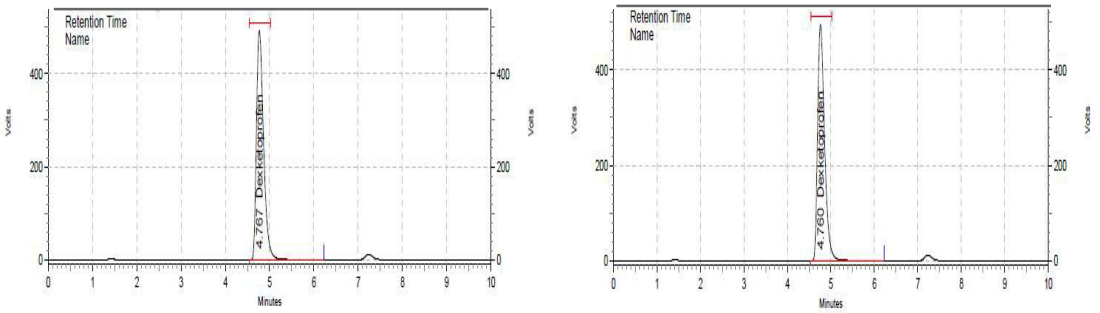


Fig. No. 37 : Chromatogram of Wavelength Minus

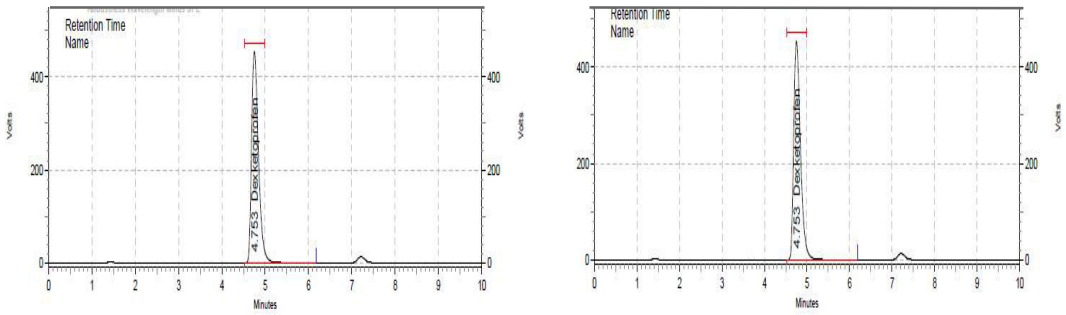


Fig. No. 38 : Chromatogram of Wavelength Plus

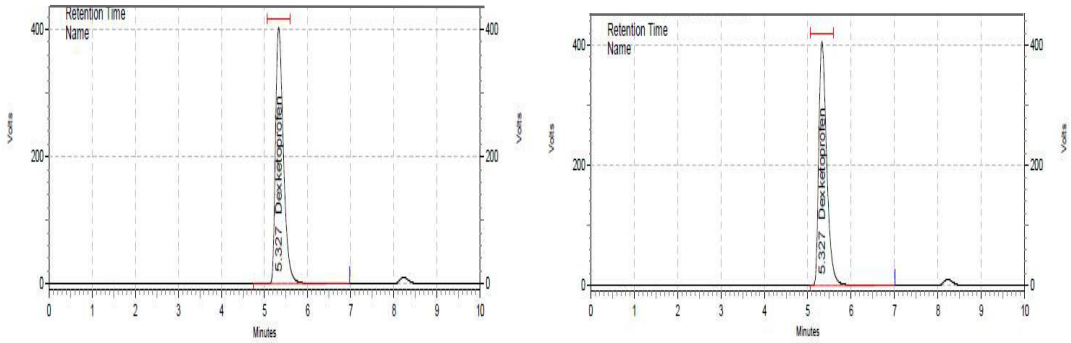


Fig. No. 39 : Chromatogram of Mobile Phase Minus

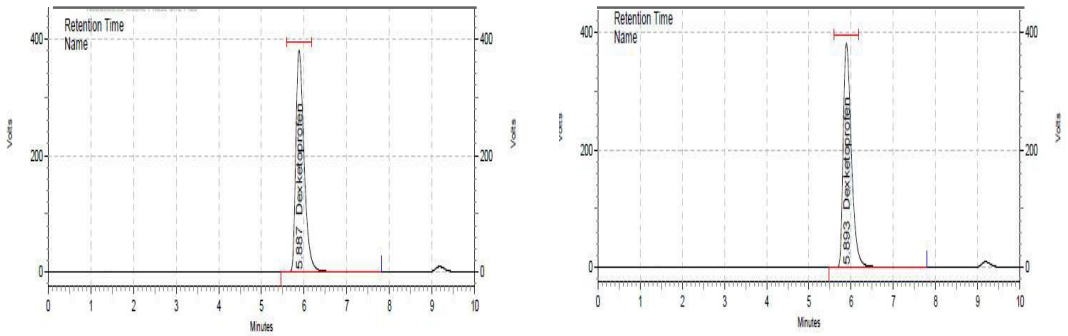


Fig. No. 40 : Chromatogram of Mobile Phase Plus

S.No.	Parameter Name	Dexketoprofen Results obtained		
		Tailing factor	Area (RSD)	Theoretical Plates
01	Wavelength 262 nm minus	1.46	0.17	4464
02	Wavelength 258 nm	1.45	0.26	4458
03	Flow rate 1.3 mL	1.50	0.20	4420
04	Flow rate 1.7 mL	1.43	0.19	4068
05	Mobile phase plus	1.51	0.03	4509
06	Mobile phase minus	1.49	0.11	4255
Acceptance criteria		**NMT 2.0**	**NMT 2.0%**	**NLT 2000**

Table No.26 System suitability Data

S.No	Parameter Name	Dexketoprofen Drug in mg	Dexketoprofen Drug in %	Acceptance Criteria
01	Change in wavelength 231 nm	25.89	103.56	
02	Change in wavelength 235 nm	25.88	103.52	
03	Robust flow rate 0.8mL	25.96	103.84	
04	Robust flow rate 1.2mL	26.20	104.80	
05	Robust mobile phase composition + 5 %	25.80	103.20	
06	Robust mobile phase composition - 5 %	26.08	104.32	

Table No.27: Peak Results for Robustness of Dexketoprofen

Report:

System suitability result passes in all the deliberately changed methods and the results obtained for all deliberately changed methods are found within the acceptance criteria. It is concluded that the deliberately changed assay methods results are remains unaffected in small variations which confirmed that all the methods are proficient to estimate Dexketoprofen Tablets.

VIII) STABILITY OF ANALYTICAL SOLUTIONS:

Solutions to be used in the analytical method should be analyzed for the study of their stability. This study should be performed by injecting standard solution and sample solution at probable time points, and minimum not less than 24 hours shall be studied.

Solution Stability for Dexketoprofen Standard Solution

S. No.	Time point	Standard solution area	Results obtained		
			Cumulative % RSD	Tailing factor obtained	Theoretical plate obtained
01	0^{th} hour	632676886	0.000	1.540	5266
02	1^{st} hour	633114563	0.049	1.520	5319
03	2^{nd} hour	633733706	0.084	1.540	5318
04	3^{rd} hour	633355442	0.070	1.530	5337
05	4^{th} hour	633537559	0.065	1.520	5342
06	5^{th} hour	633045093	0.060	1.540	5303
07	6^{th} hour	634033916	0.072	1.480	5316
08	7^{th} hour	634046484	0.077	1.520	5292
09	8^{th} hour	633745916	0.074	1.510	5300
10	9^{th} hour	633765012	0.071	1.510	5304
11	10^{th} hour	633514978	0.067	1.540	5295
12	11^{th} hour	633405833	0.064	1.520	5309
13	12^{th} hour	633968097	0.065	1.490	5359
14	13^{th} hour	633831257	0.064	1.480	5379

15	14th hour	633438369	0.062	1.470	5421
16	15th hour	633248917	0.061	1.530	5356
17	16th hour	633504053	0.059	1.450	5385
18	17th hour	633043809	0.060	1.490	5395
19	18th hour	632865627	0.062	1.480	5365
20	19th hour	632920245	0.064	1.470	5279
21	20th hour	632590841	0.069	1.440	5379
22	21th hour	632795102	0.070	1.410	5309
23	22th hour	632594622	0.073	1.430	5303
24	23th hour	632740294	0.074	1.440	5263
25	24th hour	632775828	0.074	1.390	5302
Limit			**NMT 2.0 %**	**NMT 2.0**	**NLT 2000**

Table No. 28 : Solution Stability for Dexketoprofen Standard

Solution Stability for Dexketoprofen Standard Solution

S. No.	Time point	Sample solution area	Results obtained		
			Cumulative % RSD	Tailing factor obtained	Theoretical plate obtained
01	0th hour	676698822	0.000	1.590	5230
02	1st hour	677464246	0.080	1.540	5299
03	2nd hour	677172436	0.057	1.580	5266
04	3rd hour	677651361	0.061	1.510	5380
05	4th hour	677569280	0.057	1.570	5310
06	5th hour	677809028	0.059	1.550	5286
07	6th hour	678750466	0.093	1.520	5285
08	7th hour	678860187	0.109	1.510	5281
09	8th hour	678715469	0.112	1.550	5238

10	9th hour	678299846	0.108	1.530	5270
11	10th hour	678003409	0.102	1.520	5305
12	11th hour	679195506	0.112	1.500	5304
13	12th hour	678573067	0.110	1.500	5327
14	13th hour	679411801	0.118	1.500	5362
15	14th hour	679983253	0.133	1.520	5394
16	15th hour	679306507	0.134	1.510	5367
17	16th hour	679743431	0.139	1.480	5520
18	17th hour	678884006	0.136	1.490	5359
19	18th hour	678619091	0.132	1.490	5353
20	19th hour	680201604	0.141	1.460	5237
21	20th hour	679636180	0.142	1.460	5303
22	21th hour	679752492	0.143	1.470	5284
23	22th hour	679231196	0.141	1.470	5267
24	23th hour	679794857	0.142	1.400	5219
25	24th hour	679765426	0.142	1.390	5262
Limit			**NMT 2.0%**	**NMT 2.0**	**NLT 2000**

Table No.29 : Solution Stability for Dexketoprofen Sample

Conclusion for stability of analytical solutions:

System suitability result passes and the results obtained for stability of standard solution and sample solution are found within the acceptance criteria for the minimum period of 24 hours study. Hence, it is concluded that the standard solution and sample solution as mentioned in the assay method are capable to inject into the chromatography within 24 hours from the time of preparations.

RESULT AND DISCUSSION

The objective of the proposed work was method development for the in estimation of Dexketoprofen in tablets by RP-HPLC and to validate the developed method according to USP and ICH guidelines and applying the same for use in the quality control samples in pharmaceutical industry.

As there is no official method for the estimation of Dexketoprofen , so we tried to develop a method by which we can quantify the amount of drug present in the given sample.

In RP-HPLC method, the conditions were optimized, to estimation of dexketoprofen. Initially, various mobile phase compositions were tried, to got better elution of drug. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The system with buffer and Methanol: Water (2:48:50) at gradient flow rate of 1.5ml min was found to be quite robust.

The optimum wavelength for detection was 260 nm at which better detector response for the drug was obtained. The average retention times for Dexketoprofen was found to be 4.5 min, respectively. According to United States Pharmacopeia, system suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in various concentration range with correlation.

The low values of RSD indicate that the method was precise and accurate. The mean recoveries were found in the range of 98 – 102 %. System precision is evaluated by injecting 10 injections of standard solution and low value of % RSD shows that system is precise. Precision for method is evaluated by analyzing a sample of homogenous batch six times and the low % RSD value shows the method is precise

Method robustness was evaluated by alteration of flow rate (±10%), Mobile phase organic content (±5°C), Wavelength (±2 nm) and it was found robust as % RSD was below 2.0%.

Ruggedness of the proposed method was determined by analysis of aliquots from homogeneous slot in different laboratories, by different analysts, different column, different system using similar environmental conditions, the % R.S.D. reported was found to be less than 2 %. The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in marketed formulations.

Both sample solution and standard solution are stable at 25°C for24 hrs. as the % difference in the area was found to be less than 2.0%.

Finally, it can be concluded that the assay values of formulation were the same as mentioned in the label claim with the RSD of < 1.0%. The proposed method was found to be accurate, precise, reproducible and stable, and can be successfully applied for the routine analysis of the drug in tablet dosage forms.

CONCLUSION

This assay method by HPLC is validated for statistical parameter and it is found that the analytical procedure for **Dexketoprofen tablets 25 mg** meets system suitability requirements and acceptance criteria for Specificity, Linearity, Precision, Accuracy, Robustness, Ruggedness parameters and Solution stability.

Solution stability study of the standard and sample preparations are found stable for a minimum of 24 hours. There was no deviation obtained during this validation study. Hence, this In-House analytical method is recommended to carry out the assay analysis from now on for this indistinguishable formulation on **Dexketoprofen Tablets 25 mg.**

SUMMARY

Validation Parameter	Acceptance Criteria	Results
System Suitability	The RSD Should be **≤2%** for 5 replicate injections for each peak	**0.172**
Specificity	The peaks of diluents and placebo should not interfere with the main peaks	**The peaks of diluents and placebo did not interfering with the peaks of Dexketoprofen**
	Precision	
System Precision	The %RSD of 5 replicate injections should be**≤2.0%**	**0.071**
Method Precision	The% RSD calculated on 6 determinations of assay value should be ≤ **2%**	**0.494**
Linearity	The correlation coefficient should be ≥**0.998**	**0.99947**
Solution Stability	RSD**≤2.0%**	Standard and sample solutions are stable at 25^0c for 24hrs

Table. No. 30 : Summary

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