METHOD DEVELOPMENT AND VALIDATION OF DEXIBUPROFEN FOR RELATED SUBSTANCES IN TABLET DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Chennai - 600032

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

MASTER OF PHARMACY

(Pharmaceutical Analysis)

Submitted by

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MAY- 2011.

CERTIFICATE

This is to certify that the dissertation entitled **METHOD DEVELOPMENT AND VALIDATION OF DEXIBUPROFEN FOR RELATED SUBSTANCES IN TABLET DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY''** is a bonafide and genuine research work carried out at Department of Pharmaceutical Analysis, K. K. College of Pharmacy, Chennai – 600122, by **MRS.K.SUGANYA SRI** during the academic year 2011-2012 under my direct guidance and supervision. This dissertation submitted in partial fulfillment for the award of **Degree of Master of Pharmacy (Pharmaceutical Analysis)** to The Tamil Nadu Dr. M.G.R Medical University, Chennai – 600032.

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ABBREVIATIONS

%	-	Percentage
ºC	-	Degree Celcius
CONC.	-	Concentration
g/mol	-	Gram per mole
H_2O_2	-	Hydrogen Peroxide
HCL	-	Hydrochloric acid
HPLC	-	High Performance Liquid Chromatography
ICH	-	International Conference on Harmonisation
LOD	-	Limit of Detection
LOQ	-	Limit of Quantification
mg	-	Milligram
ml	-	Milliliter
µg	-	Microgram
µl	-	Microliter
nm	-	Nanometer
NaOH	-	Sodium Hydroxide
pH	-	Negative Logarithm of Hydrogen Ion
r^2	-	Correlation coefficient
RRF	-	Relative Response Factor
RSD	-	Relative Standard Deviation
SD	-	Standard Deviation
UV	-	Ultra Voilet Spectroscopy
w/w	-	Weight/weight

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INTRODUCTION

DRUG PROFILE

1. INTRODUCTION

1.1. ANALYTICAL METHOD DEVELOPMENT

HPLC method development and validation is important for the analysis of drugs in any formulation. The method which is used for quantitation or identification of drug should be a validated one. It must be able to detect or quantitate the particular drug in the presence of other components. Before starting any method development one should have knowledge about the information of nature of the sample, separation goals, number of compounds present, chemical structures, molecular weights, pKa values, solubility and UV spectrum of the compounds. Perhaps maximum method development involves the trial and error procedures.

The most difficult problem usually occurs in method development is where to start, what type of column is worth trying with what kind of mobile phase. While there are a number of HPLC methods available to the development chemist, perhaps the most commonly applied method is Reversed Phase Chromatography method.

Alliance HPLC System.

A typical pharmaceutical compound is considered to be an active pharmaceutical ingredient (API) of less than 1000 Daltons, either soluble in water or in an organic solvent. The water soluble drug is further differentiated as ionic or nonionic which can be separated by reverse phase. Similarly, the organic soluble drugs can be classified as polar and non polar and equally separated by reverse phase. In some cases the non polar API may have to be separated using adsorption or normal phase HPLC, in which mobile phase would be non polar organic solvent. The other chromatographic modes may need to be considered for separation. These include ion exchange, chiral and size exclusion chromatography. Samples like proteins, peptides nucleic acids and synthetic polymers analyzed by using some special columns or ion pair reagents (i.e. 0.1% TFA).

1.1.1. General conditions to initiate HPLC method development

In general, one begins with reversed phase chromatography, when the compounds are hydrophilic in nature with many polar groups and are water soluble. The organic phase concentration required for the mobile phase can be estimated by gradient elution method. For aqueous sample mixtures, the best way to start is with gradient reversed phase chromatography. Gradient can be started with 5-10% organic phase in the mobile phase and the organic phase concentration can be increased up to 100% within 20-30 min. Separation can be optimized by changing the initial mobile phase composition and the slope of gradient according to the chromatogram obtained from preliminary sample run. The initial mobile phase composition can be estimated on the basis of where the compounds of interest were eluted, namely at what mobile phase composition. Changing the polarity of a mobile phase can alter elution of drug molecules.

Table-1: Column & mobile phase selection shall be done as per the table given below

Method/Description/Column	**When the method preferred**
Reverse-Phase HPLC	
Water/organic mobile phase	For neutral and non ionized compounds that dissolve in water/organic mixtures.
Column: C18,C8,Phenyl,Cyano, and Trimethylsilys (TMS) columns.	
Normal Phase HPLC	
Mixture of organic solvents as mobile phase	For samples that do not dissolve in water/organic mixture.
Column: Silica, Cyano and Amino columns.	

The elution strength of a mobile phase depends upon its polarity, the stronger the polarity, higher is the elution. Ionic samples (acidic and basic) can be separated, if they are present in associated form. Dissociation of ionic samples may be suppressed by proper selection of pH. The optimized chromatogram is the one in which all the peaks are symmetrical and are well separated in less run time.

The peak resolution can be increased by using a more efficient column (column with higher theoretical plate number), which can be achieved by using a column of smaller particle size, or a longer column in length. These factors will increase the analysis time. Flow rate does not influence resolution, but it has a strong effect on the analysis time.

The parameter that are affected by the changes in chromatographic conditions are,

- Capacity factor (K').
- Selectivity (α).
- Column efficiency (N).
- Peak asymmetry factor or Tailing factor (As).

1.1.2. Selection of mobile phase

The selection of the mobile phase mainly based on the solubility and polarity of the compound. Usually, in RP-HPLC method water and organic solvents are used as the mobile phase. In NP-HPLC method non polar solvents like Hexane and THF were used. If the sample contains ionic or ionizable compounds, then use of a buffered mobile phase to ensure the reproducible results.

In many cases, a silanophilic interaction causes tailing, mainly for the basic compounds due to ion-exchange interaction. This can usually be reduced or suppressed by the use of mobile phases modifiers (0.1% v/v triethylamine for basic analyte or 1% v/v glacial acetic acid for the acidic analyte), or a combination thereof. Whenever buffers or other mobile phase are used, check the solubility in mobile phase. This is especially true for gradient applications. Acetonitrile is the preferred organic modifier in reversed phase chromatography. The elution strength increases in the order methanol, acetonitrile and tetrahydrofuran. The retention changes by roughly 10% for every 1% change in the concentration of organic modifier.

1.1.3. Mobile phase composition

In reverse phase chromatography, the separation is mainly controlled by the hydrophobic interaction between drugs molecules and the alkyl chains on the column packing materials. Most chromatographic separation can be achieved by choosing the optimum mobile phase composition. This is due to the fact that a fairly large amount of selectivity can be achieved

by choosing the qualitative and quantitative composition of aqueous and organic portions.Most widely used solvents in reverse phase chromatography are Methanol and Acetonitrile. Tetrahydrofuran is also used but to a lesser extent.

A drug solution having all possible known impurities can be used for checking the extent of separation with different mobile phase ratios. Alternatively, solution of stressed drug substance can be used to check for separation of impurities.Silica based column with different crosslinkings in the increasing order of polarity are as follows.

<-----Non-polar-----Moderately polar----Polar----->

C18<C8<C6<Phenyl<Amino<Cyano<Silica.

Experiments are to be conducted using different columns with different mobile phase to achieve best separation in chromatography. A column which gives separation of all the individual impurities and degradants from each other and from API peak and which is rugged for variation in mobile phase shall be selected.

1.1.4. Selection of column

The HPLC column is the heart of the method, performing the critical separation. The column must possess the selectivity, efficiency and reproducibility to provide good separation. Commonly used reversed phases are crosslinking the Si-OH groups with alkyl chains like, C8 (octylsilane), C18 (octadecylsilane) and nitrile groups (CN), phenyl groups (-C6H6) and amino groups (-NH2). They are chemically different bounded phases and demonstrate significant changes in the selectivity using the same mobile phase. During method development selection of column can be streamlined by starting with shorter column (150, 100 or even 50 mm long). By selecting a shorter column with an appropriate phase run time can be minimized so that an elution order and an optimum mobile phase can be quickly determined. The following are the parameters of a chromatographic column which are to be considered while choosing a column for separation of impurities and degradants.

- Length and diameter of the column.
- Packing material.
- Shape of the particles.
- Size of the particles.
- % of carbon loading.
- Pore volume.
- Surface area.
- End capping.

1.1.5. Selection of Column temperature

Temperature variation over the course of a day has quite significant effect on HPLC separations. This can even occur in air conditioned rooms. While temperature is a variable that can affect the selectivity, its effect is relatively small. Always it is preferable to optimize the chromatographic conditions with column temperature as ambient. However, if the peak is symmetry could not be achieved by any combination of column and mobile phase, then the column temperatures above ambient can be adopted. The increase in column temperature generally will result in reduction in peak asymmetry and peak retentions. When found necessary, the column temperatures between 30ºC and 80ºC shall be adopted. If a column temperature of above 80ºC is found to be necessary, packing materials which can withstand to that temperature shall be chosen.

1.1.6. Selection of flow rate

Flow rate, more for isocratic than gradient separation, can sometimes be useful and readily utilized to increase the resolution, although its effect is very modest. The slower flow rate will also decrease the column back pressure.

Flow rate shall be selected bases on the following data.

- Retention times.

- Column back pressures
- Separation of impurities.
- Peak symmetries.

Preferably the flow rate shall be not more than 2.5 ml/min. check the ruggedness of the method by varying the flow rate by ± 0.2 ml from the selected flow rate. Select the flow rate which gives least retention times, good peak symmetries, least back pressures and better separation of impurities from each other and from API peak.

The mobile phases are pumped at different flow rates so as to achieve the required composition and then mixed in a chamber and then introduced into the column. While optimizing the separation of impurities, it is to be decided whether Low-pressure gradient [not more than 80% organic phase is to be pumped] or High-pressure gradient [more than 80% of the organic phase is to be pumped]. While optimizing the gradient program, especially using low viscous solvents like acetonitrile and phosphate buffers, it is recommended to mix about 10% aqueous portion preferably the same buffer used in mobile phase to avoid pumping problems.

1.1.7. Selection of detector wavelength

Selection of detector wavelength is a critical step in finalization of the analytical method for impurities and degradants. Inject the impurity and API standard solutions into the chromatographic system with photodiode array detector and collect the spectra. Also conduct forced degradation studies and collect the UV spectra of all the major degradation products. Overlay the spectra of all the compounds and select a wavelength which is most common and gives higher responses for all compounds.

1.1.8. Selection of Diluents for Test preparation

Diluent for test preparation is selected initially based on solubility of the drug substances and known impurities.Finalization of diluent is based on its extraction efficiency peak symmetries and resolution of Impurities and diluent blank injection interference. Inject the diluent blank and test solution spiked with known impurities into the chromatographic

system and establish the non-interference of blank in estimation of impurities and the effect of diluent on resolution of impurities and peak symmetries.

Conduct experiments to optimize the extraction of API in presence of recipients at different test concentrations using the diluent chosen based on solubility and select the test concentration at which the extraction is most efficient. Select a diluent in which all the known impurities/degradants and drug substances are soluble, in which the extraction is complete, due to which there is no blank interference, in which the peak symmetries and resolution between impurities is found to be satisfactory.

1.1.9. Selection of Test concentration, Injection volume

The test concentration is generally chosen based upon the response of API peak and impurities at the selected detector wavelength. The test concentration shall be finalized after it is proved that API is completely extractable at the selected test concentration. Generally an injection volume of 10 to 20µl is recommended for estimation of impurities.

If the extractions are found to be difficult, then the test concentrations can be kept low and the injection volume can be increase up to 50 µl but it is to be ensured that at the selected injection volume the column is not overloaded, resolution between individual impurities and the peak symmetries are not compromised. After the test concentration and the diluent is finalized, prepare a test solution and keep the filtered solution in closed condition in a stoppered flask on the bench top and observe for any precipitation or turbidity after 24 hours. The solution should not show any turbidity/precipitation.

1.2.ANALYTICAL METHOD VALIDATION[4]

1.2.1. Types of Analytical Procedures to be validated

The validation of analytical procedures is directed to the four most common types of analytical procedures.

- Identification tests
- Quantitative tests for impurities' content
- Limit tests for the control of impurities
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below.

- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

This list should be considered typical for the analytical procedures cited but occasional exceptions should be dealt with on a case-by-case basis. It should be noted that robustness is not listed but should be considered at an appropriate stage in the development of the analytical procedure.

Furthermore revalidation may be necessary in the following circumstances

- Changes in the synthesis of the drug substance.
- Changes in the composition of the finished product.
- Changes in the analytical procedure.

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.

1.2.2. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

This definition has the following implications:

Identification: to ensure the identity of an analyte.

Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Assay (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

1.2.3. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

1.2.4. 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: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a

homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

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

Reproducibility: Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

1.2.5. Detection limit

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

1.2.6. Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit 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.

1.2.7. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

1.2.8. Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

1.2.9. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

2. REVIEW OF LITERATURE

2.1. Hassan Y.Aboul-Enein, et al[5],(2011) validated enantioselective HPLC assay of dexibuprofen tablet formulations. Mobile phase was composed of 0.025 M potassium phosphate dibasic (pH 4.5) – methanol- ethanol (85:10:5 v/v/v).The method was linear over the range 15-35 µg/ml (r^2= 0.9995) ; accuracy and precision were acceptable with % RSD < 2.0%.

2.2. P.Balan, et al[6],(2011) validated Rp-HPLC Method for estimation of dexibuprofen and paracetamol in combined tablet dosage form. The method was carried out on a C-18 ,250× 4.5 mm consisting of acetonitrile: water in ratio of 50:50 (pH-7.8 adjusted with triethylamine) as mobile phase at a flow rate of 1.0 ml/ min. Detection was carried out at 230nm. The retention times of dexibuprofen and paracetamol were found to be 1.7 and 2.4 .Linear in the range of 2-10 µg/ml for dexibuprofen and paracetamol.

2.3. SelvaduraiMuralidharan, et al[7],(2011) developed the validation of HPLC and an UV Spectrophotometric Methods for determination of Dexibuprofen in pharmaceutical Preparations. Mobile phase was composed of acetonitrile and 0.5% triethylamine (pH adjusted with ortophosphoric acid (30:70,v/v)) with RP-18 column .UV was performed at 222 nm. No spectral or chromatographic interferences from the tablet excipients were found in UV and HPLC.

2.4. Pritesh G. Dhartarkar, et al[8],(2011) developed the validation of UV Spectrophotometric Methods for estimation of dexibuprofen in bulk and dosage form. Measurement of absorption at maximum wavelength in phosphate buffer pH 6.8 was found to be at 221 nm by using 5% methanol. Beers law was obeyed in concentration range 0-60 µg/ml having line equation y = 0.046x +0.017 with r^2 = 0.999.

2.5 XIE Bin,et al[9],(2008) Determination of dexibuprofen and its related substances in Dispersible Tablets by HPLC. Methods ODS-C18 column was applied and mobile phase was acetonitrile and potassium phosphate and detected at 263 nm. Results calibration curve was linear over the range of 4×10^{-3} ~ 100×10^{-3}g/lit (r = 1).The mean recovery rate of contents was 99.69 % with RSD as 0.92% (n=9).

2.6 Chul Soon Yong,et al[10],(2009) Enhanced oral bioavailability of Dexibuprofen by a novel solid Self-emulsifying drug delivery system(SEEDS).The solid SEEDS was

characterised by SEM,DSC and XRD studies.The liquid SEEDS was a system that consisted of dexibuprofen , labrasol,capryol 90 and labrafil. The particle size analysis revealed no difference in the z-average particle diameter of the reconstituted emulsion between liquid and solid SEEDS.

2.7 S.Agatonovic –Kustrin, et al[11],(2000) Determination of enantiomeric composition of ibuprofen in solid mixtures of the two by DRIFT Spectroscopy. Sample mixtures were dispersed as a 5% (w/w) mix in KBr and spectra were measured. T he original spectra were sampled between 650.16 & 3999 cm^{-1}. A working range of 1-100 % of the R(-) enantiomer present as an impurity in S(+) enantiomer was established with a minimum quantifiable level of 1.67 % and a limit of detection of 0.5 %.The average recovery values were 100.95 & 98.02 for R(-) & S(+) enantiomer.

2.8 Xie Bin, et al [12] ,(2008) Determination of dexibuprofen and its related substances in dexibuprofen sustained – release suppositories by HPLC. Hypersil ODS column with mobile phase acetonitrile and potassium dihydrogen phosphate was used and detected at 264 nm. The linearity was 4~128 mg/lit. The average recovery was 99.9% with RSD 0.3 %.

2.9 WANG Wen-ging, et al [13] ,(2008) Content Determination of dexibuprofen in the gel Preparation by HPLC. Hypersil ODS2 column was used, Mobile phase was acetonitrile and water and detected at 263 nm, flows at a rate of 1.0 ml/min. Linearity was performed in the range of 25.1- 251.0 µg/ml(r = 0.999). The average recovery was 101.6% (n =9).

2.10 ByranGowramma, et al[14], (2011) developed the validation of Direct chiral separation of Ibuprofen Enantiomers of sustained release dosage form. Enantiomeric separation was achieved on Lux 5 cellulose 1 column as stationary phase and Mobile phase consisting of perchloric acid and acetonitrile (50 :50) at a flow rate of 1.0 ml/min and detected at 254 nm. RT for (R) and (S) enantiomers were 6.3 and 10.4 min. Linearity was performed in the range of 0.5-3.0µg/ml.

2.11 Rao, et al[15], (2011)developed the validation of an UPLC Method for rapid determination of Ibuprofen and Diphenhydramine citrate in the presence of impurities in combined dosage form. The method was developed using C18 Column with mobile phase containing a gradient mixture of solvent A&B and monitored at 220 nm. The linear

in the range of 0.20-6.00 & 0.084-1.14 µg/ml for Ibuprofen and Diphenhydramine citrate. LOD were ranged from 0.200-0.320 and 0.084-0.099 µg/ml for Ibuprofen and Diphenhydramine citrate impurities. LOQ were ranged from 0.440-0.880 and 0.258-0.372 µg/ml for Ibuprofen and Diphenhydramine citrate impurities.

2.12 Sohan S. Chitlange, et al[16],(2009) Reported stability indicating HPTLC method for analysis of dexibuprofen in bulk and dosage form. The separation was carried out using n-Hexane : ethylacetate: glacial acetic acid (7.5:2.5:0.2v/v/v).254 plate was used as stationary phase.

2.13 Tao, et al[17], (2009) determined dexibuprofen-β- cyclodextrin inclusion complex by HPLC. The sample was dissolved with 75% methyl alcohol and prepared by ultrasonication. The mobile phase was methyl alcohol – 0.2%, phosphoric acid solution (75:25) and detected at 220nm. The linearity range of dexibuprofen was 10.01~50.05 g/lit (r = 0.9999) . The average recovery was 98.91 % (RSD =1.38 %).

2.14. MandalUttam,et al[18], (2008) developed Boiequivalence study of two formulations containing 400 mg Dexibuprofen in Healthy Indian Subjects. The concentration of dexibuprofen in plasma was determined by a validated HPLC method with UV detection using carbamazepine as internal standard. The formulations were compared using the parameters AUC, $C_{max,}$ t_{max},. Both preparation were well tolerated with no reactions observed throughout the study.

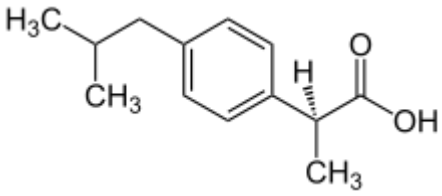
3. DRUG PROFILE

3.1. DEXIBUPROFEN:

(2S)-2-[4-(2-methylpropyl)phenyl]propanoic acid

Dexibuprofen is a non-steroidal anti-inflammatory drug. It is the dextrorotatory enantiomer of ibuprofen.Most ibuprofen formulations contain a racemic mixture of dexibuprofen [(+)-ibuprofen] and (-) – ibuprofen.

Structure:



Molecular weight: 206.281 g/mol

Molecular formula: $C_{13}H_{18}O_2$

Description: Dexibuprofen is a white, crystalline powder.

Dosage form: Tablets.

Dosage : 200 to 500 mg

Solubility: Soluble in isopropyl alcohol, ethanol absolute, ethyl acetate, n-hexane.

Indications :

- Pain and inflammation associated with musculoskeletal, joint and soft tissue disorders.
- Primary dysmenorrhea.

Special Precautions:

- History of bronchial asthma
- renal or hepatic disorders

- bleeding disorders
- CV disease
- elderly
- lactation

Adverse Effects :

- GI bleeding
- Heartburn
- epigastric pain
- dyspepsia
- peptic ulcer
- nausea
- vomiting
- diaorrhea
- jaundice
- hepatitis
- visual disturbances
- depression

Pharmacokinetics:

Dexibuprofen is absorbed from the gastrointestinal tract and peak plasma concentrations occur about 1 to 2 hours after ingestion. Dexibuprofen is also absorbed on rectal use. There is some absorption after topical application to the skin. Dexibuprofen is 90 to 99% bound to plasma proteins and has a plasma half-life of about 2 hours. It is rapidly excreted in the urine mainly as metabolites and their conjugates. About 1% is excreted in urine as unchanged dexibuprofen and about 14% as conjugated dexibuprofen. There appears to be little if any distribution into breast milk.

Mechanism of Action: Dexibuprofen is a NSAID. It acts by inhibition of cyclo-oxygenase, which is involved in prostaglandin synthesis.

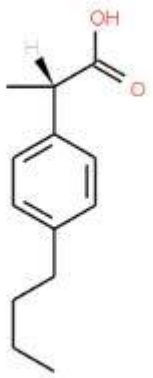
Bioavailability : 49-73 %

Plasma protein : 90-99 %

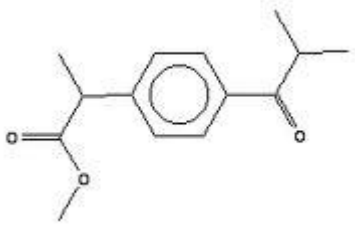
Half Life : 1.8 - 2 hours

3.2.IMPURITIES OF DEXIBUPROFEN:

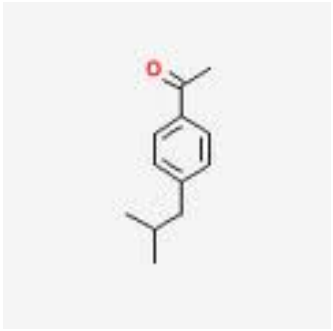
- **2-(4-butylphenyl)propionic acid (Impurity -B)**



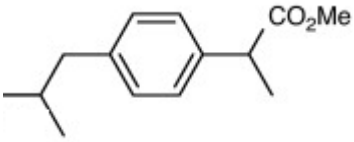
- **2-(4-isobutyrylphenyl)propionic acid(Impurity-J)**



- **4-Isobutylacetophenone(Impurity-E)**



❖ **Ibuprofen Methylester**



4. AIM AND PLAN OF WORK

4.1. NEED FOR METHOD DEVELOPMENT

The exhaustive literature survey revealed that none of the most recognized pharmacopoeias or any journals includes the determination of related substances of Dexibuprofen. So it is felt essential to develop a liquid chromatographic procedure which will serve a reliable, accurate, sensitive, and stability indicating method for the simultaneous determination of related substances of Dexibuprofen from tablets.

4.2. AIM OF THE STUDY

To develop and validate stability indicating related substances analytical method for the Dexibuprofen tablets using HPLC.

4.3. PLAN OF THE PRESENT STUDY

Development and optimization of the proposed chromatographic conditions

- Selection of wavelength.
- Selection of initial separation condition.
- Nature of stationary phase (column).
- Nature of mobile phase (peak modifier, solvent strength, ratio and flow rate).

Validation of the developed method using various parameters

- Specificity.
- Limit of detection and limit of quantitation.
- Determination of relative retention factor.
- Linearity and range.
- Accuracy.
- Precision.
- Filter compatibility.
- Solution stability
- Robustness.

5. EXPERIMENTAL WORK

Instruments used :

- HPLC with UV/PDA detector [waters]
- Analytical balance [Sartorious]
- P^H meter [Lab India]
- Sonicator [BandelinSonorex Super]

Chemicals used :

- HPLC grade [Millpore] water
- Orthophosphoric acid HPLC grade [Merck]
- Methanol HPLC grade [Fisher Scientific]
- Sodium Hydroxide [Merck]
- Hydrogen peroxide [Fisher Scientific]
- Hydrochloric acid [Merck]
- Acetonitrile [Merck]

Drug Sample :

Dexibuprofen drug sample obtained from Shasun Chemicals, Puducherry.

Initialization of the instrument:

Initially, the column was placed on the instrument and switch on the instruments and washed with methanol, isopropyl alcohol and then finally with water for 30 min. Then the system was made to run with the mobilephase for 30 min .

5.1.TRAILS :

5.1.1.TRAIL- I

Preparation of mobile phase A

Prepared a mixture of 500 ml of acetonitrile ,500 ml of Milli Q Water and 0.5 ml of ortho phosphoric acid . Mixed well and degassed.

Preparation of mobile phase B

Mixed 0.5 ml of ortho phosphoric acid in 1000 ml of acetonitrile and degassed.

5.1.2.TRAIL-II

Preparation of mobile phase A

Prepared a mixture of 400 ml of acetonitrile ,600 ml of Milli Q Water and 1.0 ml of ortho phosphoric acid . Mixed well and degassed.

Preparation of mobile phase B

Mixed 1.0 ml of ortho phosphoric acid in 1000 ml of acetonitrile and degassed.

5.1.3.TRAIL-III

Preparation of mobile phase A

Prepared a mixture of 350 ml of acetonitrile ,650 ml of Milli Q Water and 0.5ml of ortho phosphoric acid . Mixed well and degassed.

Preparation of mobile phase B

Mixed 0.5 ml of ortho phosphoric acid in 1000 ml of acetonitrile and degassed.

Hypersil ODS column was used as stationary phase .

5.1.4. TRAIL-IV

Preparation of mobile phase A

Prepared a mixture of 350 ml of acetonitrile ,650 ml of Milli Q Water and 0.5ml of ortho phosphoric acid . Mixed well and degassed.

Preparation of mobile phase B

Mixed 0.5 ml of ortho phosphoric acid in 1000 ml of acetonitrile and degassed. Flow rate was 2.0 ml/min.

5.2. FINALISED CHROMATOGRAPHIC CONDITIONS

- Column : Waters symmetry, C18(150mm×4.6mm, 5µm)
- Wavelength : 214 nm
- Flow rate : 2.0 mL/minute
- Column temperature : Ambient
- Injection volume : 20 µl
- Pump Mode : Gradient
- Run time : 75 minutes

Table 2: Gradient Program

Time (Minutes)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
25	100	0
55	15	85
70	15	85
75	100	0

5.3. PREPARATION OF SOLUTION

Mobile phase preparation

Preparation of mobile phase A

Prepared a mixture of 350 ml of acetonitrile, 650ml of Milli Q Water and 0.5ml of ortho phosphoric acid. Mixed well and degassed.

Preparation of mobile phase B

Mixed 0.5 ml of ortho phosphoric acid in 1000 ml of acetonitrile and degassed.

Preparation of diluent

" Mobile phase A" as a diluent.

Standard stock solution

Weighed accurately and transferred about 25.0 mg of Dexibuprofen reference standard/ working standard into a 100 ml volumetric flask. Added about 50 ml of methanol and sonicated for about 10 minutes and made up to volume with methanol.(≈ 250.0 µg/ml of Dexibuprofen).

Standard solution

Transferred 2.0 ml of standard stock solution into 100.0 ml volumetric flask and made up the volume with diluent and mixed well. (≈ 5.0 µg/ml of Dexibuprofen))

Note : Standard solution stable for 48 hours at ambient condition (25 ºC).

Impurity –B Stock Solution [2-(4- butyl phenyl) propionic acid]

Weighed accurately and transferred about 2.5 mg of Impurity –B[2-(4- butyl phenyl) propionic acid] standard and transferred into a 25.0 ml volumetric flask, added about 10 ml of methanol and sonicated for about 2 minutes and made up to volume with methanol and mixed well. (≈ 100.0 µg/ml of Impurity –B).

Resolution solution

Weighed accurately and transferred about 50.0 mg of Dexibuprofen reference standard/ working standard into a 25.0 ml volumetric flask. Added about 10 ml of methanol and sonicated for about 10 minutes to dissolved the material. To this added 1.0 ml of the impurity stock solution and made up to volume with methanol and mixed. (≈ 2000.0 µg/ml of Dexibuprofen and ≈ 4.0 µg/ml of impurity-B).

Sample solution

Determined the average weight of 20 tablets, Crushed the tablets to fine powder using mortar and pestle. Accurately weighed and transferred the sample powder equivalent to about 200 mg of Dexibuprofen into a 100 ml volumetric flask. Added about 10 ml of methanol and sonicated for about 10 minutes and made up the volume with diluent. Filtered the solution through 0.45µm nylon syringe filter and discarded first 5 ml of the filtrate. . (≈ 2000.0 µg/mL of Dexibuprofen)

Note: Filtered sample solution stable for 48 hours at ambient condition (25 ºC).

Procedure

Separately injected each 20 µL of blank (diluent), standard solution (6 replicates) and sample solution into the chromatograph, recorded the chromatograms and measured the peak response for all peaks. Disregarded any peak corresponding to the blank.

Note: After every six sample injections, inject the blank, standard solution as Bracketing standard.

Table-3: The relative retention time and correction factor of known impurities

S.NO	Name	RRT	RRF
1	2(4-isobutyryl phenyl) propionic acid (Impurity : J)	0.20	0.92
2	2(4-butylphenyl) propionic acid (Impurity :B)	1.04	0.91
3	4-Isobutylacetophenone (4-IBAP) (Impurity-E)	1.09	1.37
4	Ibuprofen methyl ester	1.34	0.90
5	Dexibuprofen	1.00	-

5.4. EVALUATION OF SYSTEM SUITABILITY

- Relative standard deviation for six replicate injections of standard area should be not more than 10.0%.
- Resolution between Dexibuprofen and Impurity –B[2-(4- butyl phenyl) propionic acid] peaks is not less than 1.5 in resolution solution .
- Theoretical plate count for Dexibuprofen should not be less than 5000.

5.5. CALCULATIONS

For *Known Impurity*

$$\frac{A_{T1}}{A_D} \times \frac{S_D}{100} \times \frac{2}{100} \times \frac{100}{T_W} \times \frac{Avg.Wt}{LC} \times \frac{P}{100} \times 100 \times \frac{1}{RRF}$$

For Unknown Impurity

$$\frac{A_{T2}}{A_D} \times \frac{S_D}{100} \times \frac{2}{100} \times \frac{100}{T_W} \times \frac{Avg.Wt}{LC} \times \frac{P}{100} \times 100$$

Where,

A_{T1}: Area of any known impurity peak in the chromatogram of sample solution

A_{T2}: Area of unknown impurity peak in the chromatogram of sample solution.

A_D: Average peak area of Dexibuprofen in the chromatogram of standard solution.

S_D: Weight of Dexibuprofen reference standard/working standard in mg.

T_W: Weight of Dexibuprofen tablets powder sample taken in mg.

Avg.Wt: Average weight per tablet in mg.

P: % Purity of Dexibuprofen reference standard/working standard (as is basis)

LC: Label claim of Dexibuprofen tablets in mg.

RRF: Relative Response factor

Total impurities: Sum of Known and Unknown impurities

6.1.ANALYTICAL METHOD DEVELOPMENT

TRAIL-1

Dexibuprofen and impurities :

System suitability results

1.) Theoretical plates obtained from trail-1 were 1872 and 2748.

2.) Resolution obtained from trail-1 was 0.7.

3.)% RSD obtained from trail -1 was 17.3 and 22.7.

TRAIL-2

Dexibuprofen and impurities:

System suitability results

1.) Theoretical plates obtained from trail-2 were 3327 and 1482.

2.) Resolution obtained from trail-2 was 0.3.

3.)% RSD obtained from trail -2was 18.6 and 21.3.

TRAIL-3

Dexibuprofen and impurities:

System suitability results

1.) Theoretical plates obtained from trail-3 were 3228 and 1985.

2.) Resolution obtained from trail-3 was 0.45.

3.) % RSD obtained from trail -3 was 14.9 and 21.2.

TRAIL-4

Dexibuprofen and impurities:

System suitability results

1.) Theoretical plates obtained from trail-4 were 87652.

2.) Resolution obtained from trail-4 was 2.8.

3.) % RSD obtained from trail -4 was 0.5.

TRAILS DISCUSSION

On evaluation of the above results, it can be concluded that trail 1, 2, 3 shows poor resolution and affected the method significantly. Trail -4 shows good system suitability results and also in the limit so the trail 4 were accepted and trail 1, 2, 3 were rejected.

6.2. ANALYTICAL METHOD VALIDATION

Method validation is the process of demonstrating the analytical procedures are suitable for their intended use and that they support the identity, strength, quality, purity and potency of drug substances and drug products.

Purpose of Method Validation

- Identification of sources and quantitation of potential errors.
- Determination if method is acceptable for intended use

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- Establish proof that a method can be used for decision making.
- Satisfy regulatory requirements.

Table-4: Specification of Impurities

Impurities	*Specification limit (%)*
Impurity J	***0.20***
Impurity B	***0.20***
Impurity E	***0.20***
Methyl ester	***0.30***
Any other impurity	***0.17***
Total impurities	***0.80***

Preparation of sample composite for validation

Weighed 20 tablets and calculated the average weight of a tablet. Transferred the tablets into a mortar; crushed the tablets into fine powder. Use this powdered sample for validation.

6.1. SYSTEM SUITABILITY

The system suitability of the method has been demonstrated as indicated by the tailing factor for Dexibuprofen, Resolution for Dexibuprofen and Impurity – B and the percentage RSD that is not more than 10.0 from six replicate injections of standard solution. The obtained results were shown in Table-5.

Table-5: System suitabilityResults

S.No.	***Observed results***	***Acceptance criteria***

1	***Dexibuprofen– 64460***	***The theoretical plate for peak due to Dexibuprofen should be not less than 5000.***
2	***Impurity – B - 2.7***	***Resolution between Dexibuprofen and 2(4-butylphenyl)propanoicacid peaks is not less than 1.5.***
3	***System precision***	***Dexibuprofen Peak area***
	Injection	
	1	***121407***
	2	***120231***
	3	***120096***
	4	***120580***
	5	***120293***
	6	***119487***
	Average	***120349***
	% RSD	***0.5***

6.2. PRECISION

6.2.1. System precision

Procedure

Six replicate injections of standard solution were injected. The mean and percentage relative standard deviation (%RSD) for peak areas of Dexibuprofen were calculated.

Acceptance criteria

Percentage relative standard deviation (%RSD) for peak areas of Dexibuprofen was not more than 10.0.

Results and discussion

The method has been determined to be precise as demonstrated by % RSD that was not more than 10.0 for the determinations of related substances of Dexibuprofen tablets. The obtained results were shown in Table-6.

Table-6: System Precision data

Injection No.	***Peak area***
1	***121407***

2	***120231***
3	***120096***
4	***120580***
5	***120293***
6	***119487***
Mean	***120349***
Percentage relative standard deviation (%RSD)	***0.5***

6.2.2. Method precision

Prepare adequate quantity of mobile phase required for the continuation of mobile phase stability, standard and sample solution stability along with method precision and filter validation tests. These tests should be preferably continued in the same HPLC instrument. If different HPLC instrument is used run the required system suitability as appropriate.

Impurities spiked sample solution

Weighed accurately and transferred about 420.0 mg of powdered sample (equivalent to 200 mg of Dexibuprofen) into a 100 ml volumetric flask. Added 50 ml of diluent, and sonicated for 10 minutes, cooled to room temperature and added each 5.0 ml of impurity stock solutions and diluted to the volume with diluent. Mixed well and filtered through 0.45

µm nylon filter. Discarded first 5 ml of the filtrate. (≈ 2000 µg/ml of Dexibuprofen, ≈ 4.0 µg/ml of impurity J,B&C).

Note:(i)Prepared six replicate sample solutions using the same procedure.

(ii) Allocated spiked sample solution 1 for filter validation.

(iii) Allocated standard and spiked sample solution for solution stability study.

Procedure

Separately injected the blank (diluent), standard solution (6 replicates), each impurities spiked sample solution, blank (diluent) and standard solution (bracketing) into the chromatograph and recorded the peak response.

Calculation:

For known impurity :

$$\frac{A_{T1}}{A_D} \times \frac{S_D}{100} \times \frac{2}{100} \times \frac{100}{T_W} \times \frac{Avg.Wt}{LC} \times \frac{P}{100} \times 100 \times \frac{1}{RRF}$$

For Unknown Impurity :

$$\frac{A_{T2}}{A_D} \times \frac{S_D}{100} \times \frac{2}{100} \times \frac{100}{T_W} \times \frac{Avg.Wt}{LC} \times \frac{P}{100} \times 100$$

Where,

A_{T1}: Area of any known impurity peak in the chromatogram of sample solution

A_{T2}: Area of unknown impurity peak in the chromatogram of sample solution.

A_D: Average peak area of Dexibuprofen in the chromatogram of standard solution.

S_D: Weight of Dexibuprofen reference standard/working standard in mg.

T_W: Weight of Dexibuprofen tablets powder sample taken in mg.

Avg.Wt: Average weight per tablet in mg.

P: % Purity of Dexibuprofen reference standard/working standard (as is basis)

LC: Label claim of Dexibuprofen tablets in mg.

RRF: Relative Response factor

Total impurities: Sum of Known and Unknown impurities

Acceptance criteria

The percentage RSD of each individual known impurity and total impurities from six impurities spiked samples should be not more than 10.0.

Results and Discussion

The method has been determined to be precise as demonstrated by % RSD that is not more than 10.0 for individual known impurity and total impurities from six determinations of impurities spiked samples at specification level. The obtained results were shown in Table-7.

Table-7: Method precision data

Sample No.	***Percentage (w/w)***				
	Impurity-J	***Impurity-B***	***Impurity -E***	***Methyl ester***	***Total impurities***

1	***0.198***	***0.178***	***0.191***	***0.372***	***0.974***
2	***0.194***	***0.179***	***0.190***	***0.350***	***0.945***
3	***0.189***	***0.180***	***0.189***	***0.350***	***0.939***
4	***0.194***	***0.185***	***0.194***	***0.358***	***0.958***
5	***0.189***	***0.177***	***0.191***	***0.351***	***0.937***
6	***0.189***	***0.182***	***0.191***	***0.350***	***0.941***
Avg.	***0.192***	***0.180***	***0.191***	***0.355***	***0.949***
% RSD	***0.0***	***2.3***	***0.0***	***0.0***	***1.2***

6.2.3. Intermediate Precision (Ruggedness)

Demonstrate the intermediate precision by performing the impurities spiked sample solution described under the method precision using different instrument, different column and second analyst on different day.

Note: Prepared six replicate impurities spiked sample solutions as per method precision parameter.

Procedure

Separately injected the blank (diluent), standard solution (6 replicates), each impurities spiked sample solution, blank (diluent) and standard solution (bracketing) into the chromatograph and recorded the peak response.

Calculation

For known, unknown and total impurities refer method precision parameter.

Acceptance criteria

- The percentage RSD of each individual known impurity and total impurities from six impurities spiked samples should be not more than 10.0.
- The % RSD of both the analysts of impurities spiked samples for each individual known impurity and total impurities should be not more than 10.0.

Results and Discussion

The method has been determined to be repeatable and precise as demonstrated by an RSD that was not more than 10.0 of individual known impurity and total impurities respectively from six impurities spiked samples at specification level of individual and combined results of both the analysts. The obtained results were shown in Table-8.

Table-8:Intermediate precision data

Spl. No.	**Percentage (w/w)**									
	Impurity-J		**Impurity-B**		**Impurity-E**		**Methyl ester**		**Total**	
	Analyst		**Analyst**		**Analyst**		**Analyst**		**Analyst**	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
1	0.24	0.22	0.22	0.21	0.16	0.14	0.14	0.15	0.86	0.83
2	0.24	0.22	0.22	0.21	0.16	0.14	0.14	0.15	0.87	0.86
3	0.24	0.22	0.22	0.21	0.16	0.14	0.14	0.16	0.88	0.87
4	0.24	0.22	0.22	0.21	0.16	0.14	0.14	0.15	0.88	0.87
5	0.24	0.22	0.23	0.22	0.16	0.14	0.14	0.16	0.89	0.88
6	0.24	0.22	0.23	0.23	0.16	0.14	0.14	0.15	0.88	0.88

Avg.	0.24	0.22	0.22	0.22	0.16	0.14	0.14	0.15	0.9	0.9
RSD	0.0	0.0	2.3	3.9	0.0	0.0	0.0	3.4	1.2	2.2
RSD (12 result)	4.5		3.6		7.0		5.3		1.7	

**Analyst 1 data taken from method precision*

6.3. SPECIFICITY

6.3.1. Interference from blank, placebo & impurities

Placebo solution

Weighed accurately and transferred equivalent to 200 mg of Dexibuprofen (subtracted the 200 mg from the obtained equivalent weight) into a 100 ml volumetric flask. Added about 50 ml of diluent, sonicated for 10 minutes, cooled to room temperature and diluted to the volume with diluent. Mixed well and filtered through 0.45 µm nylon membrane filter, discarded first 5 ml of the filtrate.

Resolution standard:

Weighed accurately and transferred about 50.0 mg of Dexibuprofen working reference standard into 25 ml volumetric flask, added about 10ml of methanol and sonicated for 10 minutes, cooled to room temperature and added 1 ml of impurity B Stock solution and diluted to the volume with diluent. Mixed well and filtered through 0.45 µm nylon membrane filter, discarded first 5 ml of the filtrate.

Impurities spiked sample solution (at specification level)

Weighed accurately and transferred about 420.0 mg of powdered sample (equivalent to 200 mg of Dexibuprofen) into a 100 ml volumetric flask. Added 50 ml of diluent, and solicited for 10 minutes, cooled to room temperature and added each 5.0 ml of impurity stock solutions and diluted to the volume with diluent. Mixed well and filtered through 0.45 µm nylon filter. Discarded first 5 mL of the filtrate. (≈ 2000 µg/ml of Dexibuprofen, ≈ 4.0 µg/ml of impurity J,B&C).

6.3.2. Interference from forced degradation study

S.No.	*Forced degradation study*	*Volume and strength of reagent*
1	*Acid hydrolysis*	*10 mlof 5 N Hydrochloric acid*
2	*Alkali hydrolysis*	*10 ml of 5 N Sodium hydroxide*
3	*Peroxide oxidation*	*10 mlof 30 % Hydrogen peroxide*

Placebo Procedure for acid, alkali, Peroxide oxidation

Weighed accurately and transferred about 220.0 mg of placebo into 3 separate 100 ml volumetric flask, added 50 ml of diluent and sonicated to dissolve the content. To the first 100 ml flask, added 10 ml of 5N HCL. Added 10 ml of 5N NaOH into second and added 10 ml of 30% Hydrogen peroxide into third 100ml flask, kept on water bath / reflux for 12 hours. Cooled and diluted to the volume with diluent and mixed well. Filtered through 0.45 µm nylon filter, discarded first 5 ml of the filtrate and collected the filtrate.

Sample Procedure for acid, alkali, peroxide oxidation

Weighed accurately and transferred about 420.0 mg of Dexibuprofen into 3 separate 100 ml volumetric flask, added 50 ml of diluent and sonicated to dissolve the content. To the first 100 ml flask, added 10 ml of 5N HCL. Added 10 ml of 5N NaOH into second and added 10 ml of 30% Hydrogen peroxide into third 100ml flask, kept on water bath / refluxed for 12 hours. Cooled and diluted to the volume with diluent and mixed well. Filtered through 0.45 µm nylon filter, discarded first 5 ml of the filtrate and collected the filtrate.

Note: After heating/ refluxion, neutralized the solution with sodium hydroxide and hydrochloric acid solution in acid and alkali hydrolysis respectively .(Check and adjust the pH, if required, in the range of about 5 to 7).

Procedure for thermal and photo degradation

Spread evenly about 1 g of placebo, Dexibuprofen powdered sample in separate glass petridish for each degradation condition and exposed as per the following condition.

Table: 9 ***Thermal and photo degradation***

S.No.	***Sample degradation***	***Stressing condition***	***Hours to be exposed***
1	***Thermal (oven)***	***105° C***	***24***
2	***Photo***	***Controlled (covered with aluminum foil)***	***1.2 million lux***
		Uncontrolled (direct exposure)	

Thermal and photo light condition:

Sample preparation

Sample was kept in an oven at 105° C for about 24 hours for thermal condition and Sample was exposed to light of 1.2 million lux hours for photo light condition. Weighed accurately and transferred about 420.0 mg of Dexibuprofen into a separate 100 ml volumetric flask, added 50 ml of diluent and sonicated for 10 minutes to dissolve the content. Cool and dilute to the volume with diluent and mix well. Filtered through 0.45 µm nylon filter, discarded first 5 ml of the filtrate and collected the filtrate.

Placebo Preparation

Weighed accurately and transferred about 220.0 mg of Placebo into a separate 100 ml volumetric flask and followed the same procedure as mention above for sample.

6.3.3. Evaluation of the sample chromatograms

The degradation of analyte peaks shall be in the range of 5 to 30 % by area normalization method. If the degradation level is higher than 30 %, stress condition can be altered (less concentration or less volume of stressing agent or reduced heating/refluxing period) in acid, alkali, water hydrolysis and peroxide oxidation. For other degradation, report the obtained degradation level as highest stress condition.

Ascertain the peak purity, with the help of software; for each individual peak(preferably peak height of the analyte should be less than 1.0 AU at the highest absorbance) in the chromatogram. If required, adjust the test solution concentration accordingly to achieve the desired peak height. If the main peak is not degraded in highest stress condition, then conclude the sample is stable in that particular condition.

Procedure

Separately injected blank (diluent), standard solution (6 replicates), blank (diluent), placebo solution, each individual impurity identification solution, impurities spiked sample solution, blank (diluent), each stressed placebo solution, unstressed sample solution, each stressed sample solution, blank (diluent), standard solution (bracketing) into the chromatograph and recorded the peak response.

Acceptance criteria

- The analyte peaks (impurity peaks and main peak) should be separated from placebo peaks, blank peaks and from each other.

- Peak purity of known impurities and main peaks in standard solution, sample solution, each impurity identification solution and impurities spiked sample solution should pass (purity angle should be less than purity threshold). Report the peak purity values.
- Report the relative retention time of known impurities with respect to Dexibuprofen peak from impurities spiked sample solution.
- Major degradation peaks should be separated from the analyte peaks in stressed sample.
- Peak purity should pass for Dexibuprofen peak. Report the peak purity value of known impurity peaks,Dexibuprofen peak from each stressed sample.

6.3.5. Results and Discussion

The method was found to be specific as demonstrated by forced degradation studies that all degradation impurities were resolved from the analyte peaks, no interference was observed from the placebo and diluent with respected to Dexibuprofen and related impurities.

In addition, Photo diode array detector (PDA) was used to demonstrate the peak spectral homogeneity with the aid of peak purity results. The related substances method has been demonstrated as suitable for monitoring the long term stability of Dexibuprofen tablets. The obtained results were shown in Table-10 to 13

Table-10: Placebo interference data

Sample Name	***Interference Peak found (Yes/No)***	***Acceptance criteria***
Blank	***No***	***Placebo solution should not show any peak at the retention time of known impurity peak and also peak due to Dexibuprofen***
Unstressed placebo	***No***	
Acid hydrolysed placebo	***No***	

Alkali hydrolysed placebo	***No***	
Peroxide oxidised placebo	***No***	
Thermal stressed placebo	***No***	
Photo stressed placebo	***No***	

Table-11: Relative Retention Time of impurities from impurities spiked sample

S.No.	***Name of the impurity***	***Relative retention time***
1	***Impurity-J***	***0.20***
2	***Impurity-B***	***1.04***
3	***Impurity-E***	***1.09***
4	***Methyl ester***	***1.34***

Table-12: Peak purity data of impurities spiked sample

S.No	***Peak Name***	***Retention time***	***Purity angle***	***Purity threshold***	***Purity flag***
1	***Impurity-J***	***6.113***	***2.129***	***7.605***	***No***
2	***Dexibuprofen***	***30.779***	***0.211***	***6.097***	***No***

3	*Impurity-B*	*32.075*	*2.779*	*9.564*	*No*
4	*Impurity-E*	*33.463*	*0.998*	*7.191*	*No*
5	*Methyl ester*	*41.285*	*1.605*	*8.225*	*No*

Table-13: Peak purity data of Dexibuprofen from forced degradation studies

S.No	*Condition*	*% Purity*	*Purity angle*	*Purity Threshold*	*Purity Flag*
1	*Unstressed Sample*	*99.98*	*0.437*	*0.625*	*No*
2	*Acid stressed Sample*	*99.97*	*0.516*	*0.701*	*No*
3	*Alkali stressed Sample*	*99.97*	*0.384*	*0.606*	*No*
4	*Peroxide stressed Sample*	*99.95*	*0.419*	*0.633*	*No*
5	*Heat stressed Sample*	*97.78*	*0.454*	*0.669*	*No*
6	*Photo light stressed Sample-Controlled condition*	*99.98*	*0.366*	*0.578*	*No*
7	*Photo light treated Sample-Uncontrolled condition*	*99.98*	*0.349*	*0.565*	*No*

6.4. DETERMINATION OF LIMIT OF DETECTION AND LIMIT OF QUANTITATION

Determine the limit of detection (LOD) and limit of quantitation (LOQ) for all specified impurities, Dexibuprofen based on signal to noise ratio method.

Procedure:

Separately injected the blank (diluent), standard solution (6 replicates), blank (diluent) (2 replicates), each LOD & LOQ test solution (from lowest to highest concentration) into the chromatograph and recorded the peak response.

LOD & LOQ calculation

The limit of detection and (LOD) and limit of quantification (LOQ) was determined by signal to noise ratio method by using the formula.

Signal to noise ratio (S/N) =2H/h

H – Height of the analyte peak

h- Height of the noise

LOD and LOQ value was verified by giving six replicate injections of solution containing impurities and Dexibuprofen at this level. The percentage relative standard deviation (%RSD) calculated for the peak areas and tabulated in table 14.

Results and Discussion of LOD & LOQ

The LOD and LOQ of known impurities, Dexibuprofen has been determined by signal to noise.The obtained results were shown in Table-14

Table-14: Results for LOD and LOQ

Name of the compound	***Limit of Detection***		***Limit of Quantification***	
	S/N	***%***	***S/N***	***%***
Impurity J	***6***	***0.004***	***21***	***0.01***
Dexibuprofen	***5***	***0.004***	***17***	***0.01***
Impurity B	***3***	***0.004***	***13***	***0.01***
Impurity E	***4***	***0.002***	***22***	***0.005***
Methyl ester	***8***	***0.004***	***18***	***0.01***

6.5. LINEARITY

The linearity of response of known impurities and Dexibuprofen should be established over a range of concentrations between LOQ level to 150 % of specification limit.

Linearity stock solution1 :

Weighed accurately and transferred about 10.0 mg of Dexibuprofen reference standard/working standard into a 25 ml volumetric flask, dissolved and dilute to the volume with acetonitrile and mixed well. (≈ 400.0 µg/ml). (same procedure for Impurity-J,B,&E, Methyl ester).

LOQ Level :(For Dexibuprofen, Impurity –J,B& Methyl ester)

Pipetted 1 ml from stock solution I to 100 ml volumetric flask, dissolved and diluted to the volume with diluent and mixed well. Transferred 1 ml of this solution to 20 ml volumetric flask and made up with diluent (0.2 µg/ml).

For Impurity E:

Pipetted 1 ml from stock solution I to 100 ml volumetric flask, dissolved and diluted to the volume with diluent and mixed well. Transferred 0.5 ml of this solution to 20 ml volumetric flask and made up with diluent (0.1 µg/ml).

Stock solution II:

Pipetted 5 ml from stock solution I to 50 volumetric flask, dissolved and diluted to the volume with diluent and mixed well.

Stock solution III :

Pipetted 5 ml from stock solution I (Methyl ester) to 100 ml volumetric flask, dissolved and diluted to the volume with diluent and mixed well.

***25 % Level* :** :(For Dexibuprofen, Impurity –J,B& E)

Transferred 0.5 ml of stock solution II to 20 ml volumetric flask and made up with diluent (1.0 µg/ml). **For Methyl ester**: Transferred 1.5 ml of stock solution III to 20 ml volumetric flask and made up with diluent (1.5 µg/ml).

***50 % Level* :** :(For Dexibuprofen, Impurity –J,B& E)

Transferred 1.0 ml of stock solution II to 20 ml volumetric flask and made up with diluent (2.0 µg/ml). **For Methyl ester:** Transferred 3.0 ml of stock solution III to 20 ml volumetric flask and made up with diluent (3.0 µg/ml).

***75%Level* :** :(For Dexibuprofen, Impurity –J,B& E)

Transferred 1.5 ml of stock solution II to 20 ml volumetric flask and made up with diluent(3.0 µg/ml). **For Methyl ester**: Transferred 4.5 ml of stock solution III to 20 ml volumetric flask and made up with diluent (4.5 µg/ml).

***100 % Level* :** (For Dexibuprofen, Impurity –J,B& E)

Transferred 2.0 ml of stock solution II to 20 ml volumetric flask and made up with diluent (4.0 µg/ml). **For Methyl ester**: Transferred 6.0 ml of stock solution III to 20 ml volumetric flask and made up with diluent (6.0 µg/ml).

***125 % Level* :** :(For Dexibuprofen, Impurity –J,B& E)

Transferred 2.5 ml of stock solution II to 20 ml volumetric flask and made up with diluent (5.0 µg/ml). **For Methyl ester**: Transferred 7.5 ml of stock solution III to 20 ml volumetric flask and made up with diluent (7.5 µg/ml).

***150 % Level* :** :(For Dexibuprofen, Impurity –J,B& E)

Transferred 3.0 ml of stock solution II to 20 ml volumetric flask and made up with diluent (6.0 µg/ml). **For Methyl ester**: Transferred 9.0 ml of stock solution III to 20 ml volumetric flask and made up with diluent (9.0 µg/ml).

Linearity test solutions

Prepared individual linearity solutions from linearity stock solution according to the table below.

Procedure

Separately injected the blank (diluent), standard solution (6 replicates), blank (diluent) (2 replicates) and each linearity solution (from lowest to highest concentration) into the chromatograph and recorded the peak response.

Linearity calculation

Plot the respective peak area obtained in each linearity solution against the concentration (in X-axis). Using suitable software, performed a linear regression analysis to generated a best-fit line. Determined the correlation coefficient (r) of the best-fit line.

Calculation for RRF value for Dexibuprofen impurities

$$RRF = \frac{\text{Slope of Impurity-J}}{\text{Slope of Dexibuprofen}} \times \frac{\text{\% Purity ofDexibuprofen}}{\text{\% Purity of impurity-J}}$$

Acceptance criteria

- The correlation coefficient (r) value should be not less than 0.990
- Report the slope, y-intercept and RRF value.

Results and Discussion

The method was found to be linear in the range of LOQ level to 150 % of specification limit of known impurities,Dexibuprofen indicated by a correlation coefficient that is ≥ 0.990. The obtained results were shown in Table-15 to 19, figure-1 to 5.

Table-15: Linearity data of impurity -J

S.No.	***% w.r.to specification limit***	***Concentration (µg/ml)***	***Peak Area***

1	***LOQ***	***0.1981***	***4282***
2	***25%***	***0.9905***	***23551***
3	***50%***	***1.9810***	***45954***
4	***75%***	***2.9714***	***63758***
5	***100%***	***3.9619***	***91132***
6	***125%***	***4.9524***	***110112***
7	***150%***	***5.9429***	***129388***

Figure-1: Linearity graph of **Impurity –J:**

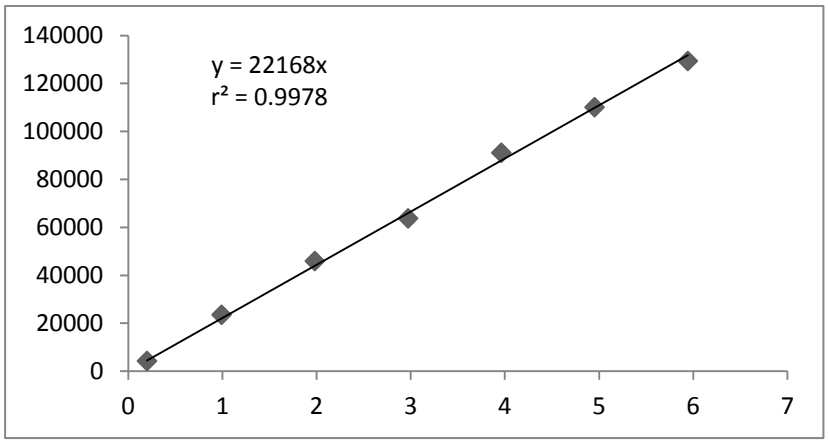


Table-16: Linearity data of Dexibuprofen

S.No.	***%w.r.to specification limit***	***Concentration (µg/ml)***	***Peak Area***
1	***LOQ***	***0.2029***	***6247***

2	***25%***	***0.8542***	***24568***
3	***50%***	***1.7084***	***43196***
4	***75%***	***2.5625***	***64711***
5	***100%***	***3.4167***	***86213***
6	***125%***	***4.2709***	***104676***
7	***150%***	***5.1251***	***123867***

Figure 2: Linearity graph of Dexibuprofen

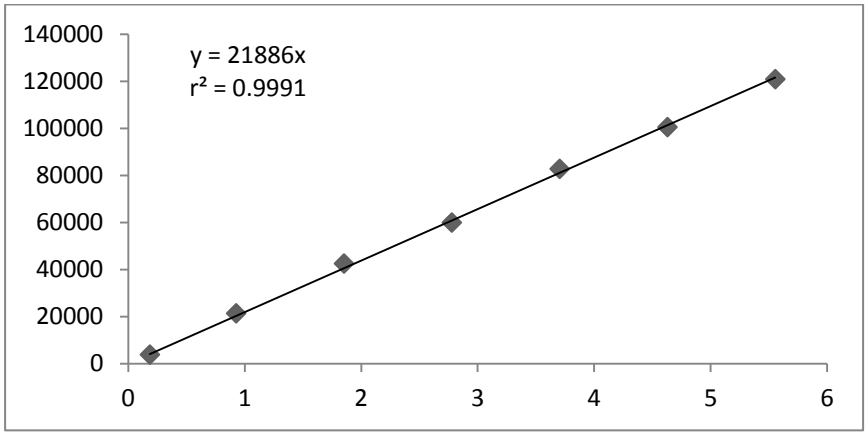


Table 17: Linearity data of impurity-B 2(4-butylphenyl)propionic acid

S.No.	***%w.r.to specification limit***	***Concentration (µg/ml)***	***Peak Area***
1	***LOQ***	***0.1852***	***3790***

2	***25%***	***0.9261***	***21333***
3	***50%***	***1.8521***	***42494***
4	***75%***	***2.7782***	***59920***
5	***100%***	***3.7043***	***82721***
6	***125%***	***4.6303***	***100480***
7	***150%***	***5.5564***	***120845***

Figure-3 : Linearity graph of Impurity-B

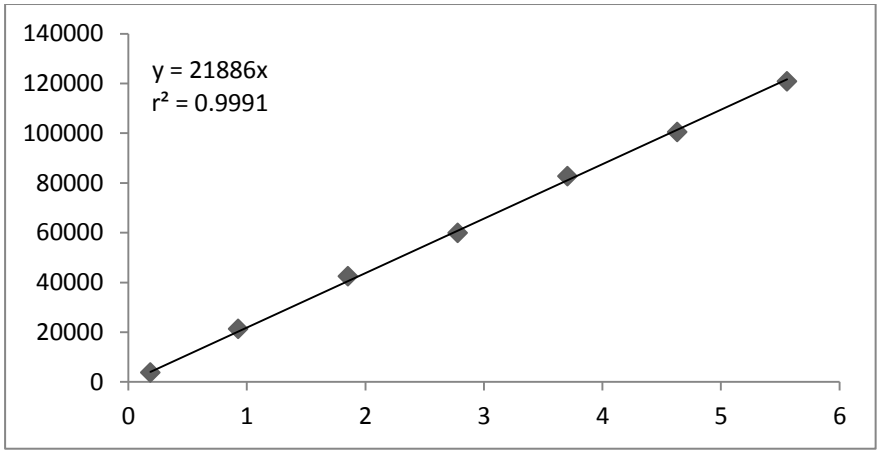


Table- 18: Linearity data of Impurity E [4-Isobutylacetophenone]

S.No.	***%w.r.to specification limit***	***Concentration (µg/ml)***	***Peak Area***
1	***LOQ***	***0.0947***	***4175***
2	***25%***	***0.9467***	***32793***

3	***50%***	***1.8934***	***65157***
4	***75%***	***2.8401***	***92261***
5	***100%***	***3.7868***	***128575***
6	***125%***	***4.7335***	***157597***
7	***150%***	***5.6802***	***18590***

Figure 4: Linearity graph of **Impurity –E**

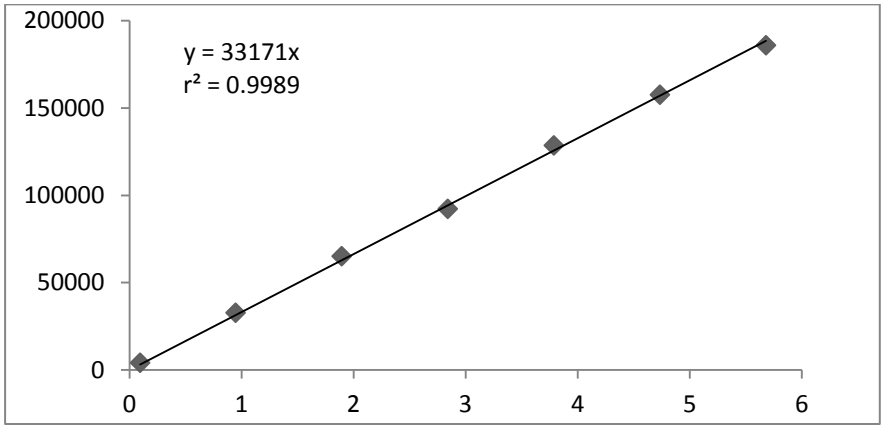


Table-19: Linearity data of Ibuprofen methyl ester

S.No.	***%w.r.to specification limit***	***Concentration (µg/ml)***	***Peak Area***
1	***LOQ***	***0.1879***	***3006***
2	***25***	***1.5660***	***31301***

3	***50***	***3.1320***	***62864***
4	***75***	***4.6980***	***99877***
5	***100***	***6.2640***	***126612***
6	***125***	***7.8300***	***163729***
7	***150***	***9.3960***	***201648***

Figure-5: Linearity graph of Methyl ester

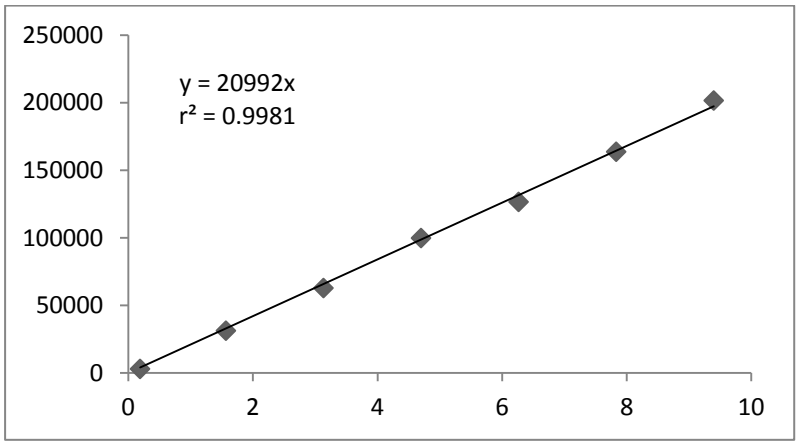


Table -20 – Slope, y-intercept & Co-efficient of correlation

Compound Name	***Slope***	***Y-Intercept***	***Coefficient of correlation (r)***
Impurity J	***21863***	***1298***	***1.000***
Dexibuprofen	***23842***	***2995***	***1.000***

Impurity B	***21654***	***922***	***1.000***
Impurity E	***32724***	***1827***	***1.000***
Ibuprofen methyl ester	***21364***	***-2506***	***1.000***

Table-21: Summary of RRF values

Name of the compound	***RRF***
Impurity J	***0.92***
Dexibuprofen	***-***
Impurity B	***0.91***
Impurity E	***1.37***
Ibuprofen methyl ester	***0.90***

6.6. ACCURACY

Preparation of Sample solution (unspiked)

Weighed and crushed 20 tablets by using mortar and pestle. Accurately weighed and transferred about 420.0 mg of powdered sample (equivalent to 200 mg of Dexibuprofen) into a 100 ml volumetric flask. Added 50 ml of diluent, and sonicated for 10 minutes, cooled to room temperature and diluted to the volume with diluent. Mixed well and filtered through 0.45 µm nylon filter. (≈ 2000 µg/ml of Dexibuprofen).

Preparation of spiked solution :

Stock Solution:

Weighed accurately and transferred about 10.0 mg of impurity into a 25 ml volumetric flask, dissolved and diluted to the volume with diluent and mixed well. (same procedure for all impurities and dexibuprofen).

LOQ LEVEL :

For impurity J,B,Methyl ester, Dexibuprofen :

Transferred 1 ml of stock solution to 100 ml volumetric flask and made up with diluent. Pipetted 5.0 ml of this solution to 100 ml volumetric flask and made up with diluent. (0.2 µg/ml).

For Impurity E :

Transferred 1 ml of stock solution to 100 ml volumetric flask and made up with diluent. Pipetted 2.5 ml of this solution to 100 ml volumetric flask and made up with diluent.(0.1 µg/ml).

100 % LEVEL :

For impurity J, B, E, Dexibuprofen:

Transferred 10 ml of stock solution to 50 ml volumetric flask and made up with diluent. Pipetted 5.0 ml of this solution to 100 ml volumetric flask and made up with diluent (4.0 µg/ml).

For Methyl ester :

Transferred 15 ml of stock solution to 50 ml volumetric flask and made up with diluent. Pipetted 5.0 ml of this solution to 100 ml volumetric flask and made up with diluent (6.0 µg/ml).

150 % LEVEL :

For impurity J,B,E, Dexibuprofen:

Transferred 10 ml of stock solution to 20 ml volumetric flask and made up with diluent. Pipetted 3.0 ml of this solution to 100 ml volumetric flask and made up with diluent(6.0 µg/mL).

For Methyl ester :

Transferred 15 ml of stock solution to 20 ml volumetric flask and made up with diluent. Pipetted 3.0 ml of this solution to 100 ml volumetric flask and made up with diluent (9.0 µg/ml).

Procedure

6.6.1. Accuracy for known impurities

Preparation of accuracy sample solutions (spiked)

Weighed accurately and transferred about 420.0 mg of powdered sample (equivalent to 200mg of Dexibuprofen) into a 100 ml volumetric flask. Added 50 ml of diluent and spiked the specified volume of each impurity stock solution to the same flask, sonicated for 10 minutes, cooled to room temperature and diluted to volume with diluent. Mixed well and filtered the solution through 0.45 µm nylon membrane filter and discarded first 5 ml of the filtrate.

Note: Prepared three accuracy samples for each level.

Procedure

Separately injected the blank (diluent), accuracy standard solution (6 replicates), sample solution (unspiked), blank (diluent), each accuracy solution for known impurities (from lowest to highest concentration), blank (diluent), each accuracy solution for unknown impurities (from lowest to highest concentration), blank (diluent) and standard solution (bracketing) into the chromatograph and record the peak response.

$$\textit{Percentage recovery} = \frac{\text{Amount recovered in µg}}{\text{Amount added in µg}} \times 100$$

Acceptance criteria

- ❖ The percentage recovery of known impurity, Dexibuprofen at each level concentration should be between 90.0 and 110.0.
- ❖ The % RSD of percentage recovery should be not more than 10.0 at each level.

Results and Discussion

The method has been determined to be accurate in the range of LOQ level to about 150 % level of specification limit, as demonstrated by the recovery of known impurities, Dexibuprofen at each level concentration should be between 90.0 and 110.0.Percentage RSD that is not more than 10.0 for triplicate results at each level. The obtained results were shown in Table-22.

Table-22: Accuracy data of Impurity-J, Dexibuprofen & Impurity-B, Impurity-E, Methyl ester

Level	***Imp-J***		***Dexibuprofen***		***Imp-B***		***Imp-E***		***Methyl ester***	
	Recovery	***RSD***	***Recovery***	***RSD***	***Recovery***	***RSD***	***Recovery***	***RSD***	***Recovery***	***RSD***
LOQ	***101.9***	***2.2***	***99.9***	***0.6***	***95.1***	***2.4***	***103.0***	***1.1***	***97.9***	***3.6***
100	***100.3***	***0.2***	***103.7***	***2.1***	***97.0***	***0.5***	***101.0***	***1.0***	***101.2***	***0.2***
150	***100.4***	***0.5***	***101.7***	***0.4***	***97.9***	***0.1***	***101.8***	***0.1***	***101.4***	***0.2***

6.7. RANGE

Derive the method range from linearity study. The linear range of analytical method was demonstrated with suitable level of accuracy and precision in the respective parameters.

Results and Discussion:

The method range has been derived from linearity study that is LOQ level to 150% level of specification concentration and demonstrated with suitable level of precision and accuracy .

6.8. STANDARD AND SAMPLE SOLUTION STABILITY

The standard and sample solution stability can be evaluated by injecting the standard solution and impurities spiked sample solution obtained from method precision at appropriate time intervals up to 48 hours of the respective solutions stored in sample compartment of HPLC instrument at 25°C (ambient) conditions.

Procedure

Separately injected the blank. (diluent) and each solution stability standard and sample solutions into the chromatograph and recorded the peak response.

Calculation

For known, unknown and total impurities refer method precision parameter.

Acceptance criteria

- The cumulative percentage RSD of peak area response due to Dexibuprofen from standard solution should be not more than 10.0.
- The cumulative percentage RSD of each individual known impurity peak area in impurities spiked sample solution should be not more than 10.0.
- The cumulative percentage RSD of total impurities from impurities spiked sample solution should be not more than 10.0.

Results and Discussion

The standard solution and impurities spiked sample solutions were monitored for stability up to 48 hours. The standard solution stable for 48 hours in ambient .As demonstrated by cumulative percentage RSD that was not more than 10.0 for peak area due to Dexibuprofen in standard and percentage of known,total impurities in spiked sample. The obtained results were shown in Table- 23&24.

Table-23: Solution stability data of standard solution

Ambient condition :

Time in hours	***Initial***	***5***	***8***	***12***	***16***	***24***	***32***	***40***	***48***
Cumulative % RSD	***-***	***0.9***	***1.2***	***1.0***	***0.9***	***0.9***	***0.9***	***0.9***	***0.9***

Table-24: Solution stability data of impurities spiked Sample solution

Time in hours	***Cumulative % RSD***				
	Impurity-J	***Impurity-B***	***Impurity-E***	***Methyl ester***	***Total***
Initial	***-***	***-***	***-***	***-***	***-***
5	***0.8***	***0.9***	***0.9***	***0.2***	***0.2***
8	***0.8***	***0.9***	***0.6***	***0.3***	***0.3***

12	***0.7***	***0.9***	***0.5***	***0.2***	***0.3***
16	***0.6***	***1.0***	***0.6***	***0.5***	***0.4***
24	***0.8***	***0.9***	***0.6***	***0.6***	***0.4***
32	***0.8***	***1.1***	***0.9***	***0.8***	***0.5***
40	***1.0***	***1.1***	***1.2***	***1.1***	***0.7***
48	***1.2***	***1.0***	***1.5***	***1.5***	***1.0***

6.9. FILTER VALIDATION

Test Preparation:

Used the impurities spiked sample solution prepared in the method precision study; filtered a portion of the sample solution through 0.45 µm nylon filter, discarded first 5 ml of the filtrate and also centrifuged a portion of sample solution and collected the supernatant solution (unfiltered).

Procedure:

Separately injected the blank (diluent), each filtered and unfiltered sample solution, blank (diluent) and standard solution (bracketing) into the chromatograph and recorded the peak response.

Calculation:

For known, unknown and total impurities refer method precision parameter. Calculated the percentage difference of individual known impurity and total impurities between the filtered and unfiltered (centrifuged) impurities spiked sample solution.

$$\% \text{ Difference} = \frac{\% \text{ result of unfiltered} - \% \text{ result of filtered}}{\% \text{ result of unfiltered}}$$

Acceptance criteria:

The percentage difference between the unfiltered (centrifuged) and filtered sample for individual known impurity and total impurities should be not more than 10.0.

Results and Discussion

The use of 0.45 µm nylon filters has been validated as indicated by percentage difference between the filtered and unfiltered samples that were not more than 10.0 for known and total impurities. The obtained results were shown in Table-25.

Table-25: Filter validation data

Name of Impurity	***Centrifuged***	***Nylon filtered***	
	% w/w	***%w/w***	***% Difference***
Impurity-J	***0.184***	***0.186***	***1.1***
Impurity-B	***0.223***	***0.225***	***0.9***
Impurity-C	***0.243***	***0.244***	***0.4***
Methyl ester	***0.353***	***0.354***	***0.3***
Total impurities	***1.030***	***1.035***	***0.5***

6.10. ROBUSTNESS

The robustness of the method shall be established by making deliberate minor variations in the following method parameters like,

- Change in flow rate by ± 10 % of actual flow rate
- Change in organic content in mobile phase ± 2% absolute.
- Change in wavelength by ± 2 nm.

Method parameter	*Lowest altered condition*	*Actual condition*	*Highest altered condition*
Flow rate (mL/min)	*1.8*	*2.0*	*2.2*
Organic content(%)	*33*	*35*	*37*
Wavelength (nm)	*212*	*214*	*216*

Procedure

Separately injected the blank (diluent), standard solution (6 replicates) into the chromatograph and recorded the peak response.

Note: Follow the same procedure for all altered method parameters and evaluated the acceptance criteria. If the acceptance criteria failed, narrow the method parameter and reported on range at which acceptance criteria passes.

***Acceptance criteria*:** It should meet the system suitability parameters.

Results and Discussion

The robustness of the method was demonstrated by making deliberate changes in flow rate, organic content and wavelength as indicated by monitoring the tailing factor for Dexibuprofenis not more than 2.0 in standard solution. Percentage RSD of Dexibuprofen area

was not more than 10.0 from six replicate injections of standard solution. The obtained results were shown in Table-26-28.

Table-26: Robustness data for flow rate variation

Flow rate (mL/min)	***Minus flow (1.8)***	***Plus flow (2.2)***
Theoretical plate count for peak due to Dexibuprofen	***88544***	***43603***
%RSD for peak due to Dexibuprofen	***0.8***	***0.4***
Resolution	***2.5***	***2.4***

Table- 27: Robustness study data for solvent variation

Organic content	***Minus solvent (35)***	***Plus solvent (37)***
Theoretical plate count for peak due to Dexibuprofen	***147541***	***9013***
%RSD for peak due to Dexibuprofen	***1.1***	***1.6***
Resolution	***2.4***	***2.9***

Table-28: Robustness study data for wavelength variation

Wavelength (nm)	***Minus wavelength (212 nm)***	***Plus wavelength (216 nm)***
Theoretical plate count for peak due to Dexibuprofen	***65200***	***64054***
%RSD for peak due to Dexibuprofen	***0.7***	***0.5***
Resolution	***2.6***	***2.6***

7. SUMMARY AND CONCLUSION

SUMMARY:

In this project, a high versatile HPLC was used for estimation of related substances of dexibuprofen in tablets. The separation was achieved by using Waters symmetry column with acetonitrile and ortho phosphoric acid as mobile phase. The retention time was 30.6 min for dexibuprofen at 214 nm. The system suitability parameters like Relative standard deviation, Resolution, Theoretical plate count were determined and found to be in acceptance limit.

The overall results obtained were summarized in table – 29

S.No.	*Parameters*	*Resolution*	*% RSD*	*Plate count*
1	*System precision*	*2.7*	*0.5*	*64460*
2	*Method precision*	*2.8*	*0.2*	*62243*
3	*LOD and LOQ*	*2.6*	*0.4*	*68144*
4	*Specificity*	*2.5*	*2.0*	*56686*
5	*Linearity*	*2.5*	*0.9*	*62484*
6	*Accuracy*	*2.6*	*1.5*	*62131*
7	*Forced degradation*	*2.6*	*1.4*	*81612*

CONCLUSION :

A stability study was carried out with an efficient HPLC method for the quantification of related substances of Dexibuprofen in tablets was developed and validated. The present study was validated as per the ICH guidelines. Validation experiments proved that the HPLC analytical method is linear in the range of 40 µg/ml as well as accurate, precise (repeatability and intermediate precision levels) and specific, being able to separate the main drug from its degradation products. The proposed method was also found to be robust with respect to flow rate, column oven temperature and composition of mobile

phase. Due to these characteristics, the method has stability indicating properties being fit for its intended purpose, it may find application for the routine analysis of the related substances of Dexibuprofen in tablets.

8. CHROMATOGRAMS

8.1. METHOD DEVELOPMENT

***Injection Volume*: 20.00 µl,** ***Run Time*: 75.0 Minutes**

***Wavelength*: 214 nm,** ***Flow-Rate* :2.0 mL/min**

Figure- 6 ***Blank [Diluents]***

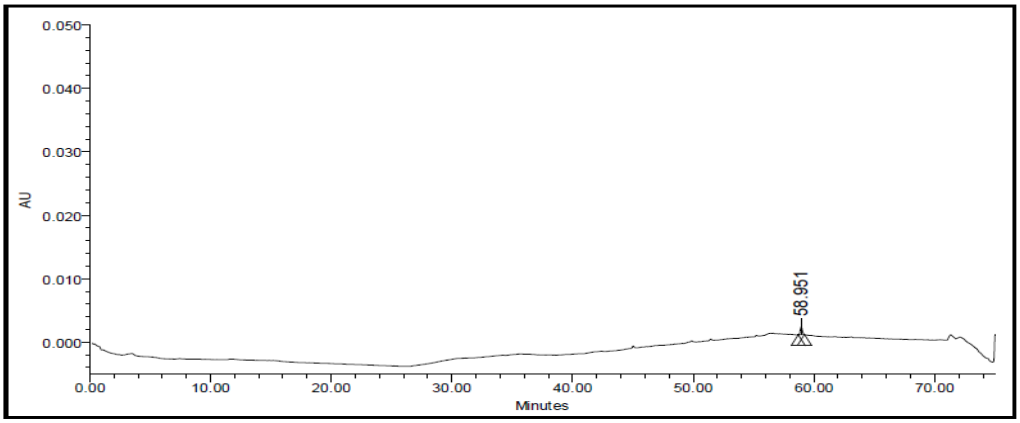


Figure- 7 ***Placebo Solution***

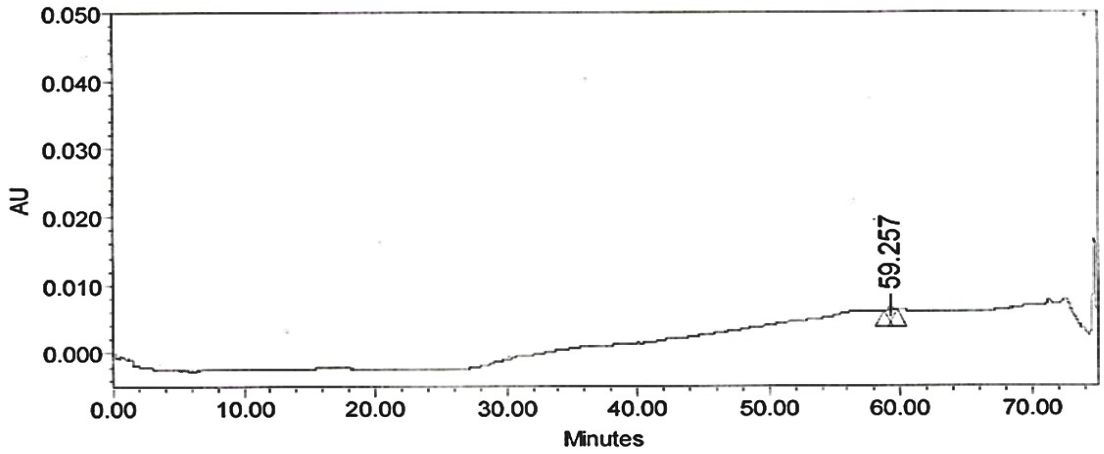
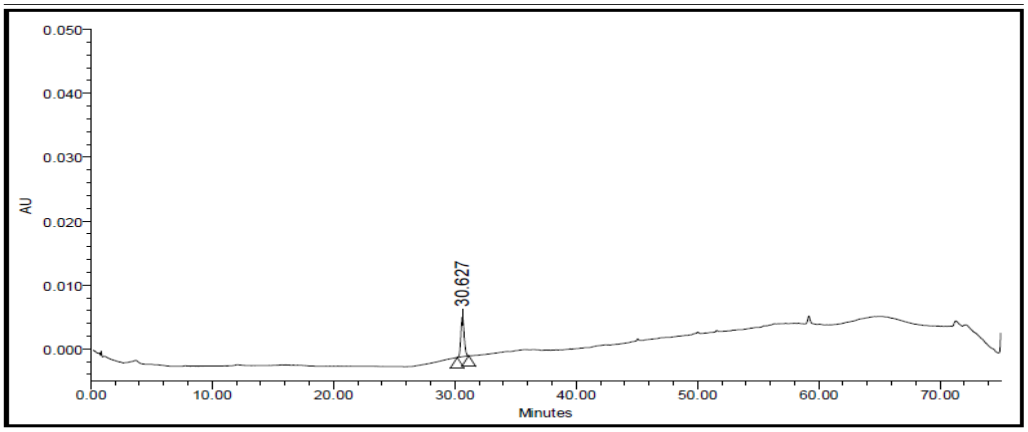


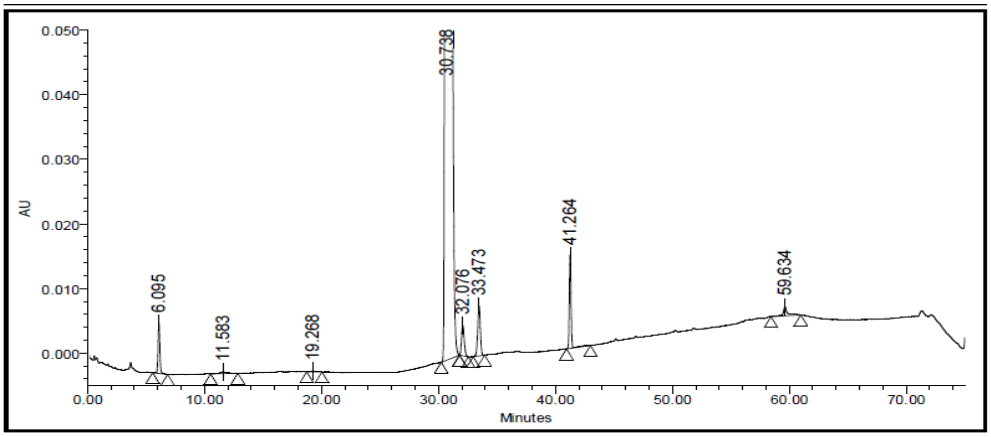
Figure – 8 Standard Solution



Standard solution data

Peak Results			
Name	***RT (min)***	***Area (µV*sec)***	***% Area***
Dexibuprofen	***30.627***	***116358***	***100.00***

Figure – 9 Sample solution

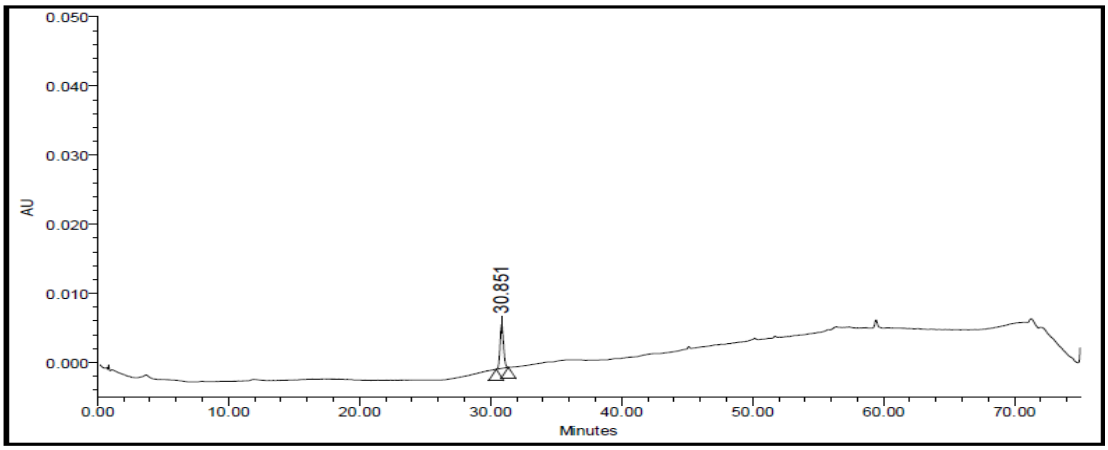


Sample Solution Data

Compound Name	*RT (min)*	*Area (µV*sec)*	*% Area*
Impurity –J	*6.095*	*84160*	*0.19*
Dexibuprofen	*30.738*	*44393567*	*98.91*
Impurity –B	*32.078*	*74644*	*0.17*
Impurity –E	*33.473*	*120949*	*0.27*
Methyl ester	*41.264*	*154374*	*0.34*

8.2. SYSTEM SUITABILITY

Figure – 10 ***System Suitability Solution***



Name	*Retention time*	*Plate count*
Dexibuprofen	*30.851*	*64460*

8.3.SPECIFICITY

8.3.1.IDENTIFICATION OF IMPURITIES

Figure – 11 ***Impurity-J***

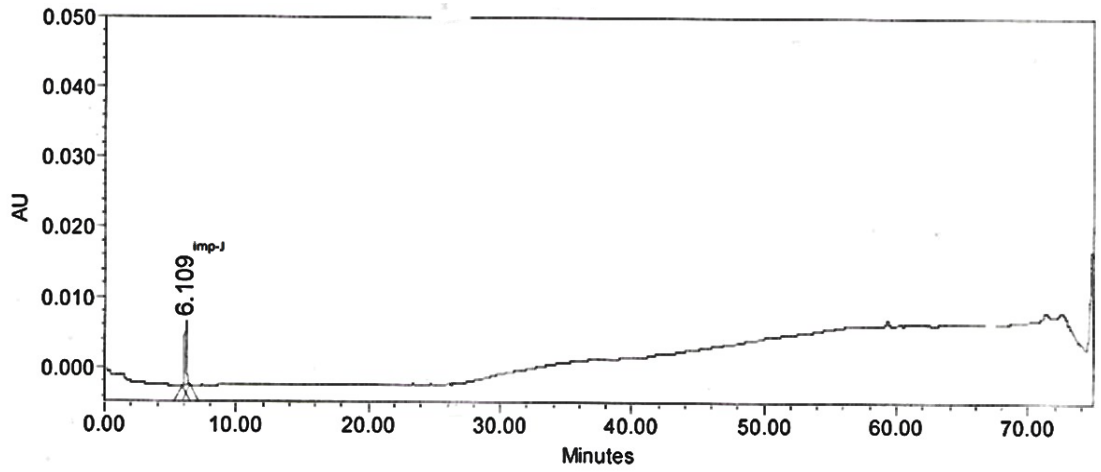


Figure – 12 **Impurity-B**

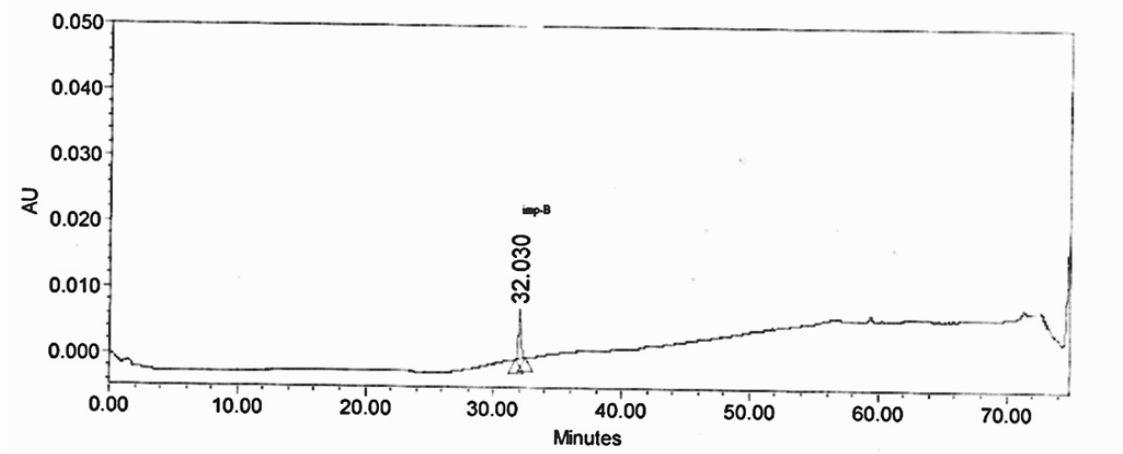


Figure – 13 ***Methyl ester***

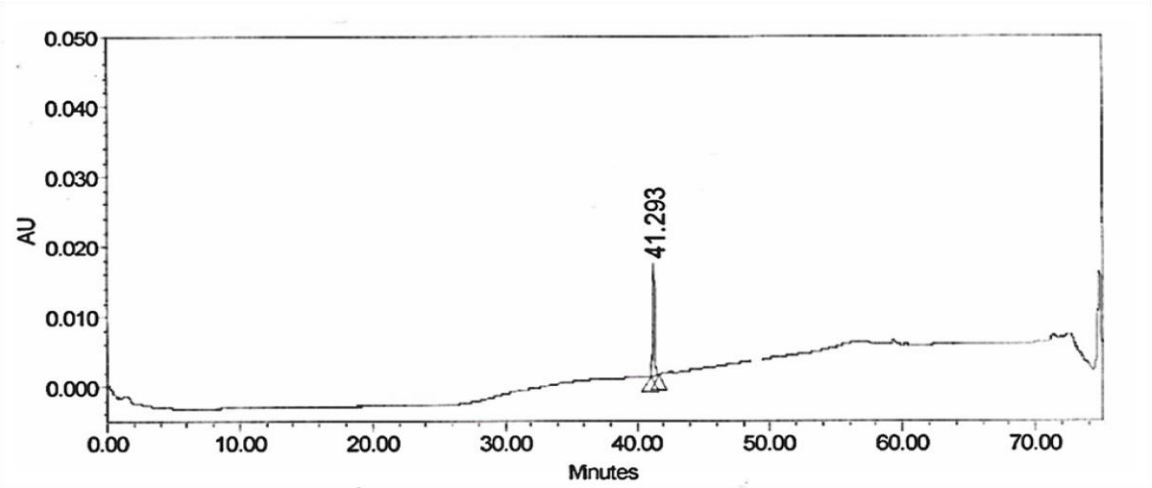
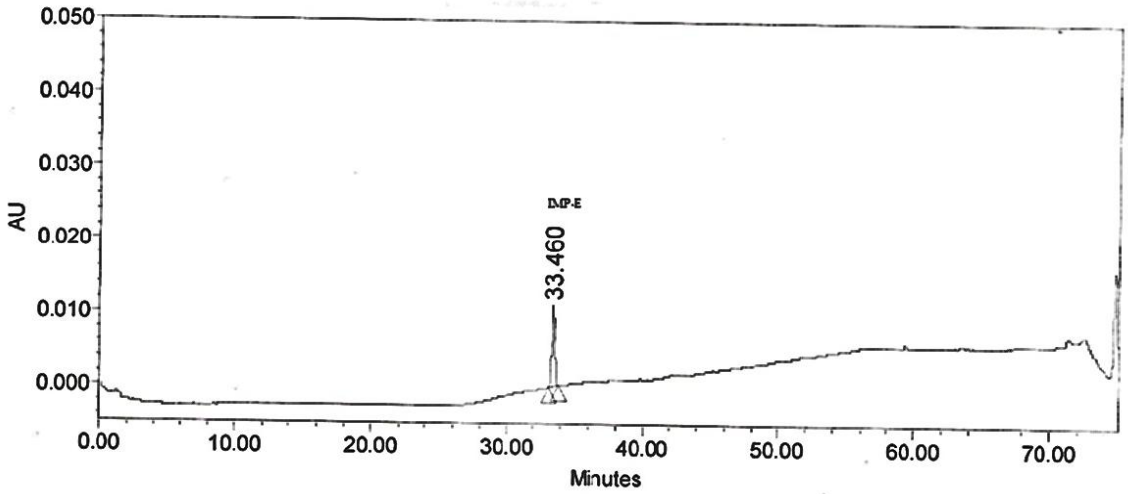


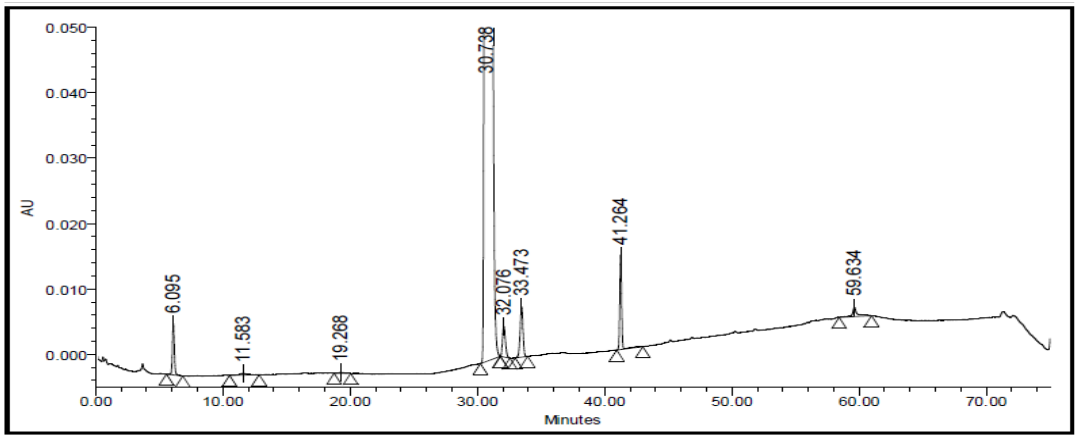
Figure – 14 ***Impurity-E***



Identification of Impurities Data

Sample Name	RT (min)	Area (µV*sec)	% Area
Impurity-J	*6.109*	*78703*	*100.00*
Impurity-B	*32.030*	*98251*	*100.00*
Methyl ester	*41.293*	*151585*	*100.00*
Impurity-E	*33.640*	*153192*	*100.00*

8.3.2. IMPURITY SPIKED SAMPLE - *Figure-15*



Impurity Spiked Sample Data

Compound Name	RT (min)	Area (µV*sec)	% Area
Impurity-J	*11.583*	*84160*	*0.19*
Dexibuprofen	*30.738*	*44393567*	*98.91*
Impurity-B	*32.076*	*74644*	*0.17*
Impurity-E	*33.473*	*120949*	*0.27*
Methyl ester	*41.264*	*154374*	*0.34*

8.3.3. FORCED DEGRADATION STUDY

Figure – 16 Acid Stressed Sample

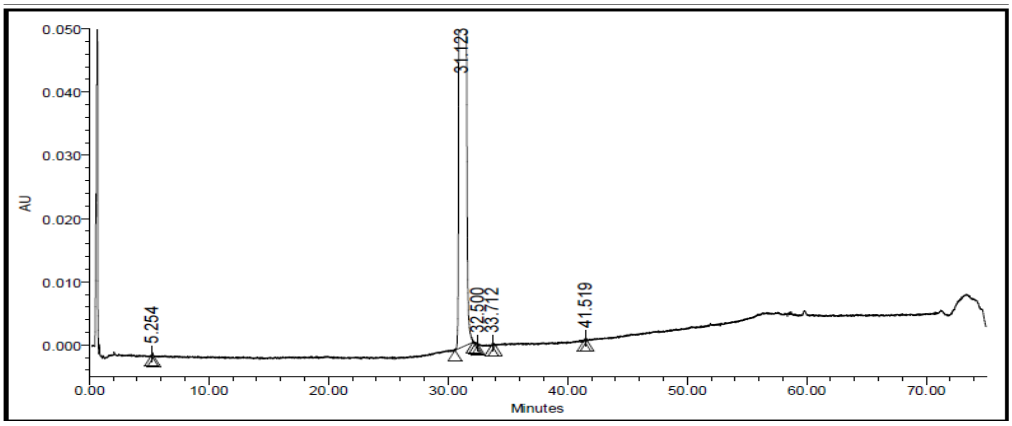


Figure – 17 Alkali Stressed Sample

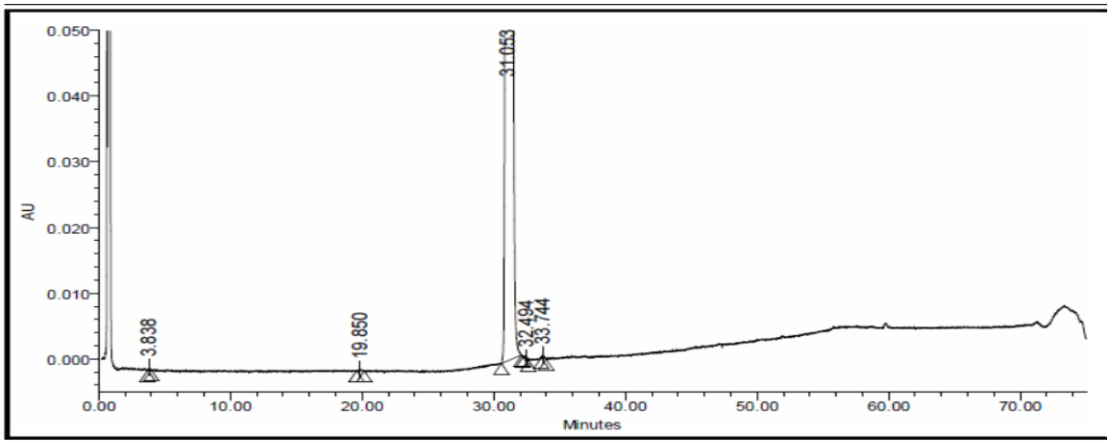
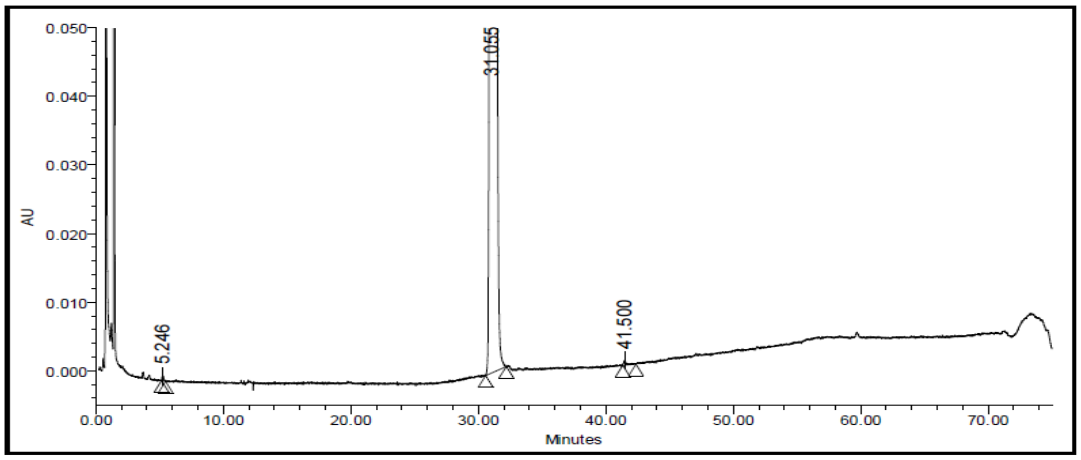


Figure – 18 Peroxide Stressed sample



Forced Degradation Study Data–Dexibuprofen

Sample Solution	*RT (min)*	*Area (µV*sec)*	*% Area*
Acid stressed	*31.123*	*35941186*	*99.97*
Alkali stressed	*31.053*	*41997216*	*99.97*
Peroxide stressed	*31.053*	*41632300*	*99.95*
Mean		*39856900*	*99.96*
%RSD		*13.9*	

8. 4. LIMIT OF DETECTION AND LIMIT OF QUANTITATION [LOD & LOQ]

Figure – 19 ***LOD Solution-1***

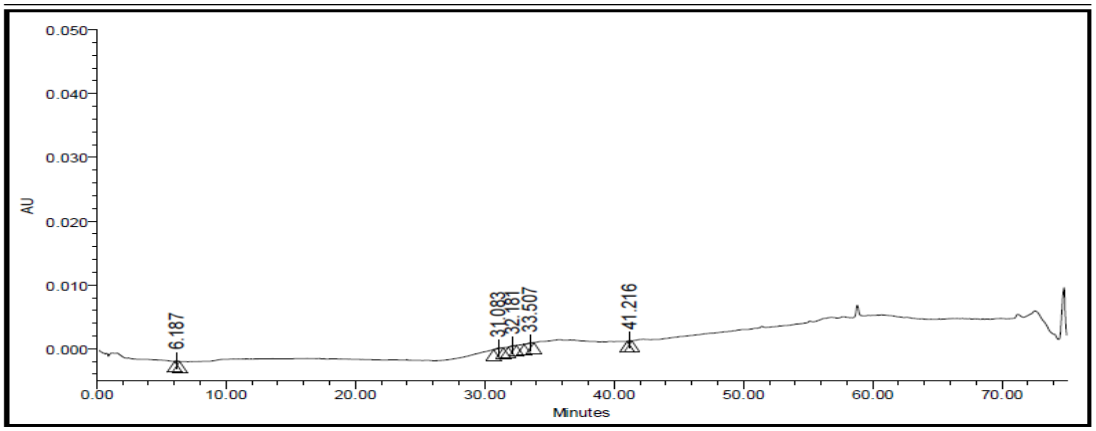


Figure – 20 ***LOD Solution-2***

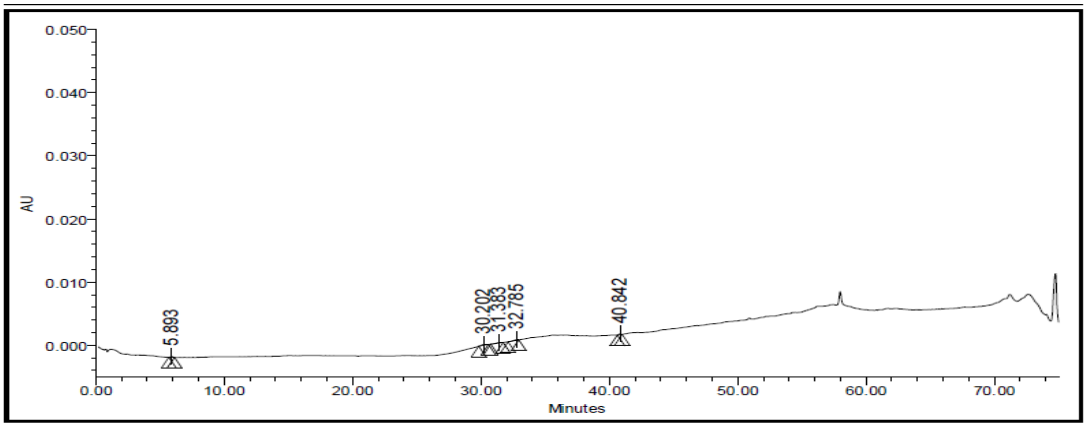


Figure – 21 ***LOD Solution-3***

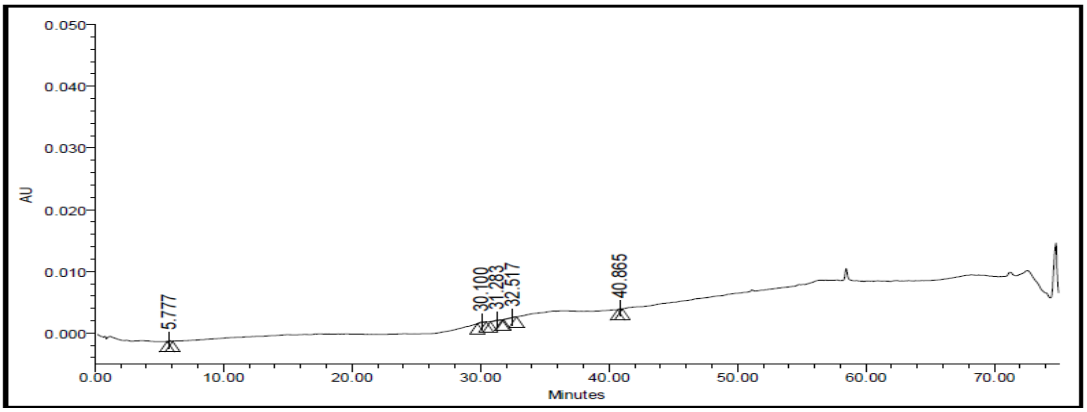


Figure – 22 ***LOD Solution-4***

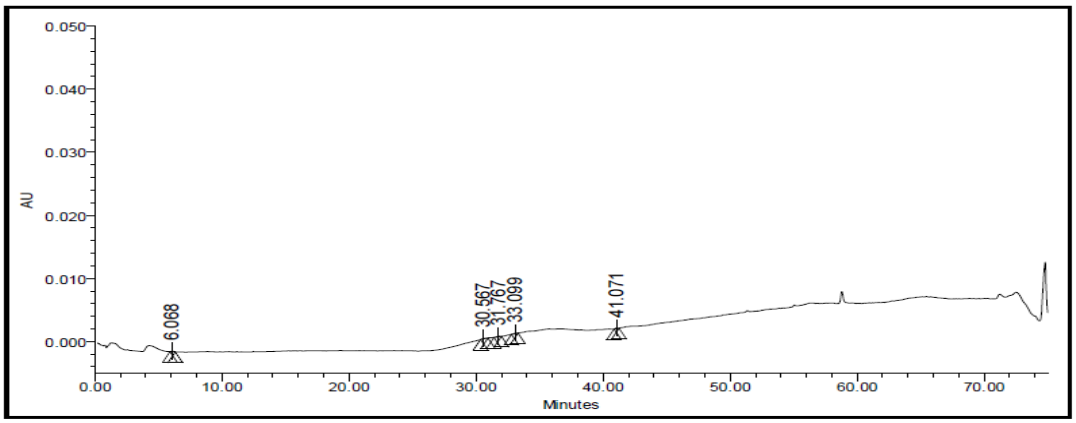


Figure – 23 ***LOD Solution-5***

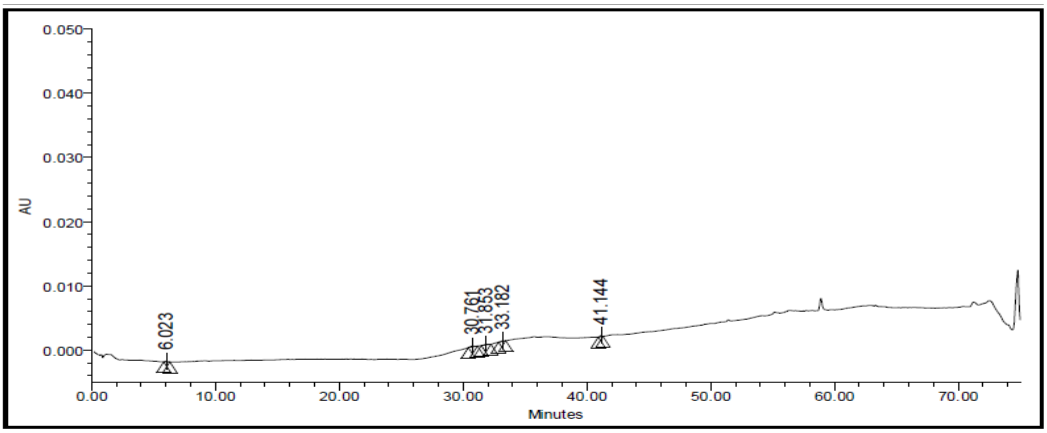


Figure – 24 ***LOD Solution-6***

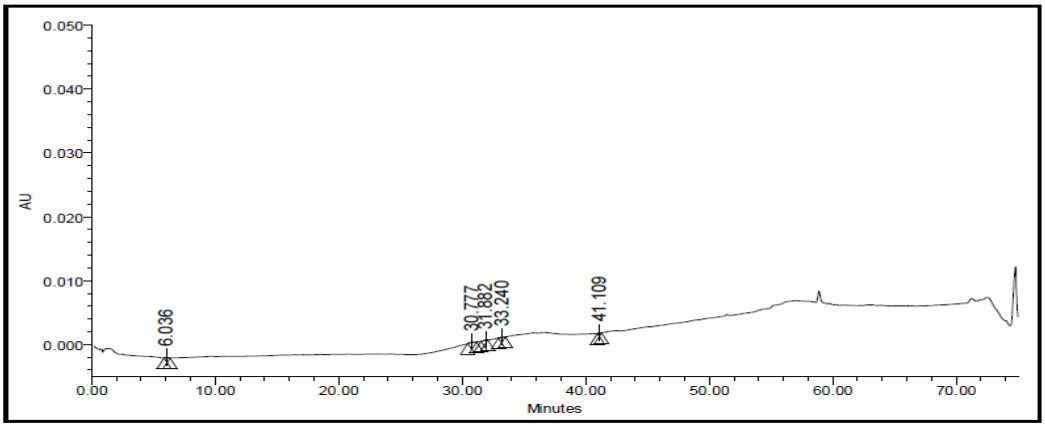


Figure – 25 ***LOQ Solution-1***

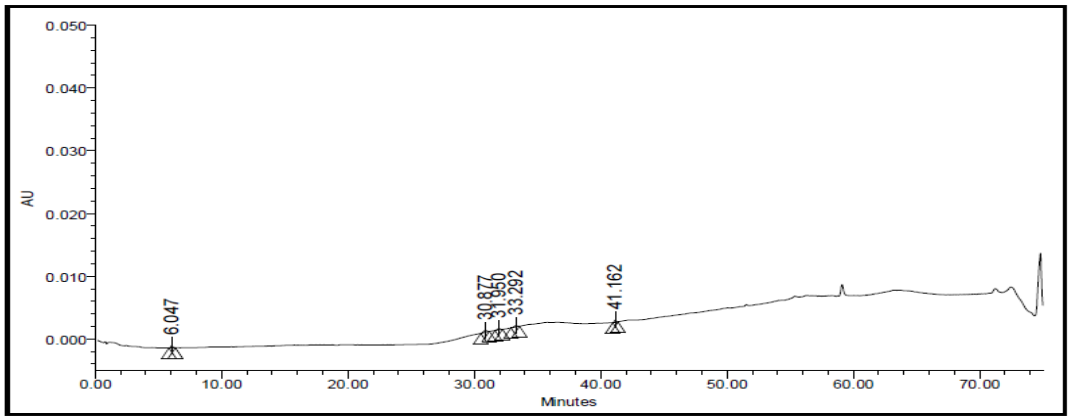


Figure – 26 ***LOQ Solution-2***

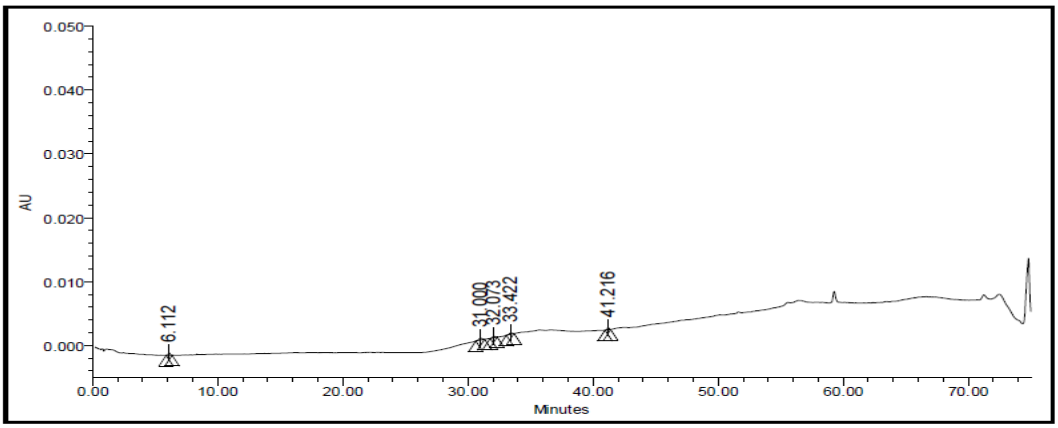


Figure – 27 ***LOQ Solution- 3***

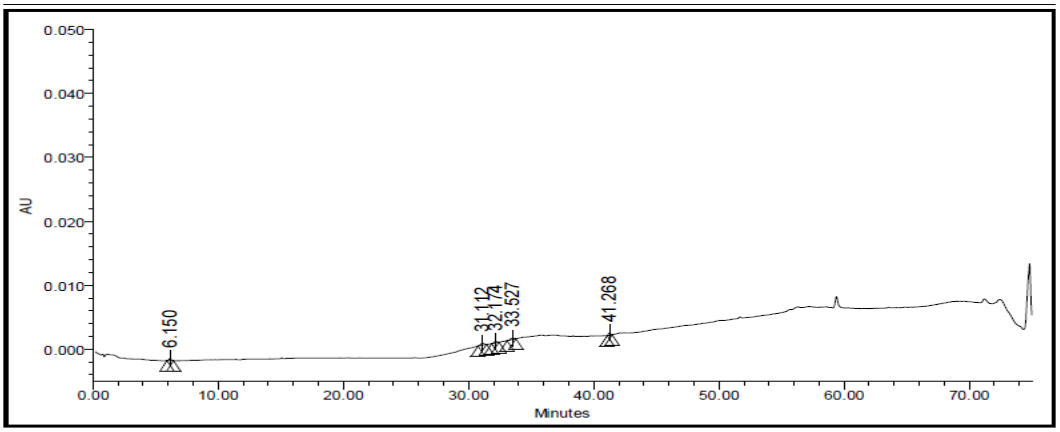


Figure – 28 ***LOQ Solution- 4***

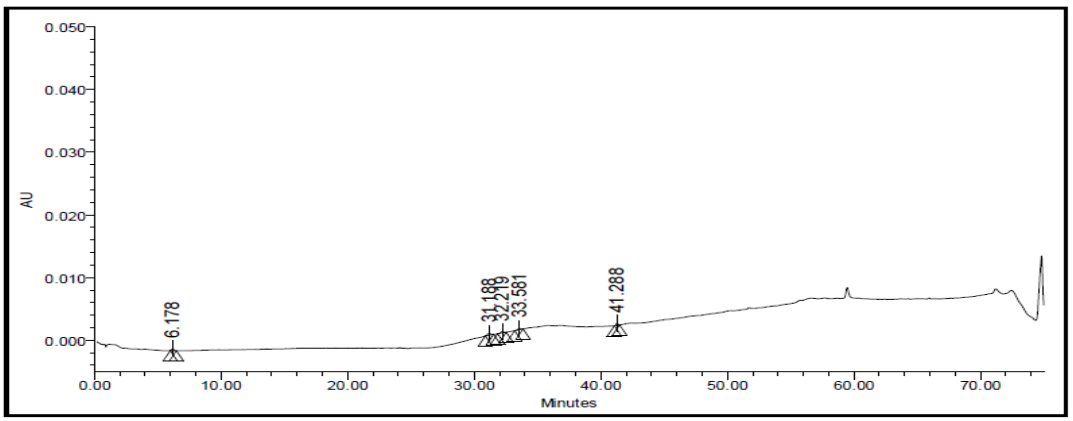


Figure – 29 ***LOQ Solution- 5***

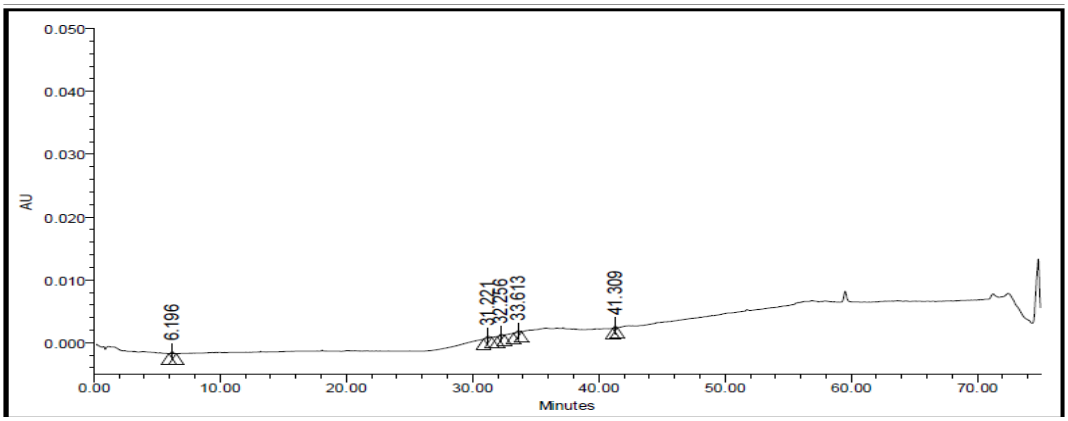
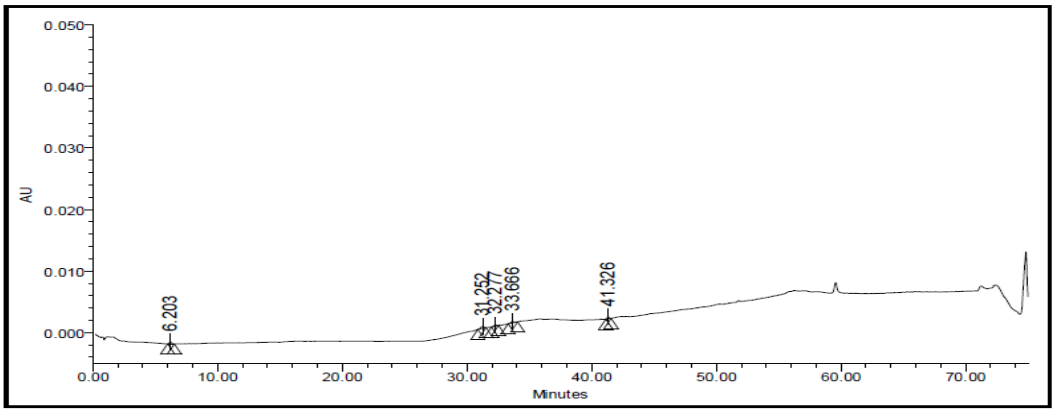


Figure – 30 ***LOQ Solution- 6***



LOD and LOQ Data for Impurity – J

Test	*LOD*			*LOQ*		
Solution	*RT min)*	*Area (µV*sec)*	*% Area*	*RT (min)*	*Area (µV*sec)*	*% Area*
1	*6.187*	*1520*	*16.22*	*6.047*	*3922*	*18.62*
2	*5.893*	*1616*	*15.67*	*6.112*	*3881*	*18.92*
3	*5.777*	*1548*	*14.48*	*6.150*	*4030*	*18.31*
4	*6.068*	*1689*	*20.06*	*6.178*	*3857*	*18.86*
5	*6.023*	*1661*	*16.86*	*6.196*	*4161*	*19.72*
6	*6.036*	*1555*	*18.75*	*6.203*	*4032*	*19.40*

LOD and LOQ Data for Dexibuprofen

Test Solution	*LOD*			*LOQ*		
	RT min)	*Area (µV*sec)*	*% Area*	*RT (min)*	*Area (µV*sec)*	*% Area*
1	*31.083*	*2952*	*31.49*	*30.877*	*5827*	*27.67*
2	*30.202*	*3365*	*32.62*	*31.000*	*6134*	*29.90*
3	*30.100*	*3561*	*33.31*	*31.112*	*5880*	*26.71*
4	*30.567*	*2399*	*28.50*	*31.188*	*5539*	*27.08*
5	*30.761*	*3291*	*33.41*	*31.221*	*5856*	*27.75*
6	*33.777*	*2797*	*33.73*	*31.252*	*6059*	*29.16*

LOD and LOQ Data for Impurity-B

Test Solution	*LOD*			*LOQ*		
	RT min)	*Area (µV*sec)*	*% Area*	*RT (min)*	*Area (µV*sec)*	*% Area*
1	*32.181*	*2052*	*21.90*	*31.950*	*3795*	*18.02*
2	*31.383*	*2113*	*20.48*	*32.073*	*3517*	*17.14*
3	*31.283*	*2616*	*24.46*	*32.174*	*3780*	*17.17*
4	*31.767*	*1714*	*20.37*	*32.219*	*3792*	*18.54*
5	*31.853*	*2100*	*21.32*	*32.256*	*3805*	*18.03*
6	*31.882*	*1445*	*17.42*	*32.277*	*3393*	*16.33*

LOD and LOQ Data for Impurity-E

Test Solution	*LOD*			*LOQ*		
	RT min)	*Area (µV*sec)*	*% Area*	*RT (min)*	*Area (µV*sec)*	*% Area*
1	*33.507*	*1555*	*16.59*	*33.292*	*3691*	*17.53*
2	*32.785*	*1782*	*17.28*	*33.422*	*3413*	*16.64*
3	*32.517*	*1419*	*13.27*	*33.527*	*4148*	*18.84*
4	*33.099*	*1266*	*15.05*	*33.581*	*3618*	*17.69*
5	*33.182*	*980*	*9.95*	*33.613*	*3806*	*18.03*
6	*33.240*	*1245*	*15.02*	*33.666*	*3829*	*18.43*

LOD and LOQ Data for Methyl ester

Test Solution	*LOD*			*LOQ*		
	RT min)	*Area (µV*sec)*	*% Area*	*RT (min)*	*Area (µV*sec)*	*% Area*
1	*41.216*	*1293*	*13.80*	*41.162*	*3825*	*18.16*
2	*40.842*	*1439*	*13.95*	*41.216*	*3568*	*17.39*
3	*40.865*	*1548*	*14.48*	*41.268*	*4177*	*18.97*
4	*41.071*	*1348*	*16.02*	*41.288*	*3647*	*17.83*
5	*41.144*	*1820*	*18.47*	*41.039*	*3476*	*16.47*
6	*41.109*	*1251*	*15.09*	*41.326*	*3466*	*16.68*

8.5.LINEARITY

Figure – 31 **LinearitySolution(LOQlevel)**

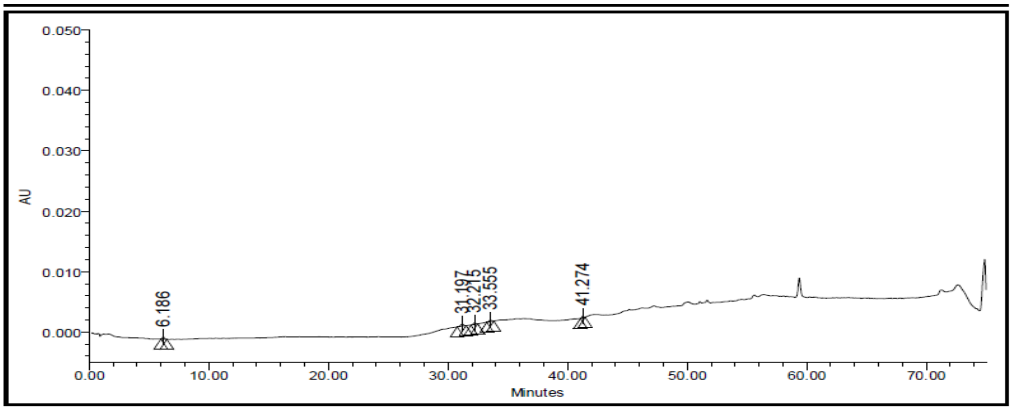


Figure – 32 **LinearitySolution(25%level)**

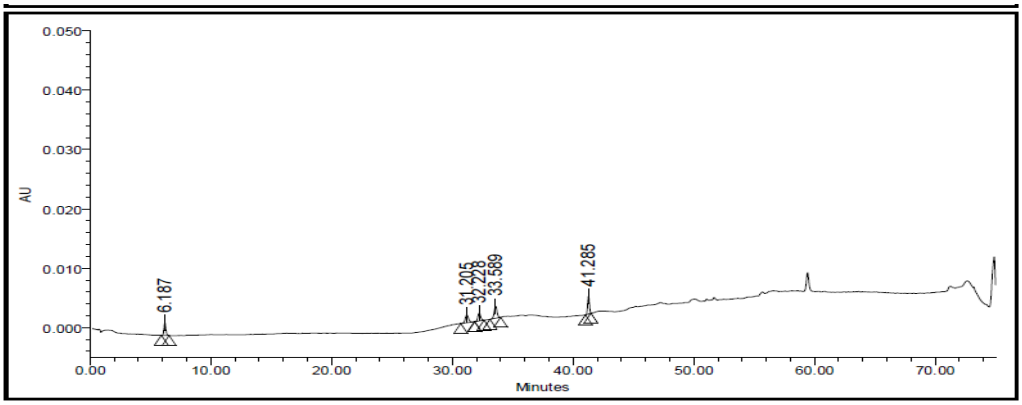


Figure – 33 **LinearitySolution(50%level)**

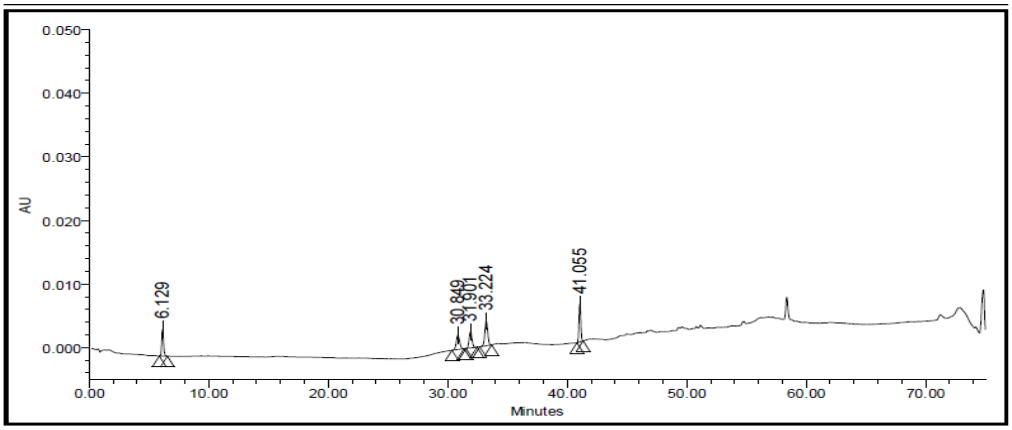


Figure – 34 **Linearity Solution(75%level)**

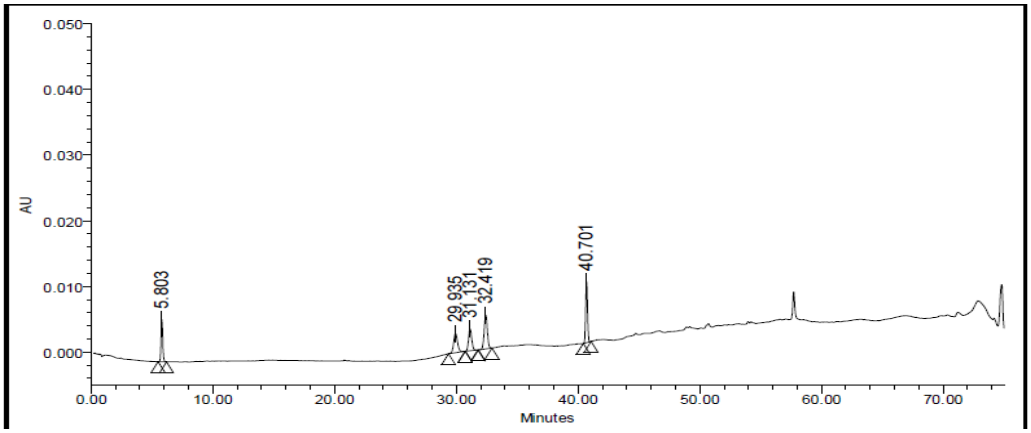


Figure – 35 **Linearity solution(100%level)**

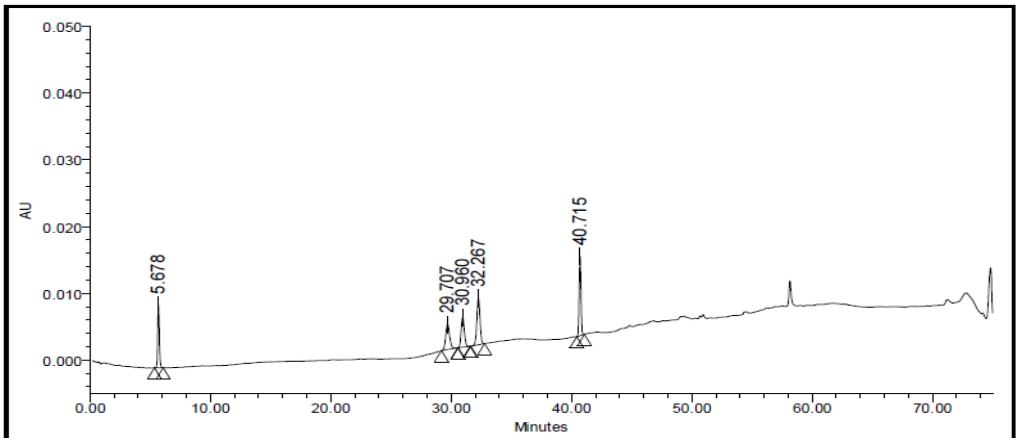


Figure – 36 **Linearitysolution(125%level)**

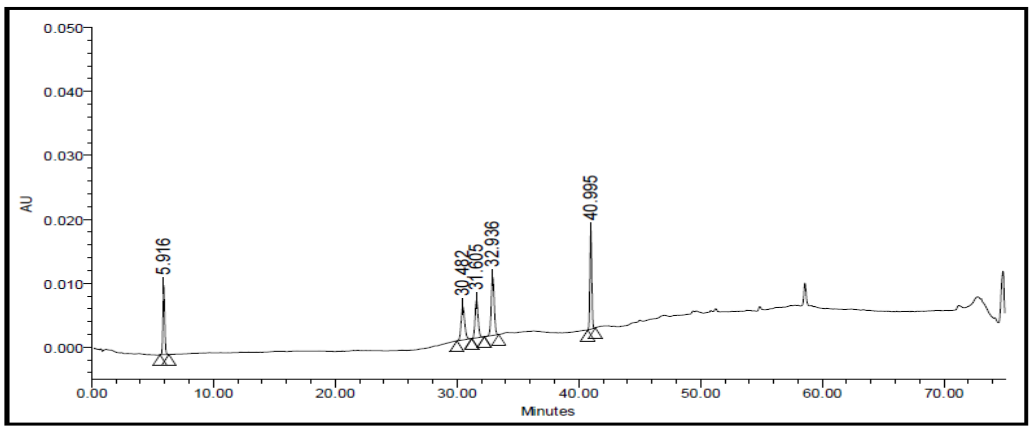
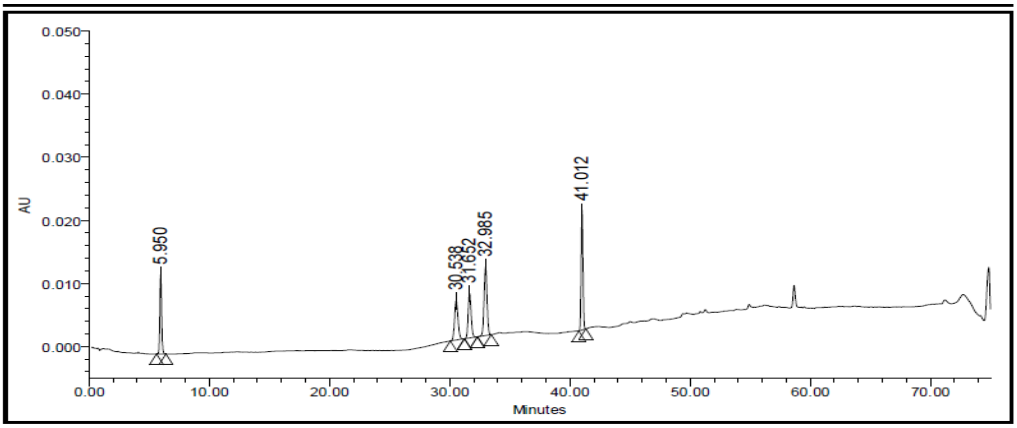


Figure – 37 **Linearitysolution(150%level)**



Linearitysolution Data [LOQ and 25%]

Compound Name	Linearitysolution(LOQlevel)			Linearitysolution(25%level)		
	RT (min)	Area (µV*sec)	% Area	RT (min)	Area (µV*sec)	% Area
Impurity – J	*6.186*	*4282*	*19.92*	*6.187*	*23551*	*17.63*
Dexibuprofen	*31.197*	*6247*	*29.05*	*31.205*	*24568*	*18.4*
Impurity - B	*32.215*	*3790*	*17.63*	*32.228*	*21333*	*15.97*
Impurity – E	*33.555*	*4175*	*19.42*	*33.589*	*32793*	*24.56*
Methyl ester	*41.274*	*3006*	*13.98*	*41.285*	*31301*	*23.44*

Linearitysolution Data [50%and 75%]

Compound Name	Linearitysolution(50% level)			Linearitysolution(75% level)		
	RT (min)	Area (µV*sec)	% Area	RT (min)	Area (µV*sec)	% Area
Impurity - J	*6.129*	*45954*	*17.7*	*5.803*	*63758*	*16.76*
Dexibuprofen	*30.849*	*43196*	*16.64*	*29.935*	*64711*	*17.01*
Impurity - B	*31.901*	*42494*	*16.36*	*31.131*	*59920*	*15.75*
Impurity - E	*33.224*	*65157*	*25.09*	*32.419*	*92261*	*24.25*
Methyl ester	*41.055*	*62864*	*24.21*	*40.701*	*99877*	*26.25*

Linearitysolution Data [100%and 125%

Compound Name	Linearitysolution(100% level)			Linearitysolution(125% level)		
	RT (min)	Area (µV*sec)	% Area	RT (min)	Area (µV*sec)	% Area
Impurity - J	*5.678*	*91132*	*17.69*	*5.916*	*110112*	*17.3*
Dexibuprofen	*29.707*	*86213*	*16.73*	*30.482*	*104676*	*16.44*
Impurity - B	*30.96*	*82721*	*16.05*	*31.605*	*100480*	*15.78*
Impurity - E	*32.267*	*128575*	*24.95*	*32.936*	*157597*	*24.76*
Methyl ester	*40.715*	*126612*	*24.57*	*40.995*	*163729*	*25.72*

Linearitysolution Data [150%]

Compound Name	Linearitysolution(150% level)		
	RT (min)	Area (µV*sec)	% Area
Impurity - J	*5.95*	*129388*	*16.99*
Dexibuprofen	*30.538*	*123867*	*16.26*
Impurity - B	*31.652*	*120845*	*15.87*
Impurity - E	*32.985*	*185904*	*24.41*
Methyl ester	*41.012*	*201648*	*26.48*

7.6.ACCURACY

Figure – 38 ***LOQ Level 1***

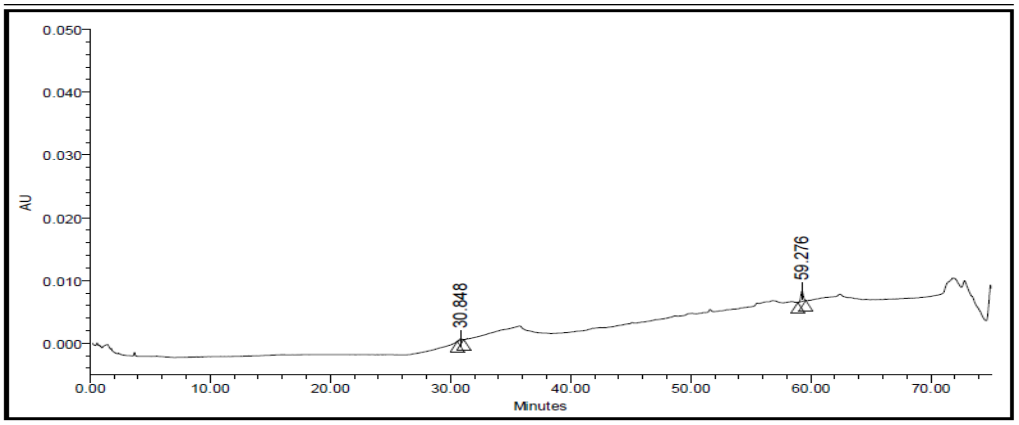


Figure – 39 ***LOQ Level 2***

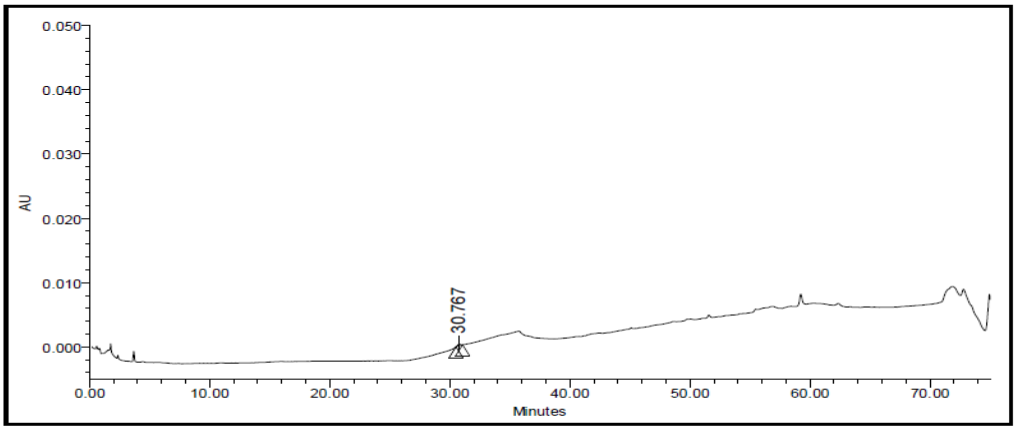


Figure – 40 ***LOQ Level 3***

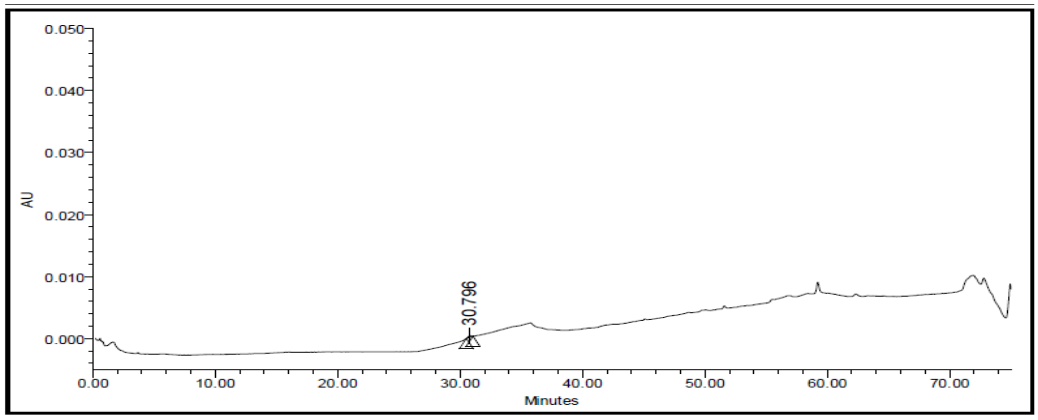


Figure – 41 ***100% Level 1***

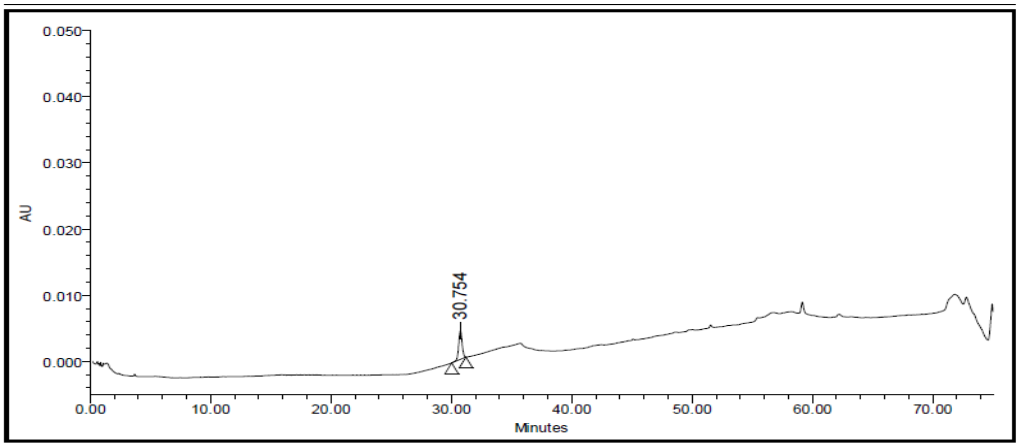


Figure – 42 ***100% Level 2***

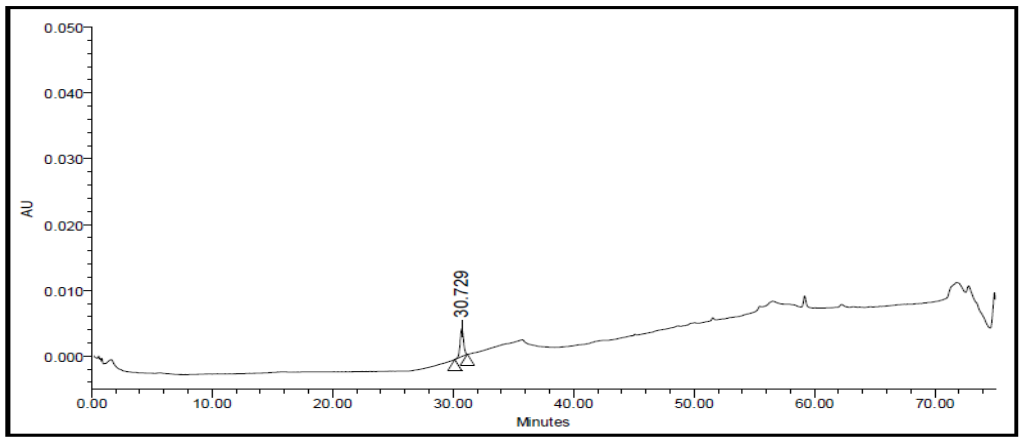


Figure – 43 **100 % Level 3**

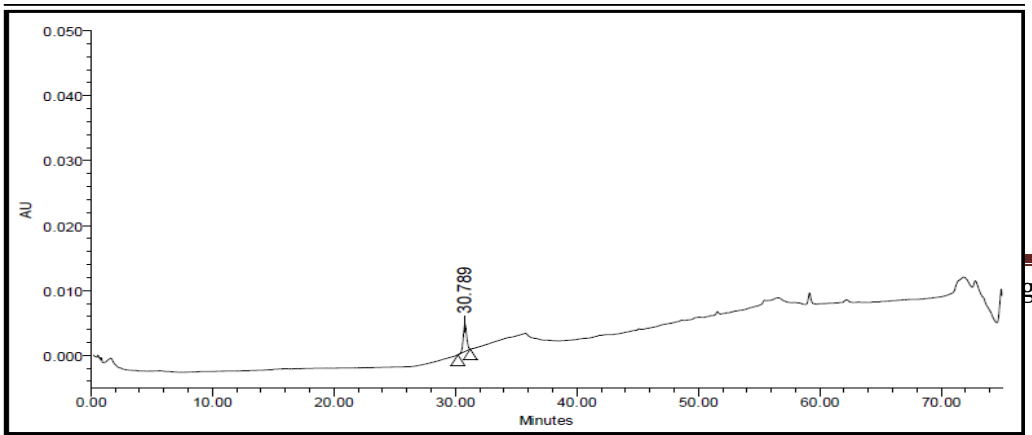


Figure – 44 **150 % Level 1**

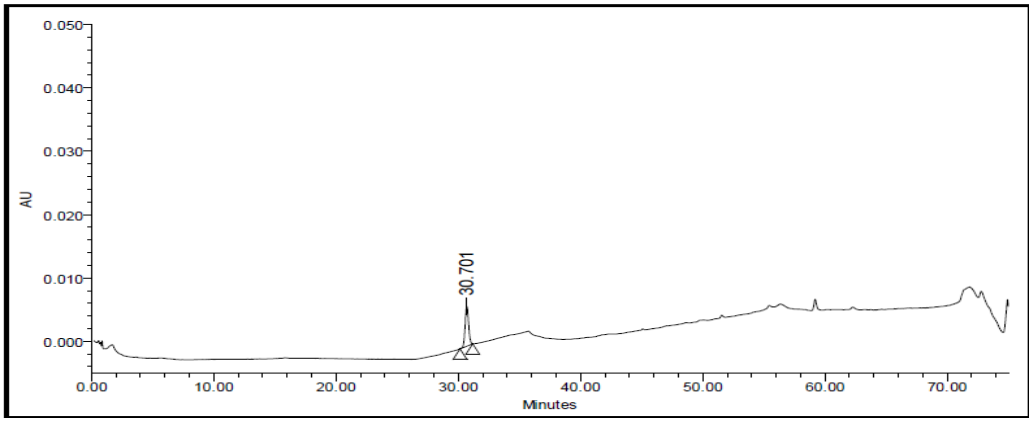


Figure – 45 **150 % Level 2**

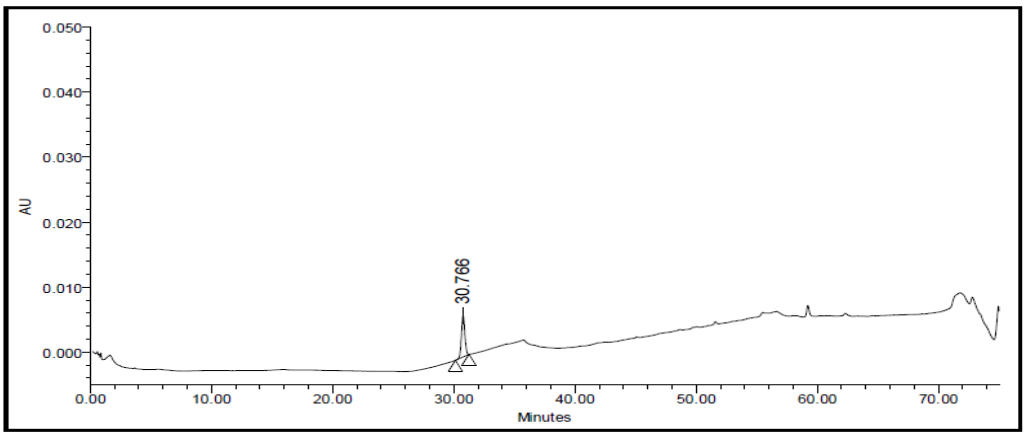
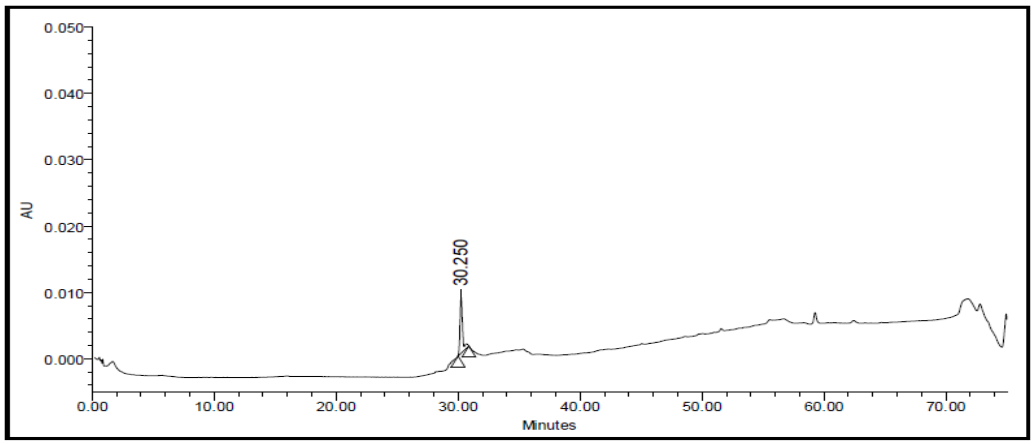


Figure – 46 **150 % Level 3**



Accuracy Data :

Solutions	RT (min)	Area (µV*sec)	% Area
LOQ Level 1	*30.848*	*4821*	*17.09*
LOQ Level 2	*30.767*	*4854*	*100.00*
LOQ Level 3	*30.796*	*4880*	*100.00*
100% Level 1	*30.754*	*85882*	*100.00*
100% Level 2	*30.729*	*83765*	*100.00*
100% Level 3	*30.789*	*82342*	*100.00*
150% Level 1	*30.701*	*123646*	*100.00*
150% Level 2	*30.766*	*123948*	*100.00*
150% Level 3	*30.250*	*123074*	*100.00*

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