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Analytical Method Development And Validation Of Etoricoxib Using Uv Spectroscopy

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INTRODUCTION

U.V SPECTROSCOPY

A UV-vis spectrophotometer is merely a tool that measures the amount of light absorbed. Initially, a detector reading indicates the amount of light reaching the detector in the absence of a sample (prior to inserting the sample between the light source and detector). When the sample is positioned in a light path, if molecules absorb light, the detector observes a decrease in the amount of light it receives. Reduction absorbance is the term we use.

The device used for ultraviolet-visible spectroscopy is known as a UV/Vis spectrophotometer. It measures the brightness of light going through a sample (I), then compares it to the brightness of light before going through the sample (I_o).

Transmittance=I/I_o.

Qualitative analysis involves using a spectrophotometer to identify organic compounds, with available data, while quantitative analysis is used to determine the amount of molecules absorbing radiation.[1]

Workflow of ultraviolet-visible spectrophotometer

For better understanding of how a ultraviolet-visible spectrophotometer works, it is important to look at the main components shown in the fig, despite the many different types available.

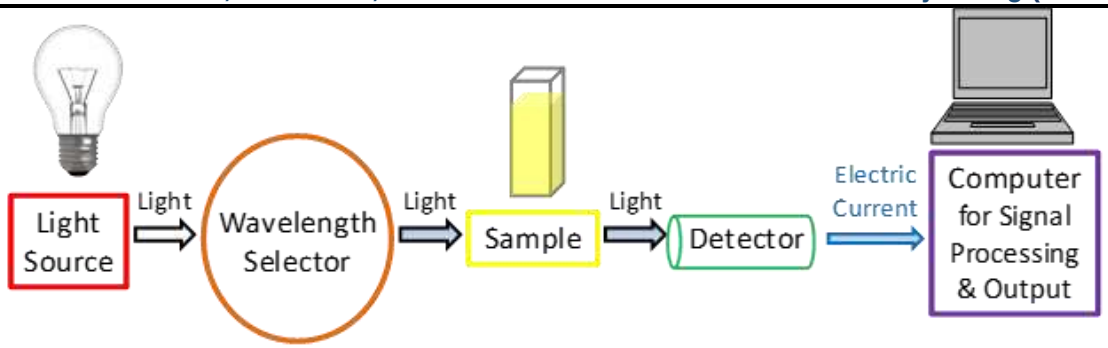

Figure No. 1 Workflow of UV-Vis Spectrophotometer

Monochromators - Monochromator divides the wavelength into limited range of light. (λ). The selection of the desired light wavelength is typically achieved by rotating diffraction gratings to select the incoming and reflected points. The groove frequency of the diffraction grating is consistently determined by counting of grooves per millimeter. A higher frequency results in reduced usable wavelength range but its improved optical resolution. Conversely, a least in number of groove frequency allows for wider usable wavelength range. Ultraviolet visible spectroscopy can use grooves ranging from 300 to 2000 per millimeter, with a minimum of 1200 per millimeter being typical. Flaws in the diffraction grating and optical arrangement affect the accuracy of spectroscopic predictions. As a result, governed diffraction gratings are more likely to have faults than laser-created holographic diffraction gratings. Damaged holographic diffraction gratings generally offer much higher quality measurements.

Sample Analysis

Its does'nt which wavelength selector is used in the spectrophotometer the light will be pass through the sample or analyte. It is necessary to include a reference sample commonly called the"blank sample"as a cuvette filled up with the same solvent as the sample being tested, in all tests and measurements. If you use a buffered aqueous solution with the sample for measurements then use a buffered aqueous solution without the substance of interest as a reference.

Detection

After the lights passed through the sample, detector converts it into a electronic signal. Typically, sensors rely on photoelectric layers or semiconductor materials.When light shines on a photoelectric coating, it releases negatively charged electrons. When the electrons are discharged, a current is generated that is directly related to the brightness of the light. A commonly used detector is photomultiplier tube UV-Vis spectroscopy, depending on the photoelectric effect, which causes electrons to be released when exposed to light and then amplified to provide a greater electrical current. Extremely low light levels can be detected very well with PMT detectors.[2]

Etoricoxib's Pharmacodynamics and Pharmacokinectics discussed

Etoricoxib shows 100 times more preference for COX-2 over COX-1.

- After a single etoricoxib dose, peak COX-2 inhibition is reached within 1.5 hours.[3]
- Long-term administration of oral doses results in a significant inhibition that remains present after 24 hours.
- Etoricoxib has high absorption rates and low clearance, leading to a half-life of 27 hours.[4]
- Etoricoxib undergoes significant liver P450-dependent oxidation to produce 6-hydroxymethyl and 6-carboxylic metabolites acid derivative (primary metabolite). Both are not active.[5]
- The majority of metabolites are eliminated through the urine, with just 20% of the dose being excreted in other ways found in excrement.[6]
- Less than 1% of etoricoxib remains unchanged in the urine when excreted.

Clinical efficacy

- Phase II or phase III trials revealed that etoricoxib 60 or etoricoxib 90 mg offers significant advantage to OA patients, demonstrating a comparable efficacy to diclofenac 150mg and naproxen 1000 mg during a year of treatment. Studies conducted during trials and regulatory submissions have demonstrated that a dose of 30 mg is efficacious in patients with osteoarthritis. Since all regulatory and professional organizations suggest using the lower dose for the limited time, it is advisable to adhere to the guidelines and promote approval of this effective dose for OA.[7]
- In the treatment of rheumatoid arthritis, 90 mg of etoricoxib showed superior statistical results compared to both placebo and naproxen.[8]
- Etoricoxib 120 mg was effective like indomethacin in reducing pain & providing pain relief in patients with gout.[9]
- In ankylosing spondylitis etoricoxib 90 mg & 120 mg showed statistical growth over both placebo and naproxen.[10]

Safety and how well something can be tolerated. The MEDAL program has evaluated how etoricoxib and diclofenac impact cardiovascular and gastrointestinal outcomes in regular clinical settings.[11]

- There were significantly fewer upper gastrointestinal clinical events with etoricoxib compared to diclofenac. The frequency of discontinuation because of liver test abnormalities was significantly lower with etoricoxib compared to diclofenac.[12]
- There were no differences seen between etoricoxib and diclofenac in terms of thrombotic events, cardiac events, cerebrovascular events, or peripheral vascular events.
- Dose of Etoricoxib of 90 mg (not 60 or 30 mg) was linked to an increased occurrence of edema and congestive heart failure (CHF).
- Higher rates of hypertension were seen with etoricoxib doses compared to diclofenac.[13]

Dosage and adminstrations

The suggested doses are:

- For osteoarthritis, the recommended dosage is either 30 mg or 60 mg once a day.[14]
- Take 90 mg of Rheumatoid arthritis medication daily.[15]
- Gouty arthritis: One daily dose of 120 mg.[16]

Objectives of validation

Primary objective are to form a written procedure for process control and production which designed to confirm that the products of drug had a unique quality, identity and purity that they are shown to process

1. They assure the quality of products.
2. Regulation of government.

Types of validation

The following are commonly required validation in pharmaceutical process

1. Validation of Equipment.
2. Validation of Process.
3. Validation of Cleaning.
4. Validation of Analytical method.
5. Validation of including utilities & Facilities.

MATERIALS AND METHODS

Table No 5 Instrument used:

S.No.	Name	Model	Supplier/Manufacturer
1	Single beam U.V spectrophotometer	Carry 60 UV-Visible	AglientTech.carry 60uv-vis
2	Digital weighing balance	TX323L	Shimadzu instrument Pvt. Ltd.

Table No 6 Chemical used:

S.no.	Name	Supplier/Manufacturer
1	Ethanol	Central drug house Pvt. Ltd.

Table No 7 Active pharmaceutical ingredient used :

Drug name	Company name
Etoricoxib	Alembic Pharmacetical Ltd., Sikkim.

Table No 8 List of marketed formulations :

Brand name	Batch No.	Mfg. Date	Exp. Date	Company name
Etoford	4361009	Dec 2023	Nov 2024	Leeford
Intacoxia	ZDT2009-33	Dec 2023	Oct 2025	Intas pharmaceutical ltd.
Etozox-90	AFB21Q95	Dec 2023	Nov 2025	Cipla

METHOD DEVELOPMENT AND VALIDATION OF ETORICOXIB AS API :

Identification & Characterstic of Etoricoxib:

Description

The physical properties (colour, odour, taste, and appearance) was discovered, white or almost white powder, odourless and tasteless.

Range of melting points

The melting point equipment is used to determine the melting range of analyte. One –ended-sealed capillary was used to hold the chemical. In the equipment, thermometer & capillary were installed properly in designed holes for capillary and thermometer, respectively. The regulator controlled the temperature, while a thermometer monitored the temperature at which the compound technique was performed.

Solubility

1gm of Etoricoxib was dissolved in 10 ml volumetric flask containing ethanol as a solvent at room temperature and flask was shake for a few minutes, and visually checked for residue.

Development of a method for estimating Etoricoxib as an active pharmaceutical ingredient using UV-spectroscopy

Weigh 10mg of drug API into a 100ml volumetric flask, and then add ethanol to make it up to 100ml. Then 1 mL of the solution was placed in a 10 mL volumetric flask, and the volume was makeup upto the mark on flask with ethanol. The drug is present at a concentration with 10µg/ml in this solution. The absorbance of

this solution is compared to ethanol as a reference in the 200-400nm visible range. The highest wavelength recorded was 235 nanometers.

Preparation of calibration cruve of Etoricoxib at 235nm:-

0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml of standard stock solution (100µg/ml) were pipette out into a 10 ml volumetric flask. Then the volume was making up to a 10ml with solvent.

The measurement was taken at 235nm against ethanol as a reference, and a graph was created showing the relationship between absorbance and solution concentration.

Method validation

Linearity and range

A linear relationship was observed between absorbance and concentration in the working range of 2-12 µg/ml.

Precision

Precision of the methods was studied as intra-day, inter-day and repeatability.

Intra-day Precision- Intra-day precision study was performed by analyzing a 6µg/ml, 8µg/ml and 10µg/ml concentration of drug for three times in a same day.

Inter-day Precision– Inter-day precision was performed by analyzing a 6µg/ml, 8µg/ml and 10µg/ml concentration of drug for three times in a gap of two hours.

Repeatability- Repeatability was performed by analyzing a 6µg/ml concentration of drug with six different replicate.

Limits of Detection and Limit of Quantification

LOD and LOQ both are used to determine the sensitivity of the proposed approaches. The limit of detection and limit of quantification of Etoricoxib were calculated by applying following equation as per ICH guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Estimation of Etoricoxib in tablet dosage form form:

To obtain a fine powder 20 Tablets were completely crushed in a mortar pestle, and weighed that powder of those crushed samples equivalent to 10mg of Etoricoxib and then transferred into a 100ml of volumetric flask. It was dissolved with 100ml ethanol. The solution was sonicate for 15 minutes, after sonicate it was filtered through whatmann filter paper. The final concentration of 100µg/ml was prepared. Then 1ml was pipette out in a 10ml volumetric flask and make up to 10ml with ethanol and the absorbance was measured at 235.

RESULT AND DISCUSSION

CHARACTERIZATION AND IDENTIFICATION OF ETORICOXIB

Table No. 9 Melting point study

The Melting point was determined using melting point apparatus

Drug	Reported melting point	Observe Melting point
Etoricoxib	116.4˚C	110.3˚C

IR Interpretation of Etoricoxib:-

The acronym FT-IR represents Fourier transform infrared, which is the most commonly used technique in infrared spectroscopy. IR radiation passes through a sample in infrared spectroscopy. A portion of the infrared radiation is taken in by the sample while the rest is transmitted. The spectrum produced shows the molecular absorption and transmission, forming a unique molecular signature of the sample. Just like fingerprints, each unique molecular structure will generate its own distinct infrared spectrum. This feature renders infrared spectroscopy beneficial for various analysis purposes. The FT-IR samples were blended with KBr at a ratio of 1:3 and compacted into pellets. Pellets were examined within a wavelength range of 4000-450cm-1, with a resolution of 4cm-1 and a specific number of scans.

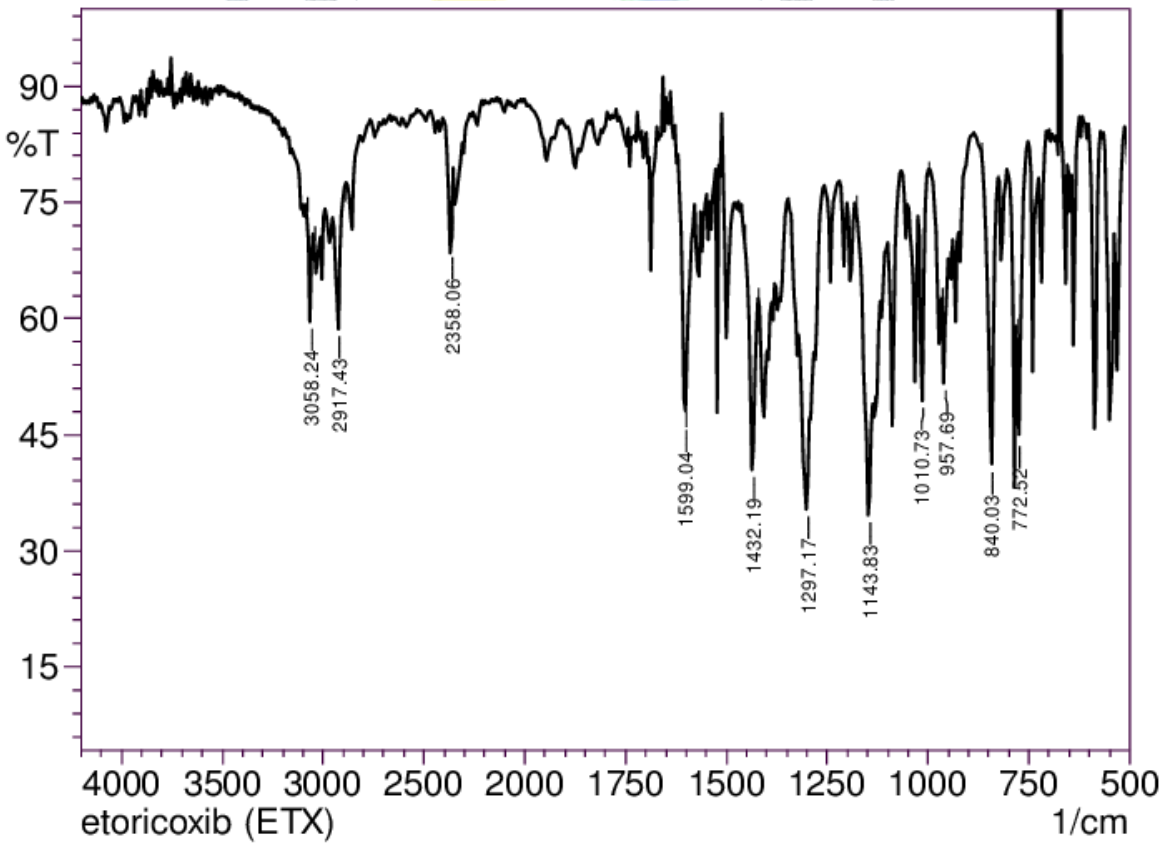

Figure No. 11 IR Interpretation of Etoricoxib

Solubility study :

Ethanol was selected for the study of Etoricoxib were freely soluble in it.

Determination of wavelength of maximum absorbance of Etoricoxib:-

Etoricoxib showed maximum wavelength absorbance (λ max) at 235nm when scanning the absorbance of 12µg/ml in the UV range of 200-400nm using Ethanol as blank.

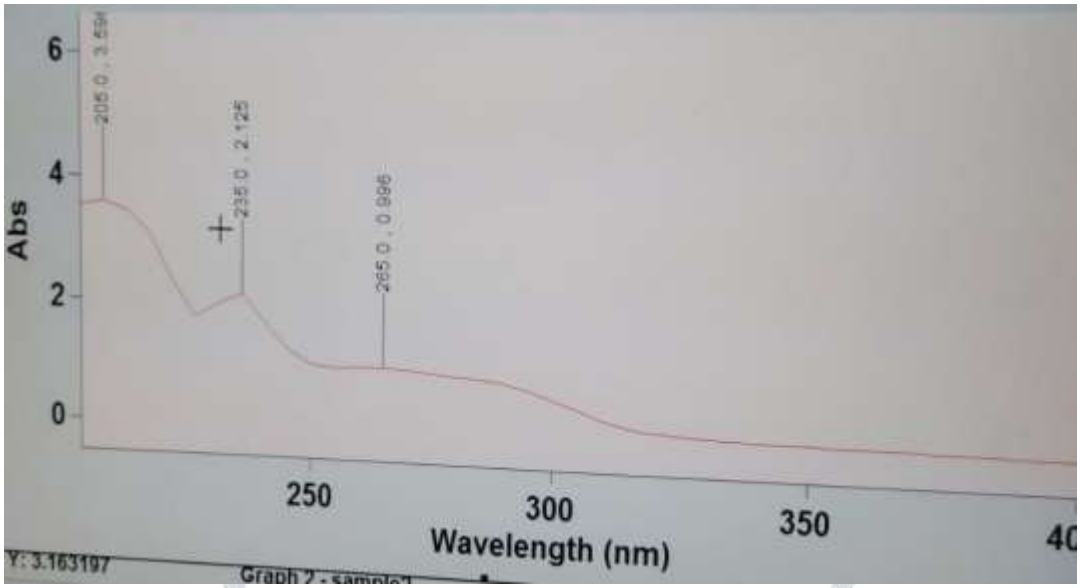

Figure No.12 Scan of Etoricoxib at 200-400nm

Preparation of calibration curve for Etoricoxib at 235nm:-

Table No. 10 Calibration curve data of Etoricoxib

Concentration(µg/ml)	Absorbance
2	0.1605
4	0.4306
6	0.5750
8	0.7672
10	1.0129
12	1.0887

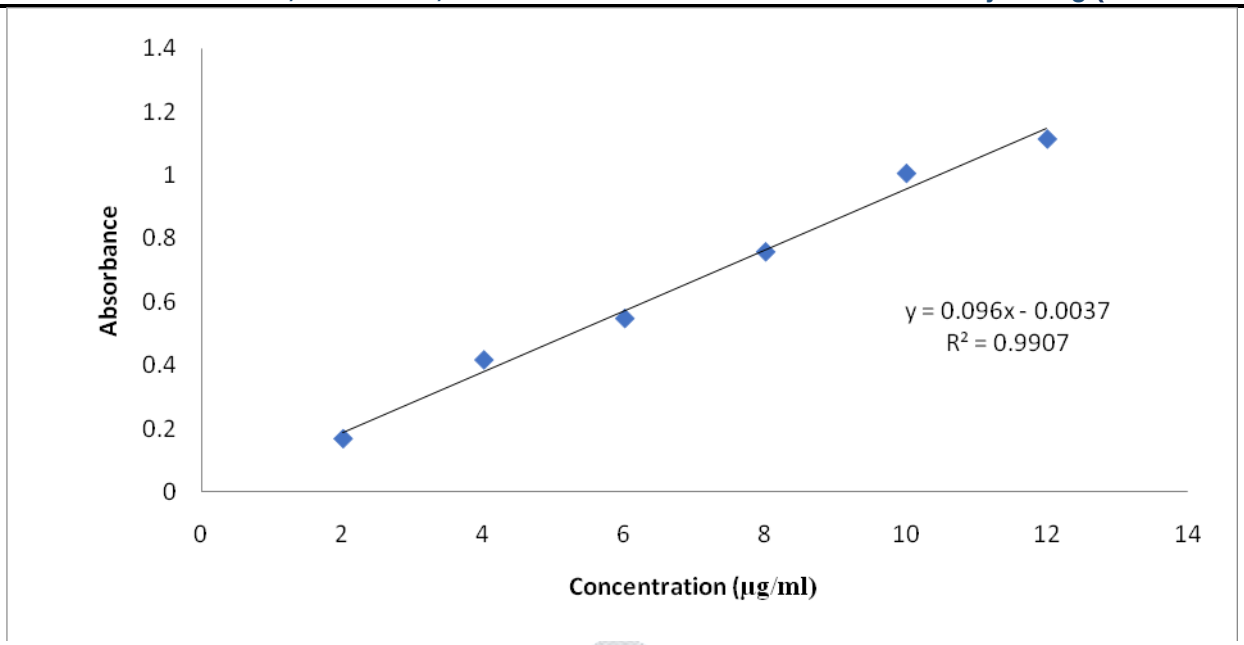

Figure No. 13 Calibration curve of Etoricoxib

Table No. 11 Absorbance, $E^{1\%}$ 1cm, Absorption and molar absorption coefficients if Etoricoxib at λ max = 235nm were determined at various concentrations.

Concentration	Absorbance	Absorbance/Conc.	$E^{1\%}$(g/100ml)	Absorptivity
2	0.1605	0.08025	802.5	80.25
4	0.4306	0.10765	1076.5	107.65
6	0.5750	0.09583	958.3	95.83
8	0.7672	0.09590	959.0	95.9
10	1.0129	0.10129	1012.9	101.29
12	1.0887	0.09072	907.2	90.72

Method Validation

Linearity and Range

The drug's response showed linearity within concentrations ranging from 2 to 12µg/ml. Linear regression analysis was used to examine the absorbance between concentration data in order to obtain the calibration curve. The equation of calibration curve for Etoricoxib obtained was Y= 0.096x-0.0037, with a linear calibration curve in the specified concentration range (R2= 0.9907).

Precision

The all three precision result indicate good reproducibility, present %RSD was less than 2%. This method was highly precise.

(a) I

ntraday

Table No. 12:- Intraday Precision result of Etoricoxib

Conc.	Absorbance			Mean	SD	%RSD	Mean % RSD
	0 hr	2 hr	4 hr				
6µg/ml	0.5313	0.5937	0.5756	0.5668	0.00103	0.18172	0.1414
8µg/ml	0.7866	0.7645	0.7430	0.7647	0.00047	0.06146	
10µg/ml	1.0904	1.0626	1.0284	1.0604	0.00192	0.18106	

(b) Inter -day Precision

Table No. 13:- Inter-day Precision result of Etoricoxib

Conc.	Absorbance			Mean	SD	%RSD	Mean % RSD
	0 hr	2 hrs	4 hrs				
6µg/ml	0.5928	0.5964	0.5936	0.5942	0.00000358	0.00060	0.04628
8µg/ml	1.0254	0.9893	0.9753	0.9966	0.00046799	0.04695	
10µg/ml	1.4246	1.4770	1.4075	1.4363	0.00131141	0.09130	

Repeatability

Table No. 14 Repeatability result of Etoricoxib (µg/ml)

Concentration	Absorbance	%RSD
6µg/ml	0.6120 0.6671 0.6626 0.5407 0.6364 0.6451	0.34953
Average	0.627316	
SD	0.002192686	

Limit of Detection and Quantification (LOD & LOQ) :

Determination of the detection and quantification limits was performed based on the standard deviation of the y- intercept and slope of the least square line parameter as defined in international conference on harmonization (ICH) guidelines. The LOD and LOQ were 3.04445µg/ml and 9.22561µg/ml respectively.

Quantification of Etoricoxib in tablet formulation:

Table No. 15 : Result for Etoricoxib in tablet dosage form by different brands :

BRAND	LABLED CLAIM	ABSORBANCE	% ASSAY
Etoford	90mg	1.1123	110.2

BRAND	LABLED CLAIM	ABSORBANCE	% ASAAY
Intacoxia	60mg	0.6571	68.8

BRAND	LABLED CLAIM	ABSORBANCE	% ASAAY
Etozox	90mg	1.0127	105.8

TABLE No. 16 : Validation parameter for Etoricoxib

S.NO	PARAMETERS	RESULTS
1	Absorption maximum	235nm
2	Beer's range (µg/ml)	2-12µg/ml
3	Standard regression equation	Y=0.096x-0.0037
4	% assay A,B & C	110.2%, 68.8% & 105.8
5	LOD	3.04445µg/ml
6	LOQ	9.22561µg/ml

RESULT:

A spectroscopic method was created and verified for estimating Etoricoxib in both API and tablet forms, simple, selective, accurate, precise. The analysis was performed on wavelength maxima (λ_{max}) 235nm. Ethanol was used as a solvent for blank and diluents. Beer's lambert law was followed with concentrations between 2-12 µg/ml. Y = 0.096x - 0.0037 was the equation of this line. The accuracy in intraday & interday was assessed and the RSD was under 2%. The suggested approach was appropriate and tailored for the examination of Etoricoxib. The technique was used on three distinct commercial products, resulting in assay percentages of 110.2% for formulation A, 68.8% for formulation B, and 105.8% for formulation C. The

limits of detection and quantification were determined to be 3.04445µg/ml and 9.22561µg/ml, respectively. The current study adhered to the ICH guidelines in all procedures conducted.

CONCLUSION

The Study of Etoricoxib, which is used to cure from the different type pain and inflamation and is it is belong from the class of NSAID's has been done and different types of method and test has been performed to validate this method as per ICH guidelines.

The method that was created was straightforward, affordable, able to be replicated, and trustworthy. Etoricoxib reached its highest absorbance at 235nm. The method follows Beer's law linearly within the concentration range of 2-12µg/ml. The examination value of Etoricoxib's available formulations was determined to be 110.2%, 68.8%, and 105.8%.The method was validated for several parameters like precision, linearity, repeatlbility, LOD & LOQ etc as per ICH guidelines. To sum up, the newly developed spectrophotometric technique is simple, accurate & reproducible, and can be consistently utilized for analyzing Etoricoxib.

It is ready to use for the futhermore steps for different departments of different industries. All the results are well examied and well performed with the help of qualified equipments and instruments and the results are within limits.

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