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MIS Determination of Etoricoxib used in Pharmaceutical Formulations

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ABSTRACT *- The present study was undertaken to develop a validated, rapid, simple, and low-cost infra-red (IR) spectrophotometric method for estimating Etoricoxib (ETX) in pharmaceutical formulations. The proposed method was validated as per International Conference on Harmonization (ICH) guidelines including parameters as linearity, accuracy, precision, reproducibility, and specificity. A Solid Inclusion Complex of Etoricoxib with β-Cyclodextrin was prepared and analysed by FTIR-ATR. Linearity range was found to be 2.0 to 16 µg/ml. The results demonstrated that the proposed methods are accurate, precise and reproducible, while being simple, economical and less time consuming than other available methods and can be used for estimation of etoricoxib in different dosage forms. The results obtained were also in comparison with that of UV-spectrophotometric analysis (validation method).*

I. INTRODUCTION

Spectroscopy is the study based on interaction between radiation and matter as a function ofwavelength(λ).Itisbasedontheuseofabsorption,emission,orscatteringofelectromagneticradiationrangingfromby mattertoqualitativelyorquantitativelytostudythe matter or physical processes. The matter can be atoms, molecules, atomic or molecularions, or solids. Mostly for the purpose of analysis, infrared spectroscopy (IR) is used as ananalytical technique, which measures the infrared intensity versus wavelength (wavenumber)of light [1]. Simply, it is the absorption measurement of different IR frequencies by a samplepositioned in the path of an IR beam. Different functional groups are obtained in accordancewithabsorption of characteristic frequencies of IR radiation.It isnoted,whenan infraredlight interacts with the matter, chemical bonds will stretch, contract and bend. As a result, achemical functional group tends to adsorb infrared radiation in a specific wavenumber rangeregardless of the structure of the rest of the molecules [2]. Based upon the wavelength,infrared light can be categorized in various regions in view of wavelength, wavenumber andfrequencywhich are as follows inTable 1.

Table1: *Different IR Regionsin View of Wavelength, Wavenumberand Frequency.*

Region	Wavelength(λ) µm	Wavenumber(ν)cm^{-1}	Frequency (ν) Hz
Near	0.78 to 2.5	12800 to 4000	3.8×10^{14}to1.2×10^{14}
Middle	2.5 to 50	4000 to 200	1.2×10^{14}to6.0×10^{12}
Far	50 to 1000	200 to 10	6.0×10^{12}to3.0×10^{11}

Based on the previous frame of reference of IR, the present research is an attempt to focus onthe middle region of IR which consists of transmission spectroscopy/MIR and reflectancespectroscopy.

For the study undertaken, transmission spectroscopy is divided into the group frequencyregionextendingfrom 4000–1300 cm−1(2.50–7.69 µm)andthefingerprintregion1300–650 cm−1 (7.69–15.38 µm). The spectrum resulting from vibrational and rotational transitionsis meant for organic chemists since the vibrations induced in organic molecules are absorbedinthisregion[3].ThreetypesofinstrumentscommonlyavailableforIRabsorptionmeasurements, viz., dispersive spectrophotometers with a grating monochromator; Fouriertransform spectrometers employing an inter-ferometer; and non-dispersive photometers usinga filter or an absorbing gas used for analysis of atmospheric gases at specific wavelengths [4,5]. So, some of the common techniques and accessories used for the preparation of samplesfor IR absorption/transmission measurements are cells (liquid samples) – liquid cells, saltplate and disposable IR cards, pellet method (solid samples), mulls (solid samples) and gascells(gases or low-boiling liquid samples)[6–9].

On the other side, reflectance spectroscopy is related to reflected or scattered light from asolid, liquid or gas. It has a number of applications, particularly dealing with solid samplesthatarediffıculttohandle,suchaspolymerfilmsandfibers,food,rubbers,agriculture

productsandmanyothers.Mid-IRreflectionspectra,althoughnotidenticaltothecorresponding absorption spectra, though appear similar in general appearance and providethe same information with respect to absorption counterparts, whereas reflectance spectra canbe used for both qualitative and quantitative analysis. These spectroscopic instruments arethesedaysofferedwithadapterswhichfitintothecellcompartmentsofIR-absorptioninstruments and make it possible to obtain reflection spectra readily [10]. It is of four types:specularreflectancespectroscopy,internalreflectionspectroscopy,diffusereflectancespectroscopy and attenuated total reflectance (ATR) spectroscopy [4]. Most commonly usedarediffuse reflectance and ATRspectroscopy.

For the undertaken study, attenuated total reflectance (ATR) spectroscopy was used as asamplingtechniqueinconjunctionwithinfraredspectroscopy.Itenablessampletobeexamined directly in the solid or liquid state including large variety of materials such aspowders, liquids, gels, pastes, pellets, slurries, fibers, soft solid materials, surface layers,polymer films, coatings, threads, opaque samples and adhesives. Further, it requires little ornosamplepreparationandisoneofthemostversatileandnon-destructivesamplingtechnique. It is commonly used in industries and institutions because of its advantages like: itis less time consuming; sampling method is easy and faster as compared to other techniqueslike FTIR (transmission), UV-Vis, etc.; and it can also be used without any destruction andpretreatmentstepstomakeasample.Thus,makesitanextremelyrobustandreliabletechniqueforquantitativestudies involvingliquidswithexcellentsample-to-samplereproducibility. Because of the above advantages, the present pharmaceutical industries likeRanbaxy,Panacea,SunPharma,PiramalHealthcareLtd.,etc.,areusingthistechniqueintheir analyses. Besides this, some renowned institutions like NIPER, etc., have this techniquewhich provides training to the students how to use this technique effectively and efficientlywhileconducting qualitativeresearchstudies.

WorkingofFTIR-ATR

A beam of infrared light passes through the ATR crystal in such a way that it reflects at leastonce off the internal surface in contact with the sample. This reflection forms the evanescentwave which extends into the sample, typically by a few micrometers. The beam is thencollected by a detector as it comes out of the crystal [11]. Evanescent effect works best if thecrystal is made of an optical material with a higher refractive index than the sample which isless dense. The sampling surface is pressed into an intimate optical contact with the topsurfaceofthe crystal such as ZnSe or Ge ordiamond[12, 13].

With this technique, IR beam is directed into a crystal which is of higher refractive index.Thus, the IR beam reflects from the internal surface of the crystal and creates an evanescentwave, which projects orthogonally into the sample in intimate contact with the ATR crystal.Some of the energy of the evanescent wave is absorbed by the sample and the reflectedradiation is returned back to the detector. This phenomenon is represented graphically inFigure 1.

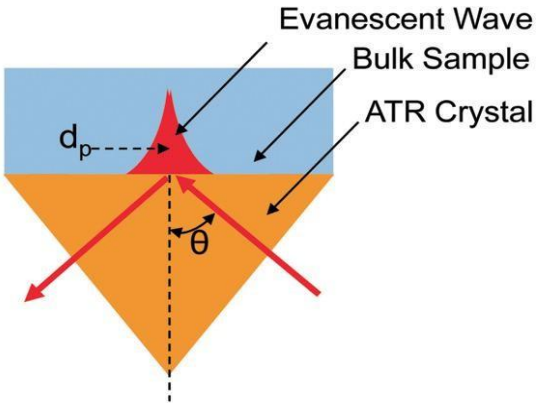


***Fig 1**: Phenomenonof* working ofFTIR-ATR

Byanalyzingthesamplethisway,finalspectrumgetsaffectedbyinfluencingthefactorssuch as:

- Refractiveindicesofthe ATRcrystalandsample
- Angleof incidence ofthe IR beam
- Criticalangle
- Depthof penetration
- Wavelengthofthe IR beam
- Effectivepathlength
- Numberofreflections
- Qualityof thesample contact withATRcrystal
- ATRcrystalcharacteristics

TherefractiveindicesofthecrystalandsampleareimportantconsiderationsintheATRsamplingtechnique by virtue of the following equation:

$\theta_c = sin^{-1}(n_2/n_1)$

where$n_2$istherefractiveindexofthesample,$n_1$istherefractiveindexofthecrystaland$\theta c$is the critical angle. To obtain internal reflectance, the angle of incidence must exceed the so-called "critical" angle to observe a purely ATR spectral result. The evanescent wave decaysinto the sample exponentially with distance from the surface of the crystal over a distance onthe order of microns. The depth of penetration of the evanescent wave *d* is defined as thedistance from the crystal-sample interface where the intensity of the evanescent decays to *1/e*(37%)of itsoriginal value. It can begiven by:

$d = \lambda / \{2\pi n_1 [\sin^2\theta - (n_2/n_1)^2]^{1/2}\}$

whereλ is the wavelength of the IR radiation. For instance, if the ZnSe crystal (n_1= 2.4) isused, the penetration depth for a sample with the refractive index of 1.5 at 1000 cm^{-1} isestimated to be 2.0 µm when the angle of incidence is 45°. If the Ge crystal (n_1= 4.0) is usedunder the same condition, the penetration depth is about 0.664 µm. The depth of penetrationand the total number of reflections along the crystal can be controlled either by varying theangle of incidence or by selection of crystals. Different crystals have different refractiveindicesofthecrystalmaterial.Bytheway,itisworthnotingthatdifferentcrystalsareappliedtodifferenttransmi ssionrangezincselenium(ZnSe)for20,000 ~ 650 cm^{-1},germanium(Ge) for 5500~800 cm^{-1} [14].

Going by the above retrospect, it can be concluded that FTIR-ATR is a well-establishedstandardmethodwhichcanbeeffectivelyusedtostudydrugreleaseinsemisolidformulations, drug penetration, and influence of penetration modifiers. Besides this, it is alsocapable of conducting *vivo* studies. Above all, the main benefit of ATR sampling can be seenwhen there is very thin sampling path length and depth of penetration of the IR beam. Thissampling is in contrast to traditional FTIR (transmission); where the sample must be dilutedwith IR transparent salt, pressed into a pellet or pressed to a thin film, prior to analysis toprevent totally absorbing bands in the infrared spectrum. In case the sample is polymer, itmeans it is too thick for transmission analysis, because most of the IR bands are totallyabsorbinginthiscase.Therefore,simply placingthethicksampleonATRcrystalandapplyingpressuregeneratesanearlyperfectspectruminlessthan1min.Thisp roveshowlesstime-consumingthismethodisincaseofthicksamples.Thisisbecauseofitscharacteristicswhich helpthis methodto eliminateexcessivesolventabsorption [15].

At last, we can say that the improved spectral acquisition and reproducibility are associatedwith FTIR-ATR technique which leads to better quality database building for more precisematerialverificationandidentification.Thus,ATRisanextremelyrobustandreliabletechniquefor quantitative and quantitative studies[5].

II. EXPERIMENTALWORK

FTIR-ATRAnalysis

MaterialRequirements

Etoricoxib, a novel, selective second-generation cyclooxygenase- 2 inhibitor, was procured asa gift sample from Piramal Healthcare Ltd., Baddi, and potassium bromide (KBr) (Uvasolquality) purchased from Merck. Two marketed tablet formulations from two manufacturesnamedas Glenmark(A) and NicholasPiramal (B)wereacquired fromlocaldrug stores.

SamplePreparation

Fourdifferentconcentrationsof25,50,75and100%preparedbydiluting2.5,5,7.5and10 mg of drug sample to 10 mg with KBr, respectively were mixed properly with the help ofpestle and mortar. These samples were used for

analysis to get standard plot. Tablets ofmarketed formulations A and B were weighed separately, average content noted down andpowdered in clean pestle and mortar. Without any addition of potassium bromide, powderedsamples of pure marketed formulationswere used for analysis to determine drug content inthe giventablet.

Apparatusand Software

All spectra were recorded over a spectral region from 4000 to 650 cm^{-1} using a Perkin ElmerModel Spectrum One FTIR spectrometer which is equipped with Perkin Elmer UniversalATR Sampling Accessory supplied with a top-plate diamond crystal that gives six internalreflections at a fixed angle of incidence of 45°. For ATR data acquisition, minimum amountof solid sample (2–3 mg) was placed onto the crystal; each sample was spread on the ATRcrystal without any prior treatment and scanned. Between each measurement, the ATR crystalwascarefullycleanedwithdistilleddichloromethaneandthenairdried.Spectraofthesamples were recorded. It was corrected against the background spectrum of the clean ATRcrystal. It was then recorded with 4 cm^{-1}resolution, 90–95 N force gauge and 16 scans weretaken in order to obtain a good signal-to-noise ratio and highly reproducible spectrum. Allspectra were obtained in the transmittance mode and done in triplicate and for each of thethreemeasurements afresh sample was used.

UV-SpectrophotometricAnalysis

Determination of etoricoxib content as a pure drug and in marketed formulations was carriedoutusingUV-Spectrophotometeranalysisasreportedearlier[16]usingPerkinElmerLambda15spectrophotometer.

MaterialRequirements

Freshly prepared 0.1 N HCl in distilled water, marketed formulations A and B containingetoricoxib(90mg)presentineachtabletasreportedonthelabelofdifferentformulations,hasbeen used for estimation of drug presentin tabletfor UVanalysis.

PreparationofStandardStockSolution

The standard stock solution was prepared by dissolving etoricoxib in 0.1 N HCl to make finalconcentration of 100 µg/mL. Different aliquots were taken from stock solution and dilutedwith0.1 NHClseparatelytoprepareseriesofconcentrationsfrom2–24 µg/mL.Theabsorbance maximum (λ_{max}) was found by taking UV spectrum of etoricoxib in 0.1 N HCl, inthe range of 200–400 nm and was found to be 233 nm. Absorbance was measured at 233 nmagainst0.1NHClasblank.Thecalibrationcurvewaspreparedbyplottingabsorbanceversusconcentration ofetoricoxib.

ProcedureforDeterminationinTablets

ThemarketedtabletformulationAofetoricoxibwasusedforthepurposeofanalysis.Twenty tablets were weighed and average weight was calculated. It was then crushed to finepowder with the help of pestle and mortar. The powder equivalent to 90 mg of etoricoxib wasweighedandtransferredtoa100-mLvolumetricflaskanddissolvedin0.1 NHClbyintermittentshaking.Thevolumewasmadeuptomarktogetfinalconcentrationof900 µg/mL. The solution prepared above was then filtered through Whatmann filter paper(No.14).Thissolutionwasusedasstocksolution.Theworkingsolutionofthedrug(9 µg/mL)was prepared from standard stock solution in 0.1 N HCl.The absorbance ofworking solution was measured and amount of etoricoxib was calculated from the calibrationcurve. The readings were taken in triplicate and same procedure was repeated with othermarketedtablet formulation B.

ComplexFormationofEtoricoxibwith β-CyclodextrinbySolidInclusionComplex

MaterialRequirement

Cyclodextrin(β-CD)($C_{42}H_{70}O_{35}$)(mwt.-1135)wasprocuredfromHimediaLaboratoriesPvt.Ltd.

Preparationof Solid InclusionComplex

The inclusion complex of pure drug etoricoxib with cyclodextrin was prepared exactly in 1:1molarratio,bywettingthephysicalmixtureinamortarwithaminimumvolumeofethanol/water (1:1, by volume) mixture and kneading thoroughly with a pestle to obtain apaste, which was then dried under vacuum at room temperature, sieved through 0.25 mmsieveand stored in a desiccatoruntil further evaluation[17].

AnalysisbyFTIR-ATR

An FTIR-ATR spectrum was recorded on sample (complex) using Perkin Elmer ModelSpectrumoneFTIRspectrometerattachedwithPerkinElmerUniversalATRSamplingAccessory.Datawascollectedoveraspectralregionfrom4000to650cm^{-1}withresolution4cm^{-1} and 16 scans.

AnalysisofData

Thespectrawereanalyzedwiththehelpof*baselinetechniquemethod,*inwhichtransmittance spectra is taken into consideration. The high concentration of solute makes theaccurate cancellation of solvent absorption very difficult, but errors may be reduced byapplying a baseline technique. The assumption is made that absorption due to solvent (or asecond component) is constant or varies linearly with wavelength over the region of theabsorption band.

All the experimental data obtained by the above method was then subjected to statisticalanalysis,usingone-wayanalysisofvariance(ANOVA)andmultiplelinearregressionanalysis to obtain quantitative information. ANOVA one-way was applied to FTIR-ATR dataof pure drug etoricoxib to build calibration of data (standard plot), which enabled predictionofetoricoxibamountinpharmaceuticalformulationsAandB,$p < 0.001$.TheresultsobtainedbyFTIR-ATRmethodwerevalidatedusingultravioletspectroscopicreferencemethod by t-test paired ($\alpha = 0.5\%$) and Scheffe test (homogenous subsets). It was introducedin order to show if there was significant difference between prediction errors between ATRandreference method.

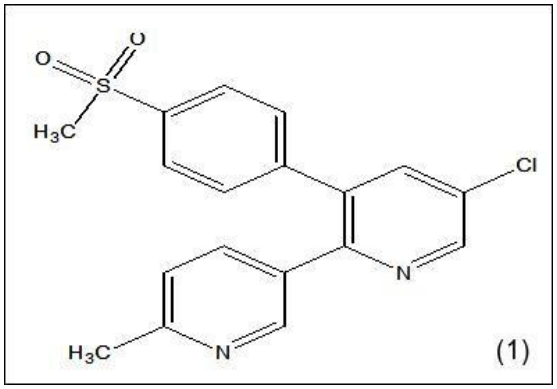
RESEARCHWORK

Theresearchworkcarried outhasbeendiscussed underthefollowingheads:

(i) Analysisof etoricoxibindifferentmarketed formulationsusing ATR
(ii) Methodvalidation
(iii) AnalysisofetoricoxibCyclodextrincomplexusingATR

AnalysisofEtoricoxibin DifferentMarketedFormulations Using ATR

Etoricoxib, 5-chloro-6'-methyl-3-[4-(methylsulfonyl) phenyl]-2, 3' bipyri-dine (1) is a novelhighly selective second generation cyclooxygenase-2 (COX-2) inhibitor administered orallyas an analgesic and anti-inflammatory drug. It is used for the treatment of osteoarthritis,rheumatoid arthritis and gouty arthritis. The spectrum below displays the infrared spectrum ofetoricoxib(Figure 2) over a frequency range of4000–500 cm$^{-1.}$



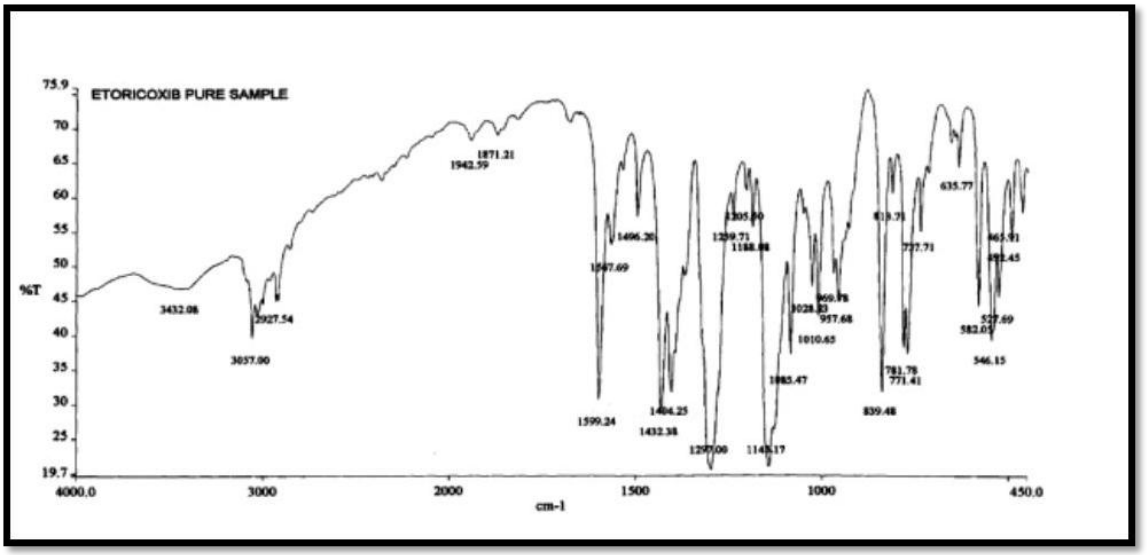


Fig.2: *IR SpectrumofEtoricoxib.*

Thecharacteristicabsorptionpeakscorrespondingtostretchingvibrationsofdifferentfunctionalgroups of etoricoxib havebeen depicted in Table 2.

Table2:*IRSpectralAnalysis ofEtoricoxib.*

Wavenumber(cm^{-1})	**Functional groupidentified**
1598.9	C= N
1431, 1300, 1143.8, 1085.8	S= O
840.9, 775.3, 638	C-Cl

Theauthors'effortstocarryoutquantitativedeterminationofetoricoxibinmarketedformulations by FTIR (transmission method) remained unsuccessful. It was observed thatintensity of peaks in transmission spectrum was not only affected by concentration of samplebutalso onthe factors such as quality ofpelletformed.

Extreme precision in sampling was also required. Clarity of spectrum at higher concentrationduetoincreasednoisesignalratiowasanotherproblem.Theseproblemswereovercomewhen quantitative determination was done with the help of ATR accessory attached to FTIRspectrophotometer.

StandardPlot

Todrawastandardplot,threedifferentconcentrationsofetoricoxib,i.e.,25,50and75%were made with the help of potassium bromide (KBr). 25% was prepared by intimatelymixing2.5mgpuredrugwith7.5mgKBr,50%waspreparedbyintimatelymixing5mgpure drug with 5 mg KBr and lastly, 75% was prepared by intimately mixing 7.5 mg puredrugwith2.5 mgKBrinpestleandmortar.Thismixingwasdonewithcareincloseenvironment,inordertopreventthepresenceofmoist ureinthesamples.Thethreeconcentrationswerethenanalyzedwiththehelpofattenuatedtotalreflectancespectroscopy

(ATR).WithFTIR-ATRspectroscopy,thepenetrationdepthoftheinfraredbeaminsampleis sufficiently large to insure a spectral reproducibility and thus representative averaging ofthe drug.

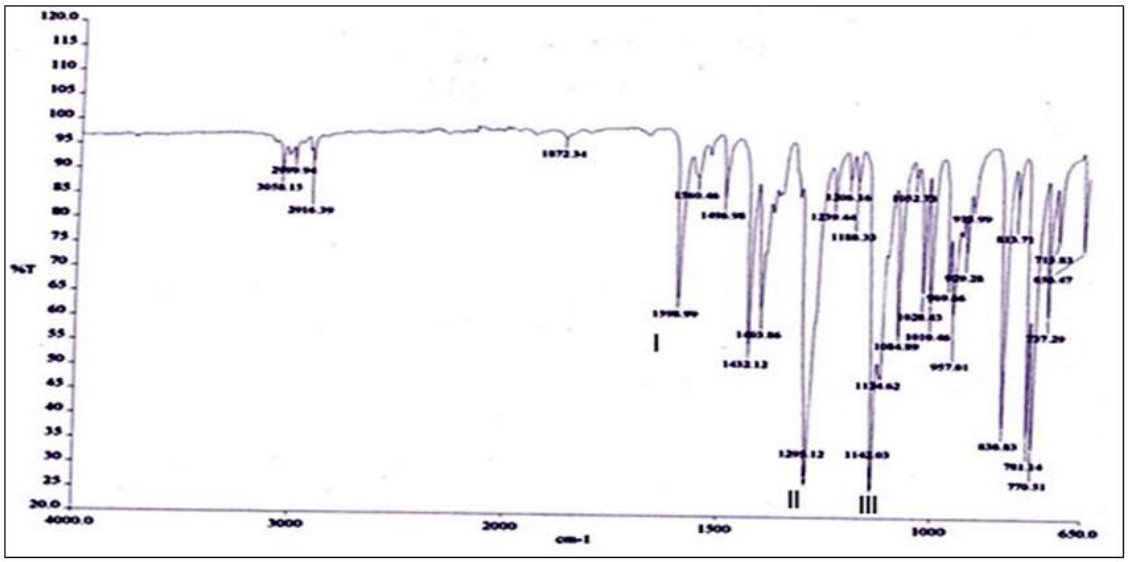


Fig.3:*TransmittanceSpectraofEtoricoxib(75%Concentration).*

The spectra of the three samples having different concentrations of etoricoxib were taken intransmittance mode with 4 cm^{-1} resolution, 16 scans and 90–95 N force gauge. In betweenanalysisofsamples,theATRcrystalwascleanedthoroughlywithdichloromethaneandwipedwithtissuecloth. Unnecessarybackgroundnoisewasremovedbytakingbackgroundofclearcrystalfirstbeforestartingtheanalysisofsamples.Itwasobservedthatincreasingthe concentration above 75% produced erroneous results. It is implied that Lambert-Beer lawis obeyed between concentration ranges of 25–75%. Analysis of three concentration sampleswas carried out in triplicate with fresh samples. Three most prominent peaks were chosen foranalytical purpose. They are marked as I, II and III in the spectra as shown in Figure 3, whichwere obtained on analyzing the three concentrations, i.e., 25, 50 and 75% as explained inTable 3.

Table3*: PeaksSelectedforAnalytical Purpose.*

Peak	**Wavenumber(cm^{-1})**	**Functionalgroups**
I	1598.9	C= N,stretching vibrations
II	1296	S= O,sulphone asymmetrical
III	1143	S= O stretching vibrations, sulphonesymmetrical

Method adopted for calculating the transmittance against each peak is baseline technique asillustrated in Figure 4. The band abc is the recorded absorption of component A and def is theabsorptioncaused by solvent andothercomponents.A line agcwasdrawn connecting thetwo minima a and c or between two suitable wavelengths on each side of the band. The pointg is obtained by dropping a line perpendicular to the zero transmittance line to meet ac to b.The absorbance is calculated from the distances I_O and I_T shown in Figure 3 by followingformulalogI_O/I_T.Thisvaluewascalculatedforthreedifferentpeaksatthreedifferent concentrations.Thedatacompiledaccordingtothistechniquehasbeensummarizedin Table4.

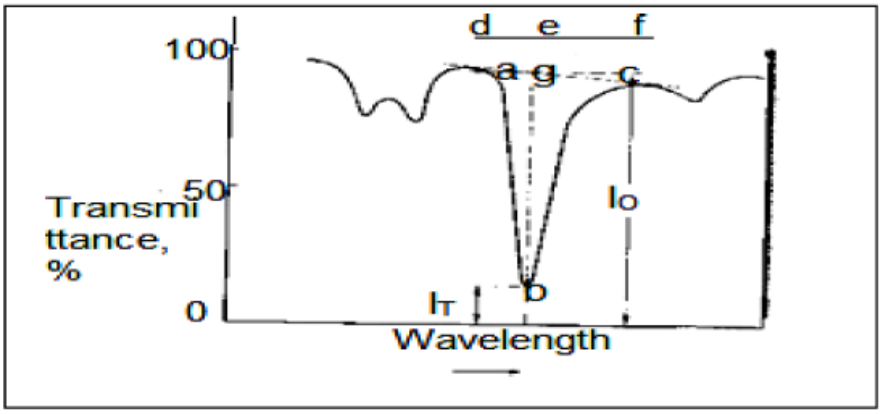
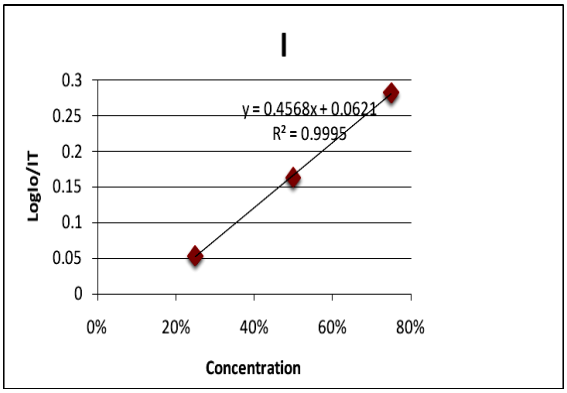


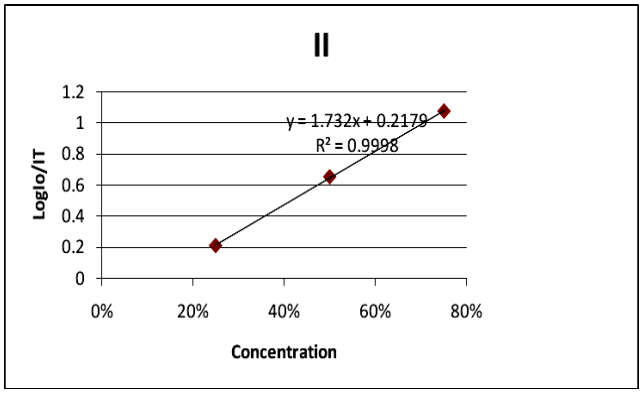
Fig.4: *The BaselineMethod for Determining theAbsorbance of an Absorption Maximum.*

Table4:*TransmittanceValuesfor VariousSelectedPeaks atDifferentConcentrations.*

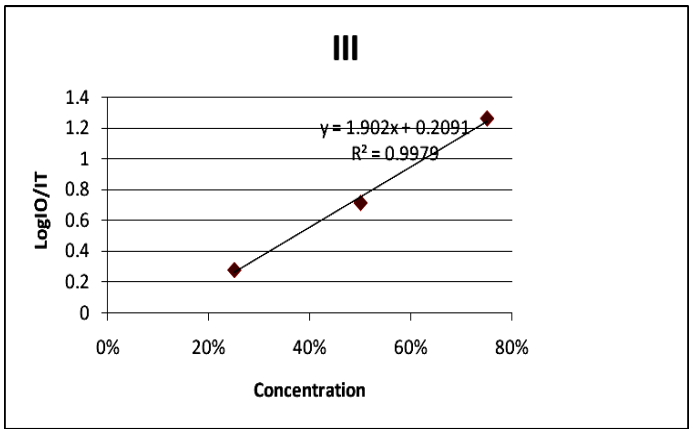
Concentration	Peak1	Peak2	Peak3
25%	0.0536	0.212	0.2799
50%	0.1634	0.6544	0.7166
75%	0.282	1.852	2.29



(i)



(ii)



(iii)

Fig.5: *StandardPlot Graphs forVarious Selected Peaks.*

ThisdatawereplottedaslogI$_O$/I$_T$versusconcentrationforallthreepeaksandstandardplotwasgraphedoutasillustratedin Figure5.Thelinearityofthedatawasestablishedwithhelp

of linear regression method. Relation coefficient was significant, i.e., $p < 0.001$ in all threepeaksand correlation coefficient turned out tobe $r^2 = 0.99$inallthreepeaks.

Analysis of Marketed Formulations Once the standard plot was obtained successfully, twomarketed formulations of etoricoxib, (A) Glenmark and (B) Nicholas Piramal were powderedand analyzed with the help of FTIR-ATR. The spectra of the above two formulations weretaken in transmittance mode with 4 cm^{-1} resolution, 16 scans and 90–95 N force gauge asshown in Figure 6.

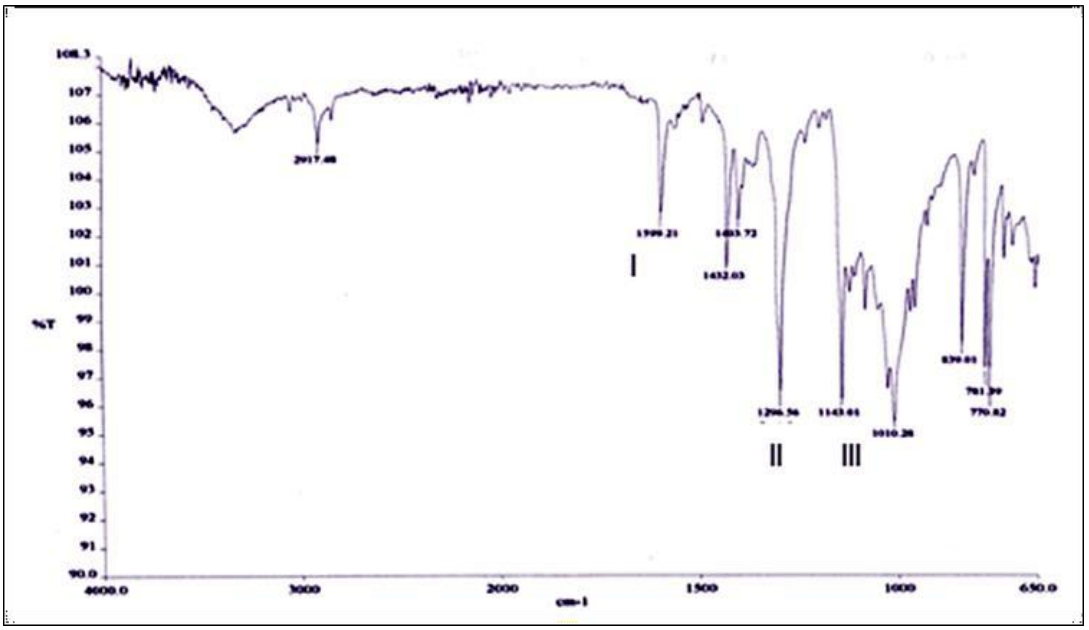


Fig.6:*Transmittance Spectraof Marketed Formulation (B)of Etoricoxib.*

Analysis of powdered samples of two formulations was done in triplicate with fresh sampleeach time. For analyzing these spectra, same three peaks were taken into consideration andwere marked as I, II and III. On these three peaks, baseline technique was applied and datawastabulated as shown in Table 5.

Table 5: *Transmittance Values of Various Selected Peaks for Drug Present in DifferentMarketedFormulations.*

Samples	Peak1	Peak2	Peak3
A	0.092	0.313	0.41
B	0.136	0.442	0.489

From the standard plot, the concentration of etoricoxib in 100 mg of tablet powder wascalculated for each formulation using the transmittance value obtained for various peaks(Table 6).

Table6:*Results oftheMarketedFormulations.*

Formulation	Etoricoxib content (in100 mg)	Peak1	Peak2	Peak3
A	34mg	34mg	30mg	29mg
B	35mg	40mg	36mg	32mg

Baseline technique data of two formulations and standard plot according to ANOVA one-waytest was found to be significant. This means all different peaks at different concentrationswere significant. This was further proved with the help of Scheffe test. Finally, t-test was alsoapplied;it wasfound that for $\alpha = 0.5\%$, datawassignificant.

MethodValidation

ThevalidationofATRassaymethodforquantitativeanalysisofetoricoxibtabletsofdifferent manufacturers was carried out using a reference UV spectrophotometric analyticalmethod[13].

The different aliquots taken from stock solution (100 µg/mL) were diluted with 0.1 N HClseparately to prepare series of concentrations from 2–24 µg/mL. The λ_{max} obtained by UVspectrum of etoricoxib in 0.1 N HCl, in the range of 200–400 nm was 233 nm as depicted inFigure 7. The absorbance range for various concentrations at λ_{max} 233 nm was found to be0.176–1.386 as showninTable 7.

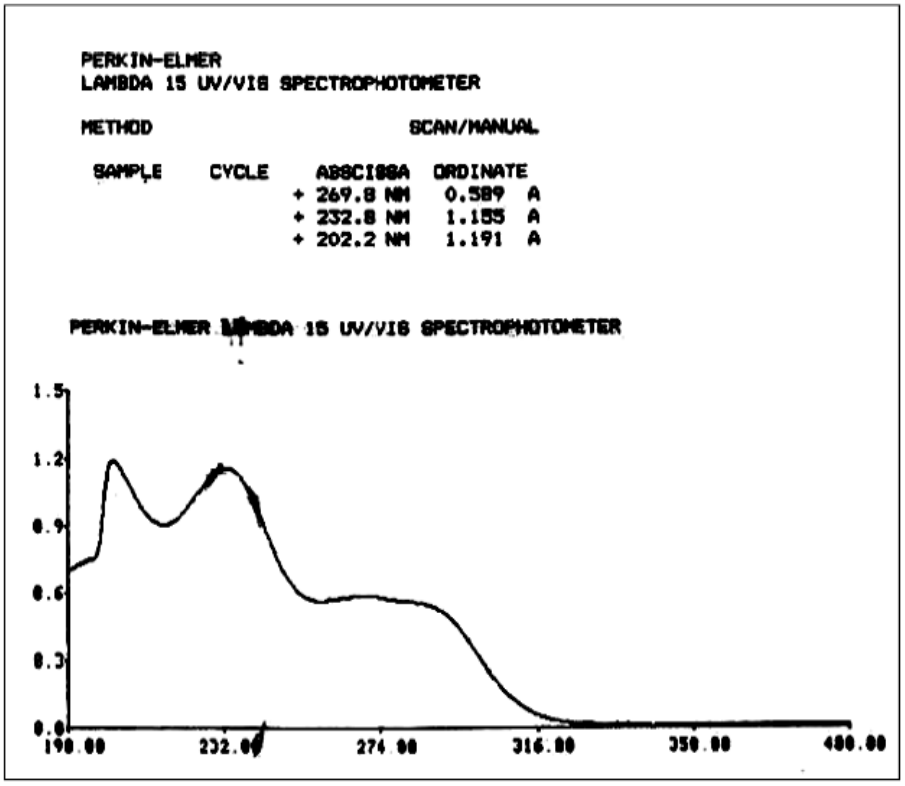


Fig.7: *UVSpectrum ofPure Drug Etoricoxib.*

ThesesolutionsobeyedLambert-Beerlawinaboveconcentrationrange(Figure8)withregressionof0.9925(Table 8).

Table7:*AbsorbanceatDifferentConcentrations.*

Concentration(µg/mL)	Absorbance(nm)
2	0.176
4	0.3463
8	0.66
14	1.17
16	1.386

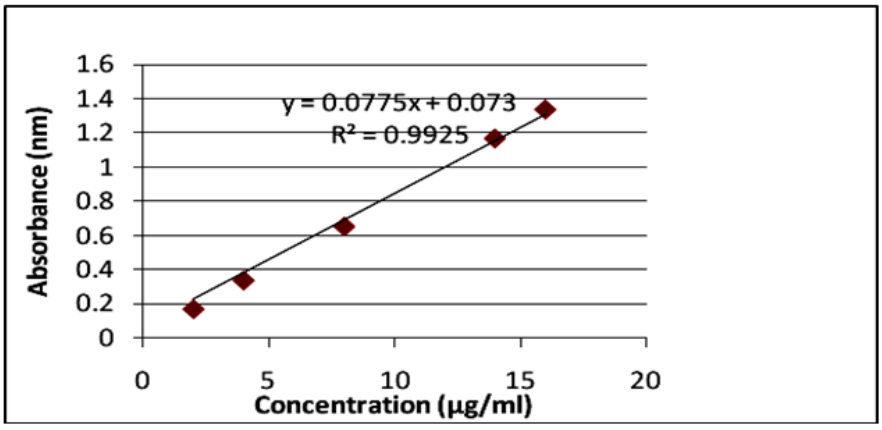


Fig.8: *Graph betweenAbsorbanceand Concentration.*

Table8: *DataforCalibration Curveof Etoricoxib.*

Parameters	**In0.1 N HCl**
Absorbancemaximum(λ_{max})innm	233
Beer'slawlimit(μg/mL)	2–24
Slope	0.073
Intercept	0.0775
Correlationcoefficient	0.9925

Absorbance of marketed formulations A and B was also scanned at λ_{max} value 233 nm. Fromthe standard plot, the amount of drug present in the different marketed formulations (Table 9)was calculated and compared with the data obtained from ATR. The readings for standardgraph and for marketed formulations were taken in triplicate every time with fresh sample.Statistical evaluation of analysis was carried out on UV spectrophotometric analysis data.Linearity was established with the help of linear regression method on the data given in Table9, from which it was seen that correlation coefficient (r^2) was significant and its value was0.9925.ANOVAone-waywasalsoappliedonTables7and9,datawassignificant($p< 0.001$)and fromt-test α = 0.5%was obtained.

Table9: *ResultsoftheMarketed Formulationsby UVSpectrophotometric Analysis.*

Formulations	**Calculated content (inonetablet,mg)**	**Absorbance(nm)**	**Obtained content(mg)**
A	90	0.728	90
B	90	0.8093	94

AnalysisofEtoricoxib-β-CyclodextrinSolidInclusionComplex

Cyclodextrins are cyclic oligomers connecting seven glucose units via α-(1, 4)-linkages,havingatoroidalshapewithanon-polarinsideandtwohydrophilicrims.Theyactasmolecular hosts for a large variety of guest molecules, polar and non-polar ones, through non-covalent interactions. They are basically used in drug formulations as solubility enhancersbecauseof their abilityto formwater solubleinclusion complexes withpoorly

watersolubledrugs[18,19].Thismethodofcomplexationmayplayaroleindrugsolubilization [20]. The detailed analysis of the solid inclusion complexes, providing theirthree-dimensional structure and lattice, can give more information about the interaction forceresponsible for their formation. Inclusion complexes are now widely used in pharmaceuticalindustry, for improving the solubility, stability and bioavailability of the guest molecules andin other areas such as the food and cosmetic industries and agrochemistry. The changesobserved in the vibrational spectra of the drug in the complex form, with respect to the purecompoundandthesolidinclusioncomplex,areindicativeoftheformationofadrug/cyclodextrin complex. In particular, when used in attenuated total reflectance (ATR)geometry, FTIR spectroscopy brings significant advantages to pharmaceutical developmentcompared with the usual technique, linked to the fact that, firstly, no sample preparation isrequiredand,secondly,FTIR-ATRspectracanbeobtainedinanon-invasiveway,i.e.,without interference due to the usual dispersion of the sample in KBr pellets. The absence ofsample manipulation guarantees rapidity in the measurement process and high reproducibilityof the spectra, making FTIR-ATR technique very adequate also in revealing differences insolid-stateformsincludinghydrationstateandpolymorphiccrystalforms,andgenerallyintheidentificationandcharacterization ofpharmaceuticals.Thecomplexformationwaschecked with the help of FTIR (Figure 8). The complex of etoricoxib with β-CD revealed ashift and slight broadening of S=O stretching vibration (1152 cm^{-1}) peak of etoricoxib. Slightshifttowardshigherfrequencywasalsoobservedat1030 cm^{-1}forabsorptionpeakcharacteristicofthecarrier.Theseobservationsmightindicatethepossibilityoftheintermolecular hydrogen bonding of the drug with the carrier. Efforts were also made toanalyzethedrug-cyclodextrincomplexwithFTIR-ATRspectroscopyusingthebaselinetechniqueandanalysis wascarriedoutina similarwayaswasdoneforthe marketedformulations. The amount of drug in the complex was found to be 20 mg in 100 mg of thecomplex, which is ± 5% range of calculated amount. FTIR-ATR spectroscopic method seemstobeeasytouseforanalyzingdrugcomplexes;however,muchworkisrequiredtobedonetoestablish this as an assay method for drug complexes.

III. CONCLUSIONS

Based on the analysis, it can be safely inferred that the amount of drug present in differentformulations obtained from FTIR-ATR method is same as that of UV-spectrophotometricanalysis (validation method). Further, this method can be used widely because of its greatprecision and added advantage in analyzing formulations containing powders, liquids, opaquesamples and rapid-absorption drugs. It also used to analyze gels, pastes, pellets, slurries,fibers,softsolid materials,surfacelayers, polymerfilms,coatings, threads,andadhesives.

It was also witnessed that it provided a high-performance approach for etoricoxib quantitativedeterminationinordertocheckthelabel-claimedcontentindifferentpharmaceuticalformulationswithinthestipulatedanalyticallimitsof90 mg ± 10%.Thismeansinpharmaceutical industry, this technique can be used most effectively because of high andpersistentdemandforqualitycontrolanalysisofpharmaceuticals.Aboveall,becauseofits improved spectral acquisition and reproducibility it can help us to determine better qualitydatabase building by following precise material verification and identification as compared toother techniques like UV-vis and IR. But, better results can be obtained when it is used inconjunctionwith other spectroscopictechniques like IR,UV, Fluorescence, Raman, etc.

Atlast,itcanbeconcludedthatitisanextremelyrobustandreliabletechniqueforquantitativeandqualitativestudies toconductexcellent sample-to-samplereproducibility.

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