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Development and Validation of UV Spectrophotometric and HPLC methods for the Concurrent determination of Tramadol and Etoricoxib

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Qualitative, Analytical, RP-HPLC, validated, UV Spectrophotometer, ICH, Quantitative

ABSTRACT:
This study's goal is to outline the creation of an analytical technique for concurrently estimating two medications, Tramadol HCL and Etoricoxib, in a combination formulation shows better analgesic effect than individual drug. As such, there is no analytical method available for Qualitative and Quantitative determination of particular drugs in both combinations. Different analytical techniques can be applied for multicomponent analysis including: spectrophotometer, chromatography and electrophoresis. The proposed approach, the analytical methods of qualitative and quantitative analysis for combination of Tramadol and Etoricoxib have been using employing a UV spectrophotometer and RP-HPLC. Absorption The subtraction technique employed in simultaneous determination utilising a UV Spectrophotometer. In high performance liquid chromatography, separation carried out using a Phenomenex C18 column with a mobile phase of acetonitrile (65:35 v/v) and phosphate buffer (20 mM) at a flow rate of 1.02 ml/min. The parameters validated in accordance with ICH requirements, and it discovered that they had acceptable levels of accuracy, precision, repeatability, and robustness. Because of this, the procedure is quick and exact, and it may be used to identify both medications at the same. The objective of validation of analytical procedures is to demonstrate that it is suitable for its intended purpose.

Introduction

Tramadol (1R,2R)-2 [(dimethylamino)methyl] A synthetic SNRI (serotonin/nor-epinephrine reuptake-inhibitor) that is primarily associated with codeine and morphine, 1- (3-methoxyphenyl) cyclohexan-1-ol [1,2] is a midway acting narcotic pain medication.

Tramadol acts through two different mechanisms: first, it inhibits the reuptake of serotonin and noradrenaline; s econd, its O-desmethyl metabolite interacts with the - opioid receptor to relieve pain.

Tramadol is effective for treating a variety of pain condi tions, such as neuropathic pain, post-operative pain, lower back pain, and labour pain Malignant growth, fibromyalgia, and osteoarthritis. (Cancer) Another inhibitor of COX-2 specifically etoricoxib,5-chloro-2-(6-methylpyridin-3yl)-3-(4 methylsulfonylphenyl)pyridine. [3,4] Rheumatoid joint inflammation, osteoarthritis, ankylosing spondylitis, persistent low back discomfort, extreme pain, and gout are now being treated. Similar to other COX-2 specific inhibitors, it focuses on impeding the enzyme cyclooxygenase compound's (COX-2) activity to reduce the generation of prostaglandins (PGs) from arachidonic acid. Individual medications, however, were said to have a weaker analgesic impact. Because of this, the globe is looking for better treatment options. In Figure 1, the structures of Tramadol and Etorcoxib are depicted.

The market is currently saturated with a huge number of different dosage form combinations. adily rising with time [2]. Pharmaceutical goods that combine the therapeutic effects of two or more medications into one product, which are commonly referred to as combination products, are intended to satisfy the needs

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of patients who have not yet been served by other treatments. The analytical chemist in charge of developing and validating analytical procedures may face formidable obstacles as a result of these combination products. For the quality assessment of novel, emerging pharmaceuticals, the development of new analytical methods is urgently needed. [3] In therapeutics, combination medication products play a venerable and crucial function. Fixed-combination medications may offer more benefits, be more affordable, and occasionally offer superior efficacy and safety when they are properly developed.

Several clinical studies have been shown that the use of combination of tramadol and etoricoxib are displayed better analgesic effect than the individual drug with occurrence of fewer side effects. As such there is no analytical method is available for qualitative and Quantitative determination of individual drug both combination. For multicomponent drug analysis, many analytical techniques, such as spectrophotometry, chromatography, and electrophoresis, can use. In this study, UV spectrophotometric techniques for simultaneous drug determination are highlighted. In the current proposal, we are developing an analytical method for RP-HPLC and UV spectrophotometer-based qualitative and quantitative analysis of the combination of tramadol and etoricoxib.

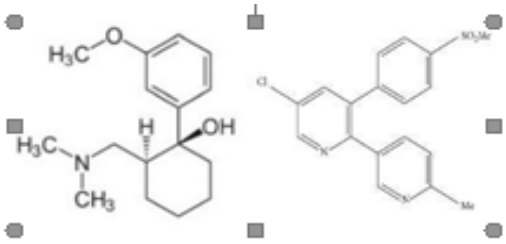

Fig.1 Structure of Tramadol and Etoricoxib.

Pre formulation

An essential step in the overall development process is pre-formulation. Prior to compounding, it is the study of the physical and chemical characteristics of the medicine. These investigations concentrate on the physicochemical characteristics of the medicine that may have an impact on its effectiveness and the creation of an effective dosage form. A full comprehension of these characteristics could eventually provide a rationale for formulation design, or support the need for molecular modification. In the simplest case, these pre-formulation investigations may merely confirm that there are no significant barriers to the compound's development. These investigations are a necessary part of the development process for a stable, reliable dosage form. By using different analytical techniques including IR spectroscopy, UV spectroscopy, melting point, etc., the drug sample that was collected was recognised (Table1).

FTIR Spectra

Any substance or medication's FT-IR (Fourier Transform Infrared) spectra can reveal the groups that are present in that specific compound. Structure analysis was conducted using FT-IR Spectroscopy. The technique used was the potassium bromide (KBr) disc method. Only the spectrum of the sample is acquired because the KBr has no absorption in the basic area of the IR spectrum. The KBr disc was prepared using 1 mg of sample in 100 mg of spectroscopic grade KBr which has been dried initially using IR lamp. KBr and respective drugs were separately mixed and subjected to hydraulic pressure to form disc. This disc was placed in FT- IR chamber. Infrared spectrum was recorded in the 4000-400 cm-1

region. FTIR of physical mixture (Tramadol Hydrochloride and toricoxib) were shown in the **figure 2** and were carried out to eliminate the possibility of interaction between drugs used in the analytical method of drug estimation. All the spectrum peaks revealed that corresponding peaks of both the drugs are present in the above spectra. Hence no interaction was observed in this mixture. [14-16].

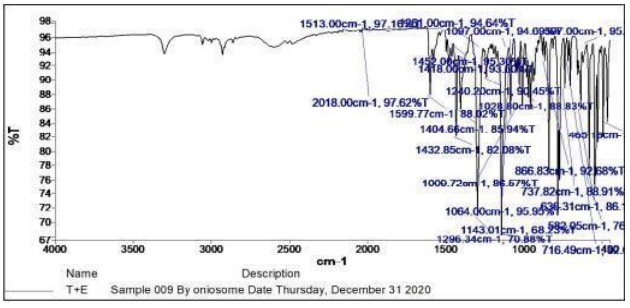

Fig. 2: FTIR spectrum of Tramadol Hcl and Etoricoxib.

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Parameters		Drugs	
		Tramadol	Etoricoxib
Colour		White	White –off white
Odour		Odourless	Odourless
Nature		Crystalline	Amorphous
Melting point (°C)		182.33±0.577	135.67±1.528
Solubility (mg/ml)	Water	1.419±0.012	0.408±0.004
	Methanol	4.912±0.049	4.337±0.021
	Ethanol	3.246±0.052	8.451±0.035
	Chloroform	11.470±0.081	4.851±0.029
Partition coefficient (noctanol:water)		1.326±0.038	3.967u±0.007

Table 1 : Preformulation Studies.

UV estimation:

Apparatus and software

UV-Visible Spectrophotometer (Shimadzu-1800, Japan) with 10 mm matched quartz cells.Analytical balance Shimadzu AUX 220), Prevalidated volumetric flasks, pipettes etc.

Reagents and Standard Solutions

Tramadol and Etoricoxib are obtained from SLR pharma pvt. Ltd, Hyderabad and Enaltec Labs, Maharashtra, resp. NaOH, NaCl, Methanol, Chloroform, n-octanol are obtained from Thermo Fischer Scientific India pvt Ltd. Ethanol (Changshu Hangsheng fine chemicals co.

Preparation of stock solutions:

From the Std. stock solutions of 1000 µg / ml, pipetted out 20 ml, placed into 100ml volumetric flask and volume was made up to mark with methanol to give a solution containing 200 µg / ml.

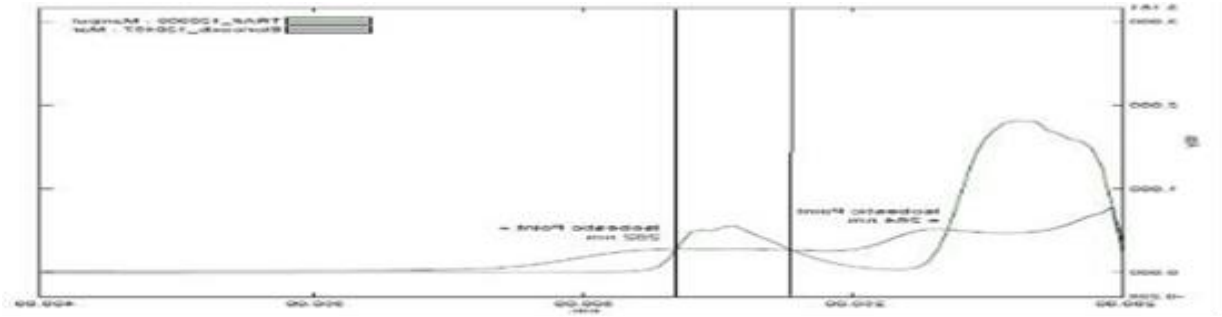
Preparation of stock solutions:

From the Std. stock solutions of 1000 µg / ml, pipetted out 20 ml, placed into 100ml volumetric flask and volume was made up to mark with methanol to give a solution containing 200 µg / ml.

Selection of analytical Wavelength:

The overlay spectrum was used to decide the isoabsorptive or isobestic point for analysis. (**Fig.**) Two Wavelengths 236.00 nm (λ max of

Etoricoxib) and 282.00 nm (Iso-absorptive point) were selected for estimation of Tramadol and Etoricoxib for Q ratio method.



Selection of analytical Concentration ranges: For the standard solution analytical concentration range were found to be 2-14 µg / ml for Etoricoxib and for Tramadol HCl, it was found to be 20-160 µg / ml. Absorbance of the solutions were recorded at 282 nm (isobestic point).

Absorbance Subtraction (AS) method [17]

For the determination of Etoricoxib and Tramadol HCl, we will utilize their isoabsorptive point at 282 nm. by

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the analysis of the recorded absorbance at the isoabsorptive point, the absorbance corresponding to Etoricoxib and Tramadol HCl, separately at isoabsorptive point 282 nm can be calculated using absorbance factor [Abs282 / Abs272] which is the average of the absorbance of different concentrations of pure Tramadol HCl using isoabsorptive point at 282 nm to that at 272 nm which shows no contribution of Etoricoxib and then the absorbance of Etoricoxib can be obtained after subtraction. Absorbance of drug in mixture at ʎ282, AbsTramadol HCl = Absorption Factor × abs ʎ272(Mixture).

AbsEtoricoxib = abs ʎ 282(Mixture) – AbsTramadol HCl

Where, abs ʎmixture is the absorbance of the binary mixture at 282 nm and abs282/ abs272 is the absorbance factor of pure Tramadol HCl at 282 nm to 272 nm and it was calculated and found to be 0.598.

Method validation (6-8) : Linearity

A calibration curve was plotted over a concentration range of 2 to 14 µg/ml for Etoricoxib at 282 nm. Accurately measured working stock solution of Etoricoxib were transferred to separate series of 10 ml volumetric flask and diluted up to the mark with methanol. The absorbance of all solution was taken at 282 nm. The Linearity was constructed by plotting concentration against absorbance. Likewise, a calibration curve was plotted over a concentration range of 20 to 160 µg/ml for Tramadol HCl at 282 nm. Accurately measured working stock solution of Tramadol HCl were transferred to separate series of 10 ml volumetric flask and diluted up to the mark with methanol. The absorbance of all solution was taken at 282 nm. The Linearity was constructed by plotting concentration against absorbance.

Precision

The term precision is defined by the ISO International Vocabulary of Basic and General Terms in Metrology (ISO-VIM) and ICH as the closeness of agreement between quantity values obtained by replicate measurements of a quantity under specified conditions. Assessing the precision implies expressing numerically the random error or the degree of dispersion of a set of individual measurements by means of the standard deviation, the variance, or the coefficient of variation. The intra-day and inter-day variation for determination of Etoricoxib and Tramadol HCl were carried out Six times in the same day and three consecutive days using concentration 2 µg/ml of Etoricoxib and 50µg/ml Tramadol HCl and % RSD was calculated. The method was found to be precise due to low values of the %RSD.

Repeatability

It is the concordance of a series of measurements of the same quantity when the experiments are conducted under same conditions (analyst, apparatus, instrument, and day) in a rapid succession. For this experiment, standard solution mixture of Etoricoxib and Tramadol HCl (2 μg/ml and 50μg/ml respectively) was prepared and analysed six times as per the proposed method.

Intermediate Precision It is the concordance of a series of measurements of the same quantity when the experiments are conducted within the same laboratory under different conditions (analyst, apparatus, instrument, and day). Standard solution mixture of Etoricoxib and Tramadol HCl (2 μg/ml and 50μg/ml respectively) was prepared and analysed as per the proposed method.

Accuracy

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the level of 80%, 100% and 120% to the preanalyzed sample. Results of the recovery study were found to be within the acceptance criteria 100±10 %, indicating a good degree of sensitivity of the method towards detection of analytes in sample. In this method the known concentration of standard drug was added to the assay sample. At each level, three determinations were performed.

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective percentage recovery was noted and the results were indicated as% RSD.

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

The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample which can

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be detected but not necessarily quantitated as an exact value. The Quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ of the proposed method were determined by using calibration curve:

LOD = $3.3\sigma/S$

LOQ = $10\sigma/S$ Where σ is the standard deviation of the response (Y intercept) and S is the slope of the calibration curve.

Robustness

Robustness is the ability to provide accurate and precise results under a variety of conditions. In order to measure the extent of method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged and

in parallel. The studied parameter was Change in wavelength. The results for robustness study in table 21 indicated that the small change in the conditions did not significantly affect the determination of Etoricoxib and Tramadol HCl.

Result & Discussion:

Upon calculation of concentration of individual drug in the mixture using simultaneous estimation method, the denominator value was found to be negative i.e. -0.0004 and therefore, another method was opted for the respective combination, which is absorption subtraction method. On calculating the Absorptivity factor of Tramadol HCl and Etoricoxib at both the isobestic points, and inputting the values for the calculation of percentage recovery, the isobestic point 282 nm provided more accurate and satisfactory results.

It was observed that there were no marked changes in the spectroscopic parameters demonstrating that spectrophotometric methods developed are rugged **(table3)**

Tramadol Hcl and Etoricoxib showed a linearity of response between 20-160, 2-14 µg/mL, respectively. The linearity was represented by a linear regression equation as summarized in **table 3** and **Fig.3&4.**

The precision of the method was demonstrated by repeatability, intraday and interday variation studies. The mean, standard deviation and the percentage of relative standard deviation were calculated and are presented in **table3**. The results reveal that a low relative standard deviation showing that the developed methods are precise.

An analysis of results shows that the percentage recovery values are close to 100% and also the relative standard deviation values are less than 1% thus establishing that the developed methods are accurate and reliable. **(Table 2)**

The results for robustness study in **(Table 4)** indicated that the small change in the conditions did not significantly affect the determination of Etoricoxib and Tramadol HCl.

The LOD was found to be 0.065µg/ml and 0.160µg/ml for Etoricoxib and Tramadol HCl respectively and LOQ was found to be 0.197µg/ml and 0.486µg/ml Etoricoxib and Tramadol HCl respectively **(table 5)** which showed that sensitivity of the method was high.

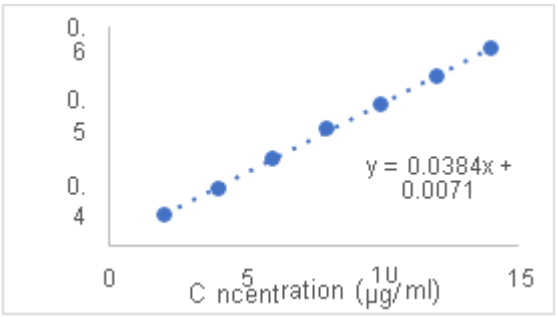

Fig. 3: Linearity graph of Etoricoxib

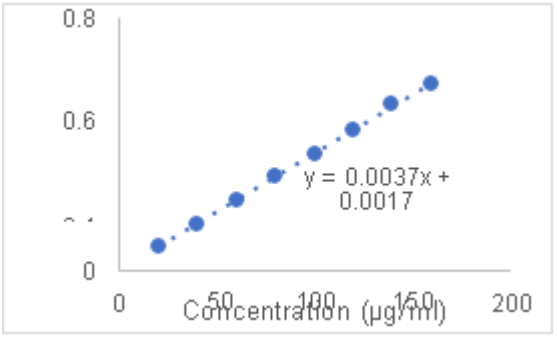

Fig.4 linearity graph of Tramadol HCl

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Drug	% Recovery level	Conc. Added (µg/ml)	%mean Recovery	%RSD
Etoricoxib	80%	1	101.158±1.088	1.075
	100%	2	99.692±0.546	0.548
	120%	3	99.305±0.819	0.824
Tramadol	80%	25	101.285±0.988	0.975
	100%	50	102.146±0.646	0.633
	120%	75	100.709±0.449	0.445

Table2: Recovery Studies

Method parameter(282nm)	**Tramadol Hcl**	**Etoricoxib**
Linearity Range	20-160	2-4
Regression equation	Y= 0.0037x+0.0006	Y= 0.0384x+0.0074
Intercept	0.0006	0.0074
Slope	0.0037	0.0384
Correlation coefficient(R^2)	0.9999	0.9992
Precision		
Repeatability (Interday)	98.860±0.593(%RSD-0.600)	99.842±1.203(%RSD-1.230)
Repeatability(Intraday)	99.075±0.569(%RSD-0.574)	101.447±0.896(%RSD-0.883)
Intermediate precision	104.568±0.670(%RSD-0.641)	102.060±1.159(%RSD-1.136)
Ruggedness		
Analyst 1	99.398±0.490(%RSD-0.493)	100.451±1.322(%RSD-1.316)
Analyst 2	96.812±0.339(%RSD-0.350)	102.123±1.058(%RSD-1.036)

Table3: Validation results obtained by applying isobestic assay spectrophotometric method. Precision, Ruggedness and Robustness at Tramadol (50 µg/mL) and Etoricoxib (2 µg/mL).

Analytical balance (Shimadzu, AUX220), Digital pH meter (Lab India, OHPL-OP_024), Bath

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Abs1 (277nm)	**0.303**	**0.005**	**1.533**
Abs2 (282nm)	**0.270**	**0.005**	**1.704**
Abs3 (287nm)	**0.211**	**0.004**	**1.978**

Table 4: Robustness Studies

Parameter		**Tramadol Hcl**	**Etoricoxib**
LOD		0.160 µg / ml	0.065 µg / ml
LOQ		0.486 µg / ml	0.197 µg / ml

Table 5 : LOD & LOQ values for Tramadol HCl and Etoricoxib.

HPLC estimation

Instrumentation

RP-HPLC instrument equipped with SPD-10AVP UV-vis detector (Shimadzu, Japan), an auto- sampler, Phenomenex, C18 (4.6 × 250 mm i.d., 5µm particle size) and an LC-solution software, Sonicator (PCI, Mumbai, NSW-133), Pre-validated volumetric flasks, pipettes etc.

Reagents and materials

Tramadol and Etoricoxib are obtained from SLR pharma pvt. Ltd, Hyderabad and Enaltec Labs, Maharashtra, resp. Potassium dihydrogen

phosphate (Thermo Fisher Scientific, India)**,** Acetonitrile (Thermo Fisher Scientific, India)**,** Double distilled water (HPLC grade)**,** Nylon 0.22 µm membrane filter (Pall corporation, Mumbai).

Chromatographic method

The isocratic mobile phase consisted of 20mM phosphate buffer (pH 4.6) and Acetonitrile in the ratio of 65:35, v/v, flowing through the column at a constant flow rate of 1.02 mL/min. A Phenomenex (C-18) Column (250 × 4.6 mm, 5 µm) was used as the stationary phase. Tramadol Hcl amd Etoricoxib have different λmax (viz 236, 272 nm, respectively), but considering the chromatographic parameter, sensitivity, and selectivity of the method for both the drugs, 262 nm was selected as the detection wavelength for UV-PDA detector. [21-22]

Standard Stock Solution Preparation Blank: Diluent was filtered through 0.22 µ millipore membrane filters and injected in HPLC system. **Standard stock solution preparation:** 10 mg of tramadol and 10 mg of etoricoxib drug was accurately weighed and separately put in to 10 ml volumetric flask containing 5 ml of diluents. The solution was sonicated for 10 min then the volume adjustment was made up to mark with diluents. **Preparation of sample solution:** Sample solution of different conc. of tramadol and etoricoxib was prepared from above stock solution and diluted with diluent and filtered through 0.22 µ millipore membrane filters and injected in HPLC system.

Method validation Linearity

A calibration curve was plotted over a concentration range of 10 to 55 µg/ml for Tramadol HCl and 1 to 10 µg/ml for Etoricoxib. Accurately measured volume of working stock solution of Tramadol HCl and Etoricoxib were transferred to separate series of 10 ml volumetric flask and diluted up to the mark with methanol. Prepared solutions was filtered through 0.22µm membrane filter and injected for HPLC analysis. The

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Linearity was constructed by plotting concentration against area.

Precision

The term precision is defined by the ISO International Vocabulary of Basic and General Terms in Metrology (ISO-VIM) and ICH as the closeness of agreement between quantity values obtained by replicate measurements of a quantity under specified conditions. Assessing the precision implies expressing numerically the random error or the degree of dispersion of a set of individual measurements by means of the standard deviation, the variance, or the coefficient of variation. The intra-day and inter-day variation for determination of Tramadol HCl and Etoricoxib were carried out Six times in the same day and three consecutive days using concentration 40 µg/ml of Tramadol HCl and 7 µg/ml Etoricoxib and % RSD was calculated. The method was found to be precise due to low values of the %RSD**.**

Repeatability

It is the concordance of a series of measurements of the same quantity when the experiments are conducted under same conditions (analyst, apparatus, instrument, and day) in a rapid succession. For this experiment, standard solution mixture of Tramadol HCl and Etoricoxib (40μg/ml and 7μg/ml respectively) was prepared and analysed six times as per the proposed method.

Intermediate Precision .

It is the concordance of a series of measurements of the same quantity when the experiments are conducted within the same laboratory under different conditions (analyst, apparatus, instrument, and day). Standard solution mixture of Tramadol HCl and Etoricoxib (40 μg/ml and 7μg ml respectively) was prepared and analysed as per the proposed method.

Accuracy

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the level of 80%, 100% and 120% to the preanalyzed sample. Results of the recovery study were found to be within the acceptance criteria 100±5 %, indicating a good degree of sensitivity of the method towards detection of analytes in sample. In this method the known concentration of standard drug was added to the assay sample. At each level, three determinations were performed. Percent mean recovery was calculated as shown in table

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective percentage recovery was noted and the results were indicated as % RSD.

LOD& LOQ

The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value. The Quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ of the proposed method were determined by using calibration curve:

$$LOD = \frac{3}{S}.3\sigma \qquad LOQ = \frac{10}{S}\sigma$$

Where σ is the standard deviation of the response (Y intercept) and S is the slope of the calibration curve.

Robustness

Robustness is the ability to provide accurate and precise results under a variety of conditions. In order to measure the extent of method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged and in parallel. The studied parameter was Change in wavelength and change in flow rate.

Result & Discussion

Method : The optimization of HPLC method was done by using the design expert software. In development of method, Box-Behnken design was employed to study the effect of independent variables over the dependent variables as they showed a wide variation among the 17 batches (R 1-R 17). Independent variables were Flow rate (ml/min) (A), mobile phase ratio (B) and wavelength (nm.) (C). Area of Tramadol (R1), Retention time of Tramadol (min) (R2), Area of Etoricoxib (R3) and Retention time of Etoricoxib (R4) were considered as dependent variables. The optimized

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HPLC method presented the experimentally observed values of Etoricoxib area of 573030 ± 639.704 with Tramadol retention time of 3.471 ± 0.0302. These experimental values of Etoricoxib area, Tramadol retention time by the optimized HPLC method solutions were found in agreement with the predicted value of Etoricoxib area (573030), Tramadol retention time (3.471 min) respectively generated by design expert software, suggesting that the optimized method was rational and reliable. Simultaneous chromatogram of standards drugs and method optimization conditions is seen in **figure 5.**

The injection volume was 20 µl and methanol was used as diluent with the run time of 12 minutes.

Optimum HPLC condition.

- Stationary Phase: C_{18}, 250 × 4.6 mm, 5µ particle size, Phenomenex
- Elution mode: Isocratic elution mode (65:35 v/v)
- Mobile phase: Solvent A was 20 mM phosphate buffer pH 4.6 and Solvent B was Acetonitrile.
- Detector: UV-visible detector
- Absorption maxima: 262 nm
- Column Temperature:30 °C
- Flow rate: 1.0 ml/min.
- Injection volume: 20 µl
- Diluent: Methanol
- Run Time:12 minutes

Tramadol HCl and Etoricoxib showed a linearity of response between 10-55 and 1-10 µg/mL, respectively. The linearity was represented by a linear regression equation as follows. (Fig.6&7) Y (Tramadol HCl) = 6415.2 conc. + 17094

(r2 = 0.9962)

Y (Etoricoxib)= 68186conc. - 5476.1

(r2 = 0.9935)

The accuracy of the method was determined by recovery experiments. Recovery studies were carried out three times and the % recovery; mean and relative standard deviations were calculated and are presented in table 43-45 for HPLC analysis. An analysis of results shows that the percentage recovery values are close to 100% and also the relative standard deviation values are less than 1% thus establishing that the developed methods are accurate and reliable. **(Table 6)**

The precision of the method was demonstrated by repeatability, intraday and interday variation studies. The mean, standard deviation and the percentage of relative standard deviation were calculated and are presented in **table7**. The results reveal that a low relative standard deviation showing that the developed methods are precise.

The ruggedness of the methods was studied by changing the experimental conditions. In the present work, two different analysts performed the method with same set of parameters and mean, standard deviation and relative standard deviation was

calculated. It was observed that there were no marked changes in the Chromatographic parameters demonstrating that the HPLC method developed are rugged. (**Table7)**

The limit of detection (LOD) and limit of quantification (LOQ) of the developed HPLC methods were determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD was found to be 0.876 µg/ml and 0.141 µg/ml for Tramadol HCl and Etoricoxib respectively and LOQ was found to be 2.655 µg/ml and 0.428 µg/ml Tramadol HCl and Etoricoxib respectively which showed that sensitivity of the method was high.

The robustness of the methods were studied by changing the experimental conditions. In the present work, wavelength (±2 nm) and flow rate (±0.2 ml/min) was altered and mean, standard deviation and relative standard deviation was calculated. It was observed that there were no marked changes in the spectroscopic parameters demonstrating that the HPLC method developed are robust. **(Table 8).**

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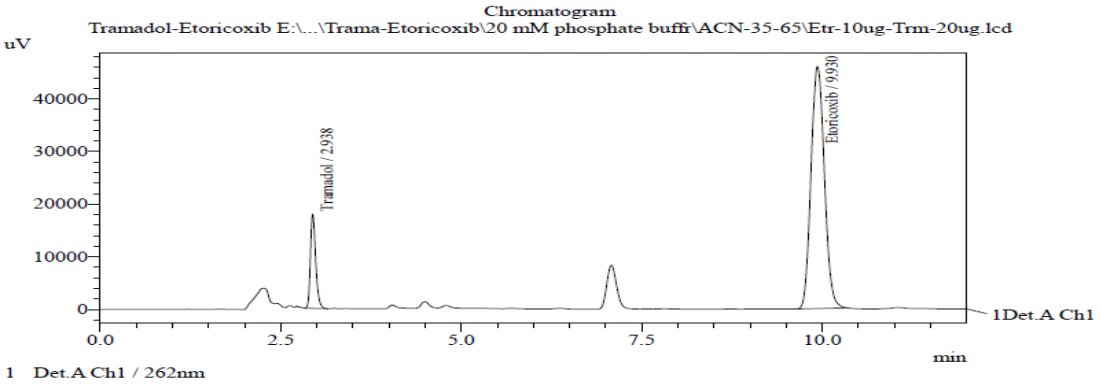

Figure 5: Simultaneous chromatogram of standards Tramadol HCl (30µg/ml) and Etoricoxib (10µg/ml).

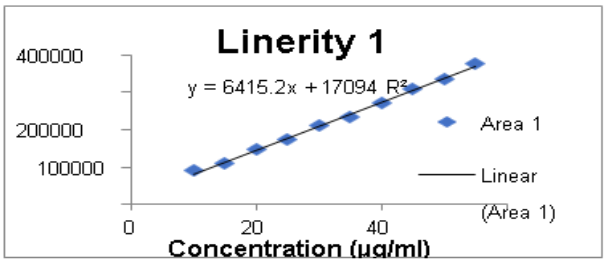

Fig. 6 Linearity Studies of Tramadol

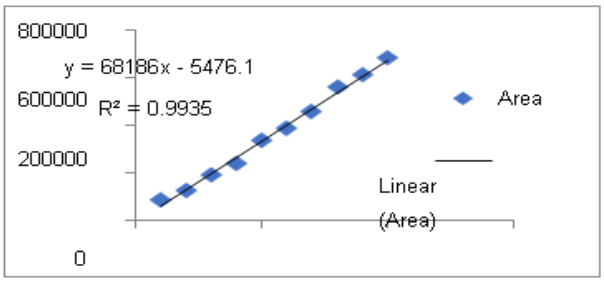

Fig. 7 Linearity Studies of Etoricoxib

Table 6 : Result of Recovery Studies.

Drug	% Recovery level	Conc. Added (µg/ml)	%mean Recovery	%RSD
Etoricoxib	80%	8	104.219±0.135	0.129
	100%	10	100.913±0.803	0.796
	120%	12	102.93±0.096	0.094
Tramadol	80%	45	102.636±0.517	0.504
	100%	55	103.157±1.09	1.06
	120%	65	99.199±0.865	0.872

Wavelength	**Mean**	**SD**	**%RSD**
Abs1 (260nm)	330117.3	1967.032	0.595
Abs2 (284nm)	440502.7	407.9036	0.092
Flow rate			
0.8ml/min	484330	1084.953	0.224
1.2ml/min	317125.3	2182.827	0.688

Table 8: Robustness Studies Gel assay

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Accurately weighed quantity of prepared gel equivalent to 10 mg Tramadol HCl and 3 mg Etoricoxib was transferred to 10 ml volumetric flask and allowed to sonicate for 5 minutes and volume was makeup to mark using diluent. Further

0.1 ml of test solution was transferred to 10 ml volumetric flask and volume was make upto mark using diluent was filtered through 0.22 µ millipore membrane filters and injected in HPLC system. From the above **table 9** the percentage assay of 101.98±0.082% and 96.28±0.876% for Tramadol HCl and Etoricoxib was found in within limits which indicated that the optimized method was accurate and precise.

Table 7: Results of Repeatability (interday & intraday) and intermediate precision.

S.no.	Validation paramet er	% mean Recovery		% RSD	
		Etoricoxib	Tramadol	Etoricoxib	Tramadol
1.	Repeatability (Inter day)	98.114±0.797	100.289±0.524	0.812	0.523
2.	Repeatability (Intraday)	98.138±0.432	100.318±0.369	0.440	0.368
3.	Intermediate precision	98.249±0.944	101.356±0.723	0.960	0.713
4.	Ruggedness (Analyst 1)	98.1797±0.834	100.882±0.455	0.849	0.451
5.	Ruggedness (Analyst 2)	97.42±1.03	100.594±0.642	1.05	0.638

Table9:AssayStudies

2% Carbopol gel of Tramadol HCl and Etoricoxib		
	Tramadol HCl	**Etoricoxib**
Claimed conc. (mg)	10	3
Observed conc. (mg ± SD)	10.19±0.008	2.88±0.026
% Assay ± SD	101.98±0.082	96.28±0.876

Conclusion:

Combination drug products occupy a time Honored and important role in therapeutics. When rationally formulated, fixed combination drugs may produce greater convenience, lower cost and sometime greater efficacy and safety.

In present proposal, the analytical method for qualitative and quantitative analysis of combination of Tramadol Hcl and Etoricoxib by UV spectrophotometer and HPLC was developed. This method is fast,

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accurate, precise and sensitive; hence, it can be employed for routine quality control of the combination of both drugs in formulation.

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