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Spectrophotometric Method Development with Green Approach for the Estimation of Tolperisone and Etoricoxib from their Dosage Form

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Abstract: There is consistently pressure from environmental department to minimise hazardous and volatile solvent content in the waste which seriously affects environment. The aim of the present research was to develop analytical method utilizing ecologically suitable common solvent which enhances solubility of analyte, sensitivity of the method etc. Chemo metric assisted absorption spectrophotometric analytical method was developed for the estimation of Tolperisone (TPS) and Etoricoxib (ECB) from their combined formulation. Simultaneous equation, Q-absorbance method were selected from the nature of spectra, solvent 0.1 N HCl was utilised; and for method 261 nm and 233 nm was the wavelength for measurement of absorbance of TPS and ECB respectively. Effect of input variables on spectrum characteristics were studied for selection of critical parameters and developed method was validated as per ICH Q 2 R1 regulatory guidelines. Linearity of the drugs was ascertained over the conc range 1-20 mcg/ml (microgram/ml) for TPS and 1-12 mcg/ml for ECB. The percentage purity of assay was found 99.76 % for TPS and 101.33 % for ECB; and the accuracy study data were varied from 98.89 to 102.34% for TPS and 98.05 to 101.39% for ECB. Precision study was shown acceptable data as SD data varied from 1.2268 to 3.7711 for TPS and from 0.2249 to 3.2143 for ECB. The developed method is rigid, robust and efficient for the estimation of TPS and ECB from the combined formulation. Commonly available solvent 0.1 N HCl made the developed method low cost, economic and environmental sound.

Keywords Tolperisone, Etoricoxib, QbD, ICH, simultaneous equation method, Q-method

1. Introduction

Tolperisone (TPS) is 2-Methyl-1- (4-methylphenyl) - 3- (1- piperidinyl) -1- propanone a central muscle relaxant used to treat cerebral arteriosclerosis and extrapyramidal movement disorders [1, 2].

Various analytical methods have been reported for the estimation of TPS alone or in combination with other drugs in pharmaceutical dosage form includes lonely UV spectroscopic methods [3], HPLC methods for lonely detection [4, 5], with other drug HPLC methods [6], stability indicating HPLC method [7].

Etoricoxib (ECB) chemically 5-Chloro-2-(6-methyl-3-pyridinyl)-3-(4-methyl-sulfonyl phenyl) pyridine [8] is an NSAID and selective inhibitor of cyclo-oxygenase-2. It is used in the symptomatic relief of rheumatoid arthritis, osteoarthritis, spondylitis and acute gouty arthritis [1, 8].

Various analytical methods for the estimation of (ECB) alone or in combination with other drugs includes lonely UV spectroscopic method [9], with other UV spectroscopic methods [10-13] , HPLC methods for lonely detection [14-16], with other drug HPLC chromatographic methods [17-21], stability indicating HPLC method [22], bio analytical LC-MS/MS method [23], GC-HS method [24], stability indicating UFLC analytical method [25] , efficacy and safety of drug study [26] have been reported. Etoricoxib is official in Indian Pharmacopoeia [27]. ICH guidelines were referred for method development and validation [28, 29]. Chemical structure of both drug molecules is shown in Fig 1.

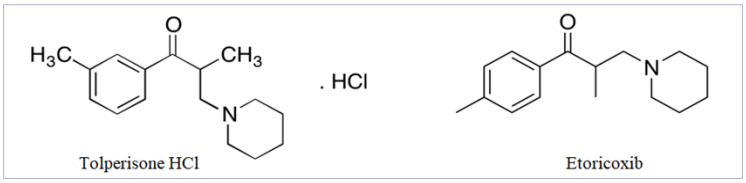

Figure 1: Chemical structure of drug molecule

2. Materials And Methods

Instrumentation

UV-1900i Shimadzu Double beam spectrophotometer (Shimadzu, Kyoto, Japan) was the instrument for method development with a spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm; and for scanning of solutions 10 mm matched Quartz cells were used. Drugs were weighed on electronic balance 'Afcoset' (The Bombay Burmah Trading corpo Ltd) with accuracy ± 0.1 mg Model No. ER 200A and Digital Ultrasonic cleaner 1.8 Ltr (Labman scientific Instruments Chennai) was used for degassing the solutions.

Reagents and Chemicals

Pharmaceutically pure samples of TPS was procured as a gift samples from Akums Drugs and Pharmaceuticals Ltd Haridwar UK, and ECB was procured as a gift samples from Macleods Pharmaceuticals Ltd, Mumbai Maharashtra, HCl acid (AR Grade) and laboratory distilled purified water was used as solvent and the commercial formulation containing tolperisone HCl 150 mg and etoricoxib 60 mg was procured from the local market.

Solvent selection

Literature survey reported solubility of TPS in water and more stability in acidic solutions [1].

ECB is freely soluble in tetrahydrofuran, DMSO and in DMF, soluble in methanol, 0.1 N HCl and in acetone and sparingly soluble in ethanol [2, 8].

Although the solubility of the procured both drugs were studied in dstilled water, 0.1 N HCl and 0.1 N NaOH separately; and found that in 0.1 N HCl both ECB and TPS were appreciably soluble, Hence 0.1 N HCl was selected as common solvent for complete analysis. Solution with known conc. of analyte was scanned in UV range of 400 nm to 200 nm. The recorded spectra in solvent are shown in Fig 2, and 3. It was found that suitable solvent is 0.1 N HCl with respect to measurable absorbance of both drug, average cost, robust and precise in producing result.

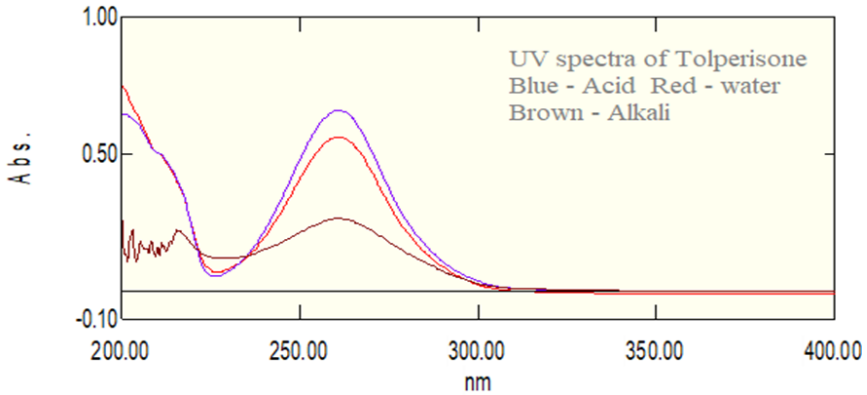

Figure 2: UV overlain spectra of Tolperisone obtained in different solvent

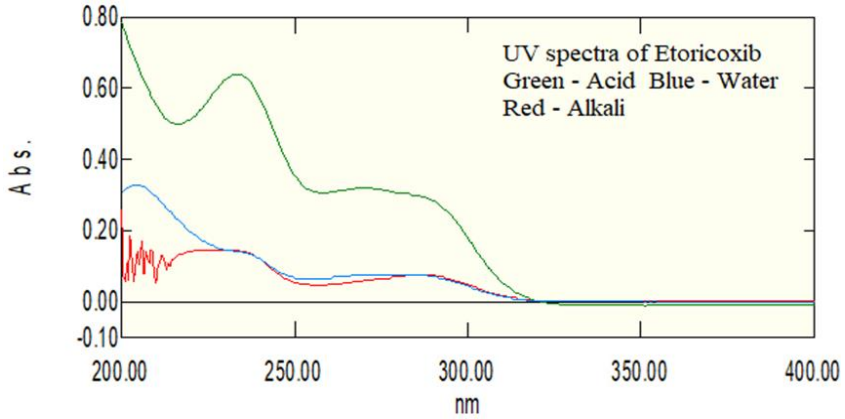

Figure 3: UV overlain spectra of Etoricoxib obtained in different solvent

Preparation of stock solutions and standard solutions

10 mg each of drug TPS and ECB were separately and accurately weighed; and transferred into separate 25 ml volumetric flasks. Dissolved into 0.1 N HCl and volume was made to 25 ml with solvent. Subsequent standard solution of each drug with conc 8µg/ml was prepared by diluting aliquot 0.5 ml of stock solution to 25 ml into 25 ml capacity volumetric flask.

Selection of wavelength and conc range

Standard solution of TPS and ECB with conc 8 μ_g/ml was prepared and scanned in the spectrum mode from 400 nm to 200 nm. From UV spectra (Fig 4) it was found that TPS has measurable absorbance at 261 nm (λ_{max}) and less interference was observed by ECB; similarly ECB has maximum absorbance at 233 nm (λ_{max}) and less interference by TPS. Chemometric methods viz. simultaneous equation method and Q method were reasonable remedy to overcome interference at each other's absorbance hence applied for estimation. From the nature of spectra to study linearity, working conc range 1 to 12µg/ml for ECB and 1 to 20µg/ml TPS was selected. Also combined drug solution was prepared simulated to marketed formulation. Selected critical parameters based upon above discussion, observations were listed and by using these; method was validated as per ICH guidelines and by analysing marketed preparations [28].

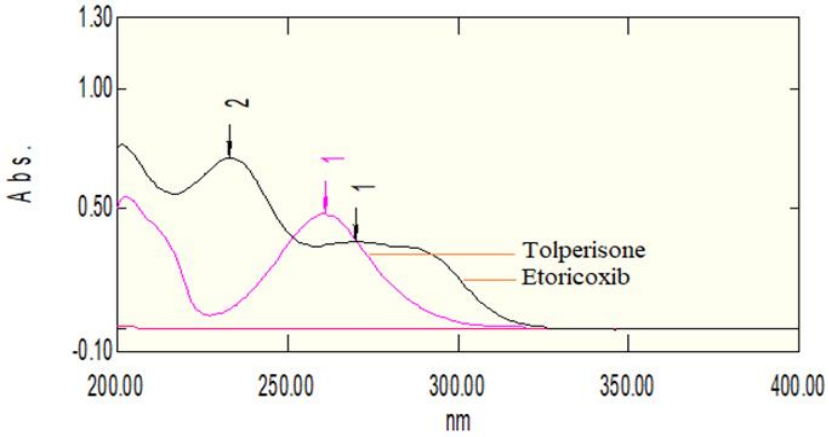

Figure 4: UV spectra of both drugs for wavelength selection

Experimental Method for estimation

From the overlain spectra it was found that many approaches of multicomponent analysis are suitable for simultaneous estimation of both the drugs. Among of this simultaneous equation method, absorption ratio methods were selected for estimation of TPS and ECB from the combined dosage form.

Method-I: Simultaneous equation method

ECB was shown absorbance at (λ_{max}) 233 nm and TPS has maximum absorbance (λ_{max}) at 261 nm. The wavelength 233 and 261 nm was considered as 1 (λ_1) and 2 (λ_2) respectively. The equation A= abc was applied for x (ECB) and y (TPS) determination. On rearranging the 2 generated equations, the conc of x and y was calculated by following formula. Working standard solutions of ECB of conc 8 μ_g /ml and TPS of conc 8 μ_g /ml were separately prepared and used for the method.

$$C_x = \frac{A_2 . a_{y_1} - A_1 . a_{y_2}}{a_{x_2} . a_{y_1} - a_{x_1} . a_{y_2}}$$

$$C_y = \frac{A_1 . a_{x_2} - A_2 . a_{x_1}}{a_{y_1} . a_{x_2} - a_{y_2} . a_{x_1}}$$

Where

Cx and Cy = Conc of ECB and TPS in sample solution

A1 and A_2 = absorbance of sample solution at 1 and 2 wavelength

ay1 and ay_2 = absorptivity of TPS at 1 and 2 wavelength of standard solution

ax1 and ax_2 = absorptivity of ECB at 1 and 2 wavelength of standard solution

Method-II Absorption ratio method

The absorption ratio method is modification of simultaneous equation method. It is based upon fact that the ratio of absorbance at any two wavelengths is constant value independent of conc. or pathlengths. Two different dilute solutions of same drug give the same absorption ratio A_1/ A_2. Two wavelengths are being selected as λ_1 (where absorptivity of both the drug remains constant) and λ_2 (λ max of one of the drug). The wavelength at which two drugs show similar absorptivity is known as iso-absorptive point (shown in the figure). There should not interference of any other component like excipients, other drug except X and Y.

The absorptivity of X Etoricoxib at λ_1 and λ_2 are aX1 and aX2 respectively.

The absorptivity of Y tolperisone at λ_1 and λ_2 are aY1 and aY2 respectively.

$$C_X = \frac{Qm - Q_Y}{Qx - Q_Y} \cdot \frac{A}{ax_1} \qquad C_Y = \frac{Qm - Qx}{Q_Y - Qx} \cdot \frac{A}{aY_1}$$

$$Q_m = \frac{A_2}{A_1} \qquad Q_X = \frac{ax_2}{ax_1} \qquad Q_Y = \frac{aY_2}{aY_1}$$

Where

Cx and CY - Concentrations of ECB and TPS respectively. (g/100 ml)

Qx - Ratio of absorptivity of ECB at 270 and 233 nm

QY - Ratio of absorptivity of TPS at 270 and 233 nm

Qm - Ratio of absorbance of sample solution at 270 and 233 nm

A - Absorbance of sample solution at Isobestic point

aX1- Absorptivity of ECB at Isobestic point

aY1- Absorptivity of TPS at Isobestic point

Validation of the Method

Selected critical parameters should meet the performance characteristics of the analytical method so as to attain analytical target profile of the method. An ICH guideline Q2 R1 was applied to study methods performance with critical parameters in order to implement part of AQbD approach. The method was validated as per ICH guidelines

System suitability

System suitability is studied to demonstrate the suitability of the developed procedure under consideration for the analytical method. Six replicates of working standard solutions with conc each of 8mcg/ml of TPS and ECB were prepared separately and absorbance was recorded, and SD and % RSD of the response were calculated.

Linearity

The linearity of an analytical method is its ability to obtain response i.e. absorbance which is directly proportional to the conc of analyte. Series of working standard solutions were prepared in conc. range of 1-12 μg/ml for ECB and 1-20 μg/ml for TPS and scanned in 200 to 400 nm range in spectrum mode of the spectrophotometer, absorbance of the standard solutions were recorded at their respective wavelength, i.e. 233 for ECB and 261 nm for TPS in spectrum order. Microsoft office excel software tool was used to obtain the standard regression curve and its analysis as slope, intercept, and correlation coefficient.

Assay of formulation

Assay was carried out by proposed methods and assay was validated by statistical parameters.

Estimation of formulations by simultaneous equation method

Tablet powder equivalent to 15 mg TPS and 6 mg ECB was weighed and transferred into 100 ml volumetric flask. Dissolved into 0.1 N HCl and volume was made to 100ml with solvent. Solution was filtered through what man filter paper and aliquots of solution were further diluted to obtain tablet solution. Solution was scanned in the range of 200 to 400 nm to obtain absorbance of tablet solution at 233 and 261 nm in spectrum order. Obtained absorbance were utilised to estimate unknown conc of formulation; and results were statistically validated to obtain % of nominal conc, standard deviation and % of RSD.

Estimation of formulations by Q method

Tablet powder equivalent to 15 mg TPS and 6 mg ECB was weighed and transferred into 100 ml volumetric flask. Dissolved into 0.1 N HCl and volume was made to 100ml with solvent. Solution was filtered through what man filter paper and aliquots of solution were further diluted to obtain tablet solution. Solution was scanned in the range of 200 to 400 nm to obtain absorbance of tablet solution at 233 and 270 nm in spectrum order. Obtained absorbance were utilised to estimate unknown conc of formulation; and results were statistically validated to obtain % of nominal conc, standard deviation and % of RSD.

Accuracy and Precision

The accuracy of an analytical method expresses the closeness of an agreement between test result and true result. Accuracy study was performed by recovery study i.e. standard addition method; diluted standard solutions of TPS and ECB were prepared and standard solutions added in 80,100 and 120% proportionate to the tablet solution. Three replicates at each of these three levels were prepared and measured and % of conc, SD and RSD were calculated.

The precision study was carried out by performing assay of tablet six times; also, the reproducibility in result was studied by inter day and intraday precision.

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

The LOD and LOQ of TPS and ECB by the proposed method were determined using calibration graph method and calculated as $3.3\sigma/s$ and $10\ \sigma/s$ for LOD and LOQ respectively; σ is the standard deviation of calibration curve and s is the slope of regression line.

Robustness and Ruggedness

It is measure of capacity of analytical procedure to remain unaffected by small but deliberate variations in method parameter.

3. Results and Discussion

Method development comprises numerous steps; of which solvent selection, method for measurement selection are significant one. Uses of eco-friendly solvents have got remarkable weightage due to low cost, readily available and environmentally sound [30]. Drugs underlying analysis must have appreciable solubility in the selected solvent. Chemical structure of the drug and physico-chemical properties available in the literature guides about use of

appropriate solvent in the method. Solubility of TPS and ECB was studied in each solvent; and in 0.1 N HCl solvent both drugs were shown maximum and consistent absorbance as compare to other solvent.

System Suitability

The absorbances of six replicates of standard solutions (6 and 12 µg/ml) are reported in Table No 1. The SD was found for TPS and ECB within acceptable limit and meets the system suitability requirements indicates method was suitable for analysis.

Table 1: System suitability study of TPS and ECB

Sr No	Conc in µg /ml	Absorbance of TPS	Conc in µg /ml	Absorbance of ECB
1	12 µg /ml	0.7482	6 µg /ml	0.5222
2	12 µg /ml	0.7638	6 µg /ml	0.5581
3	12 µg /ml	0.7548	6 µg /ml	0.5486
4	12 µg /ml	0.7465	6 µg /ml	0.5655
5	12 µg /ml	0.7681	6 µg /ml	0.5616
6	12 µg /ml	0.7348	6 µg /ml	0.5537
	SD	0.01219	**SD**	0.01557

Linearity

The overlay spectra obtained in linearity study was shown in Fig No 5 and 6 and the obtained calibration curve of both analytes was found to be linear in the selected conc range as shown in Fig No 7. The regression equation of line and its parameters slope, r2 value and intercept are tabulated in Table No 2, which proved the linear relationship between conc and obtained response.

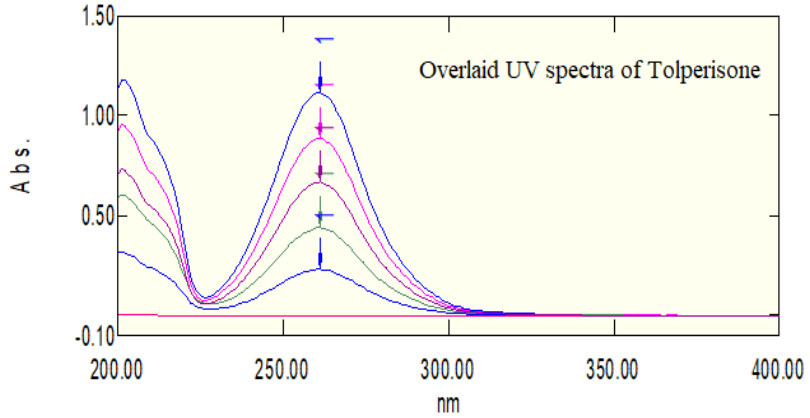

Figure 5: UV-VIS overlain spectra of TPS in linearity study

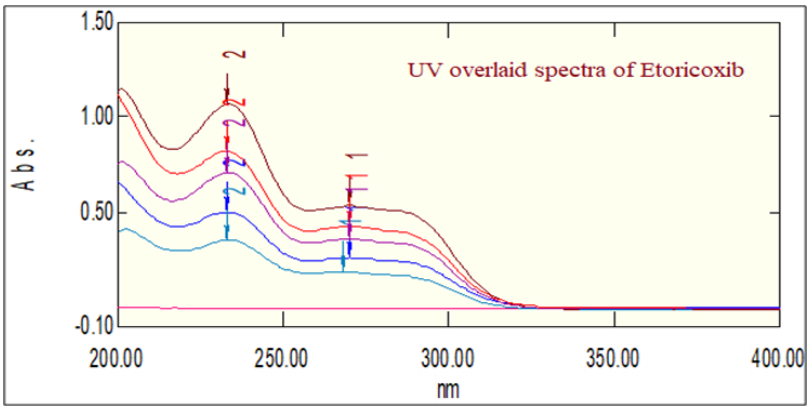

Figure 6: UV-VIS overlain spectra of ECB in linearity study

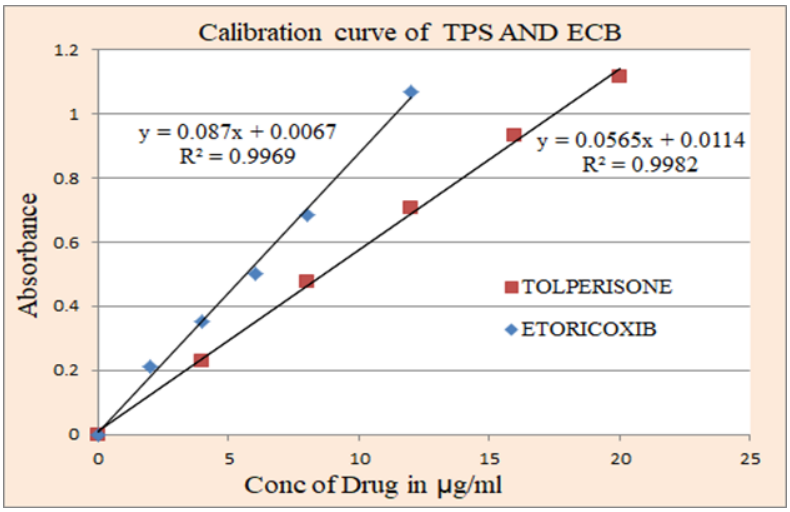

Figure 7: Calibration curve of both drug

Table 2: Parameters of regression equation obtained in Microsoft excel office

Parameters	TPS	ECB
Detection wavelength	252 nm	233 nm
Solvent	0.1 N HCl	0.1 N HCl
Beer's law limit (µg/ml)	4-20 µg/ml	1-12 µg/ml
Correlation coefficient (r^2)	0.9982	0.9969
Regression equation (y = mx + c)	y = 0.0565 x + 0.0114	y = 0.087 +0.0067

Assay

The assay was carried out by calibration curve method. The spectra of formulation was obtained and calculated % of nominal conc and SD, data was found within acceptable limits are summarized in Table No 3. The results indicated applicability of the method for estimation of Formulation.

Table 3: Results of assay of formulation by proposed method

Formulation	Drug	Label Claim (mg/Tablet)	Amount found/mg; n=6	Drug Content %	Std Deviation	% RSD
METHOD-I	TPS	150 mg	154.48	102.99	3.6055	3.5005
	ECB	60 mg	59.85	99.76	0.6352	0.63.05
METHOD-II	TPS	150 mg	149.64	99.76	1.2583	1.2614
	ECB	60 mg	60.79	101.33	3.0411	3.9882

Accuracy and Precision

The results of accuracy are summarised in Table No 4 and 5, the obtained results were within acceptable limit; and methods accuracy was justified by calculating % drug content. The precision study was carried out by performing assay of solutions; further the reproducibility in result was studied by interday and intraday precision. The values obtained SD and % RSD was shown methods precision and are summarised in Table No 4 and 5.

Table 4: Results of accuracy and precision of Method I

S. No.	Parameter	Level of study	Data Title	Obtd. Data	S.D.	RSD
1	Precision study of TPS	Intraday Precision	Mean of conc n=6	101.75	3.7711	3.7061
		Interday precision		98.22	1.2051	1.2268

1	Precision study of ECB	Intraday Precision	Mean of conc n=6	100.93	3.2143	3.1846
		Interday precision		101.84	1.3421	1.3176
2	Accuracy study of ECB	80%	% Purity found	97.64	0.5448	0.5581
		100%		100.48	2.2391	2.2284
		120%		106.55	1.2692	1.1911
2	Accuracy study of TPS	80 %	% Purity	102.34	0.4041	0.3948
		100 %		98.89	2.1176	2.1412
		120 %		101.89	3.2568	3.1962

Table 5: Results of accuracy and precision of Method II

S. No.	Parameter	Level of study	Data Title	Obtd. Data	S.D.	RSD
1	Precision study of TPS	Intraday Precision	Mean of conc n= 6	102.26	1.8019	1.7621
		Interday precision		103.76	1.8342	1.7677
1	Precision study of ECB	Intraday Precision	Mean of conc n= 6	102.93	0.2315	0.2249
		Interday precision		96.05	0.2599	0.2705
2	Accuracy study of ECB	80%	% Purity found	100.83	0.8726	0.8654
		100%		98.05	0.7087	0.7228
		120%		101.39	1.5033	1.4826
2	Accuracy study of TPS	80 %	% Purity Found	100.38	0.3428	0.3414
		100 %		102.12	1.5679	1.5353
		120 %		105.21	2.7163	2.5819

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

The LOD and LOQ of TPS and ECB by the proposed method were found within acceptable limit.

Robustness and Ruggedness

Robustness was studied and capacity of analytical procedure to measure analyte was remain unaffected by small but deliberate variations in method parameter like variation in the wavelength ± 1 nm, variation in the solvent strength by ± 0.1 %. The analytical method was found rugged during development; similarity the result was produced by performing the analysis by different analyst.

4. Conclusion

The method was developed with eco-friendly and readily available aqueous 0.1 N HCl solvent. Tolperisone and Etoricoxib were estimated from the formulation by the method and satisfactory results were obtained. The isoabsorptive Q method was given reproducible results; however obtained results of both the methods were within acceptable limits given in the pharmacopoeia. The validated method is economical, precise, accurate, robust and reproducible hence can be routinely used for estimation of both the drugs from the dosage form.

Conflict of Interest

All Authors declared that there is no conflict of interest

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