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Research Article

Significance and Approach of Hydrotropic agent for the estimation of Tolperisone and Diclofenac in the combined solid dosage form By Chemometric method

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A B S T R A C T

Objective: The aim of the present research was to select common solvent for the solublisation of both drugs. Hydrotopes are surface active, highly water soluble organic salt, which imparts solubility to insoluble or sparingly soluble organic compounds in water, which is when present at moderate to higher concentration.Chemo metric assisted absorption spectrophotometric analytical methodswere developed by using hydrotropes for the estimation of Tolperisone (TPS) and Diclofenac (DFC) from their combined formulation.

Materials and Method: Simultaneous equation, Q-absorbance method method were selected from the nature of spectra, solvent 4% urea was utilized. For simultaneous equation method 261 nm and 277 nm was the wavelength for absorbance measurement of TPS and DFC respectively and for Q method wavelength 241 and 261 as λ_1 and λ_2 respectively was selected. 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 1-48 mcg/ml for DFC.

Results and Discussion: The percentage purity of assay was found 102.03% for TPS and 97.65% for DFC; and the accuracy study data were varied from 0.1123 to 0.1581for TPS and 0.0554 to 0.1409 for DFC. Precision study was shown acceptable data as SD data varied from 1.108 to 2.6721 for TPS and from 0.8119 to 3.4549 for DFC.

Conclusion: Hydrotropic solvents improved solubility of poorly water soluble drug diclofenac. The developed method is rigid, robust and efficient for the estimation of TPS and DFC from the combined formulation.

Keywords: Tolperisone, Diclofenac, hydrotropes, ICH, simultaneous equation method, Q-method

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INTRODUCTION

The objective of the present research was to increase the solubility of Tolperisone (TPS) and Diclofenac sodium (DFC) in water by hydrotropic solubilisation. Aqueous solubility of a therapeutically active substance is a key property as it governs dissolution, absorption and thus the efficacy in vivo; and also restricts use of organic solvent in method development [1]. The development of eco-friendly method by avoiding organic solvent could be termed as economical green method [2]. There is consistently pressure from environmental department to minimise hazardous and

volatile solvent content in the waste which seriously affects environment. Use of hydrotropic solutions, supercritical fluids in the organic synthesis curbs use of organic solvent in view point of green chemistry [3].Hydrotropes are capable of increasing the solubility of organic compounds up to 200 times in water. Hydrotopes are surface active, highly water soluble organic salt, which imparts solubility to insoluble or sparingly soluble organic compounds in water, which is when present at higher concentration. The potential use of hydrotopes in industry was studied in 1946 by McKee [3]. In literature review it is revealed that green analytical methods are preferred over analytical methods using harmful organic solvent for environment [4].

Tolperisone(TPS) chemically is (2-methyl-1-(4-methylphenyl)-3-(1-piperidyl)propan-1-one)[5]is a centrally acting muscle relaxant that is used for relieving spasticity of neurological origin and muscle spasms associated with painful locomotor diseases[6].

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 method [7], with other drug UV spectroscopic method [8-12], RP-HPLC impurity detection method [13], HPLC method [14], stability indicatingHPLC technique[15], with other drug HPLC method[16], HPTLC method [17], bio analytical [6], LC-MS/MS analytical method [18]werereviewed.

Diclofenac (DFC)chemically is sodium 2-[(2,6-dichlorophenyl)–amino] phenylacetate[19-21]an cyclooxygenase inhibitor, analgesic and anti-inflammatory phenyl acetic acid derivative for the relief of pain and inflammation in various conditions musculoskeletal and joint disorder such as rheumatoid arthritis, osteoarthritis and spondylitis[19].

Various analytical methods have been reported for the estimation of DFC alone or in combination with other agents in pharmaceutical dosage form includes lonely UV spectroscopic method [22-23], with other drug UV spectroscopic method [24-27], swab analysis RP-HPLC method [28], HPLC methods[29-32], cleaning validationHPLC technique[33],impurity detectionanalytical method [34], bio analytical method [35-37], HPLC method gel analysis [38]and stability indicatinganalytical method[39-40] were reviewed. DFC is official in BP and IP[20, 21]; and chemical structure of both the drugs is shown in Fig No 1.

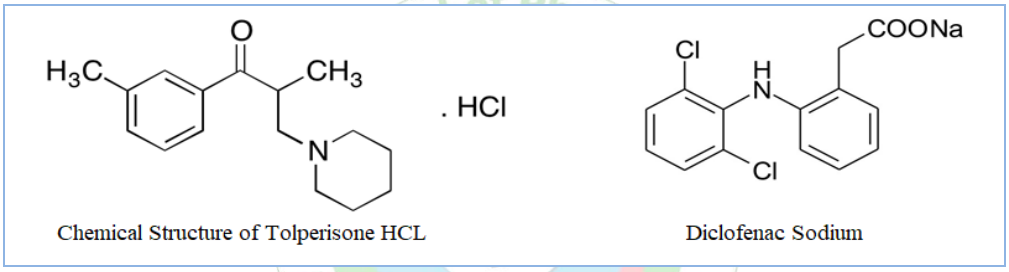

Figure 1: Chemical structure of drug molecule

MATERIALS AND METHODS

Instrumentation

Analysis was performed with a UV-1900i Shimadzu Double beam spectrophotometer (Shimadzu, Kyoto, Japan) with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with 10 mm matched Quartz cells was 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 usedfor degassing the solutions.

Reagents and Chemicals

Pharmaceutically pure samples of TPS was procured as a gift sample from Akums Drugs and Pharmaceuticals LtdHaridwar UK, and DFC was procured as a gift samples from Cure Medicines Pvt Ltd, Pune Maharashtra, Urea Analytical Grade and laboratory distilled purified water was used as solvent and the commercial formulation containing tolperisone150 mg and Diclofenac sodium50 mg was procured from the local market.

Solvent selection

Review of literature survey reported that diclofenac sodium is sparingly soluble in water, freely soluble in methanol, soluble in ethanol, slightly soluble in acetone [19, 20] practically insoluble in chloroform and ether [5].Tolperisone is soluble in water, aqueous acidic water, methanol and ethanol. Although the solubility of the procured both drugs were studied in water, 0.1HCl and 0.1 N NaOH separately; and each Solution with known conc of analyte was scanned in UV range of 400 nm to 200 nm, found that in 0.1 N HCl and water DFC was precipitating out similarly in 0.1 N NaOH tolperisone was precipitating out. Hence applicability of hydrotropic solvent was studied and 4% urea was found common solvent for complete solublisation and obtaining reasonable spectra for analysis. The recorded spectra in solvent are shown in Fig No 2 and 3. It was found that 4% urea in water is suitable solvent with respect to average cost, robust and precise in producing result.

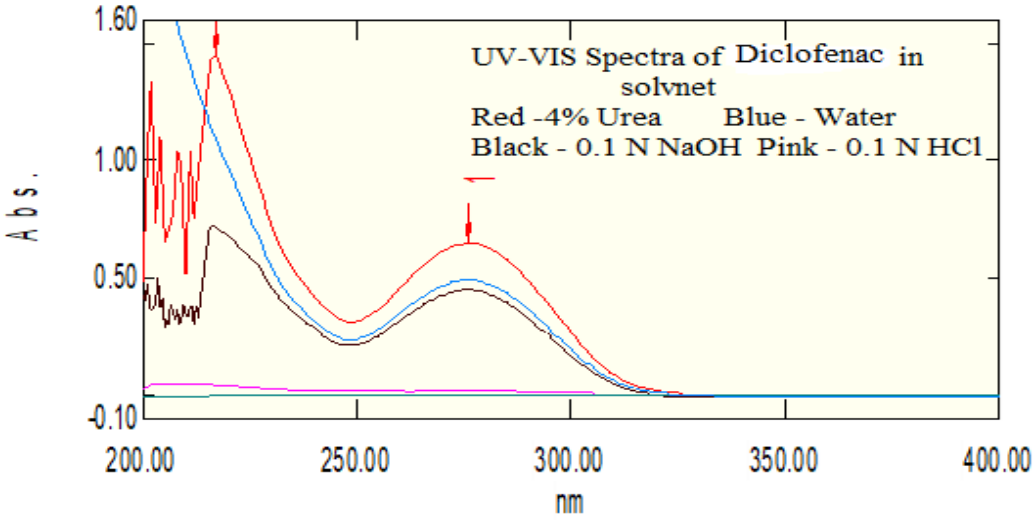

Figure 2: Spectra of Diclofenac in different solvent

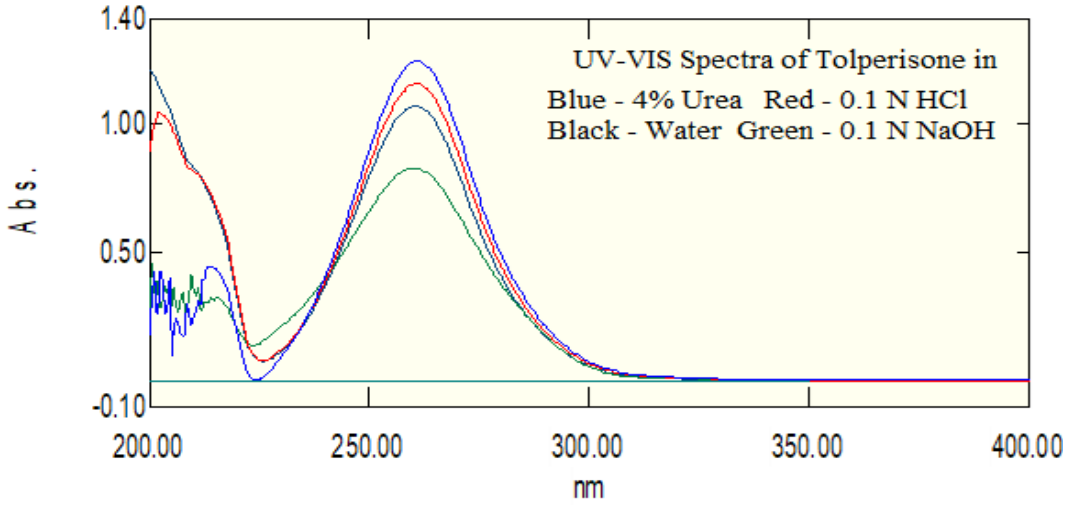

Figure 3: Spectra of Tolperisone in different solvent

Preparation of stock solutions and standard solutions

10 mg each of drug TPS and DFC were separately and accurately weighed; and transferred into separate 25 ml volumetric flasks. Dissolved into 4% Urea and volume was made to 25 ml with solvent. Subsequent standard solution of each drug with conc12µg/ml was prepared by diluting aliquot 0.3 ml of stock solution to 10 ml into 10 ml capacity volumetric flask.

Selection of wavelength and conc range

Prepared Standard solutions of TPS and DFCwere scanned in the spectrum mode from 400 nm to 200 nm.From UV spectra (Fig No 4) it was found that TPS has measurable absorbance at 261 nm (λ_{max})with absorbance interference by DFC; similarly DFC has maximum absorbance at 277nm (λ_{max}) and interference by TPS. Also the wavelength 241nm was found where both drugshave constant absorptivity; directed applicability of Q absorbance method. Chemometric method using simultaneous equation method was applied and which was reasonable remedy to overcome interference at each other's absorbance. From the nature of spectra to study linearity, working conc range 1 to 20µg/ml for TPS, 1 to 48µg/ml for DFC was selected. Also combined drug solution was prepared simulated to marketed formulation. Selected critical parameters based upon above discussion, observations were listedand by using these; method was validated as per ICH guidelines and by analyzing marketed preparations [41].

Experimental Method for estimation

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

Method-I: Simultaneous equation method

TPS was shown absorbance at (λ_{max})261 nm and DFC has maximum absorbance (λ_{max}) at 277 nm. The wavelength 261 and 277 nm was considered as 1 (λ_1) and 2 (λ_2) respectively. The equation A= abc was applied for x (TPS) and y (DFC) determination. On rearranging the 2 generated equations, the conc of x and y was calculated by following formula. Working standard solutions of TPSof conc 12µg /ml and DFCof conc16µg /ml were separately prepared and used for the method.

$$Cx = \frac{A2\ .ay1 - \ A1.ay2}{ax2.ay1 - \ ax1.ay2}$$

$$Cy = \frac{A1\ .ax2 - \ A2.ax1}{ax2.ay1 - \ ax1.ay2}$$

Where Cx and Cy = Conc of TPS and DFC in sample solution

A_1 and A_2 = absorbance of sample solution at 1 and 2 wavelength

ay_1 and ay_2 = absorptivity of DFC at 1 and 2 wavelength of standard solution

ax_1andax_2 = absorptivity of TPS 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 absorbances 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 selectedas λ_1(where absorptivity of both the drug remains constant)and λ_2($\lambda_{max}$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. TPS was shown absorbance at 261 nm considered as λ_2; and DFC has maximum absorbance at 277. From the overlain spectra of both drug iso absorptive point was found at 241nm.

The absorptivity of X Tolperisone at $\lambda_1$and $\lambda_2$are $ax_1$and ax_2 respectively.

The absorptivity of Y Diclofenac sodium at $\lambda_1$and $\lambda_2$are $ay_1$and ay_2 respectively.

$$Cx = \frac{Qm - Qy}{Qx - Qy} \frac{A}{a\,x1} \qquad Cy = \frac{Qm - Qx}{Qy - Qx} \frac{A}{aY1}$$

$Q_m = A_2 / A_1$ $Q_X = a_{X2}/ a_{X1}$ $Q_Y = a_{Y2} / a_{Y1}$

Where

Cx and C_Y - are concentrations of TPS and DFCin sample solution respectively.

Qx - Ratio of absorptivity of TPS at 261 and 241 nm

Q_Y - Ratio of absorptivity of DFC at 261 and 241 nm

Qm - Ratio of absorbance of sample solution at 261 and 241 nm

A - Absorbance of sample solution at Isobestic point

a_{X1}- Absorptivity of TPS at Isobestic point

a_{Y1}- Absorptivity of DFC 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 conc12 and 16mcg/ml each of TPS and DFC were prepared separately and absorbance was recorded, and SD and % RSD of the response was 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 1-20µg/ml for TPS and 1-48µg/ml for DFC 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. 261 for TPS and 277 nm for DFC 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 7.5 mg TPS and 2.5 mg DFC was weighed and transferred into 25 ml volumetric flask. Dissolved into 4% Urea and volume was made to 25ml 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 261 and 277 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 DFC were prepared and standard solutions added in 50,100 and 150% 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 DFC by the proposed method were determined using calibration graph method and calculated as 3.3σ/s and 10 σ/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.

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. 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 DFC was studied in each solvent; and in 4% Urea solvent both drugs were shown maximum and consistent absorbance as compare to other solvent.

System Suitability

The absorbances of six replicates of standard solutions (12µg/ml) are reported in Table No 1. The SD was found for TPS and DFCwithin acceptable limit and meets the system suitability requirements indicates method was suitable for analysis. The spectra of both the drug in selected solvent is shown in Fig No 4.

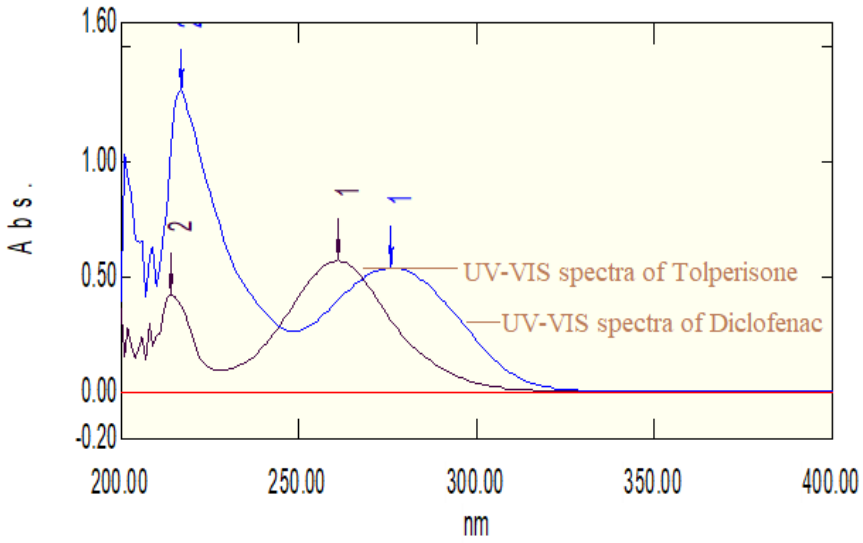

Figure 4: Spectra of Diclofenac and Tolperisone in overlain form

Table1: System suitability study of TPS and DFS

Sr No	Conc in µg/ml	Absorbance of TPS261 nm	Conc in µg/ml	Absorbance of DFS 277 nm
1	12µg/ml	0.6461	20µg/ml	0.4840
2	12µg/ml	0.6055	20µg/ml	0.4766
3	12µg/ml	0.6112	20µg/ml	05042
4	12µg/ml	0.6792	20µg/ml	0.4998
5	12µg/ml	0.6289	20µg/ml	0.4822
6	12µg/ml	0.6099	20µg/ml	0.4786
	SD RSD	0.028241 1.77958	SD RSD	0.01301 2.6488

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, r^2 value and intercept are tabulated in Table No 2, which proved the linear relationship between conc and obtained response.

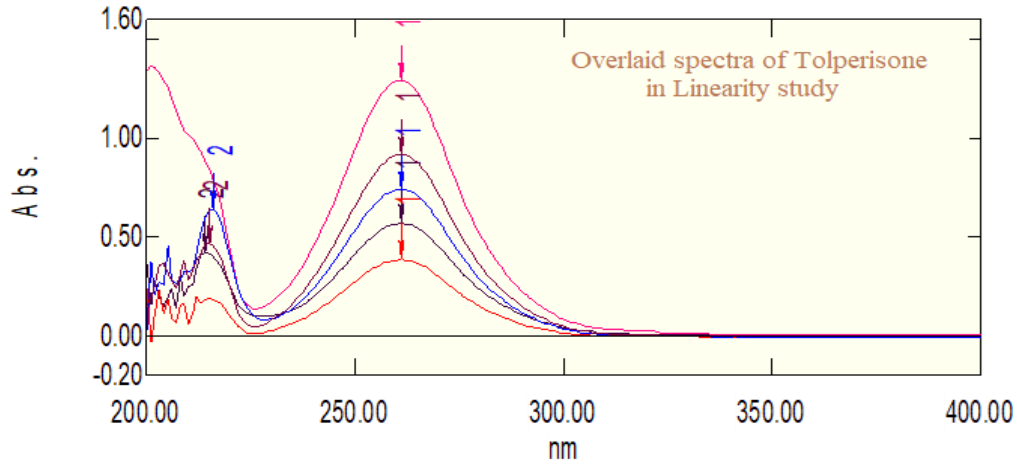

Figure 5: UV-VIS overlain spectra of TPS in linearity study

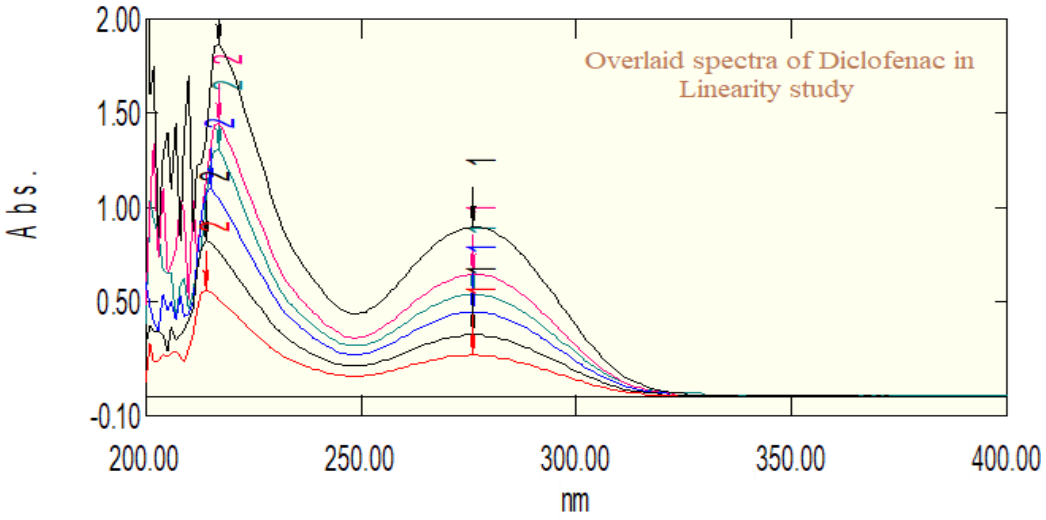

Figure 6: UV-VIS overlain spectra of DFC in linearity study

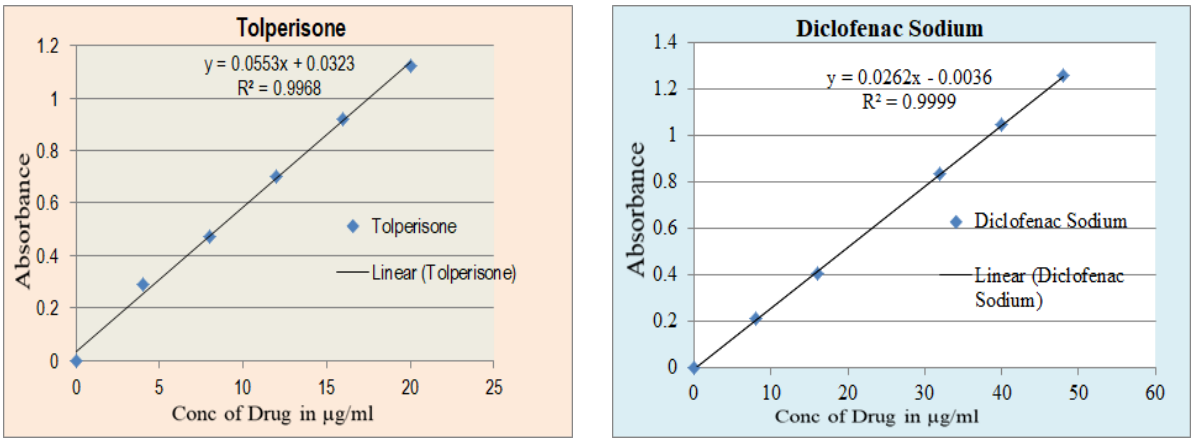

Figure 7: Calibration curve of Tolperisone and Diclofenac sodium

Table 2: Parameters of regression equation obtained in Microsoft excel

Parameters	**TPS**	**DFS**
Wavelength selected	261	277
Conc range (µg/ml)	1-20 µg/ml	1-48 µg/ml
Scan speed	Fast	Fast
Solvent	4% Urea in water	4% Urea in water
Correlation coefficient (r^2)	0.9968	0.9999
Regression equation (y = mx + c)	y=0.0553 x + 0.0323	y=0.0262 x + 0.0036

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

Name	Drug	Label Claim (mg/Tablet)	Amount found/mg; n=6	Drug Content %	Std Deviation	% RSD
Method - I	TPS	150	153.04	102.03	0.1627	0.1595
	DFC	50	48.83	97.654	1.6986	1.7394
Method - II	TPS	150	156.42	104.28	0.3959	0.3797
	DFC	50	50.66	101.33	1.8929	1.8681

Accuracy and Precision

The results of accuracy are summarized 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 summarized in Table No 4 and 5.

Table 4: Results of accuracy and precision of Method - I

Sr No	**Parameter**	**Level of study**	**Data Title**	**Obtd. Data %**	**S.D.**	**RSD**
1 (Method –I)	Precision study of TPS	Intraday Precision	Mean of Abs n= 6	103.82	2.6721	2.5701
		Interday precision		98.37	1.108	1.1301
	Precision study of DFC	Intraday Precision	Mean of Abs n= 6	104.46	3.4549	3.3119
		Interday precision		97.56	0.8119	0.8328
2(Method –I)	Accuracy study of TPS	50%	% Purity found	102.07	0.1123	1.3754
		100%		95.91	0.1350	1.1729
		150%		101.92	0.1581	1.1077
	Accuracy study of DFC	50%	% Purity	99.39	0.1409	5.2525
		100%		105.48	0.0554	1.6947
		150%		104.85	0.0682	1.6265

Table 5: Results of accuracy and precision obtained in Method - II

Sr No	**Parameter**	**Level of study**	**Data Title**	**Obtd. Data %**	**S.D.**	**RSD**
1 (Method –II)	Precision study of TPS	Intraday Precision	Mean of Abs n= 6	97.73	3.0687	3.1401
		Interday precision		97.79	1.1441	1.1709
	Precision study of DFC	Intraday Precision	Mean of Abs n= 6	103.89	1.8555	1.7907
		Interday precision		97.73	1.5547	1.5903
2 (Method –II)	Accuracy study of TPS	50%	% Purity found	103.16	2.2895	2.2191
		100%		95.62	1.8424	1.9261
		150%		103.44	1.3509	1.3051
	Accuracy study of DFC	50%	% Purity	106.36	7.2808	6.8451
		100%		103.34	2.3161	2.2412
		150%		109.28	4.7383	4.3351

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

The LOD and LOQ of TPS and DFC by the proposed method were found within acceptable limit shown in Table No 6.

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.

Table 6: Results of LOD, LOQ and Robustness

Parameters		TPS	DFS
LOD mcg/ml		0.13271	0.52938
LOQ mcg/ml		1.1296	1.6041
Robustness	(conc12mcg/ml) (± 1 nm)	259 (0.5871) 262	276 (0.2631) 278 (0.2619)
Ruggedness	Analyst 1	SD ± 0.0271 RSD ± 4.6159	SD ± 0.0153 RSD ± 5.8141
	Analyst 2	SD ± 0.01957 RSD ± 2.6548	SD = 0.0147 RSD ± 5.6128

CONCLUSION

The method was developed with eco-friendly and economical aqueous 4% urea hydrotropic solvent. Tolperisone and Diclofenac sodiumwere estimated from the formulation by the method and satisfactory results were obtained. The both chemometric method was given reproducible results; however obtained results of 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 tolperisone and diclofenacsodium from the dosage form.

CONFLICT OF INTEREST

All Authors declared that there is no conflict of interest

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