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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF TOLPERISONE HYDROCHLORIDE AND DICLOFENAC SODIUM IN TABLET DOSAGE FORM

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ABSTRACT

This present study reports the simultaneous quantification of Tolperisone hydrochloride and Diclofenac sodium in the bulk drug and tablet dosage form employing simultaneous equation and absorbance ratio method. This method allows determination of Tolperisone hydrochloride and Diclofenac sodium at their λ_{max} 254 nm and 282 nm respectively and at the iso-absorptive wavelength of 238 nm in methanol. Tolperisone at λ_{max} of 254 nm obeyed Beer's Law in the concentration range 4-12 µg/ml and Diclofenac sodium at λ_{max} of 282 nm obeyed Beer's Law in the concentration range 8-16 µg/ml. The accuracy and reliability of the method was assessed by linearity, precision (intra-day % RSD and inter-day % RSD of Tolperisone hydrochloride and Diclofenac sodium) and specificity in accordance with ICH guidelines.

KEY WORDS

Absorbance ratio, Diclofenac sodium, Simultaneous estimation, Tolperisone hydrochloride, Validation.

INTRODUCTION

Tolperisone hydrochloride (TOL), N-(2-[2-[(dimethylamino) methyl] thiazol-4-yl) methylthio] ethyl)-N-methyl-2-nitroethene-1, 1-diamine is a centrally acting muscle relaxant, widely used as spasmolytic. Diclofenac sodium (DIC), 2-[(2, 6-dichlorophenyl) amino] phenyl acetic acid monosodium salt is an NSAID act by inhibiting Cyclo-oxygenase[Cox] and suppressing the synthesis of prostaglandin F_2.

Literature review reveals that only few methods are available for simultaneous estimation of Tolperisone hydrochloride and Diclofenac sodium in two component dosage forms.

MATERIALS AND METHODS

Materials:

Tolperisone hydrochloride and Diclofenac sodium were obtained from Themis Medicare Limited, Gujarat and Wockhardt Pharmaceuticals Ltd, Aurangabad. The combined dosage form was purchased from local market (Tolperisone tablet 150 mg, marketed by Merck limited Mumbai, Methanol HPLC grade was obtained from SD Fine-chemicals Ltd Mumbai).

Instrumentation

Jasco V 560 Double Beam UV-VIS Spectrophotometer with spectral band width 2.0 nm wavelength, accuracy 0.5 nm and quartz cells of 1cm pathlength were used for all spectral and absorbance measurements. Class A Volumetric glasswares were used.

EXPERIMENTAL METHODS:

Preparation of Standard stock solution

Accurately weighed 100 mg of Tolperisone hydrochloride and Diclofenac sodium RS and were transferred to separate 100 ml volumetric flask and dissolved in 50 ml of methanol. The flask were shaken and volume was made up to the mark with methanol to give solutions containing 1000 µg/ml TOL and 1000 µg/ml of DIC.

Selection of a common solvent and selection of wavelength

Methanol was selected as the solvent for dissolving Tolperisone and Diclofenac because it had excellent solubility and the solution was stable.

Standard solution of concentration 10 µg/ml each of Tolperisone and Diclofenac were prepared and scanned separately in the entire UV region of 200-400 nm.The overlain spectra is given **Fig: 1.** From the spectra , two different wavelengths were selected for the determination of drug concentration in the mixture by simultaneous equation method [method I]ie.,254 nm (λmax of TOL) and 282 nm (λmax of DIC).

The wavelength at iso-absorptive point i.e., 238 nm and 254 nm (λmax of TOL) were chosen for the calculation of concentration of drugs by Absorbance ratio method.

Derivation of equations

Method I

Simultaneous Equation method

4-12 µg/ml solution of TOL and 8-16 µg/ml solutions of DIC were prepared in methanol and spectrum was scanned and recorded between 220 nm-350 nm.This method was based on the absorption of TOL and DIC at 254 nm and 282 nm (λ_{max} of TOL and DIC) since both drugs absorbs at the λ_{max} of each other.

The spectrum shows that both drugs absorbs at the λ_{max} of the other. Thus 254 nm (λ_{max} of TOL) and 282 nm (λ_{max} of DIC) were selected for the development of the simultaneous equations.

The absorptivity values were determined for both TOL and DIC at the selected wavelengths for different concentrations of both drugs. The absorbance of mixture of TOL and DIC at 254 nm and 282 nm was determined by using following equations. Two equations are constructed based upon the fact that at λ1 and λ2, the absorbance of the mixture is the sum of the individual absorbances of X and Y.

At λ1, $A1=ax_1bCx+ay_1bCy$ (1)

At λ2, $A2=ax2bCx+ay2bCy$ (2)

For measurements in 1 cm cells, b=1

Rearrange eq. (2). $Cy = \frac{A2-ax2Cx}{ay2}$

Substituting for Cy in Eq.(1) and rearranging gives

$$Cx = \frac{A2ay1-A1ay2}{ax2ay1-ax1ay2} \quad (3)$$

And

$$Cy = \frac{A1ax2-A2ax1}{ax2ay1-ax1ay2} \quad (4)$$

Where, Cx and Cy are the concentrations of TOL and DIC respectively

A1 and A2 are the absorbances of sample at 254 nm and 282 nm respectively

ax1 and ax2 are the absorptivity of TOL at 254 nm and 282 nm

ay1 and ay2 are the absorptivity of DIC at 254 nm and 282 nm

Method II

Absorbance ratio/Q value method

The absorption ratio method is a modification of the simultaneous equation procedure. It depends on the property that, for a substance which obeys Beer's Law at all wavelengths, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length. In the USP, this ratio is referred to as Q value.

In the quantitative assay of the two components in the mixture by the absorbance ratio method, absorbances are measured at two wavelengths one being the λmax of one of the components (λ_2) and the other being a wavelength of equal absorptivity of two components (λ_1), i.e., an iso-absorptive point. Two equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbances of X and Y.

Overlay spectra of TOL and DIC was studied and two wavelengths were selected for absorption calculations, 254 nm (λmax of TOL) and 238 nm (iso-absorptive point).The absorptivity coefficient of each drug at both the wavelengths were determined. The concentration of both the drugs in mixture and in tablet formulation were determined by substituting the absorbances and absorptivity coefficients in the equation,

$$Cx = \frac{Qm-Qy}{Qx-Qy} \times \frac{A1}{ax1} \quad , \quad Cy = \frac{Qm-Qx}{Qy-Qx} \times \frac{A1}{ay1}$$

Where,

Qm=Absorbance of sample at 254 nm/Absosorbance of the sample at 238 nm

Qx= Absorptivity of TOL at 254 nm/Absorptivity of TOL at 238 nm

Qy=Absorptivity of DIC at 254 nm/Absorptivity of DIC at 238 nm

A1=Absorbance of sample at 238 nm (iso-absorptive point)

ax1=Absorptivity of TOL at 238 nm

ay1=Absorptivity of DIC at 238 nm

Analysis of the tablet formulation

Twenty tablets of marketed formulation of TOLPIDOL D marketed by Merck Limited Mumbai containing 150 mg of TOL and 50 mg DIC were accurately weighed and powdered.

Transferred an accurately weighed portion of the mixed tablet content equivalent to 150 mg of TOL and 50 mg of DIC into 50 ml volumetric flask. Volume was made to 50 ml with methanol and shaken for 15 minutes and then sonicated for 5 minutes and filtered through the Whatman filter paper No.41.Necessary dilutions of the filtrate were made with methanol to get final concentration 5 µg/ml and 15 µg/ml of DIC and TOL respectively.

The absorbances of the solutions were measured at 254 nm and 282 nm for method I and 238 nm and 254 nm for method II. The values obtained were substituted in the respective formula of method I and II to obtain concentration of TOL and DIC. Results are shown in **Table 5.**

Method Validation

The proposed methods were validated in terms of linearity, accuracy, precision, and specificity, limit of detection and quantitation (LOD and LOQ) and robustness.

Linearity

Appropriate volume of aliquot from TOL and DIC standard solution was transferred to volumetric flask of 10 ml capacity. Volume was adjusted to the mark with methanol to give solutions containing 4-12 µg/ml and 8-16 µg/ml of TOL and DIC respectively. The absorbance of these solutions were measured at 254 nm, 282 nm and 238 nm (n=6), Calibration curves were obtained by plotting absorbance against concentration.**Fig:2**.The r2 value of > 0.995 was observed for both the methods. The statistical data are shown in **Table 1** and **2.**

Accuracy

Accuracy of the methods developed was confirmed by recovery studies as per ICH guidelines by standard addition method. Known amounts of standard solutions of TOL and DIC were added at 80,100 and 120 % level to pre quantified sample solution of TOL and DIC. Each concentration was analysed three times and average recoveries measured. The results of accuracy study are shown in **Table 3.**

Precision

Variation of results within the same day (Intraday) and between days (Interday) were analysed. The intraday precision was determined by analyzing six different samples of TOL solution (15 µg/ml) and DIC (5 µg/ml), obtained by dilution from stock solutions for three times a day.

Interday precision was determined for three days at the same time interval as in intraday. The % RSD value of < 2 % suggests that the developed methods are precise. The results are reported in **Table 4**.

Specificity

Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a known quantity of drugs and then absorbance was measured and calculations done to determine the quantity of the drugs.

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

LOD and LOQ were determined using mathematical equations.

LOD=3.3 x σ/S and LOQ= 10 x σ/S

Where, σ = Standard deviation of the response

S = Slope of the calibration curve

The results are reported in **Table 1** for method I and **Table 2** for method II.

Robustness

The robustness of both the methods was checked by performing the analysis with change in the typical analytical conditions like stability of analytical solution and results were found to be satisfactory.

CONCLUSION

The proposed UV Spectrophotometric methods for simultaneous estimation of TOL and DIC are accurate and precise. The proposed methods are simple, rapid and cost effective. The developed methods could be successfully applied for simultaneous estimation of TOL and DIC from their marketed formulations and for routine quality control of these drugs.

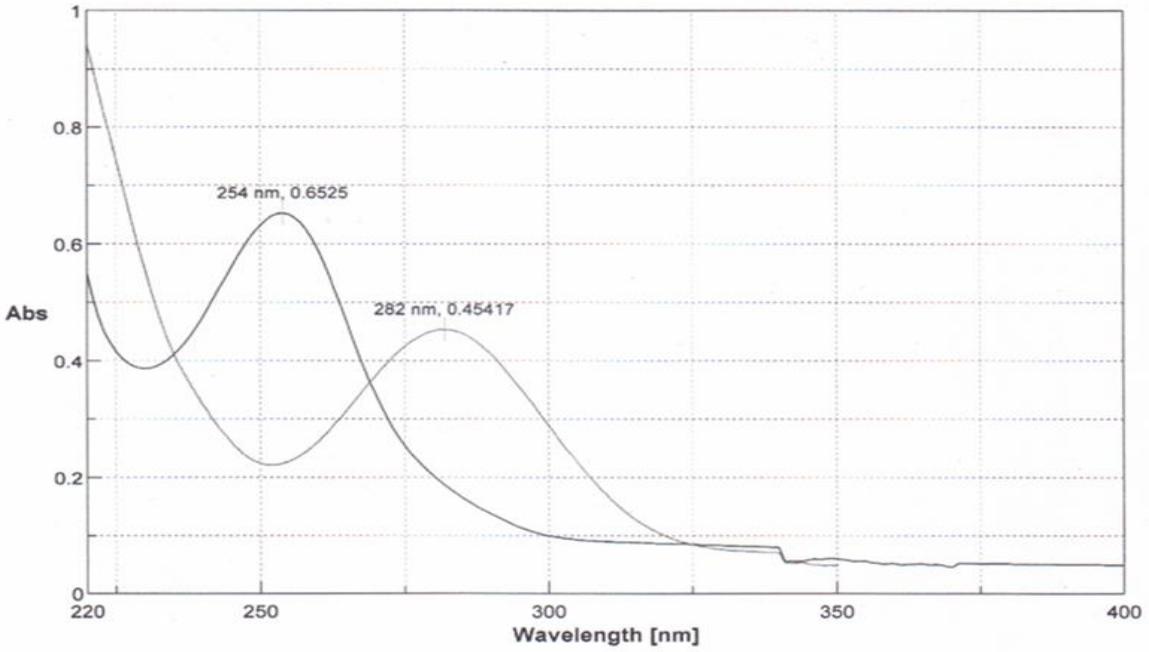
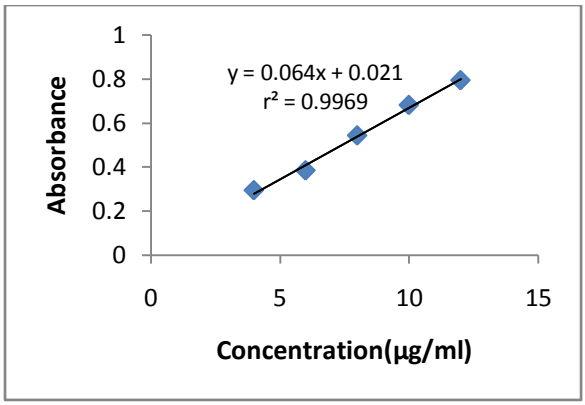
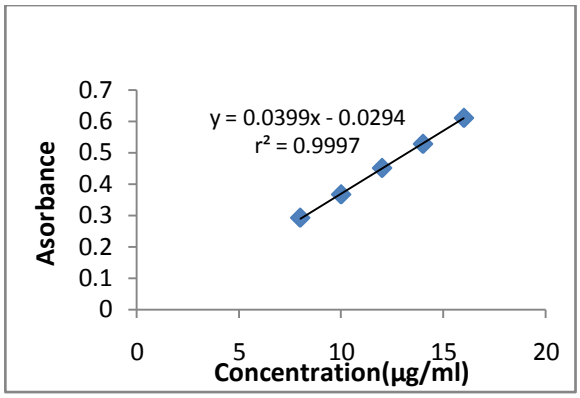


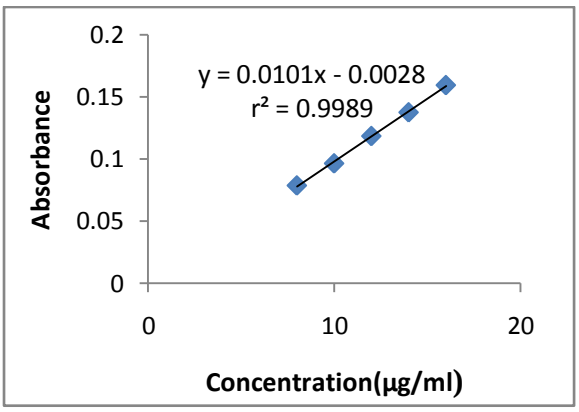
Fig. 1: UV absorption overlain spectrum of Tolperisone hydrochloride and Diclofenac sodium in Methanol



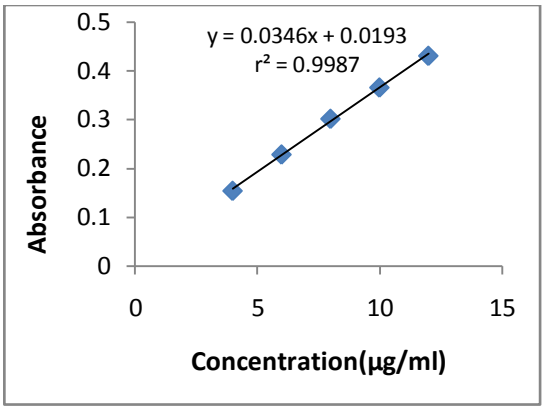
Calibration curve of TOL at 254 nm



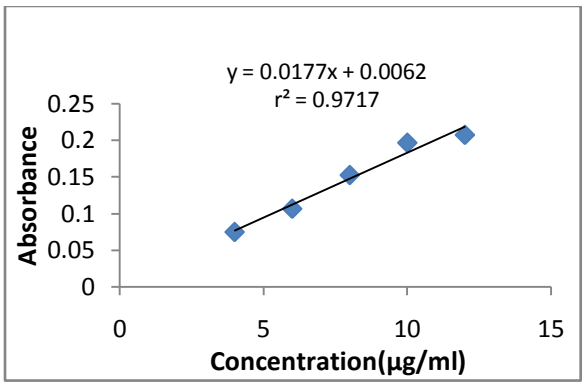
Calibration curve of DIC at 282 nm



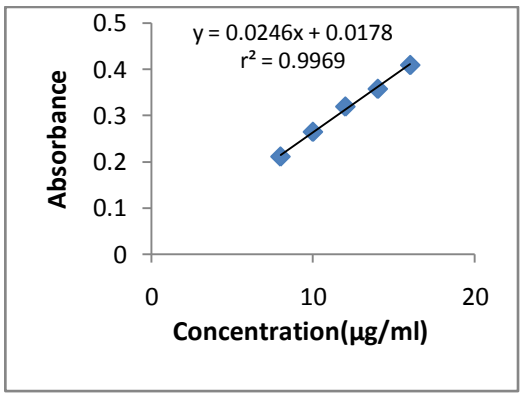
Calibration curve of DIC at 254 nm



Calibration curve of TOL at 238 nm



Calibration curve of TOL at 282 nm



Calibration curve of DIC at 238 nm

Fig. 2: Calibration curves of Tolperisone hydrochloride and Diclofenac sodium in methanol

Table 1: Statistical data of Method I

Parameters	Tolperisone hydrochloride		Diclofenac sodium	
Wavelength(nm)	254	282	254	282
Beer's law limit(µg/ml)	4-12	4-12	8-16	8-16
Regression equation	y=0.064x+0.021	y=0.017x+0.006	y=0.010x-0.003	y=0.040x-0.029
Slope	0.064	0.017	0.010	0.040
Intercept	+0.021	+0.006	-0.003	-0.029
Correlation coefficient(r^2)	0.997	0.972	0.999	1.000
Limit of detection(µg/ml)	0.019	0.036	0.043	0.023
Limit of quantitation(µg/ml)	0.059	0.117	0.130	0.072

Table 2: Statistical data of Method II

Parameters	Tolperisone hydrochloride		Diclofenac sodium	
Wavelength(nm)	238	254	238	254
Beer's law limit(µg/ml)	4-12	4-12	8-16	8-16
Regression equation	y=0.035x+0.019	y=0.064x+0.021	y=0.025x+0.018	y=0.010x-0.002
Slope	0.035	0.064	0.025	0.010
Intercept	+0.019	+0.021	+0.018	-0.002
Correlation coefficient(r2)	0.999	0.997	0.997	0.999
Limit of detection(µg/ml)	0.019	0.016	0.043	0.044
Limit of quantitation (µg/ml)	0.059	0.049	0.130	0.133

Table 3: Results of Recovery Studies

Level of % Recovery	Amount present (mg/tab)		Amount of standard drug added (mg)		Total amount of drug recovered(mg)		% Recovery	
	TOL	DIC	TOL	DIC	TOL	DIC	TOL	DIC
	30	10	24	8	23.93	7.79	99.73	97.39
80	30	10	24	8	23.88	7.89	99.51	98.74
	30	10	24	8	23.89	7.84	99.57	98.06
	30	10	30	10	29.97	9.84	99.91	98.43
100	30	10	30	10	29.92	9.89	99.73	98.97
	30	10	30	10	29.89	9.78	99.66	97.89
	30	10	36	12	35.90	11.86	99.74	98.90
120	30	10	36	12	35.88	11.78	99.68	98.22
	30	10	36	12	35.92	11.84	99.78	98.67

Table 4: Results of Precision Studies

Precision studies	Method I		Method II	
	TOL	DIC	TOL	DIC
Intraday(% RSD),n=6	0.2191	0.1823	0.0807	0.1765
Interday(% RSD),n=6	0.1910	0.2908	0.0974	0.5982

Table 5: Results of Analysis of Tablet Formulation

Brand name	Company	Label Claim	Amount Present (mg)	
			Method I	Method II
TOLPIDOL D	Themis Medicare Ltd	DIC 50mg	49.8± 0.12	50.0±0.01
		TOL 150mg	149.8±0.05	149.8± 0.07

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