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SIMULTANEOUS ESTIMATION OF DICLOFENAC SODIUM AND TOLPERISONE HYDROCHLORIDE IN COMBINED PHARMACEUTICAL FORMULATION

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Keywords:

Tolperisone Hydrochloride, Diclofenac Sodium, Q-Absorbance equation method, Simultaneous equation method (Vierodt's method), Zero crossing first derivative spectrophotometry

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ABSTRACT

Three simple, rapid, precise and accurate spectrophotometric methods have been developed for simultaneous analysis of Tolperisone Hydrochloride (TOL) and Diclofenac Sodium (DIC) in their combined dosage form. Method A, Simultaneous equation method (Vierodt's method) applies measurement of absorptivities at two wavelengths, 261.00 nm (λ_{max} of Tolperisone Hydrochloride) and 279.00 nm, (λ_{max} of Diclofenac Sodium) in zero order spectra. The concentrations can be calculated from the derived equations. Method B, Q-Absorbance equation method. It involves formation of Q-absorbance equation at 233.50 nm (isoabsorptive point) and 261.00 nm (λ_{max} of Tolperisone Hydrochloride) in zero order spectra. Method C, Zero crossing first derivative spectrophotometry involves measurement of absorbance at 249.20 nm (for Tolperisone Hydrochloride) and 227.40 nm (for Diclofenac Sodium) in first derivative spectra. Developed methods were validated according to ICH guidelines. The calibration graph follows Beer's law in the range of 6.0 to 18.0 µg/ml for Tolperisone Hydrochloride and 2.0 to 6.0 µg/ml for Diclofenac Sodium with R square value greater than 0.999. Accuracy of all methods was determined by recovery studies and showed % recovery between 98 to 102%. Intraday and interday precision was checked for all methods and mean %RSD was found to be less than 2 for all the methods. The methods were successfully applied for estimation of Tolperisone Hydrochloride and Diclofenac Sodium in marketed formulation.

INTRODUCTION: Tolperisone Hydrochloride (TOL) chemically is 1-piperidino-2-methyl-3-(p-tolyl)-3 propanone hydrochloride (**Fig. 1**), is a centrally acting Muscle Relaxant for the Symptometic treatment of Spasticity and Muscle Spasm.

Tolperisone Hydrochloride is official in Japanese pharmacopoeia JP15. JP15 describes Potentiometric Titration for its estimation [1].

Diclofenac Sodium (DIC) Chemically is sodium 2-[(2,6-dichlorophenyl)-amino]phenylacetate (**Fig. 2**), is a broadly used non-steroidal anti-inflammatory drug for the treatment of inflammatory conditions such as rheumatoid arthritis, osteoarthritis and ankylosing spondilytis. Diclofenac Sodium is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP). IP, BP, USP describes potentipmetric titration for its estimation.[2], [3, 4].

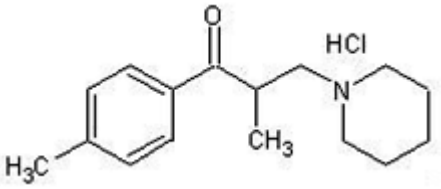

FIG. 1: TOLPERISONE HYDROCHLORIDE

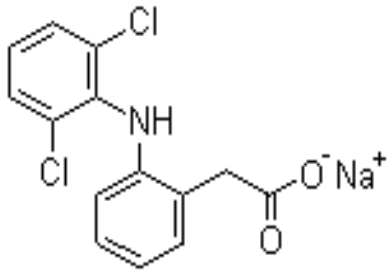

FIG. 2: DICLOFENAC SODIUM

Objective of Study: Survey of literature revealed that numbers of method have been reported in literature for the individual analysis of Tolperisone Hydrochloride and Diclofenac sodiumm by UV spectrophotometric and RP-HPLC method. UV spectrophotometric method available in literature for simultaneous determination of Diclofenac Sodium with other drugs like Peracetamol, Misoprostol and Thiocolchicoside Nimesulide [5, 6, 7, 8]. RP-HPLC method available in literature for simultaneous determination of Tolperisone Hydrochloride with Etodolac.[9] However, to our knowledge, there is no reported uv-spectrophotometric method available for simultaneous estimation of Tolperisone Hydrochloride and Diclofenac Sodium.

The aim of the present work was to develop easy, economic, accurate, specific and precise spectrophotometric methods for simultaneous estimation of Tolperisone Hydrochloride and Diclofenac Sodium in bulk drugs and combined pharmaceutical formulations and validation of newly developed analytical methods.

MATERIALS AND METHODS:

Apparatus and Software: Shimadzu UV-1700 double beam spectrophotometer connected to a computer loaded with Shimadzu UV Probe 2.10 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-400 nm. The samples were weighed on electronic analytical balance (A×120, shimadzu).

Reagents and Chemicals:

Solvent: Methanol: Water (30:70), Methanol analytical reagent grade (Spectrochem Pvt. Ltd, Mumbai, India). Water, single distilled water.

Diluent: Methanol: Water (30:70), Methanol analytical reagent grade (Spectrochem Pvt. Ltd, Mumbai, India). Water, single distilled water.

Year of Experiment– 2012.

Site- Quality Assurance Laboratory, Centre of Relevance and Excellence in Novel Drug Delivery System, G. H. Patel Building, Donor's Plaza, The Maharaja Sayajirao University of Baroda, Fatehgunj, Vadodara – 390 002, Gujarat, India.

Preparation of Stock Solution: Accurately weighed TOL and DIC (in quantities of 25.0 mg) were transferred to two separate 25 ml volumetric flasks, dissolved with the use of methanol : water (30:70) and volume was made up to the mark with methanol : water (30:70) to obtain stock solution of TOL (1000 µg/ml) and DIC (1000 µg/ml)

Preparation of Working Standard Solution: From the above solution, standard stocks solutions of TOL (50 µg/ml) and DIC (50 µg/ml) were prepared by transferring 2.5 ml aliquots to 50 ml volumetric flasks and making up the volume with methanol : water (30:70).

Preparation of Calibration Curve of Standard TOL and DIC: From working std. solution of TOL (50 µg/ml) 1.2, 1.8, 2.4, 3.0 and 3.6 ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with methanol : water (30:70). This gives 6.0 to 18 µg/ml of TOL. From working std. solution of DIC (50 µg/ml) 0.4, 0.6, 0.8, 1.0 and 1.2 ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with methanol : water (70:30). This gives 2.0 to 6.0 µg/ml of DIC.

Method A-

Simultaneous Equation Method (Vierodt's Method): If a sample containing two absorbing drug (X and Y) each of which absorbs at λmax of other. It may possible to determine both drugs by the technique of

simultaneous equations (Vierodt's method) provided that certain criteria apply. The information required is the aborptivities of X at and λ1 and λ2 ax1 and ax2 respectively (a) The aborptivities of Y at and λ1 and λ2 ay1 and ay2 respectively (b) The absorbances of the diluted sample at λ1 and λ2, A1 and A2 respectively. Let Cx and Cy be the concentrations of X and Y respectively in the diluted sample. Two equations are constructed based upon the fact that at λ1 and λ2 the absorbance of the mixture is the sum of the individual absorbance of X and Y.

From the stock solutions, working standard solutions of TOL (50 µg/ml) and DIC (50µg/ml) were prepared. By appropriate dilutions, the solutions with concentrations 6.0-18 µg/ml (for TOL) and 2.0-6.0 µg/ml (for DIC) were prepared and scanned between 200 to 400 nm **(Fig. 3).** Calibration curve of absorbance versus concentration were prepared. The calibration curves were found to be linear in the concentration range under study **(Fig. 4.1 & 4.2)**. For TOL and DIC, analytical wavelengths of 261.00 nm and 279.00 nm were selected respectively. Absorptivity of TOL and DIC were calculated at both the wavelengths.The concentrations of TOL and DIC can be calculated from following equations [10]:

Cx (DIC) = (A2 ay1 – A1 ay2) / (ax2 ay1 – ax1 ay2)

Cy (TOL) = (A1 ax2 – A2 ax1) / (ax2 ay1 – ax1 ay2)

Where; Cx & Cy are concentrations of DIC and TOL respectively in gm/100 ml in the sample solution. A1 & A2 are the absorbances of the mixture at 261.00 nm & 279.00 nm respectively; aX1 and aX2 = Absorptivity of DIC at 261.00 nm and 279.00 nm; aY1 and aY2 = Absorptivity of TOL at 261.00 nm and 279.00 nm.

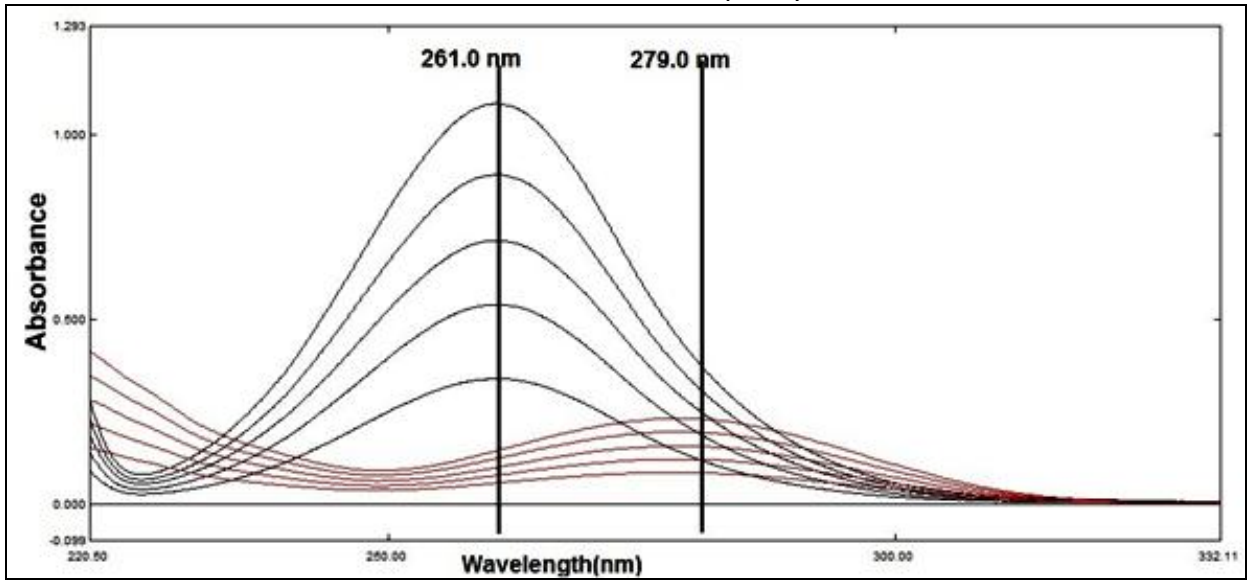

FIG. 3: ZERO ORDER OVERLAIN SPECTRA OF DIC (2, 3, 4, 5, 6 µg/ml, RED) AND TOL (6, 9, 12, 15, 18 µg/ml, RED)

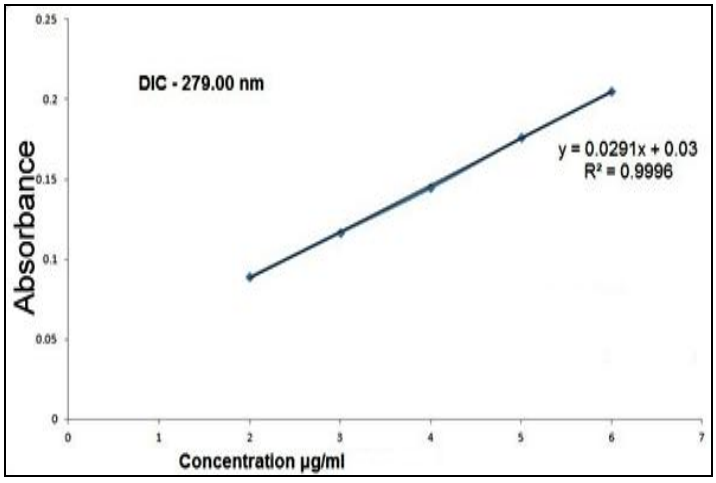

FIG. 4.1 CALIBRATION GRAPH OF DIC.

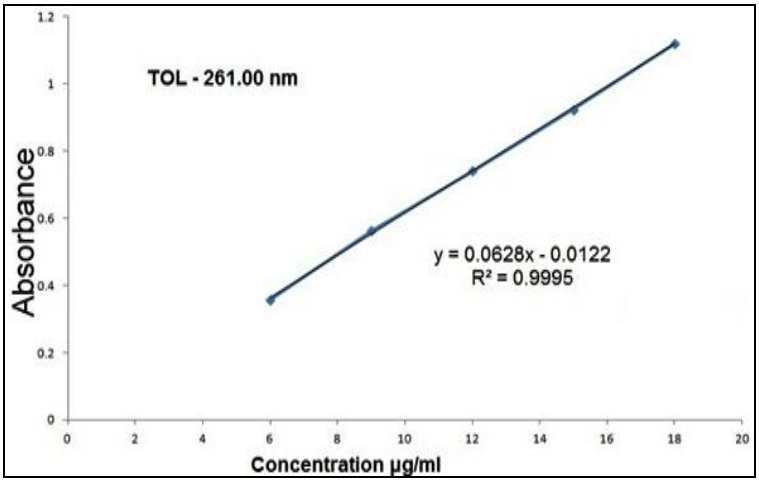

FIG. 4.2: CALIBRATION GRAPH OF TOL.

Method B-

Q-Absorbance Ratio Method: Q method uses the ratio of absorbances at two selected wavelengths, one at isoabsorptive point and other being the λmax of one of the two compounds. From the stock solutions, working standard solutions of TOL (50 µg/ml) and DIC (50µg/ml) were prepared. By appropriate dilutions, the solutions with concentrations 6.0-18 µg/ml (for TOL) and 2.0-6.0 µg/ml (for DIC) were prepared and scanned between 200 to 400 nm **(Fig. 5).** series of standard solutions ranging from 2.0-6.0 µg/ml for DIC and 6.0-18.0 µg/ml for TOL were prepared and the absorbance of solutions was recorded at 233.50 (isoabsorptive point) and 261.00 nm(λmax of TOL) to plot a calibration curve of absorbance versus concentration **(Fig. 6.1 & 6.2)**. Calibration curves were found to be linear in the concentration range under study. Absorptivity values of DIC and TOL were determined at selected wavelengths and are presented in **Table 2**. The concentration of two drugs in mixture was calculated by using following equations [10]:

$$CX = [(QM - QY) / (QX - QY)] \times A1/aX1$$

$$CY = [(QM - Qx) / (Qy - Qx)] \times A1/ay1$$

Where; Qm = A2/A1, Qx = ax2/ax1, Qy = ay2/ay1; **1** designates isoabsorptive point and **2** designates λ-max of TOL; ax1 and ax2 is Absorptivity of DIC at 1 and 2 wavelength respectively; ay1and ay2 is Absorptivity of TOL at 1 and 2 wavelength respectively; A1 and A2 are absorbances of the mixture at 1 and 2 wavelength respectively.

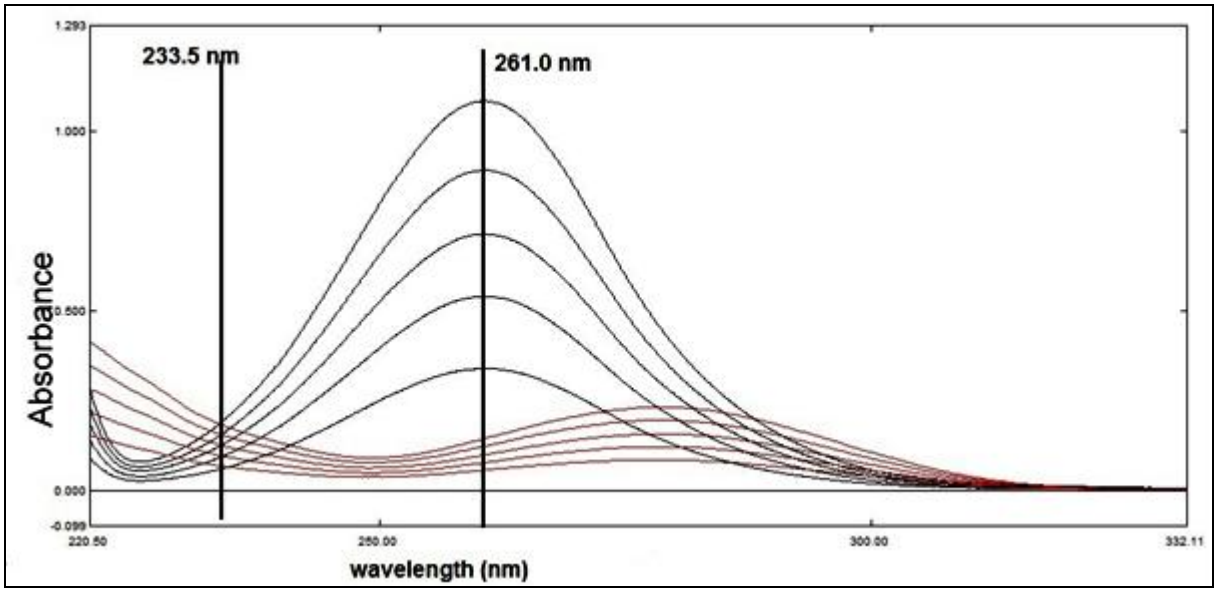

FIG. 5: ZERO ORDER OVERLAIN SPECTRA OF DIC (2, 3, 4, 5, 6 µg/ml, RED) AND TOL (6, 9, 12, 15, 18 µg/ml, RED)

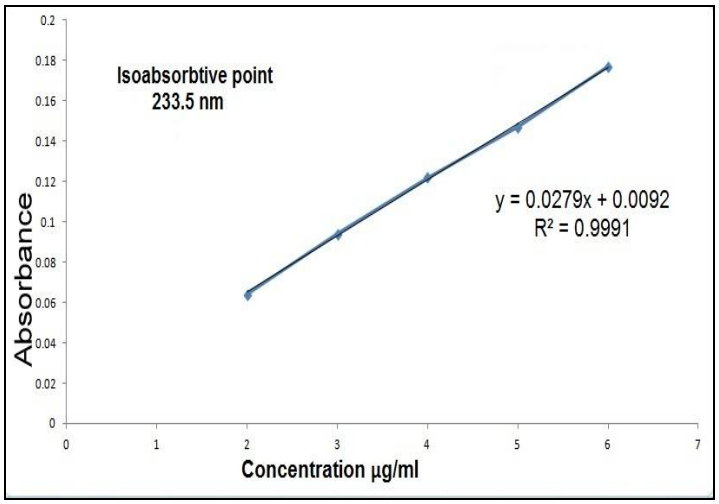

FIG. 6.1: CALIBRATION GRAPH AT ISOABSORBTIVE POINT

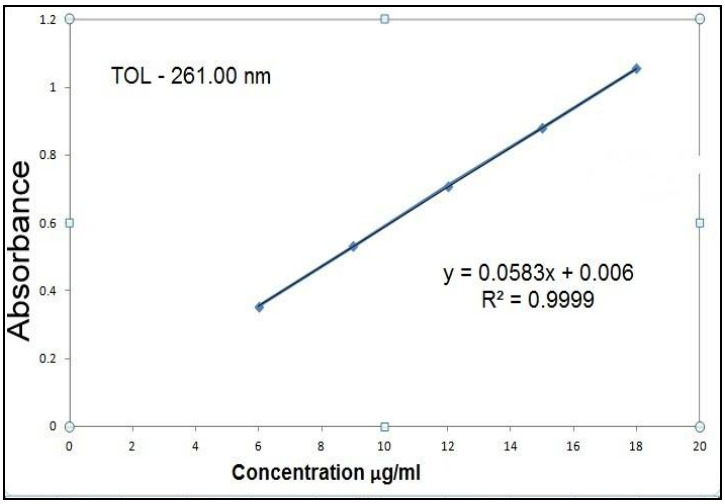

FIG. 6.2: CALIBRATION GRAPH OF TOL

Method C-

Zero Crossing First Derivative Spectrophotometry: The solutions of standard DIC and TOL were prepared in the range of 2.0 to 6.0 µg/ml and 6.0 to 18.0 µg/ml respectively. The absorption spectra of the solutions of DIC and TOL were recorded in the range of 200 nm to 400 nm and were stored in the memory of the instrument and transformed to first derivative with Δλ = 8nm and scaling factor 50 **(Fig. 7)**. At 227.40 nm, TOL is having zero crossing point and DIC can be determined. At 249.20 nm, DIC is having zero crossing point and TOL can be determined. The amplitudes at 227.40 nm were plotted against respective concentrations of DIC and the amplitudes at 249.20 nm were plotted against the respective concentrations of TOL for the preparation of calibration graph [10]. Calibration graph for DIC and TOL are shown **(Fig. 8.1 & 8.2).**

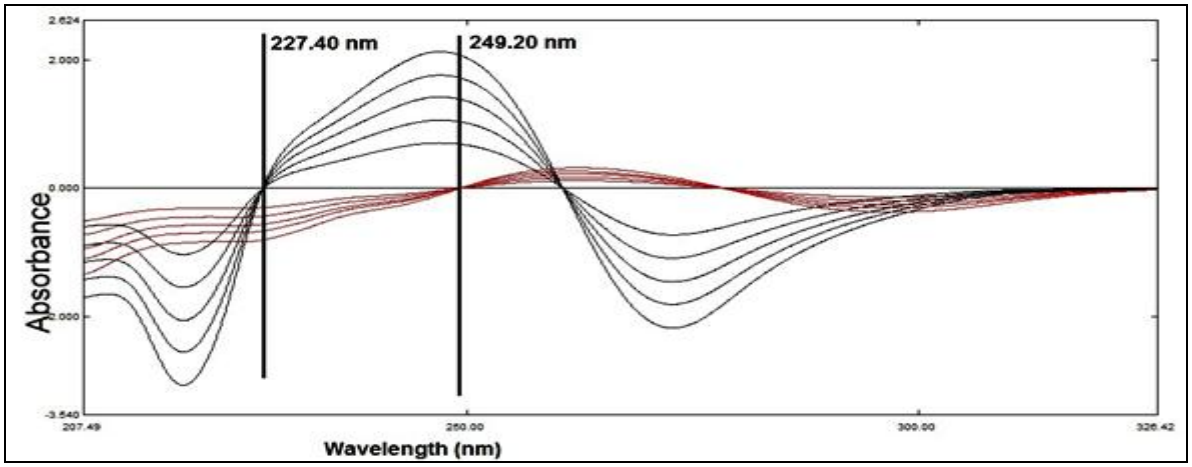

FIG. 7: FIRST ORDER OVERLAIN SPECTRA OF DIC (2, 3, 4, 5, 6 µg/ml, RED) AND TOL (6, 9, 12, 15, 18 µg/ml, RED)

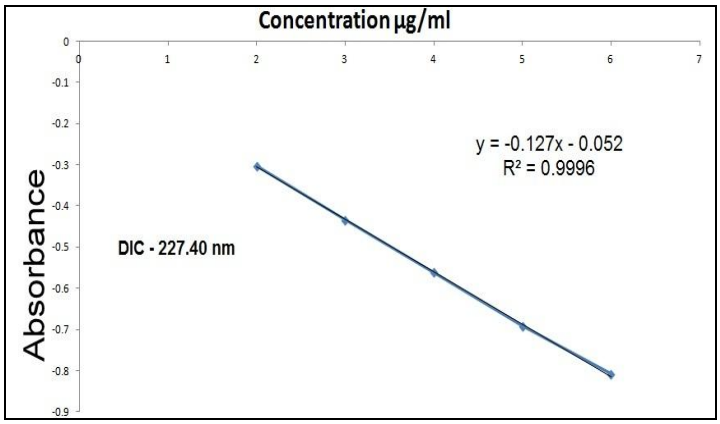

FIG. 8.1: CALIBRATION GRAPH OF DIC

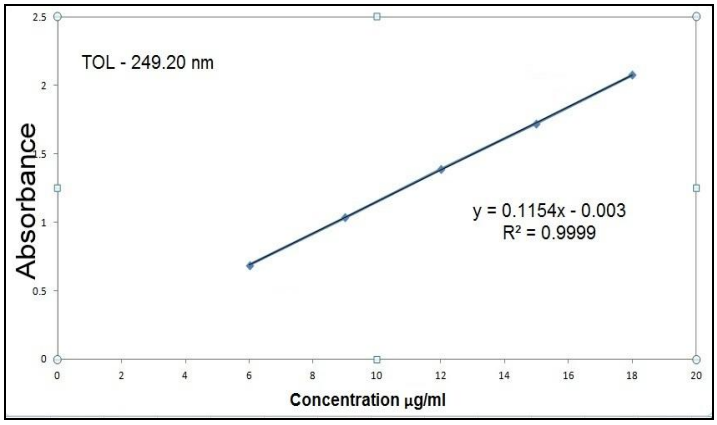

FIG. 8.2: CALIBRATION GRAPH OF TOL

Assay of Commercial Formulation by Method A, B, and C: 20 tablets were powdered and an amount equivalent to 25 mg DIC and 75 mg TOL was weighed and dissolved in 25 ml methanol: water (30:70). Solutions were filtered using whatmann filter paper grade 1. Appropriate dilutions were prepared in methanol: water (30:70) taking suitable aliquots of the clear filtrates and subjected to analysis using all the four methods described above. The result of analysis is reported **(Table 1).**

TABLE 1: RESULTS OF SIMULTANEOUS ESTIMATION OF MARKETED FORMULATION FOR METHOD A, B, AND C

Formulation:- Tolpidol – D		
Labelled Claim :- DIC : TOL (50mg : 150mg)		
Method	**DIC*±SD**	**TOL*±SD**
A	99.33±0.0308	99.67±0.0724
B	102.5±0.0353	100.83±0.0264
C	99.00±0.0316	101.00±0.0515

***Mean value of five determinations**

RESULTS AND DISCUSSION: Developed spectrophotometric methods for the simultaneous estimation of DIC and TOL were validated according to ICH guidelines and data complying with the standards were obtained [11]. The results of validation parameters for all the three developed methods are reported **(Table 2 and 3).**

TABLE 2: SUMMARY OF VALIDATION PARAMETERS BY DEVELOPED METHODS

Parameters	Method - A		Method - B		Method – C	
	DIC	TOL	DIC	TOL	DIC	TOL
Analytical wavelength (nm)	261.00 & 279.00		233.50 & 261.00		227.40	249.20
Beer's range (µg/ml)	2.0 -6.0	6.0 -18.0	2.0 - 6.0	6.0 - 18.0	2.0 - 6.0	6.0 -18.0
Slope	0.0291	0.0268	0.0279	0.0583	0.127	0.1154
Intercept	0.0300	0.0122	0.0092	0.006	0.052	0.003
Correlation coefficient	0.9996	0.9995	0.9991	0.9999	0.9996	0.9999
Intraday precision (%RSD)	0.6622	0.4484	1.2067	0.7898	1.1994	0.8171
Interday precision (%RSD)	1.3986	0.6990	1.5618	0.8453	1.3586	0.8801
LOD (µg/ml)	0.2884	0.3832	0.3248	0.3248	0.3092	0.4146
LOQ (µg/ml)	0.8652	1.1495	0.9744	1.3211	0.9276	1.2439

TABLE 3: RESULTS OF RECOVERY STUDY OF DIC AND TOL BY DEVELOPED METHODS

METHOD	% SPIKING	C_{ACTUAL} µg/ml		C_{ADDED} µg/ml		C_{FOUND}* µg/ml		%RECOVERY ± S.D.	
		DIC	TOL	DIC	TOL	DIC	TOL	DIC	TOL
A	50	2	6	1	3	3.02	9.04	100.67±0.0765	100.44±0.1061
	100	2	6	2	6	4.08	11.97	102.00±0.0406	99.75±0.1768
	150	2	6	3	9	5.03	15.18	100.60±0.1267	101.20±0.0778
B	50	2	6	1	3	3.08	9.03	102.88±0.0768	100.37±0.0644
	100	2	6	2	6	4.07	11.84	101.75±0.1353	98.67±0.1058
	150	2	6	3	9	5.12	14.99	102.4±0.0557	99.93±0.1127
C	50	2	6	1	3	3.05	9.08	101.67±0.0158	100.88±0.1389
	100	2	6	2	6	4.09	12.09	102.25±0.0308	100.75±0.0818
	150	2	6	3	9	5.02	15.19	100.4±0.0872	101.27±0.0505

* Mean of three determinations

CONCLUSION: Three Spectrophotometric methods (Q-Absorbance equation method, Simultaneous equation method, Zero crossing first derivative spectrophotometry) were developed for simultaneous estimation of DIC and TOL in their combined formulation without prior separation. Methods were found to be precise and accurate as can be reflected from validation data. Developed methods were successfully applied for estimation of DIC and TOL in marketed formulation.

REFERENCES:

1. Japanese Pharmacopoeia JP15, The ministry of health, labour and welfare, prefectural office in Japan, 2006, 1190.
2. Indian Pharmacopoeia, The Indian Pharmacopoeia Commission, Ghaziabad, 2007, Vol. - 2, 402-403.
3. British Pharmacopoeia, British Pharmacopoeia Commission, 2009, Vol. – 1 & 2, 1893-1894.
4. United state Pharmacopoeia 30 National formulary 24, United State Pharmacopoeia Convention, 2007, 1922.
5. Mukesh Chandra Sharma, Smita Sharma, Determination and validation of UV-spectrophotometric method for estimation of Paracetamol and Diclofenac sodium in tablet dosage form using hydrotropic solubilising agent, International Journal of Pharm Tech Research, 2011, 3(1), 244-247.
6. Sunil R. Dhaneshwar, Vidhya K. Bhusari, Validated HPLC method for simultaneous quantitation of Diclofenac sodium and Misopristol in bulk drug and formulation, Der Chemical Sinica, 2010, 1(2), 110-118.
7. S. V. Gandhi, M. R. Sengar, U.P. Patil, V.S. Rajmane, Simultaneous determination of Diclofenac sodium and Thiocolchicoside in fixed dose combination by spectrophotometry, Asian Journal of Pharmaceutical and Clinical Research, 2010, 3(2), 89-81.
8. S. Rawat, Akhilesh Gupta, Spectrophotometric method for simultaneous estimation of Nimesulide and Diclofenac sodium in pharmaceutical dosage forms, Asian Journal of Pharmaceutical Analysis, 2011, 1(4), 85-87.
9. Mit J. Patel, R. Badmanaban, C. N. Patel, RP-HPLC method for simultaneous estimation of Tolperisone hydrochloride and Etodolac in combined fixed dosage oral formulation, Pharm methods, 2011, 2, 124-129.
10. Beckett AH, Stenlake JB, Practical pharmaceutical chemistry, 4th edition, part -2, new delhi, CBS publication, 1997, 285-288.
11. ICH Q_2 (R_1), Validation of analytical procedure, Text and methodology, Geneva, International conference on Harmonization, 2005.

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