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Hplc Method Development and Its Validation for Simultaneous Estimation of Tolperisone Hydrochoride and Diclofenac Sodium in Combined Tablet Dosage Form

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Abstract: Multi-ingredient formulation is regularly used in the management of various ailments in order to avoid the intake of large number of doses. Tolperisone Hydrochoride and Diclofenac Sodium is one of such combination useful in the treatment of muscular pain. In the proposed project, an attempt has been made to develop and validate a HPLC method and to apply the method for determination of Tolperisone Hydrochoride and Diclofenac Sodium in tablet dosage form. A HPLC method was developed and validated successfully for simultaneous estimation of Tolperisone Hydrochoride and Diclofenac Sodium. The method utilizes a BDS hypersil C_{18}, 250mm × 4.6mm, 5µ (particle size), Thermo scientific with mobile phase of 0.05 M KH_2PO_4 Buffer : Acetonitrile: TEA (60: 40: 0.1) (%v/v) (pH 3.5 by o-phosphoric acid) with the flow rate of 1 ml/min and UV detection at 215nm. The method was validated as per ICH guidelines. Linearity was observed over concentration range of 22.5 ppm-67.5ppm for TOL and 5 ppm-15ppm for DICLO. The accuracy of the proposed method was determined by recovery studies and found to be 100.16- 100.48 % and 99.99-100.12 % for TOL and DICLO respectively. The proposed method was extended for estimation of TOL and DICLO in tablet formulation and it was found to be well within the acceptance limit. This RP-HPLC method for simultaneous estimation of TOL and DICLO was found to be linear, accurate, precise, robust and rugged. Hence it can be used for routine analysis of TOL and DICLO in tablets.

Key words: Tolperisone Hydrochoride, Diclofenac Sodium, RP-HPLC, Method development and Validation.

I. Introduction

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results[1].The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included. Other analytical procedures may be considered in future additions to this document[2].

TOL is 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl)propan-1-one Hydrochloride Typically, tolperisone is indicated in the treatment of acute muscle spasms in back pain and spasticity in neurological diseases[3]. Easily soluble in Methanol and ethanol, slightly soluble in acetone, almost insoluble in benzene or ether[4]. Melting point of TOL is 181 to 183^0C[5]. Being, centrally acting muscle relaxant, tolperisone acts at the level of spinal cord by blocking sodium channels and calcium channels. Tolperisone exerts its spinal reflex inhibitory action predominantly via a pre synaptic inhibition of the transmitter release from the primary afferent endings via a combined action on voltage-gated sodium and calcium channels. Tolperisone increases the blood supply to skeletal muscles this action is noteworthy since a muscle contracture may compress the small blood vessels and induce an ischemia leading to release of pain stimulating compounds[6]. Tolperisone is intensively metabolised by the liver and the kidneys[7]. It may cause excessive sweating, urticaria or erythema. Also may

lead to GI upset with abdominal paon, nausea, vomiting, diarrhea, flatulence or dryness of mouth[8]. Tolperisone Hydrochloride is official in Japanese pharmacopoeia[9].
Diclofenac is Nonsteroidal anti-inflammatory drugs used to relieve the inflammation, swelling, stiffness, and joint pain associated with rheumatoid arthritis, osteoarthritis[10]. Chemically is called Sodium 2-[(2,6-dichlorophenyl)amino]phenyl]acetate. Sparingly soluble in water, freely soluble in methanol, soluble in ethanol (96 per cent), slightly soluble in acetone[11].

Gastrointestinal experiences including: abdominal pain, constipation, diarrhea, dyspepsia, flatulence, gross bleeding/perforation, heartburn, nausea, GI ulcers (gastric/duodenal) and vomiting[12-14]. Diclofenac Sodium is official in Indian Pharmacopoeia (IP) [15], British Pharmacopoeia (BP)[16] and United States Pharmacopoeia (USP)[17].

II. Material and Method

Chemicals and Reagents:- Tolperisone Hydrochoride and Diclofenac Sodium were obtained from Molecule lab, Ahmedabad, India. Tolperisone Hydrochoride and Diclofenac Sodium combined dosage form tablets were purchased from local market. Tolperisone Hydrochoride (Potency 99.5 %), Diclofenac Sodium (Potency 99.7 %) and Acetonitrile, Methanol, Water, Ortho Phosphoric acid all were taken of HPLC Grade.

Instrumentation:- HPLC: Shimadu LC-2010c HT, Liquid Chromatograph: LC-20AT,UV-Visible, Detector :- ShimadzuUV-1601 PC, Column :- BDS hypersil C_{18}, 250mm × 4.6mm, 5µ(particle size), Thermo scientific, Digital pH meter (EUTEC1100), Ultra Sonicator-Spincotech Pvt. Ltd, Durasil-Pipettes of 1, 2 and 10 ml capacity and Borosil-Volumetric flasks of 10,25,50,100 mL capacity were used. (All glassware was previously calibrated). Measuring cylinder of 100 ml capacity and Hamilton 25 µl syringe were used.

Chromatographic conditions:-

Table 1: Chromatographic conditions

PARAMETER	CONDITION
Column	Thermoscientific, BDS hypersil C_{18}, 250 mm × 4.60 mm, 5µ
Flow rate	1.0 ml/min.
Mobile Phase	0.05 M KH_2PO_4 Buffer : Acetonitrile: TEA (60: 40: 0.1) (%v/v) (pH 3.5 by o-phosphoric acid)
Detection	215 nm
Injection Volume	20 µl
Runtime	10 Minute
Diluent	Mobile Phase

Diluent: Mobile Phase

Preparation of Mobile Phase

0.05 M KH_2PO_4 Buffer: Acetonirtrie : TEA (60: 40: 0.1 %v/v) pH 3.0 adjust with O – Phosphoric acid: - 680 mg KH_2PO_4 in 100ml water and than add 0.1 ml tri ethyl amine and than adjust pH at 3.0 using o-phosphoric acid. Mix this 60ml with 40ml Acetonitrile. Sonicate for 30 minute and filter through 0.20 µ size membrane filter.

Preparation of standard and stock solution of TOL: Accurately weighed quantity of TOL 45 mg was transferred into 100 ml volumetric flask, add 50 ml of diluent and it was then sonicated for 10 min and final volume of solution was made up to mark with diluent to get stock solution

containing 450 µg/ml of TOL in 100 ml volumetric flask this solution used as stock solution.

Preparation of standard and stock solution of DICLO: Accurately weighed quantity of DICLO 10 mg was transferred into 100 ml volumetric flask, add 50 ml of diluents and It was then sonicated for 10 min and final volume of solutions was made up to mark with diluent to get stock solution containing 10 µg/ml of DICLO in 100 ml volumetric flask This solution used as a stock solution.

Preparation of sample solution: Twenty tablets were weighed. The powder from twenty tablets were collected and weighed. The Powder equivalent to 45 mg of TOL and 10 mg of DICLO was transferred to a 100 ml volumetric flask and dissolved in mobile phase. The solution was ultrasonicated for 30 min and filtered through 0.20 micron membrane filter to obtain concentration about 450 µg/ml TOL and 100 µg/ml DICLO respectively. And a solution of 45 µg/ml of TOL and 10 µg/ml of DICLO solution was prepared by diluting 1 ml of sample stock solution with diluents in 10 ml volumetric flask up to the mark label the both flask.

III. Results & Discussion

The detection wavelength was chosen at 215 nm for Tolperisone Hydrochoride and Diclofenac Sodium in tablet dosage form has better absorption and sensitivity at this wavelength. However, to achieve the better separation of Tolperisone Hydrochoride and Diclofenac Sodium in the present combination, the mobile phase chromatogram was shown in Fig. 1(a), (b) and (c), which illustrate the separation of both active ingredients in this system. The isocratic HPLC method was adopted to analyze both components in a single run. Figure (b) is final optimize condition with good resolution.

System suitability and system precision:
System suitability and system precision was daily performed during entire validation of this method. The results of system suitability and system precision were presented in table 1.

Linearity and calibration curve:
The linearity parameter was performed to ensure that the test results are directly proportional to the concentration of analyte sample. The correlation coefficient was found to be 0.99 to 1.00. A linear relationship was found for all components. The results of linearity, limit of detection and limit of quantification were presented in table 2 and 3. Calibration carves of Tolperisone Hydrochoride and Diclofenac Sodium show in Fig. 4 and 5.respectively, and Overlain chromatograph show in fig. 6.

Specificity:
There was no interference from sample placebo and peak purity of Tolperisone Hydrochoride and Diclofenac Sodium were 0.999 and 1.000. It showed that developed analytical method was specific for the analysis of Tolperisone Hydrochoride and Diclofenac Sodium in tablet dosage form.

Method precision:
The precision of the method was established by carrying out the analysis of the standard analyte (n=6) using the proposed method. The %RSD found within 2% showed that the method was precise. The results obtained were presented in table 4.

Method accuracy:
This parameter was performed to determine the closeness of test results with that of the true value which is expressed as % recovery. This study was performed at 3 different levels 50, 100 and 150. The amount of Tolperisone Hydrochoride and Diclofenac Sodium recovered was calculated. The results of recovery studies were presented in table 5 and 6 respectively**.**

Method robustness:
Robustness of the method was determined by small deliberate changes in pH, flow rate, Organic phase ratio of mobile phase and column oven temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in table 7.

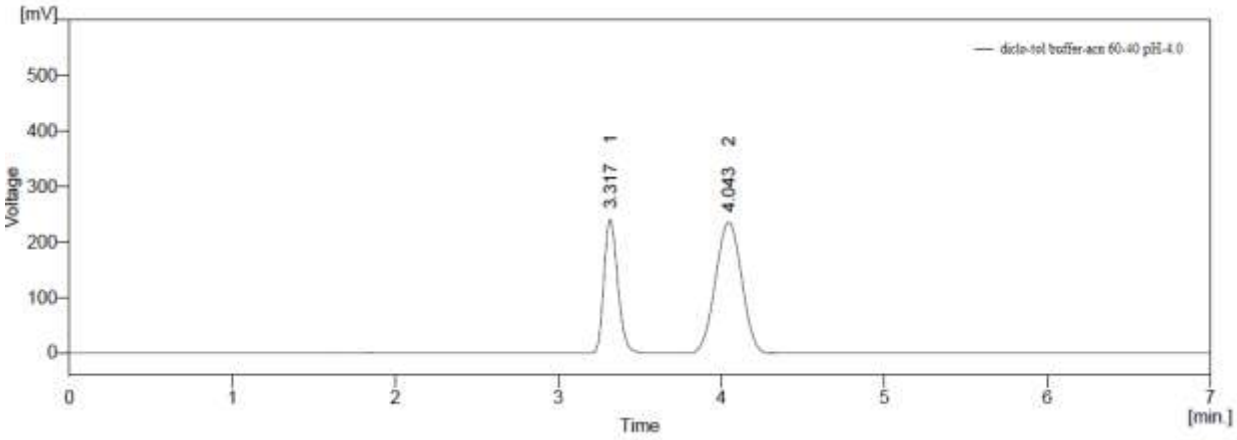

Figure 1: Buffer (phosphate) : Acetonitrile (60:40 v/v), pH: 4.0

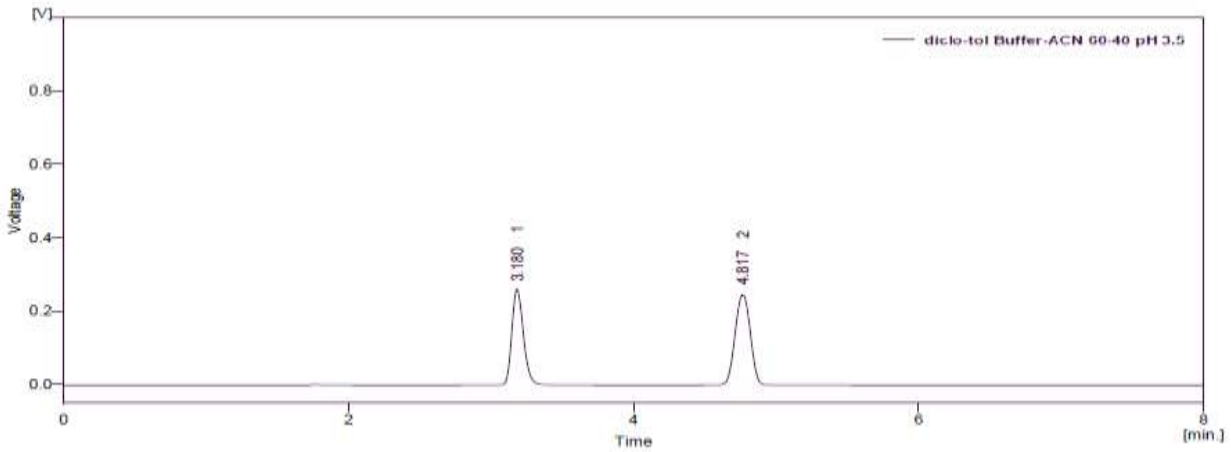

Figure 2: Buffer (phosphate) : Acetonitrile (60:40 v/v), pH: 3.5

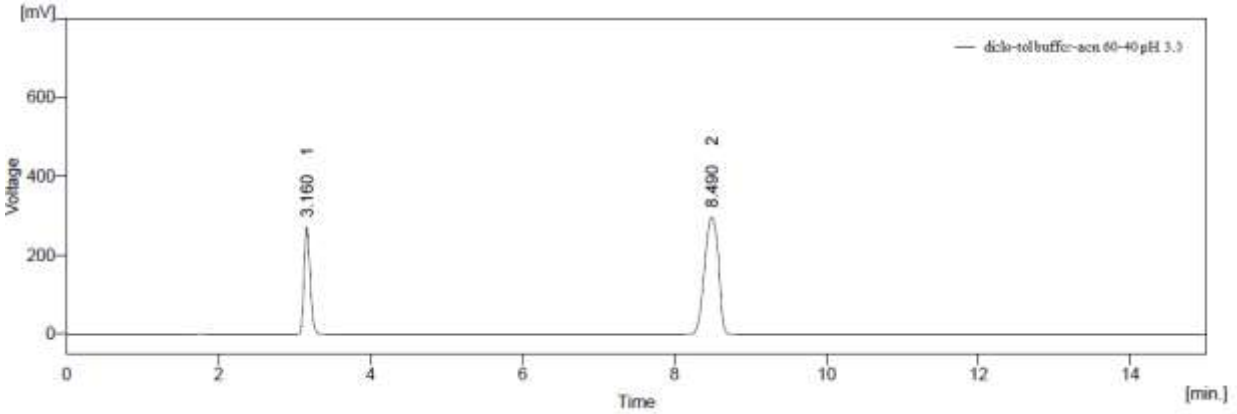

Figure 3: Buffer (phosphate): Acetonitrile (60:40 v/v), pH: 3.0

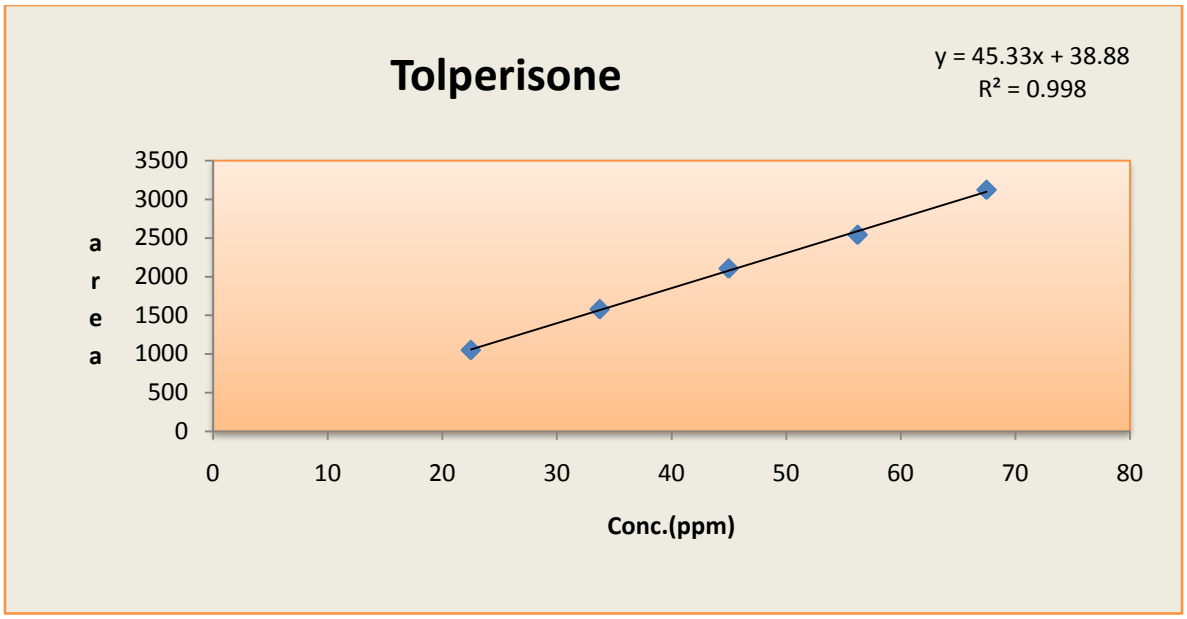

Figure 4: Calibration Curve of TOL

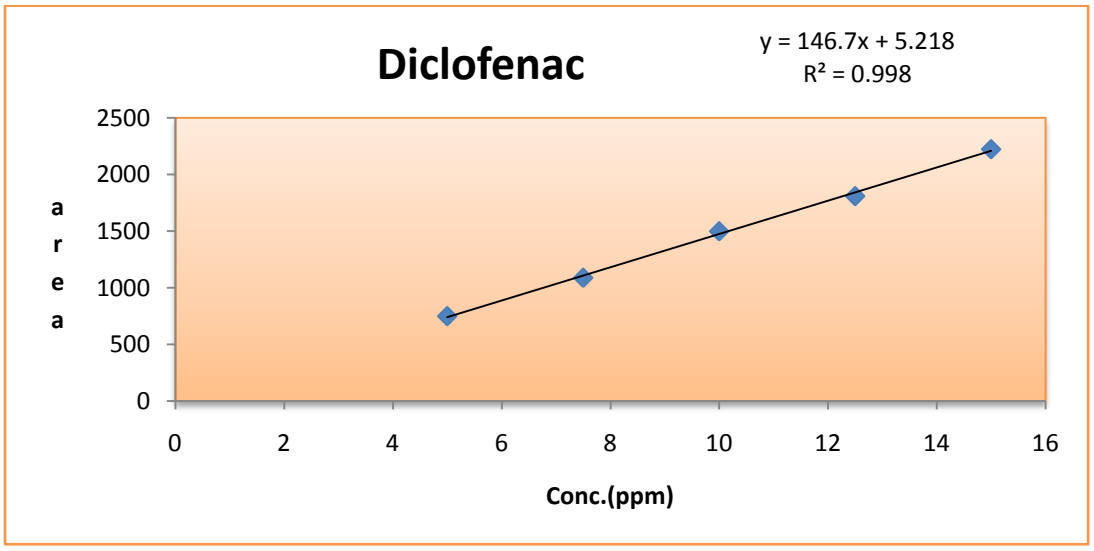

Figure 5: Calibration Curve of DICLO

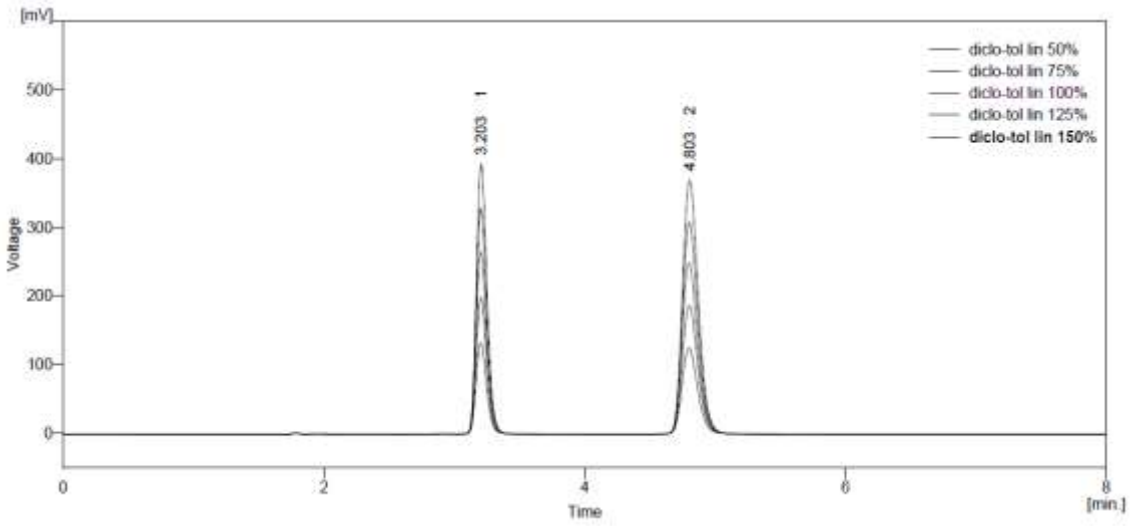

Figure 6: Overlay Chromatogram for Calibration curve of TOL and DICLO.

Table 2: System suitability parameter of TOL and DICLO

Parameter	TOL	DICLO
Retention Time	4.81 ± 0.10	3.21 ± 0.10
Resolution	8.452	-
Asymmetry Factor	1.386	1.415
Theoretical Plates	7232	7057
% RSD	1.53	1.14

Table 3: Calibration data for standard TOL

Sr. No.	***% Concentration with respect to test concentration***	***Concentration ppm(µg/ml)***	***Peak Area mean****
1	50	22.5	1051.358
2	75	33.75	1578.451
3	100	45	2104.344
4	125	56.25	2539.979
5	150	67.5	3120.696
Correlation Coefficient		0.998	
Regression Equation		Y = 45.33 x – 38.88	

***Average of Five determination**

Table 4: Calibration data for standard DILCO

Sr. No.	***% Concentration with respect to test concentration***	***Concentration ppm(µg/ml)***	***Peak Area mean****
1	50	5	748.4
2	75	7.5	1087.589
3	100	10	1497.915
4	125	12.5	1808.289

5	150	15	2222.767
Correlation Coefficient		0.998	
Regression Equation		Y = 146.7 x + 5.218	

***Average of Five determination**

Table 5: Repeatability for TOL and DICLO

TOL				**DILCO**			
Conc. ppm	**Peak Area Mean***	**SD**	**% RSD**	**Conc. ppm**	**Peak Area Mean ***	**SD**	**% RSD**
45	1509.025	13.63	0.903	**10**	2117.245	22.83	1.078

*** Average of six determination**

Table 6: Accuracy data for TOL

Conc. Level (%)	***Amount added (ppm)***	***Amount Found (ppm)***	***% Recovery***	***% Recovery mean****	***S.D.***	***%RSD***
80	36	36.60	101.67	100.48	0.74	0.74
	36	36.25	100.71			
	36	35.87	99.64			
	36	35.92	99.78			
	36	36.29	100.82			
	36	36.10	100.29			
100	45	44.53	98.96	100.16	1.15	1.15
	45	44.62	99.16			
	45	45.50	101.12			
	45	45.84	101.87			
	45	44.80	99.56			

	45	45.12	100.26			
120	54	53.75	99.55	100.29	0.64	0.64
	54	54.44	100.81			
	54	54.22	100.41			
	54	53.80	99.63			
	54	54.63	101.17			
	54	54.08	100.15			

***Average of three determination**

Table 7: Accuracy data for DILCO

Conc. Level (%)	***Amount added (ppm)***	***Amount Found (ppm)***	***% Recovery***	***% Recovery mean****	***S.D.***	***% RSD***
80	8	8.11	101.44	100.03	0.89	0.89
	8	8.04	100.51			
	8	7.98	99.76			
	8	7.96	99.59			
	8	7.90	98.80			
	8	8.00	100.11			
100	10	9.88	98.80	99.99	1.13	1.13
	10	9.90	99.01			
	10	10.09	100.95			
	10	10.16	101.66			

	10	9.94	99.40			
	10	10.01	100.10			
120	12	11.92	99.40	100.12	0.627	0.62
	12	12.08	100.66			
	12	12.02	100.22			
	12	11.93	99.48			
	12	12.11	100.97			
	12	12.00	100.00			

***Average of three determination**

Table 8: Results of Robustness parameters

CONDITION	PEAK AREA MEAN *		SD		%R.S.D.	
	TOL	*DICLO*	*TOL*	*DICLO*	*TOL*	*DICLO*
Change in the Mobile Phase Composition(± 2 ml organic Phase)						
Change in the + 2ml organic phase (62:38 v/v)	2108.847	1503.088	19.71	11.16	0.93	0.74
No Change in the organic phase (60:40 v/v)	2109.955	1505.592	29.12	14.68	1.38	0.97
Change in the - 2 ml organic phase (58:38 v/v)	2104.442	1498.321	25.48	17.61	1.21	1.17
Change pH(±0.2 unit)						
Change in the + 0.2 unit pH (3.7 pH)	2105.783	1500.83	22.25	13.12	1.05	0.87
No Change in the pH (3.5 pH)	2110.033	1503.846	34.86	21.99	1.65	1.46

Change in the -0.2 unit pH (3.3 pH)	2103.477	1497.611	36.80	25.86	1.74	1.72
Change Flow rate (±0.2 ml/min)						
Change in the + 0.2ml/min F.R.(1.2 ml/min)	2013.093	1435.577	28.51	16.70	1.41	1.16
No Change in the F.R. (1.0 ml/min)	2121.261	1511.364	14.12	9.19	0.66	0.60
Change in the - 0.2ml/min F.R.(0.8 ml/min)	2223.554	1583.566	22.80	14.91	1.02	0.94

***Average of three determination**

IV. Conclusion

The novel RP- HPLC methods has been developed for the simultaneous estimation of Tolperisone hydrochloride and Diclofenac sodium in combined tablet dosage is simple, precise, specific, accurate, quick reliable and reproducible. The method gave good resolution for both the drugs with a short analysis time below 6 minutes. The method was completely validated showing satisfactory data for all the method validation parameters tested. The results indicate that the described method can be used for analysis of the compound. The amount found in formulation well agreed with label claim in present of excipients. Thus, the reported method is considerable importance and has great industrial applicability for quality control and analysis of tolperisone hydrochloride and diclofeanc sodium in combined tablet dosage from. This method can also be used for the routine analysis of this combination in other pharmaceutical formulation.

Acknowledgement

I would like to thank my GOD and undefined power which help me to complete it. All which have cooperation to complete it.

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