Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 4| Issue 4|OCT-DEC|2014|123-133

Research Article
Pharmaceutical Sciences

DEVELOPMENTAL AND VALIDATION OF REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR ESTIMATION OF TOLPERISONE HCL AND DICLOFENAC SODIUM IN COMBINED PHARMACEUTICAL DOSAGE FORM

Brijesh Patel*[1], Neha S Mochi[2], Shirish R Patel[3], Rajesh K Patel[4]

[1, 2, 4]K. J. College of Pharmacy, Vadasma

[3]B. S. Patel Pharmacy College, Saffrony Institute of Technology, Linch, India.

*Corresponding Author Email: patelbrijesh1212@gmail.com

ABSTRACT

Tolperisone HCl and Diclofenac sodium combination are widely used for the treatment of patients with acute muscle/musculoskeletal spasm in adult. The objective of work was focused on the developmental and validation of reverse phase high performance liquid chromatography method for quantitative estimation of Tolperisone hydrochloride and Diclofenac sodium in combined pharmaceutical dosage form. Chromatographic separation was achieved on Kromasil C_{18} column (250 mm X 4.6 mm i.d., 5 µm particle sizes) using mobile phase Acetonitrile: Water (90:10) pH adjusted to 3.5 with Orthophosphoric acid, at a flow rate of 1.0 ml/min and detection was carried out at wavelength at 271 nm using UV detector. Linearity was found to be over the range of 2-10 µg/mL for Tolperisone HCl and 1-5 µg/mL for Diclofenac sodium. The retention time of Tolperisone HCl was found to be 2.93 min and the retention time for Diclofenac sodium was found to be 5.37 min. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity, and robustness.

KEY WORDS

Tolperisone HCl, Diclofenac sodium, RP-HPLC, Tablet

INTRODUCTION

Tolperisone HCl is chemically 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl) propan-1-one Hydrochloride. It is piperidine derivative and it is a centrally acting muscle relaxant. It is indicated in the treatment of acute muscle spasms in back pain and spasticity in neurological diseases. 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 presynaptic inhibition of the transmitter release from the primary afferent endings via a combined action on voltage-gated sodium and calcium channels. It 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 [1-4]. Diclofenac Sodium is chemically 2-{2-[(2, 6-dichlorophenyl)

amino] phenyl} acetic acid. It is a Nonsteroidal Anti-Inflammatory Drug (NSAID) and analgesic. It is used to treat inflammatory disorders. It has potent anti-inflammatory, analgesic, and antipyretic actions. It inhibits the enzyme, cyclooxygenase, thus resulting in reduced synthesis of prostaglandin precursors. The combined tablet dosage form of Tolperisone HCl and Diclofenac sodium are commonly used and available in market for treatment of patients with acute muscle/musculoskeletal spasm in adult [3-6]. Literature review revealed that one spectroscopy method for simultaneous estimation has been reported for Tolperisone HCl and Diclofenac sodium. No chromatographic method of Tolperisone hydrochloride & Diclofenac sodium is found. So, attempt was made on the development & validation new, simple, rapid, accurate, economic and sensitive RP-HPLC method for simultaneous estimation of Tolperisone hydrochloride & Diclofenac sodium in combined pharmaceutical dosage form [7-15].

MATERIALS AND METHOD

Instrumentation

HPLC system used was a Youngling's HPLC (YL-9100) with UV Detector; Manual injector of 20-µl loop, Column used was Kromasil C_{18} (250 mm x 4.6 mm i.d., 5 µm particle size). The output signal was monitored and processed using YL Clarity software. Digital pH meter (Elecon) and Ultrasonic bath (Frontline FS 4 ultrasonic cleaner, Mumbai) was also used.

Chemicals and Reagents

Authentic drug sample of Diclofenac sodium were obtained as a gift sample from Torrent pharmaceuticals limited and Tolperisone HCl was obtained as a gift sample from Cadila healthcare Ltd. Methanol, Acetonitrile and Water (HPLC grade) were kindly supplied by S.D. Fine Chemicals Ltd., Mumbai. Diethyl ether and Ortho Phosphoric acid were procured from Chemdyes Corporation. 0.22 µ, 47 mm Nylon membranes were purchased from Fisher scientific.

Chromatographic Conditions

Separation was achieved on a Kromasil C18 column (250 mm X 4.6 mm i.d., 5 µm particle sizes). The optimum mobile phase used was a mixture of Acetonitrile: Water (90:10) pH adjusted to 3.5 with Orthophosphoric acid, respectively. The mobile phase was pumped at room temperature, at a flow rate of 1.0 ml/min. The mobile phase was freshly prepared and filtered through 0.22 µm membrane filter before use. Detection was performed at 271 nm using UV detector.

Determination of wavelength of maximum absorbance

The standard solution of Tolperisone HCl and Diclofenac sodium was scanned in the range of 200-400 nm against mobile phase as a blank. It showed optimum iso absorptive point at 271 nm and hence this wavelength was selected for the determination of Tolperisone HCl and Diclofenac sodium.

Preparation of solutions

Preparation of standard stock of (100µg/ml)

Accurately weighed 10 mg of Tolperisone HCl and 10 mg of Diclofenac sodium were transferred to two separate 100 ml volumetric flask. 50 ml methanol was added to the flask. The drug was dissolved with sonication and the final volume was adjusted with methanol up to the mark to prepare a 100 µg/ml stock solution of both drugs.

Preparation of working standard solution (100 µg/ml)

The aliquots of the above stock solution were diluted further with diluent to get concentrations of 2-10 µg/mL for Tolperisone HCl and 1-5 µg/mL for Diclofenac sodium.

Quantification of Tolperisone HCl and Diclofenac sodium in formulation

Twenty tablets were weighed accurately and powered. Quantity of the powder equivalent to

150 mg of Tolperisone HCl and 50 mg of Diclofenac sodium was transferred into 100 ml measuring flask and sonicated for 20 minutes. The solutions were filtered through 0.22 µ, 47 mm Nylon membrane and the residue was washed thoroughly with methanol. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with methanol to obtain a concentration of 1500µg/mL for Tolperisone HCl and 500µg/mL for Diclofenac Sodium.1 ml of above solution was transferred into a 10 ml volumetric flask and diluted to mark with methanol (150 µg/mL of Tolperisone HCl and 50 µg/mL of Diclofenac Sodium). This solution was used as working sample solution.

RESULTS AND DISCUSSION

Method development [16-19]

Knowledge of survey suggests that reverse phase liquid chromatography is a suitable method for analysis of Tolperisone HCl and Diclofenac sodium. Analytical method development with C18 column (250 mm X 4.6 mm i.d., 5 µm particle size) was preferred over other columns. A Kromasil C18 was preferred as it has high carbon loading with very closely packed material to give high resolution over other C18 columns. For selection of wavelength of determination, drug solutions were scanned in the range of 200 to 400 nm and Tolperisone HCl has λ_{max} 256 nm and Diclofenac sodium has λ_{max} 276 nm. Chromatogram at 256, 276 and iso absorptive point 271 nm was taken. In that at 271 nm, both drugs give good peak height and shape. So, 271 nm was selected for simultaneous estimation of Tolperisone HCl with Diclofenac sodium in tablet dosage forms was shown in Figure 3. Resolution is the most important criteria for the method, it was imperative to achieve good resolution among compounds. As per the value of pKa and solubility of the compound, various compositions of mobile phase were tried. The compound was separated with mobile phase in different proportions of Acetonitrile, Methanol and Water. Good peak shape with clear baseline and resolution was obtained within very short retention of 2.93 minutes and 5.37 minutes with mixture of Acetonitrile and Water in proportion of 90:10 v/v (pH of mobile phase was adjusted 3.5 with ortho-phosphoric acid.), respectively as mobile phase at a flow rate of 1.0 ml/min. All other method parameters were developed to obtained good peak shape. A representative chromatogram was shown in Figure 4, which satisfies all the system suitability criteria and better resolution of peaks from solvent peak with clear base line separation.

Method validation [20]

The developed method was validated in accordance with ICH guidelines (ICH Q_2R_1) for accuracy, precision, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ) and robustness.

Linearity

The linearity for Tolperisone HCl and Diclofenac Sodium was found in the range of 2 - 10 µg/ml and 1-5 µg/ml respectively. The overlain chromatogram of Tolperisone HCl and Diclofenac Sodium was presented in Figure 5. Calibration curves of Tolperisone HCl and Diclofenac Sodium were presented in Figure 6 and 7 respectively. Regression analysis data are listed in Table 1 and 2. Responses for each drug was linear (r^2 = 0.9990 and 0.9996 for Tolperisone HCl and Diclofenac sodium, respectively).

Precision

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, Relative standard deviation, or coefficient of variance of a series of measurements.

Repeatability (Precision on replication)

The precision of the instrument was checked by repeatedly (n=6) measuring the Area of Tolperisone HCl (6 µg/mL) and Diclofenac sodium (3 µg/mL). The data of repeatability for Tolperisone HCl and Diclofenac sodium are shown in Table 3 and 4. The developed method was found to be precise as the %RSD values for repeatability study were found to be less than 2.0%.

Intermediate precision (Reproducibility)

It expresses within laboratory variations as on different days analysis or equipment within the laboratory. The data for intra-day and inter-day precision for Tolperisone HCl and Diclofenac sodium are shown in Table 5 and 6. The developed method was found to be precise as the %RSD values for reproducibility study were found to be less than 2.0%.

Accuracy (% Recovery)

It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in Table 7. Recovery greater than 98 % with low S.D. justifies the accuracy of the method.

Limit of detection and limit of Quantitation

Limits of detection for both drugs were calculated theoretically. LOD for Tolperisone HCl and Diclofenac sodium were found to be 0.028 µg/mL and 0.012 µg/mL, respectively (Table 8). Limits of quantitation for both drugs were calculated theoretically. LOQ for Tolperisone HCl and Diclofenac sodium were found to be 0.085 µg/mL and 0.038 µg/mL, respectively (Table 8).

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. In the case of assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients.

System suitability

System suitability parameter is establishing to ensure that the validity of the analytical method is maintained whenever used. Typical variations are the stability of analytical solution, different equipment, and different analyst. In case of liquid chromatography typical variations are the wavelength, the mobile phase composition and flow rate. Results were shown in Table 9.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The typical variations studied under this parameter are flow rate, mobile phase composition and change in pH. The results are shown in the Table 10. Variation seen was within the acceptable range respect to peak asymmetry and theoretical plates, so the method was found to be robust.

Analysis of marketed formulation

Applicability of the proposed method was tested by analyzing the commercially available tablet formulation. The results are shown in Table 11.

Table 1: Linearity data for Tolperisone HCl and Diclofenac Sodium

Tolperisone HCl		Diclofenac Sodium	
Concentration (µg/ml)	Area (n=3)	Concentration (µg/ml)	Area (n=3)
2	232.16	1	454.56
4	435.11	2	887.87
6	632.72	3	1289.87
8	870.59	4	1754.44
10	1079.7	5	2183.42

Table 2: Linear regression equation data of Tolperisone HCl and Diclofenac Sodium

Drug	Concentration µg/ml	Slope ± S.D, % RSD (n=3)	Intercept ± S.D, % RSD (n=3)	Corrélation coefficient ± S.D, % RSD (n=3)
Tolperisone HCl	2-10	106.52 ± 0.051, 0.048	11.03 ± 0.20, 1.85	0.9990 ± 0.00012, 0.011
Diclofenac Sodium	1-5	432.43 ± 0.026, 0.006	16.62 ± 0.31, 1.89	0.9996 ± 0.00, 0.00

Table 3: Repeatability data of Tolperisone HCl

Tolperisone HCl				
Parameters	Concentration (µg/ml)	Area	Amount Found (µg/ml)	% Assay of Tolperisone HCl
1st	6	633.02	6.044	100.74
2nd	6	630.8	6.024	100.40
3rd	6	631.62	6.031	100.53
4th	6	631.27	6.028	100.47
5th	6	630.34	6.019	100.33
6th	6	631.45	6.030	100.50
Mean		631.41	6.029	100.49
SD		0.913	0.0085	0.142
%RSD		0.1447	0.142	0.142

Table 4: Repeatability data of Diclofenac Sodium

Diclofenac sodium				
Parameters	Concentration (µg/ml)	Area	Amount Found (µg/ml)	% Assay of Diclofenac sodium
1st	3	1289.01	3.019	100.65
2nd	3	1289.83	3.021	100.72
3rd	3	1286.91	3.014	100.49
4th	3	1285.21	3.010	100.36
5th	3	1286.95	3.015	100.50
6th	3	1287.74	3.016	100.56

Mean	1287.61	3.016	100.55
SD	1.648	0.0038	0.127
%RSD	0.128	0.126	0.126

Table 5: Intra-day and Inter-day precision data of Tolperisone HCl

Concentration µg/ml	Intra-day measured mean Area, ± S.D, % RSD (n=3)	Inter-day measured mean area, ± S.D, % RSD (n=3)
4	232.16±1.596, 0.68	232.2 ± 1.712, 0.73
8	435.11±0.917, 0.21	435.3 ± 1.265, 0.29
12	632.72±0.632, 0.09	632.6 ± 0.737, 0.11
16	870.59±1.387, 0.15	870.3 ± 1.588, 0.18
20	1079.70±0.835, 0.07	1079.4±1.205, 0.11

Table 6: Intra-day and Inter-day precision data of Diclofenac Sodium

Concentration µg/ml	Intra-day measured mean Area, ± S.D, % RSD (n=3)	Inter-day measured mean area, ± S.D, % RSD (n=3)
4	454.56±1.35, 0.29	453.0 ± 1.525, 0.33
8	887.87±1.13, 0.12	885.6 ± 1.802, 0.20
12	1287.0±0.50, 0.03	1290.1 ± 1.16, 0.09
16	1754.4±0.74, 0.04	1755.3 ± 1.0, 0.05
20	2183.4±1.52, 0.06	2182.9 ± 1.80, 0.08

Table 7: Recovery data of Tolperisone HCl and Diclofenac Sodium

Drug	Amount sample taken (µg/mL)	Amount Drug added (µg/mL)	Amount found (µg/mL) ± S.D (n=3)	% Recovery ± S.D (n=3)
Tolperisone HCl	4	2	5.89 ± 0.012,0.20	98.24 ± 0.20,0.20
	4	4	7.98 ± 0.004,0.058	99.81 ± 0.05,0.05
	4	6	9.92 ± 0.011,0.11	99.22 ± 0.11,0.11
Diclofenac sodium	2	1	2.99 ± 0.002,0.096	99.90 ± 0.09,0.09
	2	2	3.97 ± 0.0044,0.11	99.39 ± 0.11,0.11
	2	3	4.95 ± 0.0061,0.12	99.09 ± 0.12,0.12

Table 8: Summary of Validation parameters

Parameters	Tolperisone HCl	Diclofenac sodium
	271 nm	271 nm
Beer's Law Limit (µg/ml)	2-10	1-5
Regression equation (y* = a + bc)		
Slope (b)	106.52	432.43
Intercept (a)	10.89	16.74
Correlation Coefficient (r^2)	0.9990	0.9996
Standard Deviation (S.D)	0.913	1.64
Relative Standard Deviation (%RSD)	0.144	0.128
LOD (µg/ml)	0.028	0.012
LOQ (µg/ml)	0.085	0.038
Precision		
Intra-day (n=3) (%RSD)	0.07-0.68	0.03-0.29
Inter-day (n=3) (%RSD)	0.11-0.73	0.05-0.33

Table 9: System suitability parameters

Drug	Retention time	Resolution	Area	Theoretical plate	Asymmetric factor (A_f)	Tailing factor
Tolperisone HCl	2.93	5.42	650.0	2274.71	0.91	1.11
Diclofenac sodium	5.37		1314.0	2718.92	0.87	1.34

Table 10: Robustness data of Tolperisone HCl and Diclofenac Sodium

Condition	Variation	Tolperisone HCl			Diclofenac Sodium		
		% Assay	S.D. (n=3)	% RSD	% Assay	S.D. (n=3)	% RSD
Flow rate (1 ml/min)	1 ± 0.1	99.16	0.3404	0.3433	99.57	0.3955	0.3972
Mobile phase ACN : Water, 90:10 (v/v)	90 : 09	99.41	0.4417	0.4443	99.09	0.1652	0.1667
	89 : 10	98.26	0.4508	0.4587	98.55	0.2364	0.2399
Wavelength (271 nm)	271±1	99.46	0.4105	0.4127	99.13	0.2013	0.2030

Table 11: Analysis of marketed formulation

Drug (Tolperitas D)	Amount Taken (µg/mL)	Amount found (µg/mL) ± S.D, %RSD (n=3)	% Recovery ± S.D, %RSD (n=3)
Tolperisone HCl	3	2.97 ± 0.013, 0.46	99.01 ± 0.37, 0.38
	6	5.98 ± 0.014, 0.24	99.68 ± 0.24, 0.24
Diclofenac sodium	1	0.99 ± 0.0035, 0.35	99.63 ± 0.28, 0.29
	2	1.99 ± 0.0023, 0.11	99.97 ± 0.09, 0.09

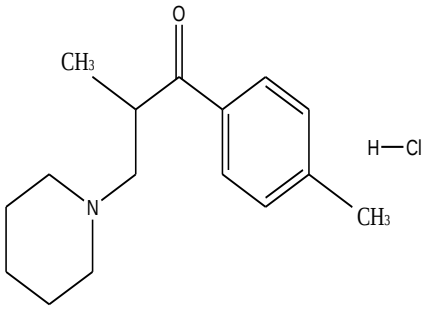

Figure 1: Structure of Tolperisone HCl

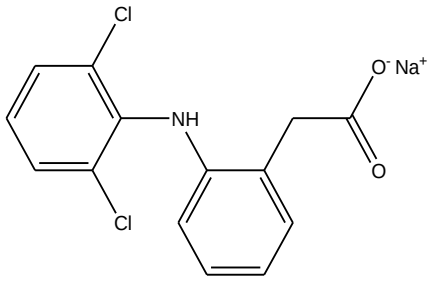

Figure 2: Structure of Diclofenac Sodium

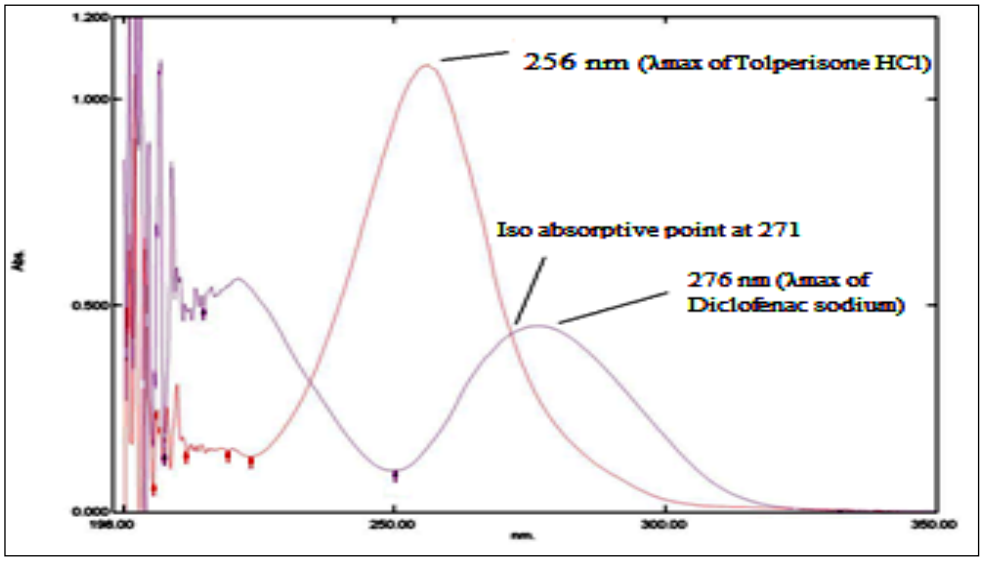

Figure 3: UV Spectra of Tolperisone HCl and Diclofenac Sodium

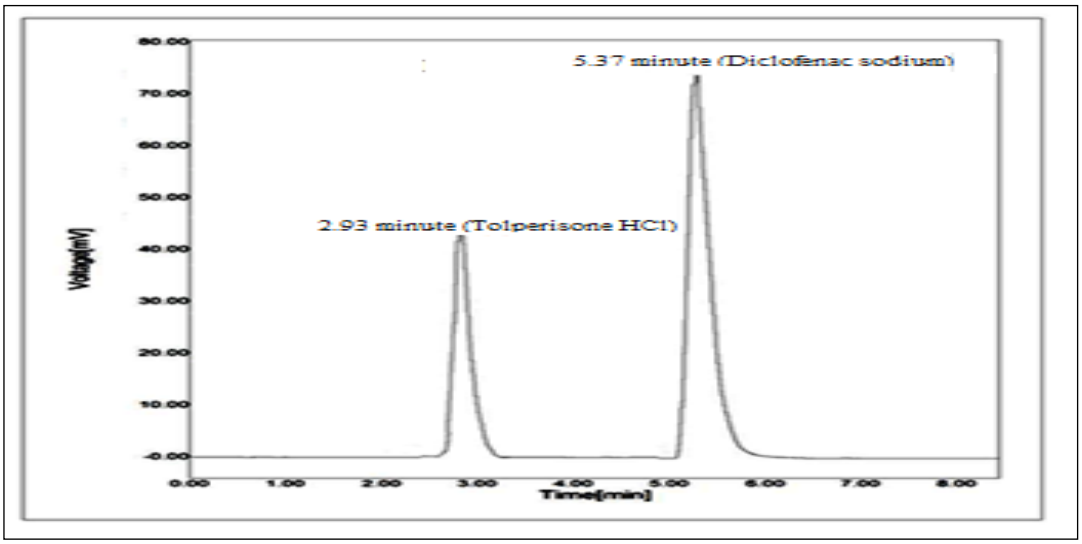

Figure 4: Chromatogram of Tolperisone HCl and Diclofenac Sodium standard

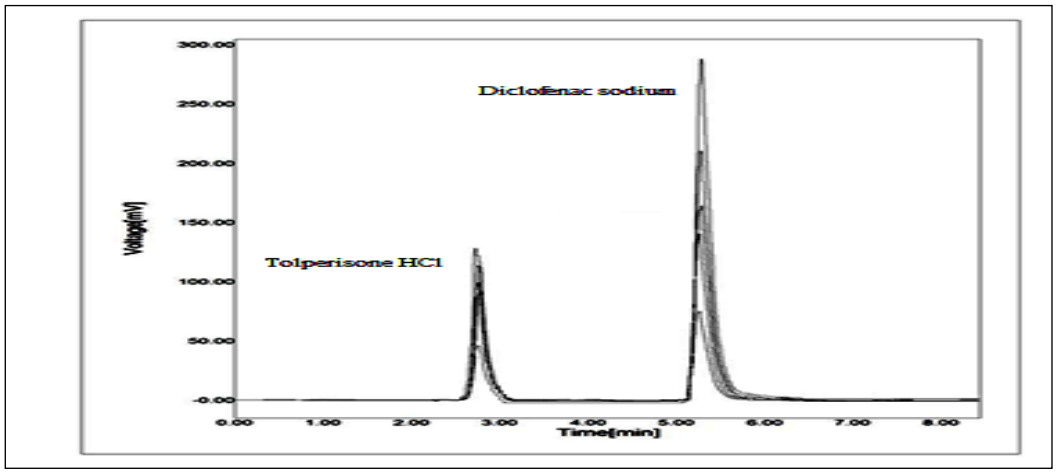

Figure 5: Overlain chromatogram of Tolperisone HCl and Diclofenac Sodium

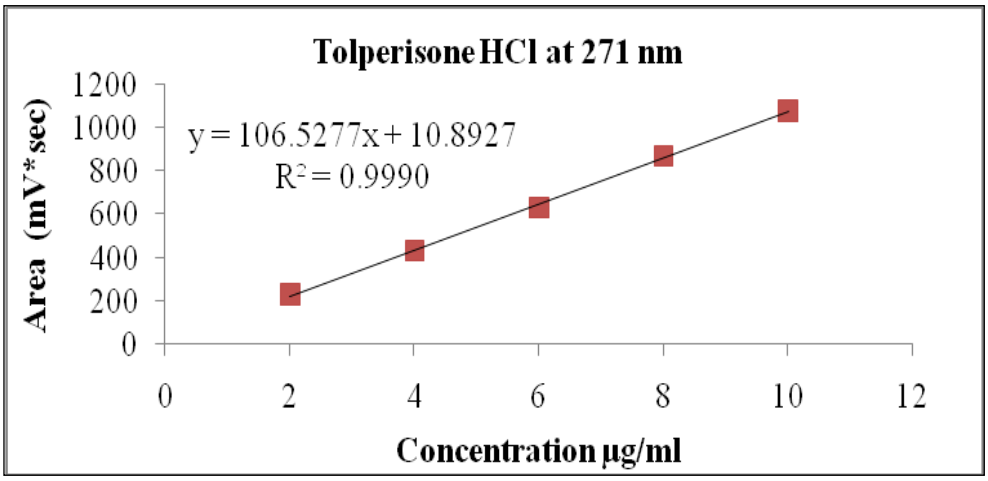

Figure 6: Calibration curve of Tolperisone HCl

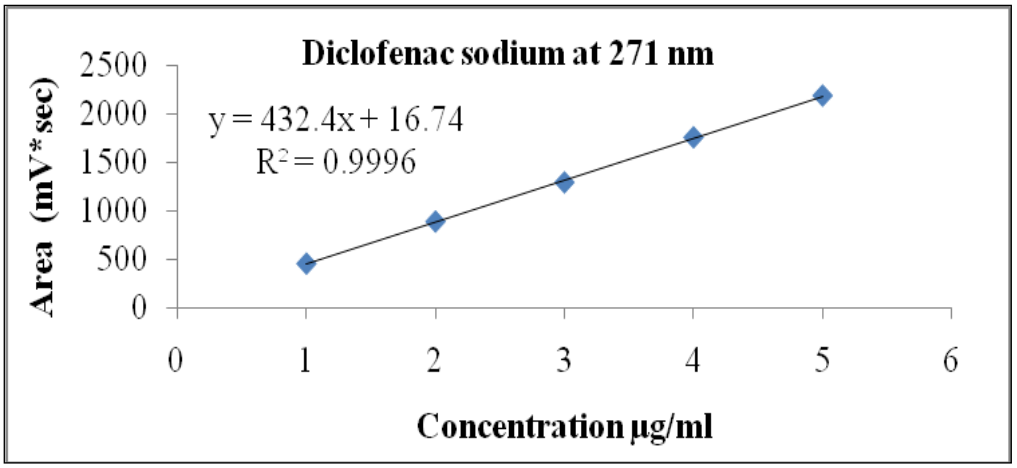

Figure 7: Calibration curve of Diclofenac sodium

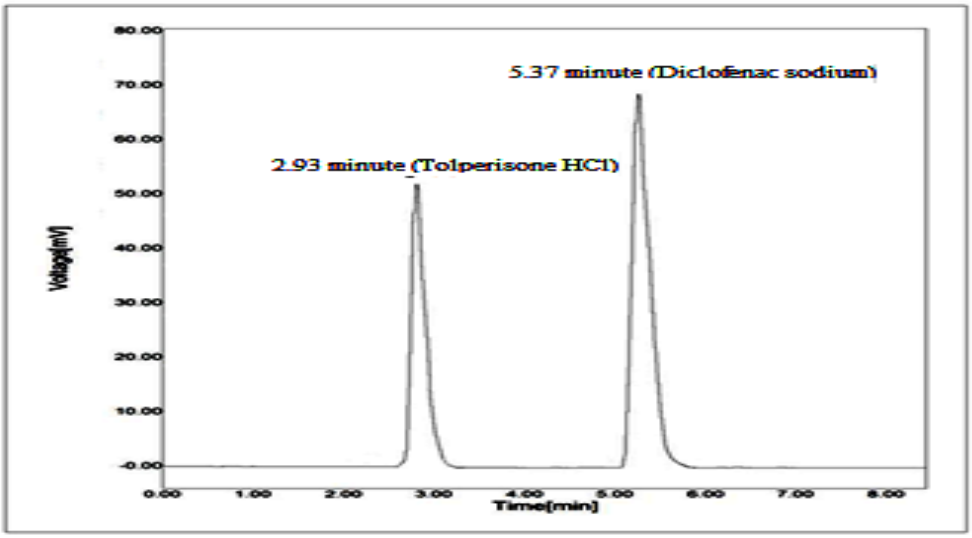

Figure 8: Chromatogram of Tolperisone HCl and Diclofenac Sodium from marketed formulation

CONCLUSION

Based on the results, obtained from the analysis of dosage form using described method, it can be concluded that there were no other co-eluting peaks of interference with the main peaks and the method is specific for the determination of Tolperisone HCl and Diclofenac sodium. The method has linear response in the range of 2-10 µg/ml for Tolperisone HCl and 1-5 µg/ml for Diclofenac sodium. Results of recovery study with low values of %RSD indicate that the developed method was accurate. Results of method precision and intermediate precision with low value of %RSD show that the developed method was precise. The developed RP-HPLC method meets the system suitability criteria and resolution for the parent drug. The analytical results demonstrated the ability of developed method to assay Tolperisone HCl and Diclofenac sodium in combined pharmaceutical dosage form.

ACKNOWLEDGEMENT

The authors are thankful to Torrent pharmaceuticals limited for providing samples of

pure Tolperisone HCl and Cadila healthcare Ltd. for providing samples of pure Diclofenac sodium.

REFERENCES

1. Rang, H.P., Dale, M.M., Ritter, J.M., Moore, P.K., Pharmacology, 5th Edn, Elsevier, New Delhi, pp. 584-587 (2007).
2. Tripathi, K.D., Essentials of Medical Pharmacology, 5th Edn, New Delhi, J P Brothers Medical Publishers Pvt. Ltd, pp. 184 (2004).
3. Maryadele. J. O. Neil, An Encyclopedia of chemicals, drugs and biological, 13th Edn, Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station, New Jersey, pp. 542, 1698 (2006).
4. Sweetman, S. C., Martindale, the Complete drug reference; 35th Edn, Pharmaceutical press, London, pp. 38-40, 1738 (2007).
5. Japanese Pharmacopoeia. 15th Edn, Society of Japanese Pharmacopeia, Shibuya Tokyo Japan, pp. 1190-1191(2006).
6. Goyal, R. K., Elements of Pharmacology, 17th Edn, B. S. Shah Prakashan, Ahmedabad, pp. 474-491(2007).
7. Koladiya, B. B., Vaghela, V. M., UV spectroscopic method for quantitative estimation of Tolperisone hydrochloride in bulk and pharmaceutical dosage form, Int. J. Pharm. Tech. Res., 4(3): 1317-1322 (2012).
8. Murali, M., Satyanarayana, P. V., Simple validated isocratic RP –HPLC method for estimation of Tolperisone in bulk and pharmaceutical dosage form, *Der.* Pharma. Chem., 3(5), 13-19 (2011). .
9. Liawruangrath, S., Liawruangrath, B., Pibool, P., Simultaneous determination of Tolperisone and Lidocaine by HPLC, J Pharm Biomed Anal., 26(5): 865-872 (2001).
10. Sireesha, K., Kumar, A. R., Simultaneous estimation of Tolperisone and Lornoxicam by RP-HPLC, Int. J. Adv. Pharma. Res., 3(7): 981-987(2012).
11. The Indian pharmacopoeia, Volume II, Ghaziabad, Published by the Indian pharmacopoeia commission, pp. 1020-1021(2007).
12. British Pharmacopoeia, Volume I & II, London, Published by stationary office on behalf of MHRA, pp. 1893 (2009).
13. Pandey, G., Spectrophotometric Methods for Estimation of Diclofenac Sodium in Tablets, Int. J. Biomed. Adv. Res., 4(2): 77-82 (2013).
14. Heda, A. A., Gadade, D. D., Kathiriya, J. M., Puranik, P. K., HPLC Method development and validation for simultaneous analysis of Diclofenac sodium and Rabeprazole sodium, J. Chem., 7(1) :386-390 (2010).
15. Mahaparale, S. P., Shinde, S. S., Nirmal, P. N., Simultaneous UV-spectrophotometric Estimation of Diclofenac and Tolperisone Hydrochloride in Tablet Dosage Form, Int. J. Rese. Deve. Sci., 2(5): 574-579 (2013).
16. Patel, R. K., Raval, B. P., Patel, B. H., Reverse Phase High Performance Liquid Chromatographic method for the simultaneous estimation of Esomeprazole and Itopride in Capsule, Der Pharma Chemica, 2: 251-260 (2010).
17. Patel, R. K., Patel, H. R., Patel, V. A., Development and validation of RP-HPLC method for simultaneous determination of Omeprazole and Diclofenac sodium in capsule dosage form, Journal of Pharmaceutical Research, 5: 1640-1642 (2012).
18. Patel, R. K., Patel, L. J., Development and Validation of RP-HPLC Method for Simultaneous Determination of Ranitidine HCl and Drotaverine in Tablet, Inventi Impact: Pharmaceutical Analysis and Quality Assurance, 2:78-81 (2011).
19. Beckett, A. H., Stenlake, J. B., Practical pharmaceutical chemistry, 14th Edn, CBS publishers and distributors, New Delhi, pp. 275-337 (1997).
20. ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1). Current Step 4 Version, Parent guideline, 1994 Oct 27. Complementary Guideline on Methodology dated 6th Nov. 1996.

***Corresponding Author Email:**
patelbrijesh1212@gmail.com