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Rp-Hplc Method for Simultaneous Determination of Tolperisone Hcl and Diclofenac Sodium in Pharmaceutical Dosage Form

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Received: March 15, 2017; Accepted: April 15, 2017; Published: May 27, 2017

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Abstract

A new method was established for simultaneous estimation of Tolperisone and Diclofenac sodium by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Tolperisone and Diclofenac sodium by using Agilent C18 column flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) ACN: phosphate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 240nm.The retention times were found to be 4.645 mins and 2.242 mins. The % purity of Tolperisone and Diclofenac sodium was found to be 100.3% and 99.27% respectively. The analytical method was validated according to ICH guidelines. The precision study was precision, robustness and repeatability. LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.

Key words: Tolperisone; Diclofenac sodium; RP-HPLC; PDA Detection; Tablet dosage form

Introduction

Diclofenac sodium (DIC) [1-9] is a non-steroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and as an analgesic reducing pain in certain conditions. Chemically it is 2-(2-(2,6- dichlorophenylamino)phenyl) acetic acid 1,2. Tolperisone hydrochloride (TOL) is a piperidine derivative, is a centrally-acting muscle relaxant used in the treatment of acute muscle spasms in back pain and spasticity in neurological diseases 1,2 Chemically it is 2-methyl-1-(4- methylphenyl)-3-(1-piperidyl) propan-1- one 1,2. Diclofenac sodium (DIC) and Tolperisone hydrochloride (TOL) and are available in tablet dosage form in the ratio 1:3. Diclofenac sodium is official in Martindale, The Extra Pharmacopoeia1, The Merck Index2, I. P.3, B. P.4 and U. S. P.5 whereas Tolperisone hydrochloride is official in Martindale, The Extra Pharmacopoeia1 and The Merck Index2.

Literature survey reveals that many analytical methods such as spectrophotometric6 and RP-HPLC7-9 methods are reported for determination of Diclofenac sodium individually from pharmaceutical dosage form and UV spectrophotometric; HPLC11-14 methods are reported for determination of DIC with other drugs in combined dosage form. Some UV spectrophotometric15-18, HPLC methods19-22 and HPTLC23,24 methods are reported for determination of Tolperisone hydrochloride individually from pharmaceutical dosage form and UV

spectrophotometric25, HPLC26 methods are reported for determination of TOL with other drugs in combined dosage form. This paper represents two simple, rapid, accurate, precise, reproducible and economic RPHPLC methods for simultaneous estimation of Diclofenac sodium and Tolperisone hydrochloride in bulk and tablet dosage form.

Materials and methods

Instrument

HPLC WATERS-2695 series containing degasser, binary gradient pump and UV detector is used.

Chemicals and Reagents

Standard gift samples of Diclofenac sodium and Tolperisone hydrochloride were procured from Dr. reddy's Pharmaceuticals Pvt. Ltd, Hyderabad. Combined Diclofenac sodium and Tolperisone hydrochloride tablets were purchased from local market. Acetonitrile and methanol (HPLC grade) was obtained from Merk Laboratories Pvt. Ltd., Mumbai.

Chromatographic Conditions

The chromatographic separation was performed on an Agilent 1120 series, which comprised a degasser, binary gradient pump and UV detector. The system was controlled through Ezchrome software using Chromasil C18 (4.6 x 250 mm, 5 µm; Advanced Chromatography Systems, Johns Island, SC) column maintained at 30°C temperature and a mobile phase flow rate of 1.0 ml/min. The mobile phase was composed of Acetonitrile: water pH adjusted to 3.0 with O- Phosphoric acid (65:35v/v). The mobile phase was kept in ultra sonicator for 30 min. and filtered

through a 0.45-µm nylon membrane filter. Measurements were made with injection volume 20 µL and UV detection at 262.0 nm.

Standard stock solutions

The stock solution (100 µg/ml) of DIC and TOL were prepared separately by dissolving accurately about 10 mg of each drug in 100 ml methanol HPLC grade in 100 ml volumetric flask.

Calibration Curve

Appropriate aliquots of standard stock solutions of DIC and TOL were diluted with mobile phase to obtain concentrations in the range of 5, 10, 20, 30, 40, 50 and 60 µg/ml of DIC and 5, 10, 20, 30, 40, 50, 60, 70 and 80 µg/ml of TOL respectively. Calibration curves of DIC and TOL were constructed by plotting peak area verses concentration separately. The linearity of DIC and TOL was found to be in the concentration ranges of 5-60 µg/ml and 5-80 µg/ml, respectively (Table 1), at their respective maxim as. The coefficients of correlation were found to be 0.9993 for DIC and 0.9990 for TOL (Table 1). The mixed standard solution containing 20 µg/ml of DIC and 60 µg/ml of TOL was prepared from standard stock solution and injected into HPLC system Figure 1.

Table 1: Analysis of Tablet formulation

Tablet sample	Label claim Mg/Tab	Amount found Mg/Tab	%Label claim found
TOL	150	149.50	99.98
DIC	50	49.79	99.67

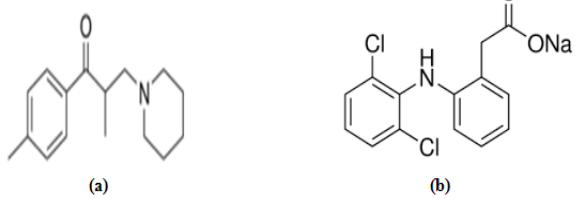

Figure 1: Chemical structure of Tolpersone and Diclofenac sodium (a and b)

Preparation of Phosphate Buffer

2.95 grams of KH2PO4and 5.45 grams of K2HPO4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with ortho phosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml of ACN (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents Preparation

Mobile phase was used as the diluent

Preparation of the Individual Tolperisone Standard Preparation

e10 mg of Tolperisone working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.5 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent. (150 ppm)

Preparation of the Individual Diclofenac Sodium Standard Preparation

10 mg of Diclofenac sodium working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2ml of diluent and sonicate to Dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 0.5 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluents (50 ppm).

Analysis of Tablet Formulation

Twenty tablets each containing 50 mg of Diclofenac sodium and 150 mg of Tolperisone hydrochloride were weighed and crushed in glass mortar to obtain fine powder. The powder sample equivalent to 10 mg of Diclofenac and 30 mg of Tolpersone was transferred into a 100 ml volumetric flask and dissolved in 50 ml methanol HPLC grade. The flask was kept in an ultrasonic bath for 20 min. The volume was adjusted to 100 ml with methanol HPLC grade. The solution was filtered through 0.2 µ nylon membrane filter. From this stock solution, 2 ml solution was pipetted out and transferred to 10 ml volumetric flask and made volume up to the mark with mobile phase to get the concentration 20 µg/ml of DIC and 60 µg/ml of Tolpersone. The solution was injected into HPLC system (Figure 2). The results of the assay of tablet formulation and its statistical validation data is given in Table 2.

Table 2: System suitability parameters

Parameter	DIC	TOL
Linearity*	37.5-225	12.5-75
Correlation coefficient	0.9995	0.9999
Slope *	9951	454.3
LOD	0.345	0.642
LOQ	0.623	0.97
Retention time*(mins)	2.4	4.6
Resolution*	-	13.7
Tailing factor	1.3	0.75
Theoretical factor*	7563	1567
*Average of six readings		

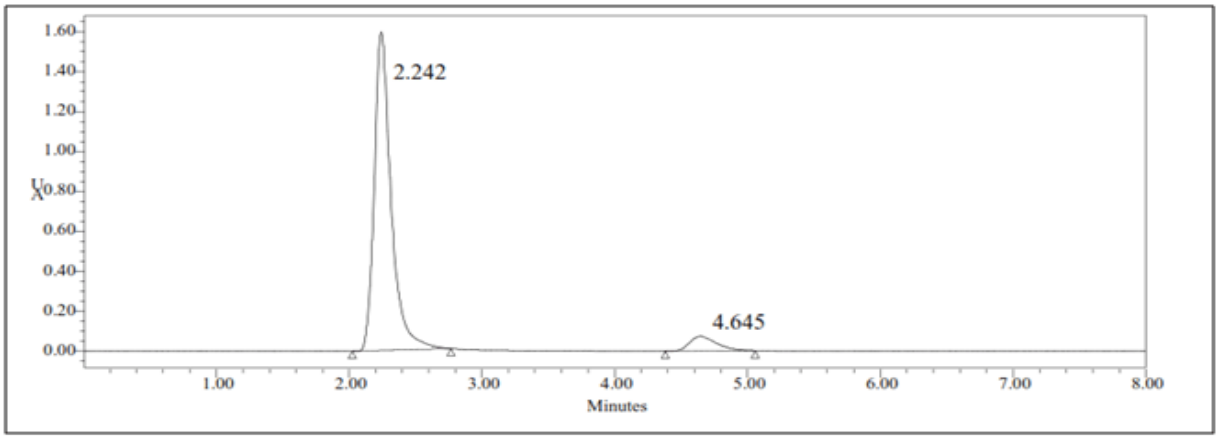

Figure 2: Chromatogram of Tolperisone and Diclofenac in standard mixture

Results and Discussion

DIC and TOL were well- Advanced resolved using mobile phase composition of Acetonitrile: water pH adjusted to 3.0 with O- Phosphoric acid (65:35 v/v) at flow rate of 1 ml/min, UV detection wavelength 240 nm and injection volume 20µl. The HPLC system was found to best for analysis. The retention time for Drotaverine hydrochloride and Paracetamol were found to be 2.3 min and 4.6 min, respectively. The resolution between two peaks was found to be 8.0.

Method Validation

Specificity

The specificity of the method is used to evaluate the homogeneity of drug peak. The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Tolperisone and Diclofenac sodium was obtained and the isobestic point of Tolperisone and Diclofenac sodium showed absorbance's maxima at 240 nm.

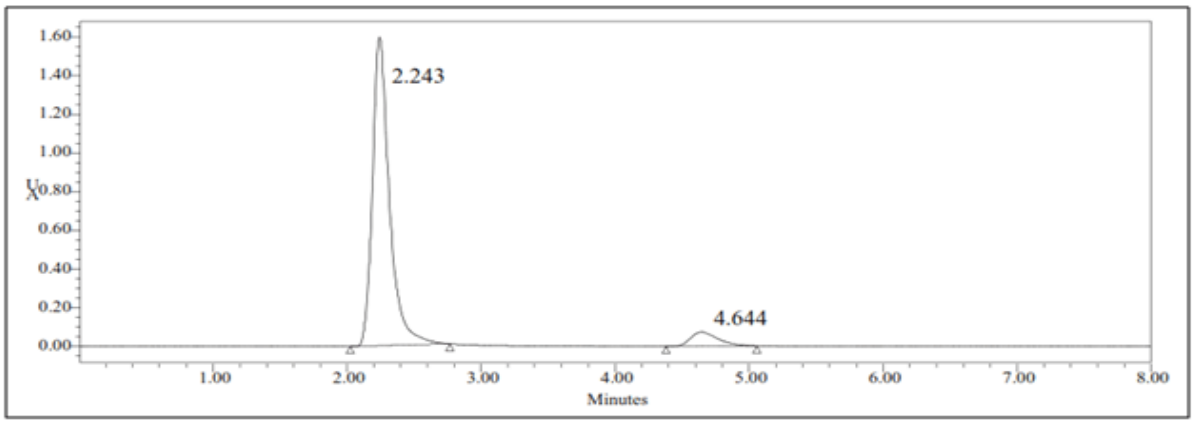

Figure 3: Chromatogram of Tolpersone and Diclofenac in Tablet Formulation

Linearity

Linearity for DIC and TOL was selected at 5-60 µg/ml and 5-80 µg/ml. The correlation coefficients were selected at 0.9993 and 0.9990 for DIC and TOL, respectively. The results are shown Table 1.

The precision of the method was studied by determining the concentrations of each drug in the tablets six times. The results of the precision study indicate that the method is reliable (% RSD < 2).

Precision (repeatability)

The precision of the method was studied by determining the concentrations of each drug in the tablets six times. The results of the precision study indicate that the method is reliable (% RSD < 2).

Citation: G Kumaraswamy, V Swapna and D Sudheer kumar.(2017) Rp-Hplc Method for Simultaneous Determination of Tolperisone Hcl and Diclofenac Sodium in Pharmaceutical Dosage Form. SOJ Pharm Pharm Sci 4(3):1-6. DOI: http://dx.doi.org/10.15226/2374-6866/4/3/00162

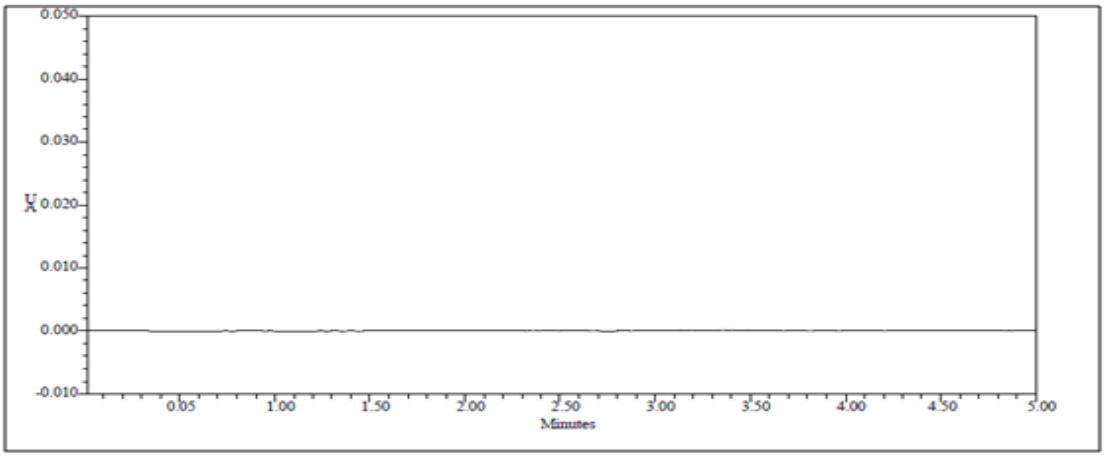

Figure 4: Chromatogram of the blank solution

Accuracy

Accuracy of the method was studied by recovery experiments. The recovery was performed at three levels, 80 %, 100 %, and 120 % of the label claim of the tablet (50 mg of DIC and 150 mg of TOL). The results are shown in Table 3.

Table 3: Linearity data of Tolpersone and Diclofenac sodium Precision (repeatability)

S.No	Linearity Level Concentration		Peak Area	
	Diclofenac	Tolpersone	Diclofenac	Tolpersone
1	37.5 ppm	12.5 ppm	56472	471543
2	75 ppm	25 ppm	73841	606277
3	100 ppm	50 ppm	84655	844999
4	187.5ppm	62.5 ppm	126541	966124
5	225 ppm	75 ppm	140567	1102139
Correlation Coefficient			0.999	0.999

Robustness

The robustness of a method is the ability of method to remain unaffected by small changes in parameters like mobile phase composition, flow rate, pH of mobile phase and temperature etc

Determination of Limits of Quantification and Detection

LOD and LOQ were determined based on the standard deviation of y-intercepts of calibration curves and slope of calibration curve. The results are shown in Table 1.

Summary and Conclusion

A new method was established for simultaneous estimation of Tolperisone Hcl and Diclofenac sodium by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Tolperisone and Diclofenac sodium by using Agilent C18 column (4.6×150mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) ACN: phosphate buffer (KH2PO4and K2HPO4) phosphate pH 3 (pH was adjusted with ortho phosphoricacid),detection wavelength was 240nm.

Table 4: Recovery studies

%Concentration (atspecification level)	Amount added (mg)		Amount found (mg)		% Recovery		Mean recovery	
	TOL	DIS	TOL	DIS	TOL	DIS	TOL	DIS
50%	75	25	74.96	24.99	99.91	99.53		
100%	150	50	149.98	49.05	99.18	99.38	99.56	99.47
150%	225	75	224.02	74.495	99.6	99.52		

The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 4.645 mins and 2.242 mins. The % purity of Tolperisone and Diclofenac sodium was found to be 100.3% and 99.27% respectively. The system suitability parameters for Tolperisone and Diclofenac sodium such as theoretical plates and tailing factor were found to be 2442, 1.1 and 2698 and 1.3, the resolution was found to be 5.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Tolperisone and Diclofenac sodium was found in concentration range of 50µg-250µg and 5µg-25µg and correlation coefficient (r2) was found to be 0.999 and

0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 1.2and 0.1, % RSD for intermediate precision was 0.4 and 0.1 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.

Hence the suggested RP-HPLC method can be used for routine analysis of Tolperisone Hcl and Diclofenac sodium in API and Pharmaceutical dosage form.

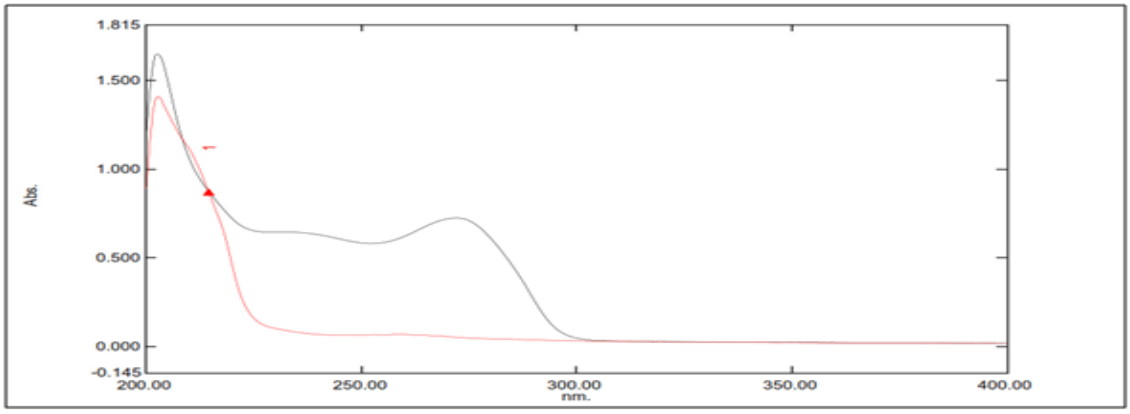

Figure 5: Spectrum showing overlapping spectrum of Tolperisone and Diclofenac

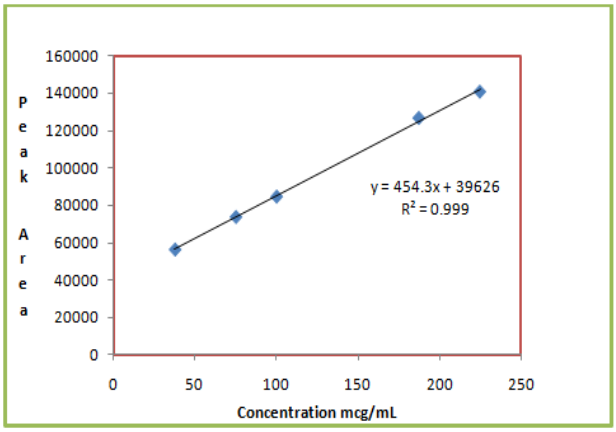

Figure 6: Calibration graph of Tolpersone

Acknowledgment

The authors are thankful to the Chairman, Care College of Pharmacy, Warangal for providing excellent research facilities and Dr. Reddy's Laboratories Pvt. Ltd., Hyderabad for providing the gift sample of Diclofenac sodium and Tolperisone hydrochloride respectively.

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