MS.ID-IJPBS-8343
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2022) 12 (3): 64-70
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

RP HPLC Method Development and Validation of Tolperisone HCl and Diclofenac Sodium in Bulk and Pharmaceutical Dosage Forms

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Received: 14 Mar 2022 / Accepted: 12 Apr 2022 / Published online: 1 Jul 2022
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Abstract
High-performance liquid chromatography is at present one of the most sophisticated tool for analysis. The estimation of Tolperisone HCl and Diclofenac sodium was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized consisting of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using a UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Tolperisone HCl and Diclofenac sodium were found to be from 100-500 µg/ml of Tolperisone hcl and 1-5µg/ml of Diclofenac sodium. Linear regression coefficient was not more than 0. 999. The values of % RSD are less than 2% indicating the accuracy and precision of the method. The percentage recovery varies from 98-102% of Tolperisone HCl Diclofenac sodium. LOD and LOQ were found to be within the limit.

Keywords
Tolperisone HCl, Diclofenac Sodium, RP-HPLC, Methanol, Validation.

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INTRODUCTION:
Tolperisone is an oral, centrally acting muscle relaxant. Its precise mechanism is not completely understood, though it blocks sodium and calcium channels. It possesses a high affinity for nervous system tissue, reaching the highest concentrations in the brain stem, spinal cord, and peripheral nerves. The anti-inflammatory effects of diclofenac are believed to be due to the inhibition of both leukocyte migration and the enzyme cyclooxygenase (COX-1 and COX-2), leading to the peripheral inhibition of prostaglandin synthesis.

The literature review reveals the few HPLC methods for the estimation of Tolperisone HCL and diclofenac sodium in combination with other drugs. Few methods are also reported for the estimation of both drugs from the formulation. we intend to develop an RP-HPLC method by simultaneous determination with simple, rapid, greater sensitivity, and faster elution.

1. Development of an HPLC method for analysis of both the drugs.
2. Validation of the method using formulations.

MATERIALS AND METHODS:

Apparatus:

An HPLC WATERS software: Empower, 2695 separation module, PDA detector and UV / VIS spectrophotometer LABINDIA UV 3000+ with a wavelength of 260 nm was used. The samples are weighed using the Afcoset ER-200A weighing balance. A calibrated digital pH meter Adwa - AD 1020 was used for pH measurement.

Preparation of Buffer and Mobile phase:

Preparation of Phosphate buffer:

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase:

Accurately measured 300 ml (30%) of the above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through a 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Preparation of the Tolperisone HCL and Diclofenac sodium Standard & Sample Solution:

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Tolperisone HCL and Diclofenac sodium10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Tolperisone HCL and Diclofenac sodium (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3 ml of Tolperisone HCL and Diclofenac sodium of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Tolperisone HCL and Diclofenac sodium peaks and calculate the %Assay by using the formulae.

RESULTS AND DISCUSSION:

Method validation

Precision:

The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits as per table 1 & 2.

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%**.**

Intermediate precision/ruggedness:

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation. The results are summarized in table 3 & 4.

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%.

The %RSD obtained is within the limit, hence the method is rugged.

Accuracy:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated and results are summarized in the table 5 & 6

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%.

The percentage recovery was found to be within the limit (97-103%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Linearity:

The linearity range was found to lie from 100µg/ml to 500µg/ml of Tolperisone HCl, 5µg/ml to 25µg/ml 0f Diclofenac sodium and chromatograms are shown below Figure 1 & 2

Acceptance Criteria:

Correlation coefficient should be not less than 0.999. The correlation coefficient obtained was 0.999 which is in the acceptance limit. The linearity was established in the range of 10% to 50% of Tolperisone HCl and 5% to 25% of Diclofenac sodium.

Limit of detection for Tolperisone HCl and Diclofenac sodium (LOD):

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio as shown below in figure 3 and (Table 8).

Signal to noise ratio shall be 3 for LOD solution

The result obtained is within the limit.

Limit of quantification for Tolperisone HCL and Diclofenac sodium (LOQ):

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio as shown below in figure 4 and (Table 9).

Signal to noise ratio shall be 10 for LOQ solution

The result obtained is within the limit.

Robustness:

The standard and samples of Tolperisone hcl and Diclofenac sodium were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor and plate count

Acceptance criteria:

Percentage RSD should be below 2.

The %RSD obtained for change of flow rate, variation in mobile phase was found to be below 1, which is within the acceptance criteria. Hence the method is robust.

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 1: Results of method precession for Tolperisone HCl

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Table 2: Results of method precession for Diclofenac Sodium:

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 3: Results of Intermediate precision for Tolperisone HCl

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	
100%	1304258	10.0	10.003	100.0%	99.84%
150%	1854608	14.4	14.224	98.780%	

Table 4: Results of Intermediate precision for Diclofenac sodium

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Table 5: Accuracy (recovery) data for Tolperisone HCl

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	
100%	124353	10	10.10	100.01%	100.51%
150%	177940	14.2	14.45	99.68%	

Table 6: Accuracy (recovery) data for Diclofenac sodium

Parameters	Tolperisone hcl	Diclofenac sodium
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Table 7: Analytical performance parameters of Tolperisone HCl and Diclofenac sodium

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Tolperisone hcl	52	522	10.03
Diclofenac sodium	52	524	10.1

Table 8: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Tolperisone hcl	52	152	2.9
Diclofenac sodium	52	156	3

Table 9: Results of LOQ

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 10: Change in Organic Composition in the Mobile Phase for Tolperisone HCl

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

Table 11: Change in Organic Composition in the Mobile Phase for Diclofenac sodium

Figure 1. Calibration graph for Tolperisone HCl

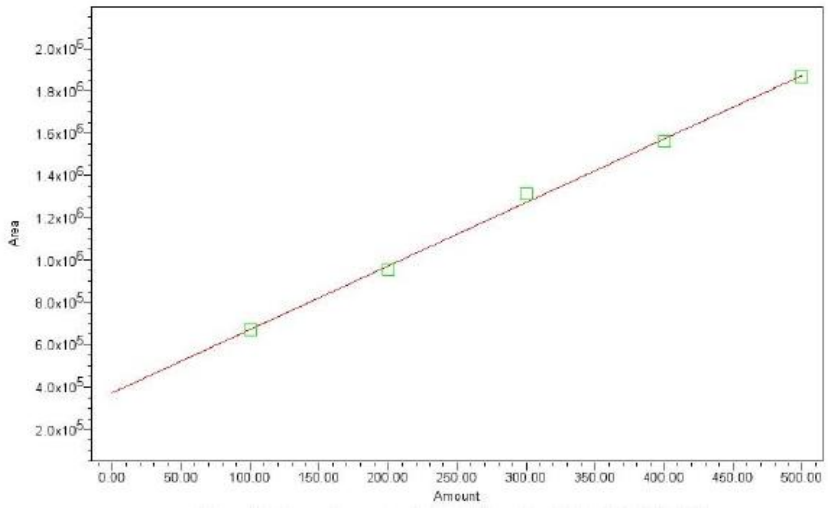


Figure 2. Calibration graph for Diclofenac sodium

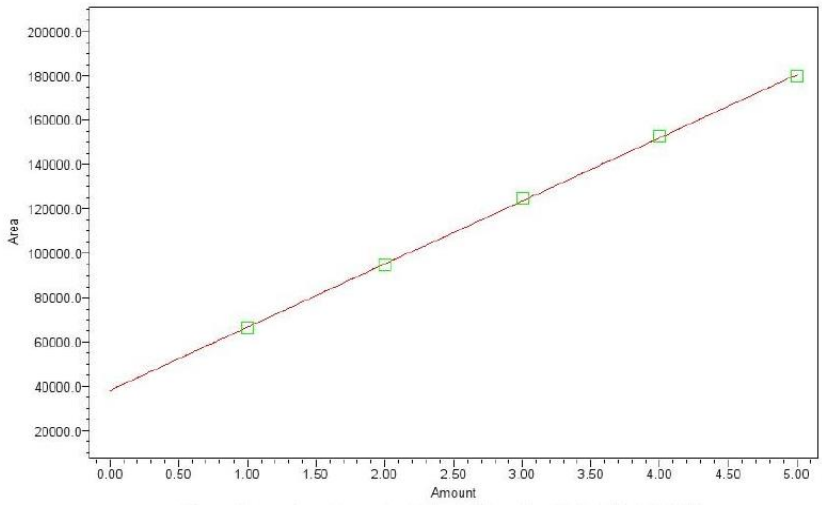


Figure 3: Chromatogram of Tolperisone HCl & Diclofenac sodium showing LOD

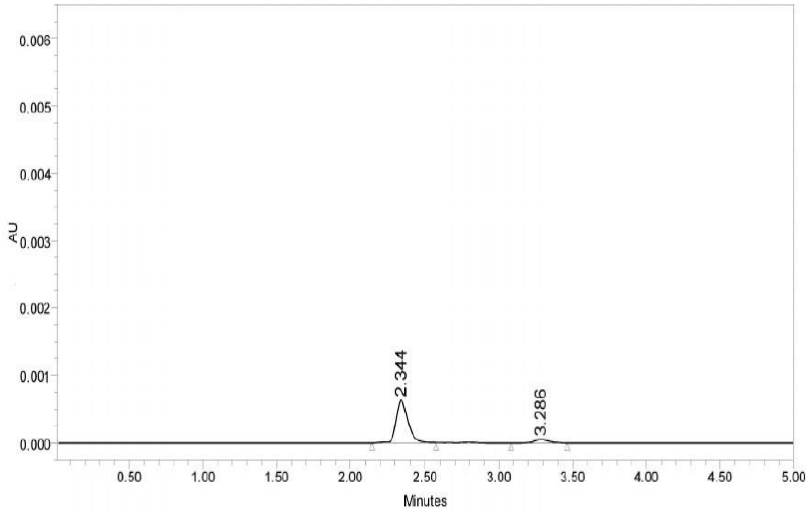
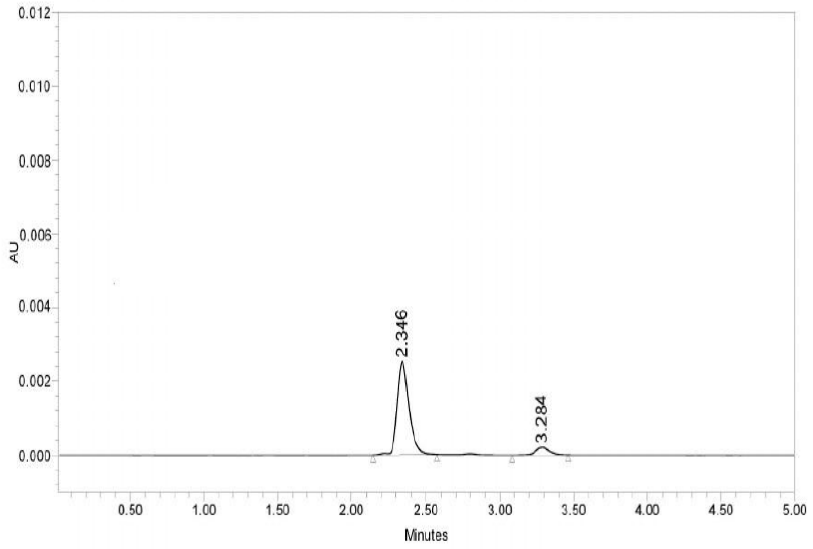


Figure 4: Chromatogram of Tolperisone HCl & Diclofenac sodium showing LOQ



SUMMARY AND CONCLUSION:

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Tolperisone hcl and Diclofenac sodium was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Tolperisone hcl and Diclofenac sodium were found to be from 100-500 µg/ml of Tolperisone hcl and 1-5µg/ml of Diclofenac sodium. Linear regression coefficient was not more than 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Tolperisone hcl and Diclofenac sodium. LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

CONFLICT OF INTEREST:

The authors declared no conflicts of Interest

ACKNOWLEDGMENTS:

I would like to thank everyone who has helped and has been on my side forever.

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