DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CENTRAL MUSCLE RELAXANT & ANALGESIC DRUG IN PHARMACEUTICAL FORMULATION

Ganesh Sonawane[1*], Kajal Pansare[1], Chandrashekhar Patil[1], Prabha Bhong[2], Deepak Somavanshi[3], Parag Pathade[3]

[1]Divine College of Pharmacy, Satana, Dist: Nashik-423301, Maharashtra, (India)
[2]Marathwada Mitra Mandal College of Pharmacy, Pune-411033, Maharashtra, (India)
[3]KBHSS Trust's Institute of Pharmacy, Malegaon-423105, Maharashtra, (India)
***Corresponding Author:** gbsonawane8@gmail.com

ABSTRACT

A robust and accurate RP-HPLC method was developed and validated for the simultaneous estimation of Diclofenac Sodium (DIC) and Tolperisone Hydrochloride (TOL) in pharmaceutical formulations. The study also employed derivative UV spectrophotometry as a complementary analytical technique. The UV spectrophotometric method utilized first-order derivative spectra with zero crossing points at 248 nm for DIC and 226 nm for TOL, ensuring accurate quantification. The method demonstrated good linearity for DIC (2-10 µg/mL) and TOL (5-25 µg/mL), with correlation coefficients close to 1. Precision, accuracy, and sensitivity were confirmed through recovery studies, with mean recoveries of 99.087-100.35% and 99.93-100.46% for DIC and TOL, respectively. The RP-HPLC method was optimized using a BDS Hypersil C18 (250mm × 4.6mm × 5µm) column with a mobile phase of 20 mM phosphate buffer (pH 3.5): acetonitrile (50:50 v/v), a flow rate of 1.0 mL/min, and UV detection at 268 nm. The retention times were 3.50 min for DIC and 5.26 min for TOL. System suitability parameters, including peak symmetry, theoretical plates, and resolution, were within acceptable limits. The method was validated according to ICH guidelines and successfully applied for the quantitative estimation of DIC and TOL in tablet dosage forms, demonstrating its suitability for routine pharmaceutical quality control.

Keywords: HPLC, Diclofenac Sodium, Tolperisone Hydrochloride, Method Development, Validation

1. Introduction

The simultaneous estimation of multiple active pharmaceutical ingredients (APIs) in pharmaceutical formulations is crucial for quality control, ensuring therapeutic efficacy, and meeting regulatory requirements. Reverse-phase high-performance liquid chromatography (RP-HPLC) is one of the most widely used analytical techniques in pharmaceutical analysis due to its high resolution, sensitivity, accuracy, and precision [1]. It enables the simultaneous quantification of multiple drugs in a single run, reducing time, cost, and solvent consumption. The present study focuses on the development and validation of an RP-HPLC method for the simultaneous estimation of Tolperisone Hydrochloride (TOL), a centrally acting muscle relaxant, and Diclofenac Sodium (DIC), a nonsteroidal anti-inflammatory drug (NSAID) with analgesic and anti-inflammatory properties, in pharmaceutical dosage forms. TOL (Figure 1) is a centrally acting muscle relaxant commonly prescribed for the treatment of muscle spasticity, musculoskeletal disorders, and neurological conditions such as stroke, cerebral palsy, and multiple sclerosis [2]. It exerts its muscle relaxant effect by inhibiting voltage-gated sodium and calcium channels, which reduces neurotransmitter release at synaptic junctions, thereby decreasing muscle hyperactivity [3]. Unlike other centrally acting muscle relaxants, TOL does not cause significant sedation, making it a preferred choice in clinical practice [4]. It is also known to have vasodilatory and analgesic properties, contributing to its therapeutic efficacy in pain management [5].

DIC (Figure 2) is a widely used NSAID that exerts analgesic, anti-inflammatory, and antipyretic effects by inhibiting cyclooxygenase (COX-1 and COX-2) enzymes, which are responsible for the biosynthesis of prostaglandins-key mediators of inflammation and pain [6]. It is extensively prescribed for the management of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, musculoskeletal disorders, and post-operative pain [7]. DIC has been formulated in various dosage forms, including tablets, injections, gels, and patches, to cater to different patient needs and conditions. However, prolonged use of DIC is associated with gastrointestinal side effects, cardiovascular risks, and renal toxicity, necessitating proper dose optimization and monitoring [8].

The combination of TOL and DIC is commonly prescribed for the management of musculoskeletal pain, post-operative pain, and inflammatory conditions due to their complementary mechanisms of action. TOL relaxes the muscles and reduces spasms, while DIC provides pain relief and reduces inflammation, offering enhanced therapeutic benefits over monotherapy [9]. The combination helps in improving patient compliance and overall clinical outcomes, as it reduces the need for multiple medications and minimizes side effects associated with high-dose NSAID monotherapy [10].

Since TOL and DIC sodium have distinct physicochemical properties, solubility profiles, and UV absorption spectra, their simultaneous estimation in pharmaceutical formulations poses analytical challenges. Several methods, including UV spectrophotometry, capillary electrophoresis, and high-performance thin-layer chromatography (HPTLC), have been reported for the quantification of these drugs individually. However, these methods often lack sensitivity, specificity, and reproducibility, making RP-HPLC a superior choice [11].

RP-HPLC provides high resolution and selectivity for separating and quantifying multiple components in a complex mixture. The development and validation of a robust RP-HPLC method for simultaneous estimation of TOL and DIC will ensure accuracy, precision, linearity, specificity, and reproducibility, as required by International Council for Harmonisation (ICH) guidelines [12].

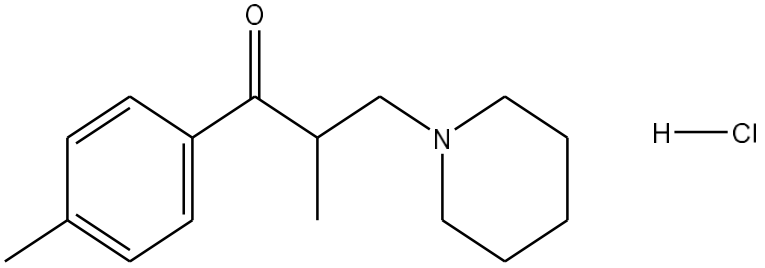

Figure 1. Chemical structure of Tolperisone Hydrochloride

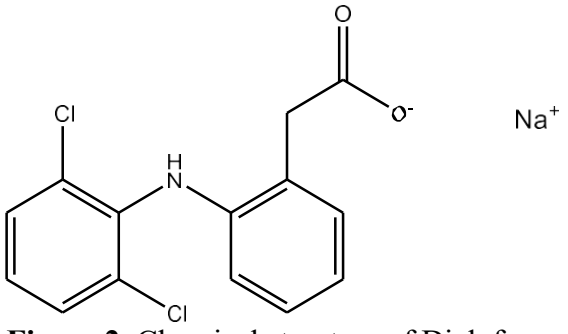

Figure 2. Chemical structure of Diclofenac Sodium

2. MATERIALS AND METHODS

Materials and Instrumentation

TOL and DIC were procured from Vidisha Analytical Laboratories, Nashik. HPLC-grade methanol and water were used as solvents, while all other chemicals were of analytical reagent grade. Chromatographic analysis was performed using an Agilent Technologies HPLC system equipped with a UV detector and OpenLab EZ Chrome software for data acquisition. Additionally, a Shimadzu UV-Visible double beam spectrophotometer (Model 1800) was used for the development and validation of the first-order derivative spectroscopic method [13].

Development and Validation of First Order Derivative Spectroscopic Method for Analysis of TOL and DIC in Tablet

Method Development

Determination of the Zero Crossing Points (Selection of Wavelength)

The overlaid first-order derivative spectra of DIC and TOL were analyzed to determine their zero crossing points (ZCPs). DIC showed a ZCP at 248 nm, whereas TOL had a ZCP at 226 nm. At 248 nm, DIC exhibited zero absorbance, while TOL displayed reasonable absorbance. Similarly, at 226 nm, TOL showed zero absorbance, and DIC exhibited reasonable absorbance. Therefore, 248 nm and 226 nm were selected as the measurement wavelengths for DIC and TOL, respectively, for further analysis [14].

Method Validation

The validation of the proposed method was performed in accordance with ICH Q2 (R1) guidelines by evaluating parameters such as linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) [15].

Linearity

The linearity of DIC was assessed at 226 nm using standard solutions of 2, 4, 6, 8, and 10 µg/ml, while the linearity of TOL was determined at 248 nm using standard solutions of 5, 10, 15, 20, and 25 µg/ml. The first derivative (D1) absorbance values were plotted against concentration to obtain a linear regression equation [16].

Precision

Repeatability was evaluated by measuring the first derivative absorbance of DIC (6 µg/ml) and TOL (15 µg/ml) six times (n=6). The intra-day and inter-day precision were determined by analyzing three different concentrations of DIC (2, 6, and 10 µg/ml) and TOL (5, 10, and 15 µg/ml) on the same day and across three different days within a week. The results were expressed as relative standard deviation (RSD) [17].

Accuracy (Recovery Study)

The accuracy of the method was assessed using the standard addition method. Known amounts of DIC (0, 2, 4, and 6 µg/ml) and TOL (0, 5, 10, and 15 µg/ml) were added to a pre-quantified sample solution (4 µg/ml DIC and 10 µg/ml TOL). The percentage recovery was calculated based on measured absorbances at 226 nm (DIC) and 248 nm (TOL) [18].

Limit of Detection and Limit of Quantification

The Limit of detection (LOD) and limit of quantification (LOQ) were calculated using the standard deviation of intercept (σ) and slope (S) of the calibration curve.

LOD = 3.3 x σ/S LOQ

=10 x σ/S

Where, σ = the standard deviation of the response and S = slope of the calibration curve [19].

Development and validation of RP-HPLC method for Simultaneous Estimation of TOL and DIC

Linearity

Standard diluted stock solutions equivalent to 2.0, 4.0, 6.0, 8.0, and 10.0 µg/ml of DIC and 6.0, 12.0, 18.0, 24.0, and 30.0 µg/ml of TOL were prepared. Each solution (20 µl) was injected into the HPLC system under optimized chromatographic conditions. The calibration curve was plotted using peak area versus concentration, and regression equations were calculated [20].

Precision

The method's repeatability was determined by injecting the solution containing DIC (6 µg/ml) and TOL (18 µg/ml) six times (n=6). Intra-day and inter-day precision were analyzed by measuring responses at three different concentrations of DIC (3.0, 6.0, and 9.0 µg/ml) and TOL (9.0, 18.0, and 27.0 µg/ml) over three different days. The results were expressed as relative standard deviation (RSD) [21].

Accuracy

The accuracy of the method was evaluated using the standard addition method by adding known concentrations of DIC (0, 4.8, 6, and 7.2 µg/ml) and TOL (0, 14.4, 18, and 21.6 µg/ml) to pre-analyzed solutions (6 µg/ml DIC and 18 µg/ml TOL). The percentage recovery was calculated by measuring the peak areas and fitting them into the calibration curve regression equation [22].

Limit of Detection and Limit of Quantification

Limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of intercept (σ) and slope (S) of the calibration curve.

LOD = 3.3 x σ/S

LOQ =10 x σ/S.

Where, σ is the standard deviation of the intercept, and S is the slope of the calibration curve [23].

3. RESULTS AND DISCUSSION

Method Development

The working standard solution of DIC and TOL were prepared separately in distilled water. They were scanned in the wavelength range of 200-400 nm. From the overlaid first order derivative spectra of both the drugs, it was observed that DIC and TOL show a zero crossing point at 248 nm and 226 nm respectively. These two wavelengths were employed for the determination of DIC and TOL. Overlain derivative spectra of both the drugs are shown in Figure 3.

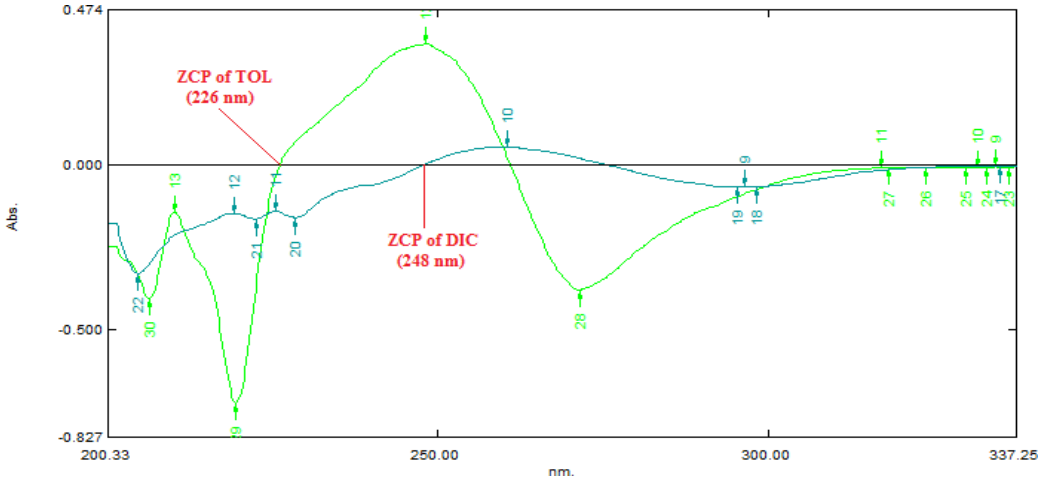

Figure 3: Overlain first order derivative absorption spectra of tablet DIC (5 µg/ml) and TOL (15 µg/ml) in distilled water

Validation of the Derivative Spectroscopy Method

Linearity

The Beer's law was obeyed. Linear correlation was obtained between D1 absorbance and concentration of DIC (2-10 µg/ml) and TOL (5-25 µg/ml) (Figure 4 & 5). The linearity of the calibration curve was validated by the value of correlation coefficient of the regression (r). The optical and regression characteristics are listed in Table 1.

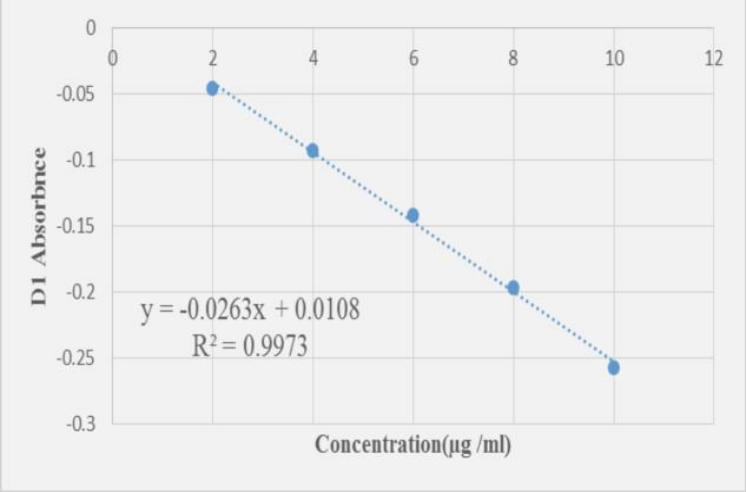

Figure 4: Calibration curve of DIC at 226 nm

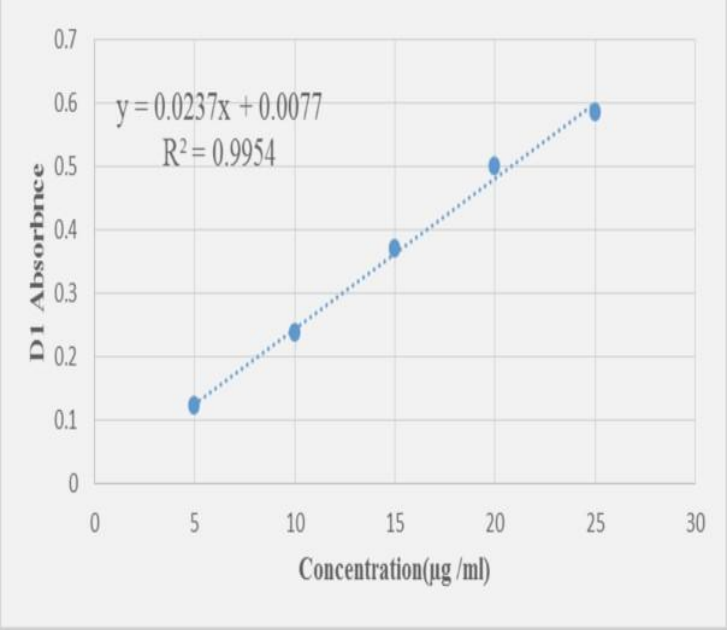

Figure 5: Calibration curve of TOL at 248 nm

Table 1: Optical and regression characteristics (n=3)

Parameter	DIC	TOL
Linearity range (µg/ml)	2-10	5-25
Linearity equation	y = -0.0263x + 0.0108	y = 0.0237x + 0.0077
LOD (µg/ml)	0.1886	0.3111
LOQ (µg/ml)	0.5659	0.9429
Correlation coefficient (r)	0.9973	0.9954

Precision

The % RSD for repeatability of DIC and TOL (Table 2) were found to be 1.8618 and 0.8999 respectively. The value of % RSD for intra-day precision (Table 3) was found to be in the range of 0.93-1.06% and inter-day precision (Table 4) was found to be in the range of 1.19-1.31%, which indicated that the method was precise.

Table 2: Repeatability Data (n=6)

Sr. No.	Concentration (µg/ml)		D1 Absorbance	
	DIC	TOL	DIC	TOL
1	6	15	-0.142	0.369
2	6	15	-0.145	0.372
3	6	15	-0.141	0.365
4	6	15	-0.140	0.371
5	6	15	-0.145	0.374
6	6	15	-0.144	0.367
Mean			0.369	
SD			0.0033	
%RSD			0.8999	

Table 3: Intraday precision data for DIC and TOL

DIC			TOL		
Conc. (µg/ml)	D1 Abs Mean ± S.D. (n=3)	% RSD	Conc. (µg/ml)	D1 Abs Mean ± S.D. (n=3)	% RSD
2	-0.045 ± 0.00057	1.273	5	0.120 ± 0.001	0.8333
6	-0.139 ± 0.0015	1.096	15	0.366 ± 0.0035	0.95778
10	-0.255 ± 0.0030	1.194	25	0.586 ± 0.0045	0.7686

Table 4: Intraday precision data for DIC and TOL

Conc. (µg/ml)	D1 Abs Mean ± S.D. (n=3)	% RSD
2	-0.041 ± 0.00057	1.385
6	-0.142 ± 0.0015	1.073
10	-0.257 ± 0.0035	1.364

Accuracy

The recovery experiments were performed by the standard addition method. The mean recoveries were found to be 99.087-100.35 % and 99.93-100.46% for DIC and TOL, respectively. The recoveries results indicate that the proposed method is accurate. Results of recovery studies are shown in Table 5 and 6.

Table 5: Recovery data of DIC (n=3)

Level	Sample Conc. (µg/ml)	Amt. of Drug added (µg/ml)	Total Conc. (µg/ml)	Amt. of Drug recovered (µg/ml)	Recovery %	Mean ± SD (%)	% RSD
50%	4	2	6	5.922	98.716	100.35 ± 1.457	1.452
	4	2	6	6.091	101.517		
	4	2	6	6.049	100.817		
100%	4	4	8	8.047	100.599	99.401 ± 1.068	1.074
	4	4	8	7.883	98.545		
	4	4	8	7.924	99.059		
150%	4	6	10	10.013	100.13	99.087 ± 0.984	0.993
	4	6	10	9.895	98.957		
	4	6	10	9.817	98.174		

Table 6: Recovery data of TOL (n=3)

Sample Conc. (µg/ml)	Amt. of Drug added (µg/ml)	Total Conc. (µg/ml)	Amt. of Drug recovered (µg/ml)	Recovery %	Level	Mean ± SD (%)	% RSD
10	5	15	14.851	99.00	50%	99.93 ± 0.803	0.806
10	5	15	15.054	100.36			
10	5	15	15.065	100.43			
10	10	20	20.173	100.86	100%	100.88 ± 1.637	1.623
10	10	20	19.853	99.265			
10	10	20	20.508	102.54			
10	15	25	25.477	101.90	150%	101.46 ± 1.942	1.914
10	15	25	24.836	99.34			
10	15	25	25.789	103.15			

LOD and LOQ

LOD and LOQ values for DIC found to be 0.1886 and 0.5659 µg/ml at 226 nm, and TOL were found to be 0.3111 and 0.9429 µg/ml at 248 nm. Low value of LOD & LOQ indicates that the method is sensitive. Results are shown in Table 7.

Analysis of Tablet Dosage Form

The proposed UV spectrophotometric method was successfully applied for determination of DIC and TOL in tablet dosage form. The percentage of DIC and TOL were found to be satisfactory, which was comparable with the corresponding label claim.

Table 7: Analysis of DIC and TOL in Tablet dosage form (n=3)

Sr. No.	Label Claim (mg)		Amount Found (mg)		% Label Claim (mg)	
	DIC	TOL	DIC	TOL	DIC	TOL
1	50	150	4.93	14.52	98.6	98.00
2	50	150	5.01	14.90	100.2	99.34
4	50	150	5.08	14.99	101.6	99.93
Mean					100.13	99.09
SD					1.501	0.988
% RSD					1.49	0.99

Trials for The Selection of Different Mobile Phase

To select the mobile phase for method development, the trials were performed on different ratio of solvents (Table 8). Figure 6 showed chromatogram of DIC-TOL, Mobile Phase-20 mM Phosphate Buffer (pH 3.5): ACN (50: 50 v/v).

Table 8: Trials for the selection of different mobile phase

Sr. No.	Mobile Phase Ratio
1	DIC Water : Methanol (50 : 50)
2	TOL Water : Methanol (50 : 50)
3	DIC - TOL Water : Methanol (30 : 70)
4	DIC - TOL Water : ACN (30 : 70)
5	DIC - TOL Water : ACN (15 : 85)
6	TOL Buffer (pH 4.5) : ACN (30 : 70)
7	DIC - TOL Buffer (pH 4.5) : ACN (30 : 70)
8	DIC - TOL Buffer (pH 4.5) : ACN (40 : 60)
9	DIC - TOL Buffer (pH 4.5) : ACN (50 : 50)
10	DIC - TOL Buffer (pH 4.5) : ACN (60 : 40)
11	DIC - TOL Buffer (pH 5.0) : ACN (60 : 40)
12	DIC - TOL Buffer (pH 4.0) : ACN (60 : 40)
13	DIC - TOL Buffer (pH 4.0) : ACN (70 : 30)
14	DIC - TOL Buffer (pH 3.5) : ACN (60 : 40)
15	DIC - TOL Buffer (pH 3.5) : ACN (50 : 50)

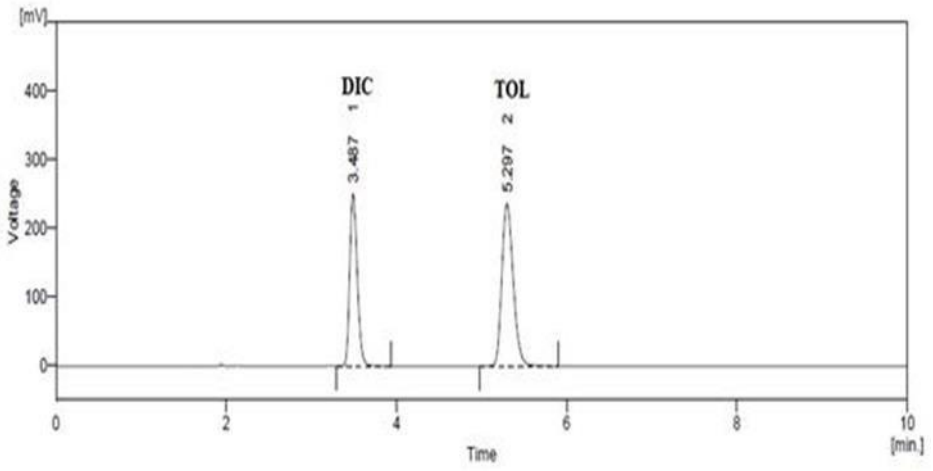

Figure 6: Chromatogram of DIC –TOL Mobile Phase –20 mM Phosphate Buffer (pH 3.5): ACN (50: 50 v/v)

Method Development and Optimization

The optimized RP-HPLC method for simultaneous estimation of TOL and DIC was developed using a BDS Hypersil C18 (250mm × 4.6mm × 5µm) column for efficient separation (Table 9). A 20 mM phosphate buffer (pH 3.5 ± 0.02, adjusted with orthophosphoric acid) and acetonitrile (50:50 v/v) was used as the mobile phase to achieve sharp and well-resolved peaks (Figure 7). The flow rate was set at 1.0 mL/min, and detection was carried out at 268 nm using a Shimadzu SPD-20AT UV detector. The injection volume was 20 µL, with a run time of 20 minutes, ensuring complete drug elution. A column temperature of 26 ± 2°C was maintained to improve peak reproducibility and minimize variability. The method was optimized for high sensitivity, accuracy, and robustness, making it suitable for routine pharmaceutical quality control.

Table 9: Optimized chromatographic conditions

Parameter	Chromatographic Conditions
Stationary phase	BDS hypersil C18, (250mm × 4.6mm × 5µm)
Mobile phase	20 mM Phosphate Buffer (pH 3.5 ± 0.02 with OPA) : ACN (50:50 v/v)
Flow rate	1.0 ml/min
Wave length	268 nm
Run time	20 min
Injection volume	20 µl
Pump	LC-20AT
Detector	UV detector, SPD-20AT
Temperature	26 ± 2°C

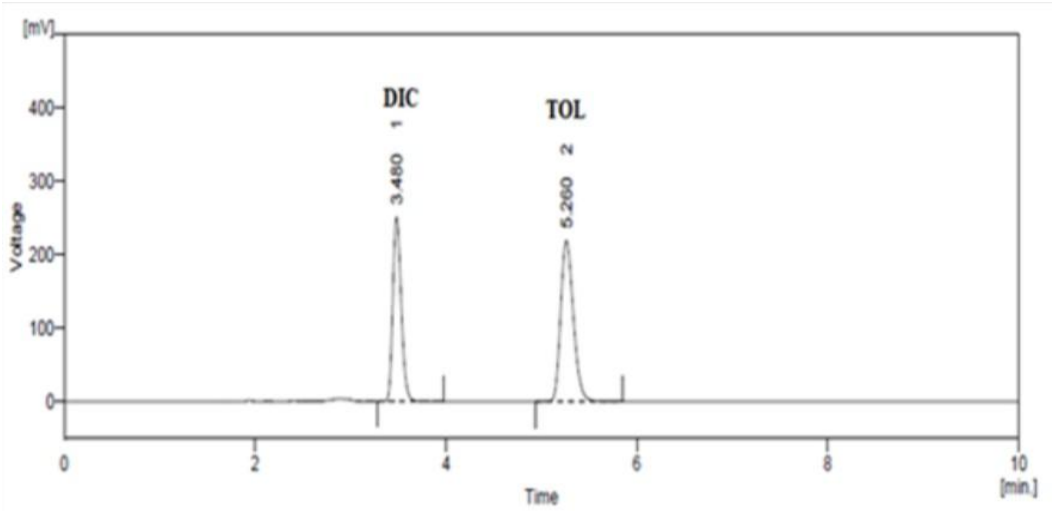

Figure 7: Chromatogram of TOL and DIC sample solution in 20 mM Phosphate Buffer (pH 3.5): ACN (50:50 v/v)

Validation of the HPLC method Linearity

Linear correlation was obtained between peak area and concentration of DIC and TOL in the range of 2-10 µg/ml and 6-30 µg/ml respectively (Figure 8), the linearity of the calibration curves were validated by the value of correlation coefficient of the regression (r), the regression analysis of the calibration curves is listed in Table 10-12.

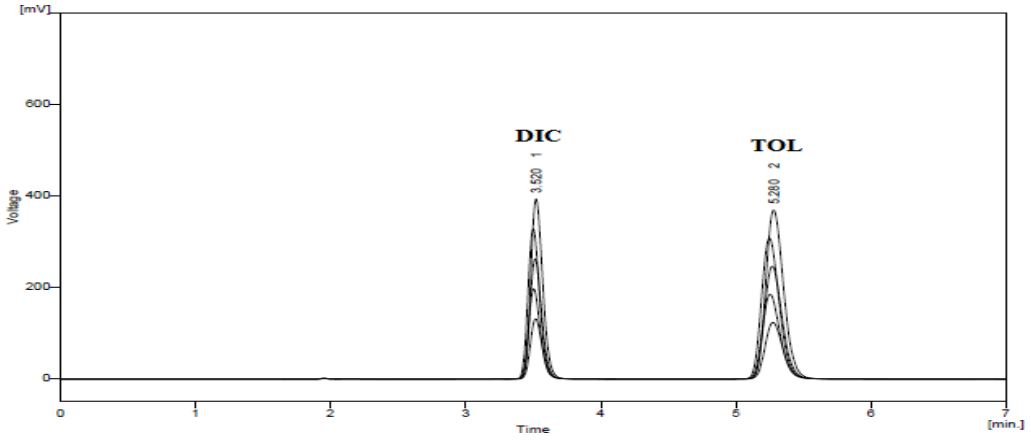

Figure 8: Overlain Chromatograms of DIC (2-10 µg /ml) and TOL (6-30 µg /ml)

Table 10: Linearity data for DIC

Sr. No.	Conc. (µg)	Area Mean ± S.D. (n=6)	% RSD
1	2	815.013 ± 6.832	0.8421
2	4	1281.084 ± 8.952	0.6954
3	6	1629.355 ± 11.398	0.6983
4	8	2007.033 ± 13.347	0.6609
5	10	2450.012 ± 17.556	0.7218

Table 11: Linearity data for TOL

Sr. No.	Conc. (µg)	Area Mean ± S.D. (n=6)	% RSD
1	6	1144.916 ± 6.132	0.5329
2	12	1711.452 ± 10.208	0.5938
3	18	2289.258 ± 16.663	0.7236
4	24	2805.833 ± 18.129	0.6427
5	30	3445.196 ± 25.019	0.7273

Table 12: Optical and regression characteristics (n=3)

Parameter	DIC	TOL
Linearity range (µg/mL)	2-10	6-30
Linearity equation	202.95x + 406.25	94.916x + 570.85
LOD (µg/mL)	0.141	0.347
LOQ (µg/mL)	0.429	1.053
Correlation coefficient (r)	0.9995	0.9992

System Suitability Test

The system suitability parameters confirm the efficiency and reliability of the developed RP-HPLC method for simultaneous estimation of DIC and TOL. The tailing factor for both drugs (DIC: 1.455, TOL: 1.424) indicates symmetrical peak shapes, ensuring accurate quantification. The number of theoretical plates (DIC: 7290, TOL: 7126) confirms high column efficiency, while the retention times (DIC: 3.50 min, TOL: 5.26 min) show proper elution order. A high resolution value (8.480) ensures excellent peak separation, reducing interference. These results validate the method's accuracy, precision, and robustness, making it suitable for routine pharmaceutical analysis. Results for system suitability of RP-HPLC method was mentioned in Table 13.

Table 13: Data of system suitability parameters

System Suitability Parameters	DIC	TOL
Tailing Factor	1.455	1.424
Theoretical Plates	7290	7126
Retention Time (minutes)	3.50	5.26
Resolution	8.480	

Precision

The % RSD for repeatability of DIC and TOL were found to be 1.86 and 0.89 respectively. The results are shown in Table 14. The value of % RSD for intra-day precision was found to be in the range of 0.850-1.003% and 0.851-1.010% while inter-day precision was found to be in the range of 1.049-1.151% and 1.050-1.153% for DIC and TOL respectively, which indicated that the method was precise. The results are shown in Table 15 and 16.

Table 14: Repeatability data for DIC and TOL

Sr. No.	Concentration (µg/ml)		D1 Absorbance	
	DIC	TOL	DIC	TOL
1	5	15	-0.142	0.369
2	5	15	-0.145	0.372
3	5	15	-0.141	0.365
4	5	15	-0.140	0.371
5	5	15	-0.145	0.374
6	5	15	-0.144	0.367
Mean	-0.1428		0.369	
SD	0.0021		0.0033	
% RSD	1.8618		0.8999	

Table 15: Intraday precision data for DIC and TOL

DIC			TOL		
Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% RSD	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% RSD
2	812.839 ± 8.156	1.003	9	1142.421 ± 11.539	1.00
6	1627.357 ± 16.312	1.002	18	2286.221 ± 22.905	1.06
10	2445.919 ± 20.809	0.850	27	3439.557 ± 29.293	1.10

Table 16: Inter-day precision data for DIC and TOL

DIC			TOL		
Conc. (µg/ml)	**Area Mean ± S.D. (n=3)**	**% RSD**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% RSD**
3	815.549 ± 9.00	1.103	9	794.730 ± 12.843	1.00
6	1633.328 ± 17.140	1.049	18	2296.086 ± 24.127	1.06
9	2457.381 ± 28.292	1.151	27	3455.172 ± 39.871	1.10

Accuracy (Recovery)

The accuracy study was carried out by the standard addition method. The percent recoveries were found in the range of 100.01-100.12% and 99.61-100.31% for DIC and TOL respectively, which indicated accuracy of the method. The results are shown in Table 17-19.

Table 17: Accuracy Data for DIC

Level	Sample Conc. (µg/ml)	Amt. of Drug Added (µg/ml)	Total Conc. (µg/ml)	Amt. of Drug Recovered (µg/ml)	Recovery %	Mean ± SD (%), (n=3)	% RSD
80%	6	4.8	10.8	10.789	99.712	100.06 ± 0.34	0.348
	6	4.8	10.8	10.819	100.409		
	6	4.8	10.8	10.803	100.066		
100%	6	6	12	11.935	98.925	100.12 ± 1.32	1.323
	6	6	12	12.092	101.547		
	6	6	12	11.995	99.916		
120%	6	7.2	13.2	13.121	98.907	100.01 ± 1.02	1.025
	6	7.2	13.2	13.215	100.217		
	6	7.2	13.2	13.266	100.930		

Table 18: Accuracy Data for TOL

Level	Sample Conc. (µg/ml)	Amt. of Drug Added (µg/ml)	Total Conc. (µg/ml)	Amt. of Drug Recovered (µg/ml)	Recovery %	Mean ± SD (%), (n=3)	% RSD
80%	18	14.4	32.4	32.394	99.962	99.613 ± 1.21	1.21
	18	14.4	32.4	32.488	100.615		
	18	14.4	32.4	32.510	98.264		
100%	18	18	36	35.833	99.073	100.31 ± 1.37	1.36
	18	18	36	36.321	101.784		
	18	18	36	36.013	100.074		
120%	18	21.6	39.6	39.324	98.724	100.05 ± 1.21	1.21
	18	21.6	39.6	39.677	100.357		
	18	21.6	39.6	39.836	101.094		

Table 19: Data for robustness (change in pH of mobile phase)

Drug	Parameter	Change 1	Change 2
		pH 3.7 (n=3)	pH 3.3 (n=3)
DIC	Area	1621.92	1630.597
	SD	11.519	16.287
	% RSD	0.710	0.998
TOL	Area	2279.995	2292.093
	SD	15.963	22.863
	% RSD	0.700	0.997

Limit of Detection and Limit of Quantification

The Limit of detection (LOD) was found to be 0.141 and 0.347 µg/mL while the Limit of quantification (LOQ) was 0.429 and 1.053 µg/ mL for DIC and TOL respectively.

Assay of the Tablet dosage form

The proposed RP-HPLC method was successfully applied for determination of DIC and TOL from combined tablet dosage form. The percentage of DIC and TOL were found to be satisfactory; which was comparable with the corresponding label claim. The results are shown in Table 20.

Table 20: Assay data of pharmaceutical formulation (n=3)

Drug	Marketed Preparation	Label claim	Amount of Drug Estimated	% Label Claim	S.D.	% RSD
DIC	TOLPERITAS-D®	50 mg	49.879	99.759	0.5466	0.544
TOL		150 mg	150.737	100.491	0.4799	0.481

5. CONCLUSION

The developed and validated RP-HPLC method provides a precise, accurate, and reproducible approach for the simultaneous estimation of Diclofenac Sodium and Tolperisone Hydrochloride in pharmaceutical formulations. The optimized chromatographic conditions ensured excellent peak resolution, minimal interference, and high sensitivity. Validation parameters, including linearity, precision, accuracy, LOD, and LOQ, confirmed the method's reliability. The successful application of the method to tablet dosage forms further supports its potential for routine use in quality control laboratories. Additionally, the derivative UV spectrophotometric method offers a simple and effective alternative for preliminary analysis. Both methods adhere to regulatory guidelines, making them valuable tools for pharmaceutical analysis.

REFERENCES

1. Kazakevich Y, Lobrutto R. HPLC for pharmaceutical scientists. John Wiley & Sons; 2007.
2. Baghel R, Tiwari V, Pathak AK. Tolperisone: A centrally acting muscle relaxant with unique properties. J Pharm Sci Res. 2016;8(8):905-12.
3. Kadam PP, Patil MJ, Bhusari KP. Mechanism of action and pharmacokinetics of tolperisone: An overview. Int J Pharm Sci Res. 2018;9(5):1734-40.
4. Müller H, Váczi D, Gaspar K, et al. Tolperisone: Pharmacological profile and clinical applications. Drugs. 2019;79(10):1105-18.
5. Chou R, Huffman LH. Nonsteroidal anti-inflammatory drugs and muscle relaxants for low back pain: Systematic review. Ann Intern Med. 2007;147(7):505-14.
6. Gan TJ. Diclofenac: An update on its mechanism of action and safety profile. Curr Med Res Opin. 2010;26(7):1715-31.
7. McGettigan P, Henry D. Cardiovascular risk and inhibition of cyclooxygenase: A systematic review of the observational studies of selective and non-selective inhibitors. BMJ. 2013;342:c7086.
8. Kaur G, Kulkarni GT, Singh M. Gastroprotective effect of polyherbal formulation against NSAID-induced gastric ulcer. Indian J Pharm Sci. 2015;77(5):522-8.
9. Patel PM, Patel NM, Patel SA. Simultaneous estimation of tolperisone hydrochloride and diclofenac sodium in tablet dosage form by RP-HPLC method. Asian J Pharm Clin Res. 2015;8(4):101-5.
10. International Council for Harmonisation (ICH). ICH guideline Q2(R1): Validation of analytical procedures: Text and methodology. 2005.

11. Meyer VR. Practical high-performance liquid chromatography. 5th ed. John Wiley & Sons; 2010.
12. Swartz ME, Krull IS. Analytical method development and validation. CRC Press; 2018.
13. Agilent Technologies. HPLC System and Software.
14. ICH Q2 (R1). Validation of Analytical Procedures: Text and Methodology.
15. Shimadzu UV-Visible Spectrophotometer Model 1800. Instrument Manual.
16. Patel MM, Shah DA. Derivative Spectrophotometry for Drug Analysis. J Pharm Sci. 2020;109(3):765-772.
17. Rahman N, Ahmad Y, Azmi SN. Spectrophotometric Methods for Simultaneous Estimation of Drugs. Indian J Pharm Sci. 2019;81(5):823-829.
18. Das S, Nayak AK. Validation Strategies for Analytical Methods. Anal Chem Res. 2021;29:101-110.
19. European Pharmacopoeia Commission. Guidelines for Chromatographic Analysis. Eur J Pharm Sci. 2022;152:1-15.
20. Roy S, Patel A. HPLC Method Validation for Drug Analysis. Int J Pharm Res. 2021;13(1):345-352.
21. USP 43-NF 38. United States Pharmacopeia.
22. Sahoo S, Pal TK. RP-HPLC Method Development and Validation for Simultaneous Estimation of Drugs. J Chromatogr B. 2018;1084:23-30.
23. International Conference on Harmonization. Analytical Method Validation Guidelines.