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Development And Validation Of HPTLC Method For Simultaneous Determination Of Tolperisone Hydrochloride And Diclofenac Sodium In Combined Dosage Form

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1. Abstract: A new, simple, precise, accurate and selective high performance thin-layer chromatographic (HPTLC) method has been developed and validated for the simultaneous determination of Tolperisone hydrochloride and Diclofenac sodium in a tablet dosage form. Chromatographicseparation was carried out on Merck TLC aluminium sheets of silica gel $60F_{254}$ using Toluene: Ethyl acetate: Methanol (4: 4: 2 ml (v/v/v)) as mobile phase followed by densitometric analysis at 282 nm. The reliability of the method was assessed by evaluation of linearity (120-720 ng/spot forTolperisone hydrochloride and 40-240 ng/spot for Diclofenac sodium), accuracy (99.65 % for Tolperisone hydrochloride and 100.75 % for Diclofenac sodium), precision, repeatability and specificity, in accordance with International Conference on Harmonization (ICH) guidelines. Statistical analysis of the datarevealed that the method is precise, accurate, reproducible, sensitive and selective. The method can be successfully employed for the simultaneous determination of Tolperisone hydrochloride and Diclofenac sodium in pharmaceutical formulation.
Key words: Tolperisone hydrochloride, Diclofenac sodium, HPTLC, simultaneous determination, validation.

2. INTRODUCTION:

Tolperisone hydrochloride (TOL) is chemically (*2RS*)-2-Methyl-1-(4-Methylphenyl)-3-Piperidine-1-ylpropan-1-one monohydrochloride (Figure 1) is an antispasmodic drug[1, 2, 3]. It is official in Japanese Pharmacopoeia (JP). It is assayed by potentiometric method as per JP. Literature review reveals HPLC[4] and UV[5]spectrophotometric and HPTLC[6] methods for estimation of TOL pharmaceutical dosage forms. Diclofenac sodium (DICLO) is chemically Sodium 2-[(2, 6-dichlorophenyl) amino] phenyl acetate (Figure 2) used as cyclooxygenase inhibitor, analgesic, and anti-inflammatory[1, 2, 3, 7]. It is official in IP, BP[8], USP[9] and JP and is estimated by potentiometric titration method as per IP, BP, USP and JP. In IP a liquid chromatographic method for DICLO is given for injection dosage form. Literature review also reveals HPLC[10], UV[11]spectrophotometric and HPTLC[12] method for the estimation of DICLO with other drugs. Literature survey does not reveal any simple HPTLC method for simultaneous determination of TOL and DICLO in Pharmaceutical dosage form. The present developed method is new, simple, precise, accurate and selective for simultaneous determination of both drugs in their tablet dosage form as per International Conference on Harmonization (ICH) guidelines[13].

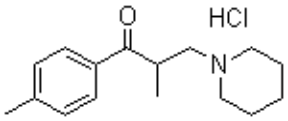

Figure 1: Structure of Tolperisone hydrochloride

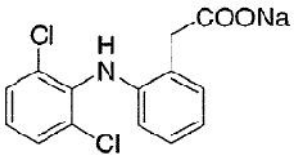

Figure 2: Structure of Diclofenac sodium

3. EXPERIMENTAL:

3.1 Material and reagents

Tolperisone hydrochloride was kindly supplied by Themis Medicare, Vapi, Gujarat, India, as gratis sample and Diclofenac sodium was obtained as gratis sample from Lincoln Pharma, Ahmedabad, Gujarat, India. Toluene, ethyl acetate and methanol were used as solvents to prepare the mobile phase. All reagents used were of analytical reagent grade (Allied Chemical Corporation, Vadodara). The tablet samples were obtained from local market (TOLPIDOL^{TM} D, Themis Medicare).

3.2 Instrumentation and chromatographic conditions

The HPTLC system (Camag, Muttenz, Switzerland) consisted of Linomat V autosprayer connected to a nitrogen cylinder, a twin trough chamber (20 × 10 cm), a derivatization chamber, and a plate heater. Pre-coated silica gel 60 F254 TLC plates (10 × 10 cm, layer thickness 0.2 mm (E. Merck KGaA, Darmstadt, Germany) was used as stationary phase. TLC plates were pre-washed twice with 10 mL of methanol and activated at 80^0C for 5 min prior to sample application. The standard and formulation samples of TOL and DICLO in mixture were spotted on Pre-coated TLC plates in the form of narrow bands of lengths 6 mm. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nL/s. The mobile phase consists ofToluene: Ethyl acetate: Methanol (4:4:2, v/v/v). Linear ascending development was carried out in twin trough chamber (20 x 10 cm). The optimized chamber saturation time for mobile phase was 30 min, at 25^0C ± 2); the length of chromatogram run was 7 cm and TLC plates were air dried. Densitometric scanning was performed on CAMAG TLC scanner III in absorbance mode and operated by winCATS planar chromatography version 1.3.4. The source of radiation utilized was deuterium lamp. The spots were analyzed at a wavelength of 282 nm. The slit dimensions used in the analysis were length and width of 5 mm and 0.45 mm, respectively, with a scanning rate of 20 mm/s. The parameters were selected as recommended by the CAMAG TLC scanner III manual. Evaluation was performed using linear regression analysis via peak areas.

3.3Standard solutions and calibration curves

Standard stock solution of combined drugs was prepared containing 0.3 g/L of TOL and 0.1 g/L of DICLO in methanol. Which were further diluted with methanol to obtain 60 µg/mL of TOL and 20 µg/mL of DICLO. Calibration was done by applying mixture of standard solutions ranging from 2.0 – 12.0 µL by Hamilton syringe with the help of Linomat V autosprayer on TLC plate that gave concentration 120-720 ng/spot for TOL and 40-240 ng/spot for DICLO, respectively. Each concentration was spotted six times on TLC plates. From the developed plates calibration curve was plotted as peak areas versus corresponding concentrations (Figure 5 and 6).

3.4 Method Validation

3.4.1 HPTLC method development

In trial phase ethyl acetate and methanol in ratio of 5:5 (v/v) was used but it shows dragging of peaks. Then toluene was incorporated to the mobile phase composition and tried with different ratios. In ratio of mobile phase consisting toluene, ethyl acetate and methanol (4:5:1, v/v/v) shows tailing. By increasing the methanol in mobile phase composition tailing was resolved and ultimately, mobile phase consisting of Toluene: Ethyl acetate: Methanol (4: 4: 2, v/v/v) gave good results. Both the peaks were symmetrical in nature and no tailing was observed when plate was scanned at 282 nm. The chamber was saturated with the mobile phase for 30 min at room temperature.

3.4.2 Linearity

Linearity responses for TOL and DICLO were assessed in the concentration range 120-720 ng/spot and 40-240 ng/spot of standard solutions, respectively.

3.4.3 Precision

Precision of the method was determined in the terms of intra-day and inter-day variation (%RSD). Intra-day precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days.

3.4.4 Accuracy

To the pre-analyzed sample a known amount of standard solution of pure drug (TOL and DICLO) was spiked at three different levels. These solutions were subjected to re-analysis by the proposed method.

3.4.5 Sensitivity

The sensitivity of measurement of TOL and DICLO by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by equation.

3.4.6 Specificity

Specificity of the method was ascertained by analyzing standard drug and sample. The mobile phase resolved both the drugs very efficiently as shown in Figure 7. The spot for TOL and DICLO was confirmed by comparing the R_f and spectra of the spot with that of standard. The wavelength 282 nm for detecting peak purity of TOL and DICLO was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

3.4.7 Repeatability

Repeatability of sample application was assessed by spotting 4µL (240 ng/spot of TOL and 80 ng/spot of DICLO) of drug solution six times on a TLC, followed by development of plate and recording the peak area for six spots.

3.5 Analysis of TOL and DICLO in marketed formulation

To determine the content of TOL and DICLO simultaneously in conventional tablets (label claim 150 mg TOL and 50 mg DICLO); twenty tablets were accurately weighed, average weight determined and ground to fine powder. A quantity of powder equivalent to 150 mg (TOL) and 50 mg (DICLO) was transferred into 100 mL volumetric flask containing 50 mL methanol, sonicated for 30 min and diluted to mark with same solvent. The resulting solution was filtered using 0.45 µm filter (Millifilter, MA). From the above solution 2 mL was transferred into 10 mL volumetric flask and diluted to mark with same solvent. 1µL of this solution applied on TLC plate followed by development and scanning as described in section 3.2. The analysis was repeated for three times.

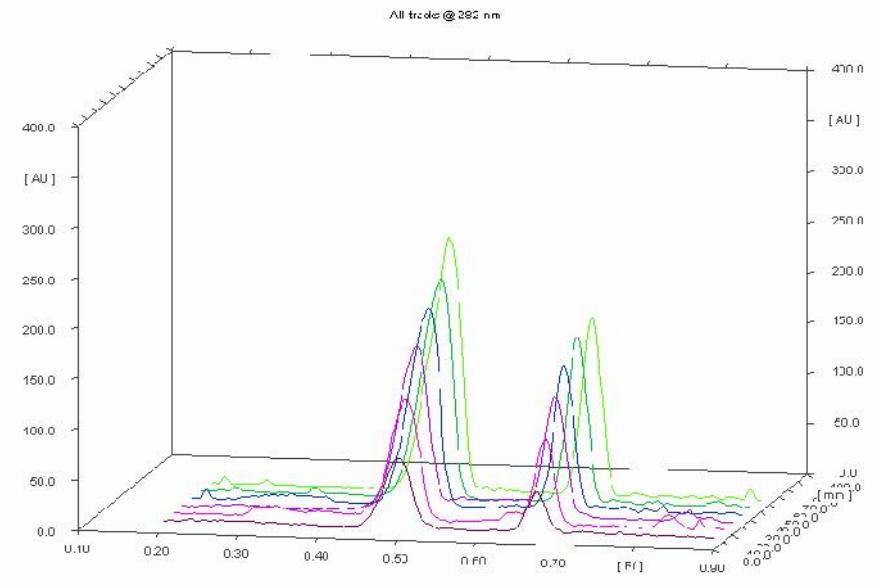

Figure 3: 3D Representation of Densitogram for Calibration curve of TOL and DICLO

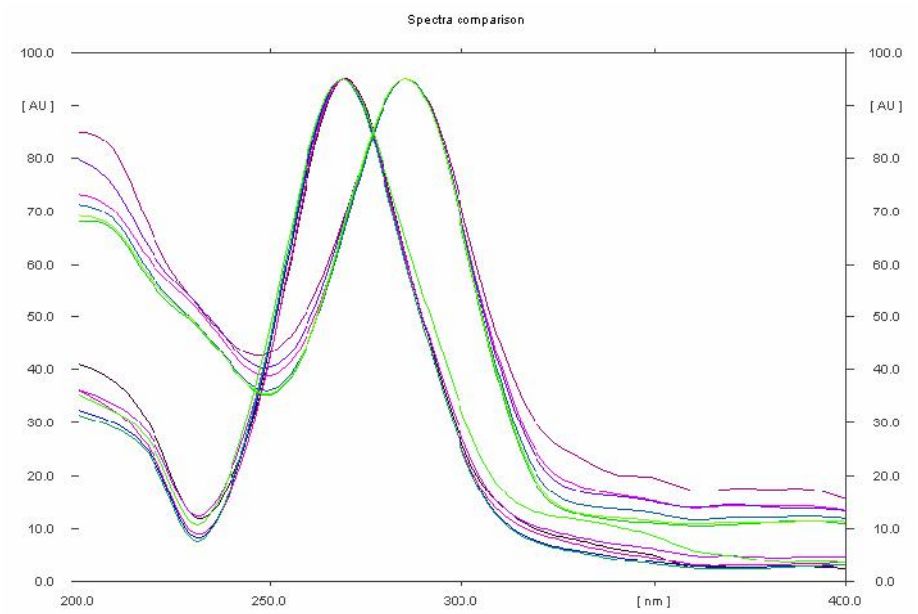

Figure 4: UV Absorption (Reflectance Mode) of the corresponding spots for TOL and DICLO

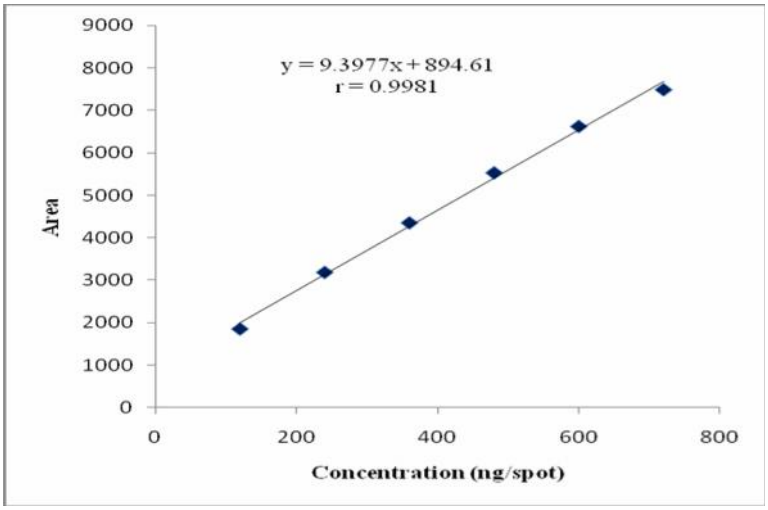

Figure 5: Calibration curve of TOL in Methanol at 282 nm

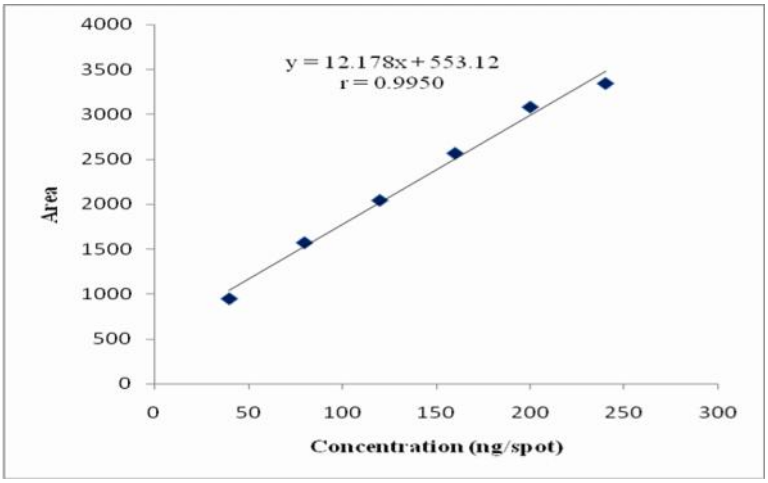

Figure 6: Calibration curve of DICLO in Methanol at 282 nm

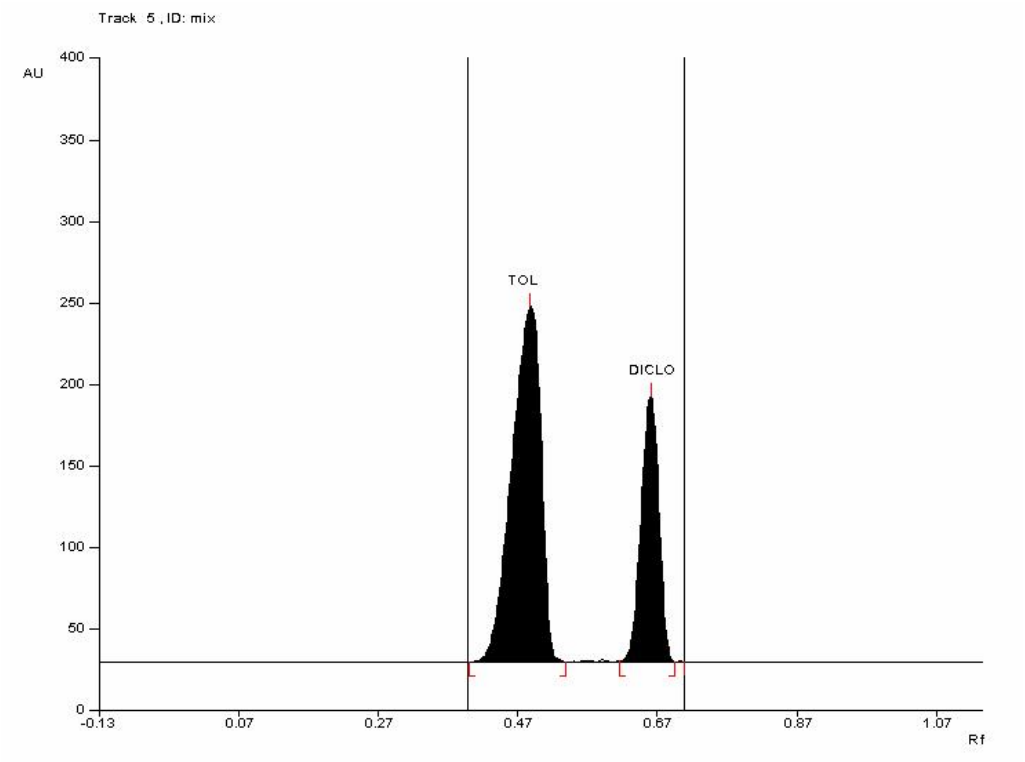

Figure 7: HPTLC Chromatogram of Standard TOL and DICLO in mixture

4. RESULT AND DISCUSSION:

4.1 Method development

The TLC procedure was optimized for simultaneous determination of TOL and DICLO. The mobile phase Toluene: Ethyl acetate: Methanol (4: 4: 2 v/v/v) resulted in good resolution and sharp and symmetrical peaks of R_f 0.48 ± 0.005 for TOL and 0.66 ± 0.005 for DICLO. It was observed that pre-washing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 30 min (optimum chamber saturation time) ensured good reproducibility and peak shape of both drugs (Figure 3 and 7).

4.2 Validation

4.2.1 Linearity

Linear regression data for the calibration plots revealed good linear relationships between area and concentration over the ranges 120-720 ng/spot for TOL and 40-240 ng/spot for DICLO. The linear equations for the calibration plots were y = 9.3977x + 894.61 and y = 12.178x + 553.12 with correlation coefficient (r) being 0.9981 and 0.9950 for TOL and DICLO, respectively (Table 1, 2 and 3).

4.2.2 Precision

The precision of the method was expressed as relative standard deviation (RSD,%). The results listed in (Table 4 and 5) reveal the high precision of the method.

4.2.3 Accuracy

When the method was used for accuracy and subsequent analysis of both drugs from the pharmaceutical dosage forms, and spiked with 50, 100, and 150% of additional drug, the recovery was 99.46- 99.83% for TOL and 100.36- 101.13% for DICLO (Table 6 and 7).

4.2.4 Sensitivity

The LOD and LOQ were calculated by equation. The LOD and LOQ value were 9.90 and 30.00ng/spot for TOL and 1.54 and 4.66ng/spot for DICLO.

4.2.5 Specificity

The peak purity of TOL and DICLO was assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9994 and r (M, E) =0.9990 for TOL and r (S, M) = 0.9986 and r (M, E) = 0.9996 for DICLO. Good match was obtained between standard and sample spectra of TOL and DICLO respectively. (Figure 4)

4.2.6 Repeatability

The % RSD for peak area values of TOL and DICLO was found to be 0.885 and 0.978, respectively as given in Table 8.

4.3 Analysis of TOL and DICLO in marketed formulation

When the TOLPIDOL™ D tablets were analyzed, TOL and DICLO gave sharp and well defined peaks at R_f 0.48 and 0.66, respectively, when scanned at 282 nm. The results in Table 9 indicate that there was no interferences from the excipients commonly present in the tablets. The % purity was 99.16% for TOL and 99.56% for DICLO.

Table 1: Result of Calibration reading for TOL

Concentration (ng/spot)	R_f	Area Mean (n=6) ± SD	%RSD
120	0.49	1854.05± 24.976	1.347
240	0.48	3188.15± 40.817	1.280
360	0.48	4357.38± 21.228	0.487
480	0.49	5536.63± 18.278	0.330
600	0.49	6631.12± 15.114	0.228
720	0.49	7495.50± 10.857	1.145

Table 2: Result of Calibration reading for DICLO

Concentration (ng/spot)	R_f	Area Mean (n=6) ± SD	%RSD
40	0.66	947.80± 8.065	0.851
80	0.66	1566.10± 19.965	1.275
120	0.66	2035.05± 14.475	0.771
160	0.66	2566.93± 14.325	0.558
200	0.67	3078.28± 14.715	0.478
240	0.67	3348.57± 20.522	0.613

Table 3: Statistical Data of TOL and DICLO

Parameters	Results	
	TOL	DICLO
Linear Range(ng/spot)	120-720	40-240
Slope	9.3977	12.178
Intercept	894.61	553.12
St. Deviation of Slope	0.0549	0.0804
St. Deviation of Intercept	28.196	5.669
Limit of Detection(ng/spot)	9.90	1.54
Limit of Quantification(ng/spot)	30.00	4.66
Regression Equation	y = 9.3977x + 894.61	y = 12.178x + 553.12
Co-Relation Co-Efficient (r)	0.9981	0.9950
Co-Efficient of Determination (r^2)	0.9962	0.9901

Table 4: Intra Day and Inter Day study of TOL

Concentration (ng/spot)	Intra Day Area Mean (n=3) ± SD	%RSD	Inter Day Area Mean (n=3) ± SD	%RSD
240	3208.86± 12.530	0.390	1568.63± 16.574	1.056
360	4367.80± 21.579	0.494	2037.06± 12.228	0.600
480	5540.96± 24.871	0.449	2559.43± 16.859	0.659

Table 5: Intra Day and Inter Day study of DICLO

Concentration (ng/spot)	Intra Day Area Mean (n=3) ± SD	%RSD	Inter Day Area Mean (n=3) ± SD	%RSD
80	3167.43± 52.159	1.647	1563.56± 26.505	1.695
120	4356.96± 30.057	0.699	2033.03± 19.030	0.936
160	5552.3± 40.304	0.726	2561.10± 24.551	0.959

Table 6: Determination of Accuracy for TOL

Concentration of Sample Taken (ng/spot)	Concentration of Pure API spiked (ng/spot)	Total Concentration (ng/spot)	Mean Total Concentration Found (n=3) (ng/spot)	%Recovery Mean (n=3)	%RSD
240	120	360	358.05	99.46	0.665
	240	480	479.18	99.83	0.442
	360	600	597.93	99.66	0.324
Average				99.65	

Table 7: Determination of Accuracy for DICLO

Concentration of Sample Taken (ng/spot)	Concentration of Pure API spiked (ng/spot)	Total Concentration (ng/spot)	Mean Total Concentration Found (n=3) (ng/spot)	%Recovery Mean (n=3)	%RSD
80	40	120	120.36	100.30	0.384
	80	160	161.54	100.96	0.245
	120	200	201.95	100.98	0.634
Average				100.75	

Table 8: Repeatability study of TOL and DICLO

Concentration	TOL (240 ng/spot)	DICLO 80 (ng/spot)
Area	3205.1	1569.8
	3155.2	1587.5
	3215.4	1592.9
	3198.5	1601.7
	3179.1	1577.6
	3236.2	1559.8
Mean	3198.25	1581.55
± SD	28.289	15.472
%RSD	0.885	0.978

Table 9: Assay Result of Marketed Formulation

Formulation	TOLPIDOL™ DTAB	
	TOL	DICLO
Actual Concentration (ng/spot)	300	100
Concentration Obtained (ng/spot)	297.06	98.82
%Purity	99.16	99.56
%RSD	0.669	0.518
Limit[3, 7]	Not less than 98.5%	98.5% - 101.0%

Table 10: Validation Parameters

Summary of Validation Parameters		
	TOL	DICLO
Recovery (%)	99.65	100.75
Repeatability (%RSD)	0.885	0.978
Precision (CV)		
Intra-day (n=3)	0.0044	0.0077
Inter-day (n=3)	0.0102	0.0196
Specificity	Specific	Specific
Selectivity	Selective	Selective

5. CONCLUSION

The developed HPTLC method is simple, precise, accurate and reproducible and can be used for simultaneous determination of TOL and DICLO in tablets. The method was validated as per International Conference on Harmonization (ICH) guidelines.

6. ACKNOWLEDGEMENT

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