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Analytical Approach to Develop and Validate Stability Indicating HPLC Method for Simultaneous Estimation of Tolperisone Hydrochloride and Diclofenac Sodium In Combined Pharmaceutical Dosage Form

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ABSTRACT

A simple and precise stability-indicating high performance liquid chromatography method was developed and validated for the simultaneous estimation of Tolperisone Hydrochloride and Diclofenac Sodium in commercial tablet dosage form. Separation was achieved by using C_{18} (250mm × 4.6 mm, 5µm) column and mobile phase comprising of potassium dihydrogen phosphate buffer (pH: 6.0): acetonitrile (70:30% v/v) at a flow rate of 1.0ml/min. The detection wavelength was 275 nm. The retention times for Tolperisone Hydrochloride and Diclofenac Sodium were 5.26min and 3.59min, respectively. Linearity of Tolperisone Hydrochloride and Diclofenac Sodium were found in range of 15-52.5µg/ml and 5-17.5µg/ml. The % recovery of Tolperisone Hydrochloride was found to be 99.58%-100.88% and 100.75%-101.33% for Diclofenac Sodium. The values of Limit of detection and limit of quantification were found to be 0.203µg/ml and 0.616µg/ml for Tolperisone Hydrochloride and 0.003µg/ml and 0.010µg/ml for Diclofenac Sodium, respectively. The linear regression coefficient for both drugs was found to be 0.999. The method was found to be robust since the retention times and areas were within the limits even after little deliberate variations in pH, flow rate, mobile phase ratio. Stress studies were conducted on the drug substance and product under the ICH prescribed stress condition viz. hydrolysis, oxidation, photolysis and thermal stress. The drugs showed sufficient decomposition under acidic hydrolysis, alkaline hydrolysis, and oxidation. The drug was found to be moderately sensitive to thermal studies and sunlight studies. This method can be successfully employed for simultaneous quantitative analysis of Tolperisone Hydrochloride and Diclofenac Sodium in pharmaceutical dosage form.

Keywords: Tolperisone Hydrochloride, Diclofenac Sodium, Stress degradation, Stability Indicating, Validation.

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INTRODUCTION

Tolperisone Hydrochloride (TOL) is piperidine derivative, is a centrally acting muscle relaxant[1]. Chemically it is (figure-1), 2-Methyl-1-(4-methylphenyl)-3-piperidin-1-ylpropan-1-one hydrochloride. It has molecular formula of $C_{16}H_{23}NO.HCl$ and molecular weight is 281.82 g/mol. Tolperisone Hydrochloride acts at the level of spinal cord and exerts its spinal reflex inhibitory action and predominantly via pre inhibition of the transmitter release from the primary different endings via a combined action on voltage gated Sodium and calcium channel[2]. TOL is official in Japanese Pharmacopoeia[3].

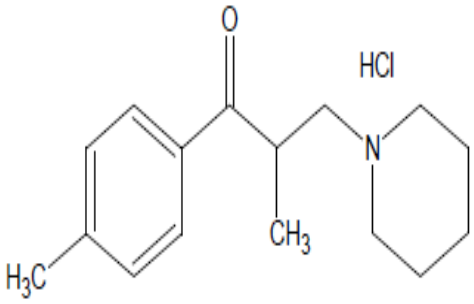

Figure 1: Chemical structure of TOL

Diclofenac Sodium

Diclofenac Sodium (DIC) is non-steroidal anti inflammatory drug. Chemically it is (figure-2), Sodium; 2-[2-(2, 6-dichloroanilino) phenyl] acetate. It has molecular formula of $C_{14}H_{10}Cl_2NNaO_2$ and molecular weight of 318.13 g/mol. During injuries arachidonic acid is converting to prostaglandins in the presence of enzymes cyclo-oxegenases (COX-1and COX-2).These prostaglandins sensitize pain receptors. Diclofenac works by blocking the effects of chemicals called cyclo-oxygenase enzymes on arachidonic and responsible for the analgesic effects of Diclofenac. DIC is official in I.P, B.P, U.S.P, and J.P[4].

Central Drug Standard Control Organization (CDSCO) has approved Tolperisone Hydrochloride and Diclofenac Sodium combination in July 2013 for the treatment of acute skeletal muscle spasm. Very few analytical methods have been reported for estimation of TOL and DIC as a single as well as their combination with other drugs[5, 6]. Literature survey reveals that many analytical methods such as UV Spectrophotometric, RP-HPLC and TLC methods are reported for estimation of TOL and DIC individually or in combined pharmaceutical dosage form. So, presented work was carried out stability indicating method for simultaneous estimation of Tolperisone Hydrochloride and Diclofenac Sodium in combined pharmaceutical dosage form.

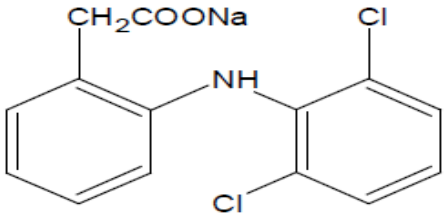

Figure 2: Chemical structure of DIC

MATERIALS AND METHOD

Chemicals and reagents

Marketed formulation TOLIFAST-D was procured from the local market. Methanol and Acetonitrile HPLC grade (Merck, India Limited), Potassium dihydrogen phosphate, Orthophosphoric acid, Hydrochloric acid, Sodium Hydroxide, Hydrogen peroxide AR grade (Merck, India Limited) were used.

Instrumentation and Chromatographic conditions

The HPLC system analysis was performed with shimadzu, An SPD-20AT UV detector and spinchrome software for data handling using C_{18} column. Before analysis the mobile phase was degassed by use of a sonicator (sonorax). In addition, an electronic balance (scale Tec, MS015) a pH meter (LAB India, Pico+), IR Affinity-1 (shimadzu) and pump (LC-20AT) were used in the study. The column was maintained at ambient temperature and injection volume of 20 µl was used.

Preparation of mobile phase

Mobile phase was prepared by mixing 700 ml of phosphate buffer (pH 6.0) and 300 ml Acetonitrile, filtered through 0.45 µm whatman filters and sonicated for 10 min.

Preparation of standard stock solution (TOL 30 µg/ml and DIC 10 µg/ml)

An accurately weighed 30 mg and 10 mg of TOL and DIC and transferred to individual 10 ml of volumetric flask, dissolve and volume make up with methanol. Further 1 ml TOL and 1 ml DIC were taken in individual 10 ml volumetric flask and the volume adjusted up to mark with methanol to get a concentration of TOL 30µg/ml and DIC 10µg/ml.

Preparation of working standard solution

1 ml of TOL and 1 ml of DIC standard stock solutions were transferred to a 10 ml of volumetric flask and volume was making up to the mark with methanol to give solution containing TOL 30µg/ml and DIC 10µg/ml.

Preparation of sample solution

An accurately weighed amount of powder mixture equivalent to 30 mg TOL and 10 mg DIC was transferred to 100 ml volumetric flask dissolve with methanol. The content was filtered through

whatman filter paper and dilute up to mark with methanol. This solution contains 300µg/ml TOL and 100µg/ml DIC. From above solution, 1 ml transferred to 10 ml volumetric flask and diluted up to mark with methanol to get concentration of 30µg/ml TOL and 10µg/ml DIC.

Forced degradation studies

The proposed stability-indicating method was carried out by as per ICH Q1A (R2) guideline[7]. Forced degradation is a process that involves degradation of drug products and drug substances at condition more severe than accelerated conditions and thus generate degradation product that can be studied to determine the stability of the molecule. The FDA and ICH guidance's state the requirement of stability testing data to understand how the quality of drug substance and drug product changes with time under the influence of various environmental factors.

Degradation conditions[8]

Hydrolytic condition

Hydrolysis is a chemical process that includes decomposition of a chemical compound by reaction with water. Acid or base stress testing involves forced degradation of a drug substance by exposure to acidic or basic conditions which generates primary degradants in desirable range. Hydrochloric acid or sulfuric acids (0.1–1 M) for acid hydrolysis and Sodium hydroxide or potassium hydroxide (0.1–1M) for base hydrolysis are suggested as suitable reagents for hydrolysis.

Oxidation condition

Hydrogen peroxide is widely used for oxidation of drug substances in forced degradation studies but other oxidizing agents such as metal ions, oxygen, and radical initiators (e.g., azobisisobutyronitrile, AIBN) can also be used. The oxidative degradation of drug substance involves an electron transfer mechanism to form reactive anions and cations. It is reported that subjecting the solutions to 0.1–3% H_2O_2 at neutral pH and room temperature for seven days or up to a maximum 20% degradation could potentially generate relevant degradation products.

Photolytic condition

Photo stability studies are performed to generate primary degradants of drug substance by exposure to UV or fluorescent conditions. Light stress conditions can induce photo oxidation by free radical mechanism. Functional groups like carbonyls, nitro aromatic, N-oxide, alkenes, aryl chlorides, weak C–H and O–H bonds, sulfides and polyenes are likely to introduce drug photosensitivity.

Thermal condition

Thermal degradation (e.g., dry heat and wet heat) should be carried out at more strenuous conditions than recommended ICH Q1A accelerated testing conditions. Studies may be conducted

at higher temperatures for a shorter period. Samples of solid-state drug sub- stances and drug products should be exposed to dry and wet heat, while liquid drug products should be exposed to dry heat.

Humidity:

Humidity is the Key factor in establishing the potential degradants in the finished product and active pharmaceutical ingredient. Normally 90% Humidity for duration of one week shall be recommended for the establishment of forced degradation samples.

Preparation of solutions

Preparation of 0.1 N HCl

A solution of 0.1 N HCl 0.86 ml prepared by taking 0.86 ml concentrated HCl in 100 ml volumetric flask and diluted up to mark with water.

Preparation of 0.1 N NaOH

A solution of 0.1 N NaOH was prepared by dissolving 0.4 gm NaOH pallets in 100 ml water.

Preparation of 3% H_2O_2

A solution of 3% H_2O_2 was prepared by taking 3 ml of H_2O_2 in 100 ml volumetric flask and diluted up to mark with water.

Method validation

Validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

According to International Conference on Harmonization ICH Q2 (R1) guidelines[9], validation of the method was carried out by using linearity, accuracy, precision and robustness.

RESULTS AND DISCUSSION

Method development

Method development was started with initial chromatographic conditions. Various compositions of water, methanol, acetonitrile and phosphate buffer were tested for better separation of the analytes. HPLC method development requires the scientific approach for developing the method for the chemical substances and drug products. Chromatogram is shown in (Figure-3). Optimized chromatographic condition shown in (Table-1)

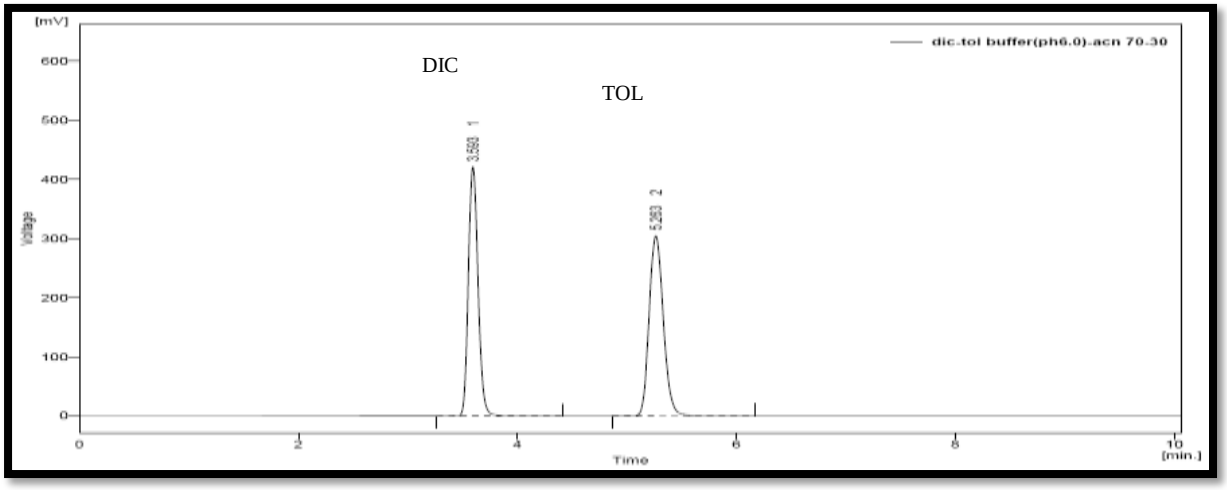

Figure 3: Chromatogram of standard solution containing TOL 30µg/ml and DIC 10µg/ml

Table 1: Final optimized chromatographic conditions

Parameters	Optimized conditions
Mobile phase	Phosphate buffer(pH:6.0): Acetonitrile (70:30%v/v)
Column	BDS hypersil$C_{18,}$ 250mm×4.6mm,5µ(particle size), Thermo scientific
Column temperature	Ambient(25°C)
Injection volume	20µl
Flow rate	1.0ml/min
Wavelength	275nm
Retention time	Tolperisone Hydrochloride: 5.263 min Diclofenac Sodium: 3.593 min

Forced degradation studies

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acidic, alkaline, oxidative, and thermal and sunlight degradation. (Table-2)

Table 2: Results of forced degradation study for TOL & DIC

Stress type	Time	%Degradation standard		%Degradation sample	
		TOL	DIC	TOL	DIC
Acid, 0.1 N HCl	4 Hour	24.92	22.31	24.05	21.95
Basic, 0.1 N NaOH	4 Hour	24.01	20.47	23.03	22.58
Oxidative, 3% H_2O_2	4 Hour	21.96	22.32	20.87	22.99
Thermal, 105°C	30 min	24.55	28.25	25.45	27.22
Sunlight	4 Hour	17.53	20.86	17.45	15.10

Acidic degradation

The acid degradation was done by sample was treated with 2 ml of 0.1 N Hydrochloric acid and kept for 4 hour at room temperature. After 4 hour the solution was neutralized with 2 ml 0.1 N Sodium hydroxide, made the volume up to the mark with mobile phase and analyzed using HPLC. (Figure-4)

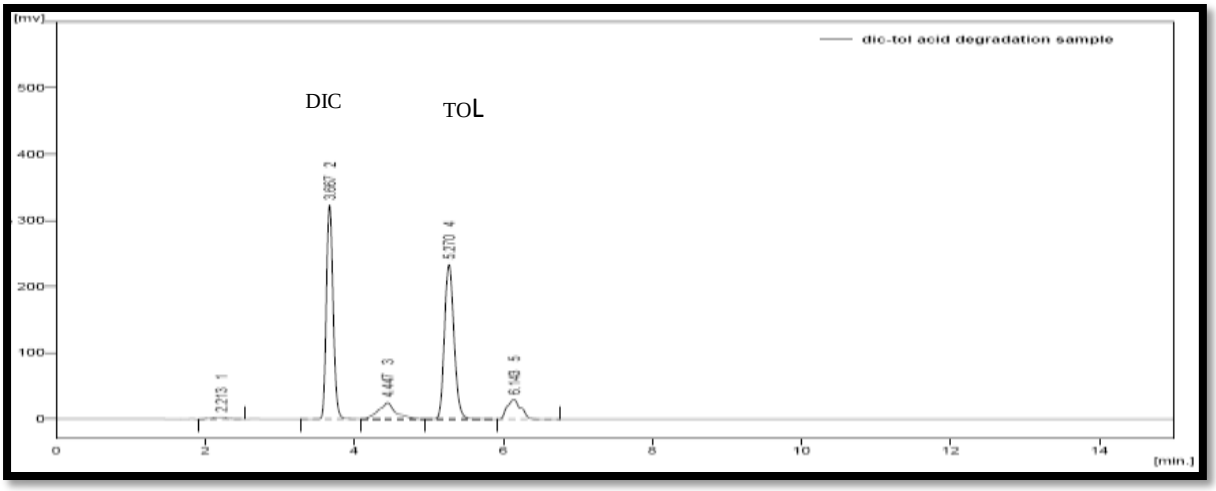

Figure 4: Chromatogram of sample mixture TOL and DIC in acid degradation (0.1N HCl, 4 Hour)

Alkaline degradation

The alkaline degradation was done by sample was treated with 2 ml Sodium hydroxide and kept for 4 hours at room temperature. After 4 hour the solution was neutralized with 2 ml 0.1 N Hydrochloric acid, made the volume up to the mark with mobile phase and analyzed using HPLC. (Figure-5)

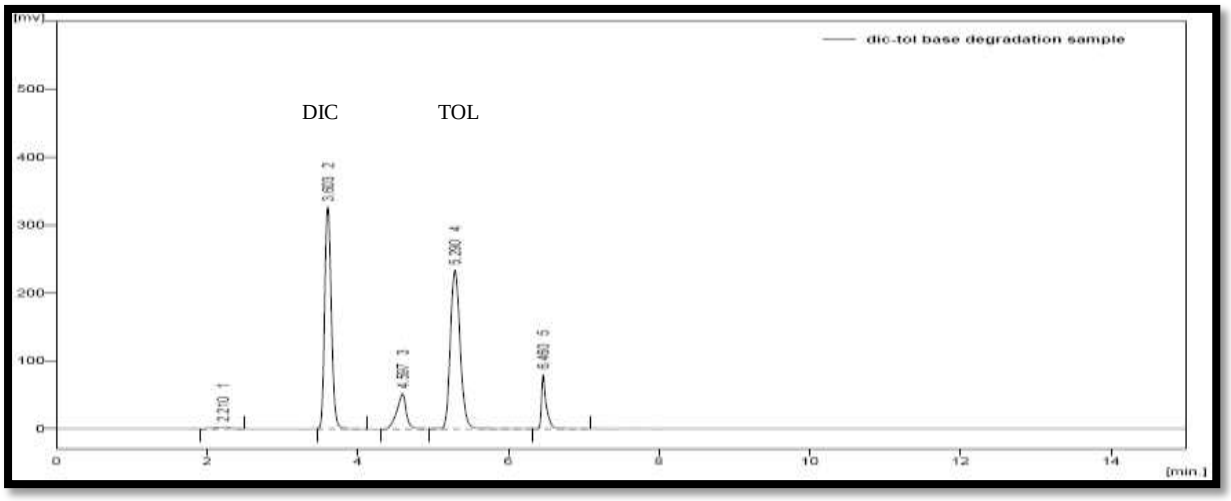

Figure 5: Chromatogram of sample mixture TOL and DIC in basic degradation (0.1 N NaOH, 4 Hour)

Oxidative degradation

The oxidative degradation was done by sample was mixed with 2 ml of 3% hydrogen peroxide solution, kept for 4 hour. After 4 hour made the volume up to the mark with mobile phase and analyzed using HPLC. (Figure-6)

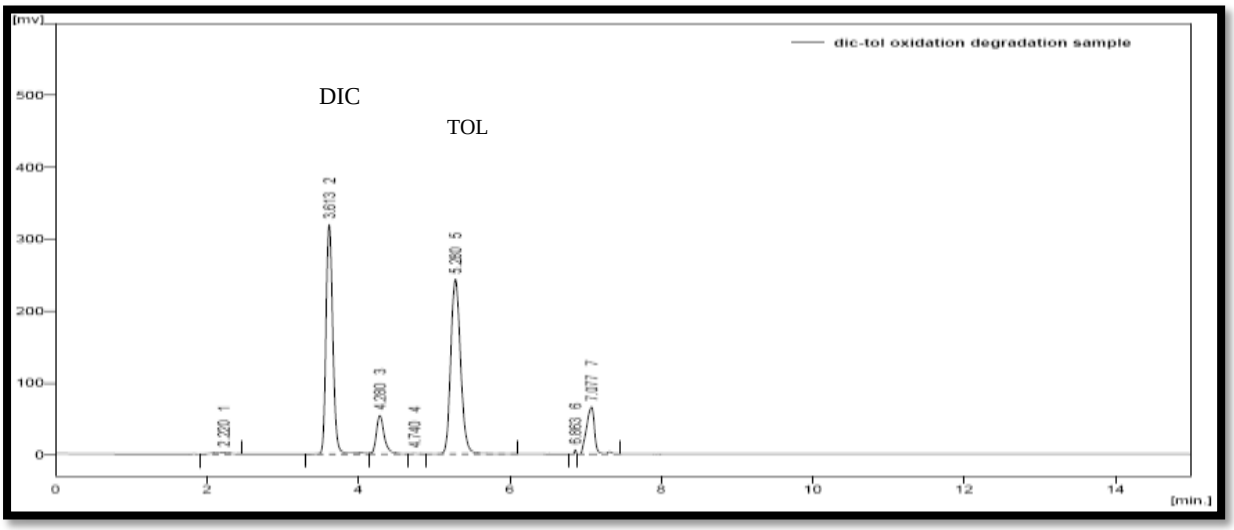

Figure 6: Chromatogram of sample mixture TOL and DIC in oxidative degradation (3% H_2O_2, 4 Hour)

Thermal degradation

The thermal degradation is performing by the exposing the solid drug at the 105°C temperature for 30 min in an oven. After 30 min made the volume up to the mark with mobile phase and analyzed using HPLC. (Figure-7)

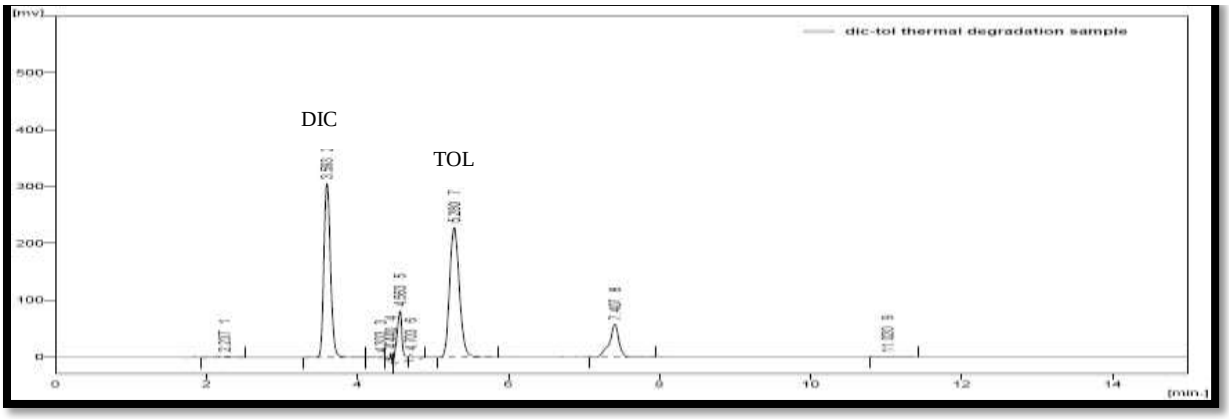

Figure 7: Chromatogram of sample mixture TOL and DIC in thermal degradation (105°C, 30 min)

Sunlight degradation

The sunlight degradation was done by exposing of drug content under the sun light for 4 hour. After 4 hour made the volume up to mark with mobile phase and analyzed using HPLC. (Figure-8)

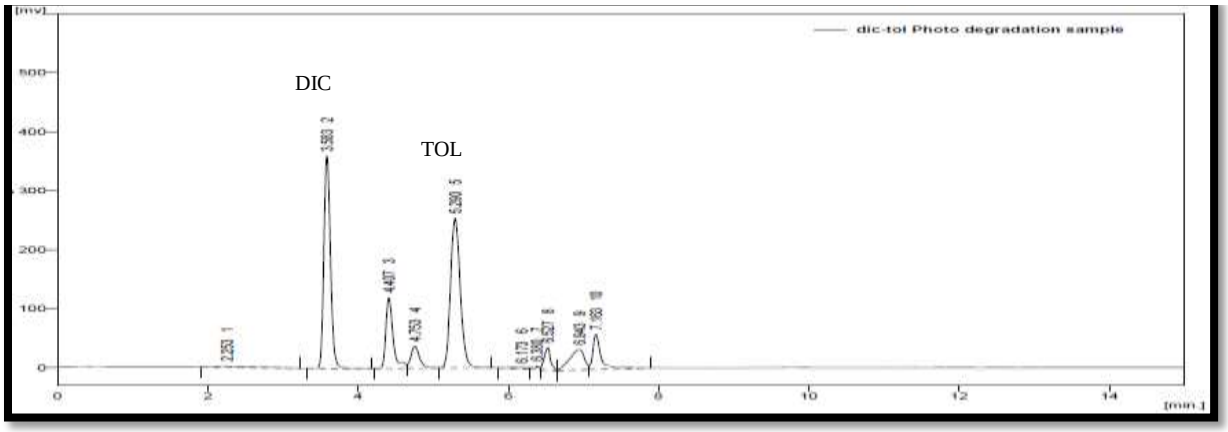

Figure 8: Chromatogram of sample mixture of TOL and DIC in sunlight degradation (4 Hour)

Method validation

Linearity

The calibration curve was plotted between peak area versus known concentration of TOL and DIC. The method shows linearity a concentration range of 15-52.5 µg/ml for TOL, the correlation coefficient was found to be 0.999. For DIC concentration range of 5-17.5 µg/ml, the correlation coefficient was found to be 0.999. (Table- 3)

Table 3: Linearity data of TOL and DIC (n=3)

TOL		DIC	
Concentration (µg/ml)	**Mean Peak Area**	**Concentration (µg/ml)**	**Mean Peak Area**
15	1429.941	5	1335.235
22.5	2061.327	7.5	1924.705
30	2828.973	10	2626.723

37.5	3481.936	12.5	3270.819
45	4176.048	15	3899.678
52.5	4865.364	17.5	4572.919
Correlation coefficient	0.999	**Correlation coefficient**	0.999

n=number of replicate injected

Accuracy

The accuracy of the test method was demonstrated by % recovery across its images by making three different concentrations at 80%, 100% and 120% levels using standard addition method where sample preparations were spiked with known amount of standard and then each concentration was injected three times in to the chromatographic system. (Table-4)

Table 4: % Recovery data of TOL and DIC

Drug	% Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total conc. (µg/ml)	Amount of recovered	% Recovery
	80	15	12	26	11.95	99.58
TOL	100	15	15	30	14.92	99.46
	120	15	18	33	18.16	100.88
	80	5	4	9	4.03	100.75
DIC	100	5	5	10	4.98	99.60
	120	5	6	11	6.80	101.33

Precision

Repeatability, intraday, and interday precision were evaluated by injecting the sample solution in to the HPLC system in triplicate on the same day and on different days. The results were reported in terms of RSD%. The results are given in (Table-5, 6).

Table 5: Repeatability data for TOL and DIC

Concentration	TOL (30µg/ml)	DIC (10µg/ml)
Area	2876.415	2685.470
	2838.857	2694.054
	2861.594	2688.740
	2898.597	2706.052
	2912.828	2719.509
	2908.874	2715.745
Mean	2882.861	2701.595
SD	29.16	14.30
%RSD	1.01	0.52

Limit: %RSD for area NMT 2.0%

Table 6: Intra-day and Inter-day study of TOL and DIC

Drug	Concentration (µg/ml)	Intra-day area mean (n=3)	%RSD	Inter-day area mean (n=3)	%RSD
	15	1431.450	0.016	1420.293	1.659
TOL	30	2086.796	0.466	2057.817	0.755

	45	2819.435	0.320	2797.437	0.818
	5	1340.404	0.437	1329.235	0.954
DIC	10	1945.475	0.352	1914.467	1.331
	15	2624.780	0.813	2618.263	0.615

n=number of replicate injected

Robustness

The robustness of the proposed method was determined by analyzing aliquots from homogenous lots by differing physical parameters like mobile phase ratio, flow rate, pH. The standard solutions were injected in to the chromatograph at varied conditions of mobile phase ratio ± 2.0, mobile phase pH ± 0.2 and flow rate ± 0.2 ml/min. (Table-7)

Table 7: Robustness study for TOL and DIC

		TOL				DIC			
Factor	**Level**	**RT (min)**	**%RSD**	**Mean area (n=3)**	**% RSD**	**RT (min)**	**%RSD**	**Mean area (n=3)**	**%RSD**
Flow rate	+0.2	5.12	0.58	2714.791	0.41	3.51	0.56	2534.465	0.41
(1.0 ml/min)	-0.2	5.43	0.36	2879.456	0.59	3.72	0.53	2681.513	0.29
Mobile	+2.0	5.12	0.58	2713.884	0.42	3.51	0.56	2533.627	0.42
phase (70:30 %v/v)	-2.0	5.38	0.37	2664.337	0.44	3.69	0.54	2853.816	0.44
pH (6.0)	+0.2	5.01	0.39	2656.487	0.48	3.43	0.29	2479.985	0.48
	-0.2	5.38	0.37	2850.943	0.58	3.69	0.54	2661.704	0.58

n=number of replicate injected

Assay of marketed formulation

Twenty tablets were weighed and their average weight was determined and finally powdered. An accurately weighed tablet powder equivalent to 30 mg of TOL and 10 mg DIC was then transferred to 100 ml volumetric flask containing 10 ml methanol and sonicated for 20 min. The solution was filtered through whatman filter paper and volume was adjusted up to the mark with methanol. This solution is expected to contain 300µg/ml TOL and 100µg/ml DIC. From this solution 1 ml transferred in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a concentration of TOL 30 µg/ml and DIC 10µg/ml. (Table-8)

Table 8: Result of assay of marketed formulation

Sr.no	**Amount of drug taken (µg/ml)**		**Amount of drug found (µg/ml)**		**% Assay**	
	TOL	**DIC**	**TOL**	**DIC**	**TOL**	**DIC**
1	30	10	29.69	9.86	98.96	98.68
2	30	10	29.63	9.84	98.76	98.48
3	30	10	29.84	9.91	99.46	99.17
%RSD					0.36	0.35

CONCLUSION

The study presents a simple stability-indicating HPLC method for the simultaneous estimation of TOL and DIC in presence of their degradation product and validated as per ICH guidelines. The method was successfully used for the estimation of drugs in pharmaceutical formulation. There was no interference observed due to excipients or other components present in the tablet dosage form. The results indicated the suitability of the method to study stability of TOL and DIC under various forced degradation condition. It can be concluded that the developed method may be employed for analysis of stability samples of TOL and DIC since the method could separate the drugs from their degradation products.

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REFERENCES

1. www.ncbi.nlm.nih.gov./pubmed/18482024
2. Kumar SM and Sunitha P. Method development and validation for the simultaneous estimation of Tolperisone hydrochloride and Diclofenac Sodium by RP- HPLC. Int. J. Pharma. Ana. Research 2015; 4: 174-182.
3. Japanese Pharmacopoeia; 14th Edn; Shibuya Tokyo Japan; 2006: 816-817.
4. Indian Pharmacopoeia; 6th edition; Ministry of health and family welfare, Indian pharmacopoeial Commission, Ghaziabad, India; 2010:1199-1200.
5. Nimila IC, Balan P, Chiranjeevi N and Vinnakota VVM. Development and validation of a RP-HPLC method of simultaneous estimation of Tolperisone hydrochloride and Paracetamol in tablet dosage form. Int. J. Pharm. Pharm. Science 2012; 4: 84-88.
6. Satish PA and Kalpesh PM. Spectrophotometric method for simultaneous determination of Chlorzoxazone and Diclofenac Sodium in synthetic mixture. Int. Res. J. Pharma 2012; 3: 293-296.
7. ICH guidelines, Q1A (R2), Harmonized Tripartite Guideline, Stability Testing of New Drug Substance and Products, In: Proceeding of International Conference on Harmonization, Geneva; 2003.
8. Charde MS, Kumar J, Welankiwar AS and Chakole RD. Review: development of forced degradation studies of drugs. Int.J.Adv.in Pharma 2013; 2: 34-39.

9. International Conference on Harmonization of technical requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guidelines, Validation of Analytical Procedure: Text and Methodology Q2 (R1), ICH Geneva, Nov.2005.