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Research Article

Analytical Method Development and Validation for Simultaneous Estimation of Tolperisone Hydrochloride and Diclofenac Sodium in Bulk and Pharmaceutical Formulation

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ABSTRACT

Quantitative analysis of any drug is an important tool in an industry. It is important to determine that the raw material, intermediate products as well as final products meet its specifications and are of required quality. The number of drugs and drug formulations introduced into the market has been increasing at an alarming rate. These drugs or formulations may be either new entities or partial structural modification of the existing ones or novel dosage forms.

Spectrophotometry and HPLC methods are considered to be most suitable for estimation of the drugs present in pharmaceutical dosage forms.

· Literature review reveals that several spectroscopic and Chromatographic method have been reported for estimation of TOL and DIC alone as well as with other drugs.

· Simultaneous equation, dual wavelength difference UV spectrophotometry and First derivative spectroscopic method is available for this combination.

· The aim of work is to develop and validate cost effective First derivative method in water and RP-HPLC method for simultaneous estimation of TOL and DIC in bulk and Tablet dosage form.

· Development of UV spectrophotometric method.

Keywords: Tolperisone, Diclofenac, Analytical Method, Validation

INTRODUCTION

Muscle spasms, which can affect any part of the body, are an involuntary contraction in the muscle tissue. Depending on the muscle's size and location, it might be sharp and painful or nearly imperceptible. A series of spasms or permanent spasms are called a spasmism. A spasm may lead to muscle strains or tears of tendons and ligaments, if the force of the spasm exceeds the tensile strength of the underlying connective tissues, such as with a particularly forceful spasm, or in the case of weakened connective tissues. An effective treatment might come from physical therapy, dietary changes, medical intervention, or some combination of the three.

Most muscle spasms fall into one of two categories[1]. There may not be enough of certain chemicals necessary for a muscle to function properly, called electrolytes, which can cause nerve signals to not travel correctly. Alternately, the nerve that triggers the muscle might be at fault, whether due to a problem with the nerve itself or with the brain. The common denominator is that the muscle is contracting inappropriately and without the person's control [2].

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In medicine a spasm is a sudden, involuntary contraction of a muscle, a group of muscles, or a hollow organ such as a heart, or a similarly sudden contraction of an orifice. It most commonly refers to a muscle cramp which is often accompanied by a sudden burst of pain, but is usually harmless and ceases after a few minutes. There is a variety of other causes of involuntary muscle contractions, which may be more serious, depending on the cause.

Causes of Muscle Spasm [3]

There are a number of reasons for muscle spasms. These include:

· Muscular fatigue, overuse or excessive stretching of muscles and prolonged periods of no movement – eventually, muscle cells run out of energy and fluid, become hyper excitable and develop a forceful contraction/spasm involving part of a muscle, the whole muscle, or even adjacent muscles.

· Dehydration and depletion of electrolytes also lead to muscle spasm and cramping.

· Abnormal supply of water, glucose, sodium, potassium, calcium, and magnesium upsets protein regulation required for normal contraction causing a muscle spasm.

· Systemic illnesses like diabetes, low red blood cell count, kidney disease and other hormonal concerns are potential causes of muscle spasms.

Classification of Drug Used for Muscle Spasms [4] **:**

Table 1 : Classification of drugs used for Muscle Spasms

Peripherally acting Muscle relaxants	**Non-depolarizing agent**	**Curare alkaloids**	**Tubocurarine, Dimethyltubocurarine**
		4-Ammonium agents	**Atracurium, Cisatracurium,Gallamine**
	Depolarizing agent	Choline derivatives	Succinylcholine,
	Ach release inhibitors	Botalinum toxin	
Centrally acting Muscle relaxants	Carbamic esters	Meprobamate, Methocarbamol, Tybamate	
	Benzodiazepines	Diazepam, Lorazepam, Nitrazepam	
	Anticholinergics	Orphenadrine	
	Piperidine derivatives	**Tolperisone,** Eperisone	
	Others	Quinine, Baclofen, Thiocolchicoside	
Directly acting Muscle relaxants	Dantrolene		
NSAIDs	**Diclofenac**, Ibuprofen, Lornoxicam		

MATERIALS AND METHOD

Table 2 : Materials

Sr. No.	Ingredient	Supplier
1	Tolperisone (TOL)	Orbit pharmaceuticals ltd, Ahmedabad
2	Diclofenac Sodium (DIC)	Orbit pharmaceuticals ltd, Ahmedabad
3	Ortho Phosphoric acid	AR grade
4	Tri ethylamine	AR grade
5	Acetonitrile	HPLC grade, Merck
6	Methanol	HPLC grade, Merck
7	Water	HPLC grade

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EXPERIMENTAL METHOD

DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD FOR ANALYSIS OF TOLPERISONE HYDROCHLORIDE AND DICLOFENAC SODIUM IN TABLET

Apparatus and Instruments

- Double beam UV-visible Spectrophotometer: Shimadzu, 1800.
· System controller: UV Probe 2.31
· Mode: Spectrum
· Scan speed: Medium
· Wavelength range: 400-200 nm
- Weighing balance:Shimadzu AUX 220
- Ultra Sonicator
- Borosil-Volumetric flasks of 10, 25, 50 and 100 ml (Borosil)
- Pipettes of 1, 2, 5 and 10 ml (Borosil)

Method Development:

Determination of the zero crossing points (Selection of wavelength)

From the overlaid first order derivative spectra of both the drug, DIC and TOL showed zero crossing at 248 and 226 nm respectively. At 248 nm DIC showed zero absorbance and TOL showed reasonable absorbance, while at 226 nm TOL showed zero absorbance and DIC showed reasonable absorbance. So these two wavelengths were selected for further measurement5.

Method Validation [6-8]

As per ICH guidelines Q2 (R1), the method validation parameters studied were so, linearity, accuracy, precision, limit of detection and limit of quantitation.

Linearity:

D_1 Absorbance of standard solutions of DIC (2, 4, 6, 8, 10 µg/ml) were measured at ZCP of TOL (226 nm) and D_1 Absorbance of standard solutions of TOL (5, 10, 15, 20, 25 µg/ml) were measured at ZCP of DIC (248 nm). D_1 Absorbance for both the drugs were plotted against their respective concentrations to get linear regression line.

Precision

The repeatability was checked by repeatedly (n = 6) measuring D_1 absorbances of DIC (6 µg/ml) and TOL (15 µg/ml).

The intra-day and inter-day precisions of the proposed method was determined by measuring the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of DIC (2, 6 and 10 µg/ml) and TOL(5, 10 and 15µg/ml) respectively. The results were reported in terms of relative standard deviation.

Accuracy (Recovery study)

The accuracy of the method was determined by calculating recovery of DIC and TOL by the standard addition method. Known amounts of standard solutions of DIC (0, 2, 4 and 6) and TOL (0, 5, 10 and 15) were added to prequantified sample solution of DIC (4 µg/ml) and TOL (10 µg/ml). The solutions were measured at 226 nm for DIC and 248 nm for TOL and % recovery of the each sample was calculated.

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 = the standard deviation of the response and S = slope of the calibration curve.

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TOL AND DIC

Apparatus and instrumentation

- HPLC: Shimadzu 20-AT

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· Column: BDS hypersil C18, (250mm × 4.6mm, 5µ)
· Manual Injector: Rheodyne Injector (Fixed Capacity Loop of 20 µl)
· Syringe: Hamilton syringe
· Pump: Binary pump, (Shimadzu, LC 20 AT)
· Detector: UV detector (PET), (SPD 20 AT)
- Weighing balance: Shimadzu AUX 220
- Digital pH meter: Chemiline
- Sonicator: Ultra sonicator
- Pipettes of 1, 2, 5 and 10 ml (Borosil)
- Volumetric flasks of 10, 25, 50 and 100 ml (Borosil)
- Measuring cylinder of 100 ml. (Borosil)

Linearity

Standard diluted stock solutions (0.2, 0.4, 0.6, 0.8, and 1.0 ml equivalent to 2.0, 4.0, 6.0, 8.0 and 10.0 µg/ml of DIC and 0.6, 1.2, 1.8, 2.4 and 3.0 ml equivalent to 6.0, 1.2, 1.8, 2.4 and 3.0 µg/ml of TOL) were transferred in a series of 10 mL volumetric flasks and diluted to the mark with methanol. An aliquot (20 µl) of each solution was injected under the operating chromatographic conditions as described earlier [9]. Chromatograms were recorded. Methanol (20 µl) blank was also injected under the same conditions and chromatogram of methanol was recorded for the correction of the response of methanol in the chromatograms containing responses of DIC and TOL. Calibration curves were constructed by plotting peak areas versus concentrations, and the regression equations were calculated. Each response was average of three determinations [10]

Precision

Repeatability was checked by repeatedly (n = 6) injecting the solution containing DIC (6 µg/ml) and TOL (18 µg/ml) and recording the chromatograms [11]

Intra-day and inter-day precisions of the developed method was determined by measuring the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentration of DIC (3.0, 6.0 and 9.0 µg/ml) and TOL (9.0, 18.0 and 27.0 µg/ml).

Accuracy

Accuracy of the method was determined by calculating percentage recovery of DIC and TOL by the standard addition method. Known amount of standard solutions of DIC (0, 4.8, 6 and 7.2 µg/ml) and TOL (0, 14.4, 18 and 21.6 µg/ml) were added to a pre-analyzed sample solution of DIC (6 µg/ml) and TOL (18 µg/ml). Each solution (20 µl) was injected in triplicate and the percentage recovery was calculated by measuring the peak areas and fitting these values into the regression equations of the calibration curves [13]

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 [14].

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

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 1

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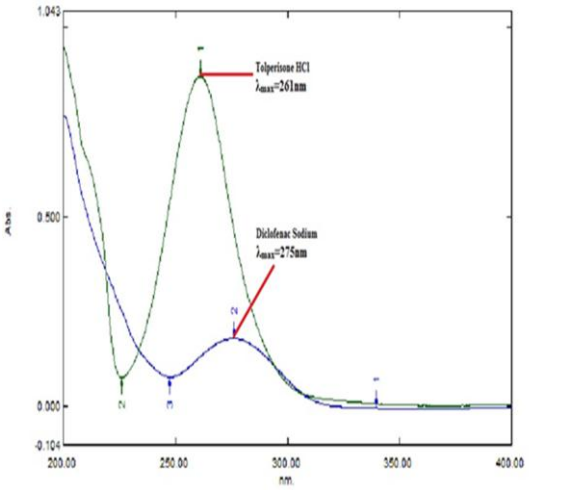

Fig: 1 Overlain zero order absorption spectra of standard DIC (4 µg/ml) and TOL (10 µg/ml) in distilled water

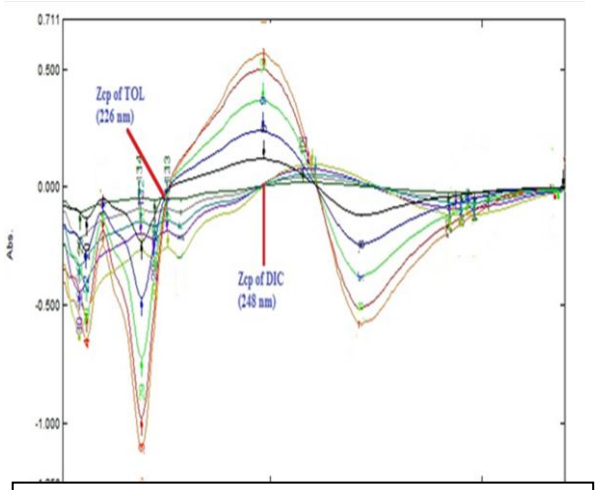

Fig: 2 Overlain first order derivative absorption spectra of standard DIC (2-10 µg/ml) and TOL (5-25 µg/ml) in distilled water

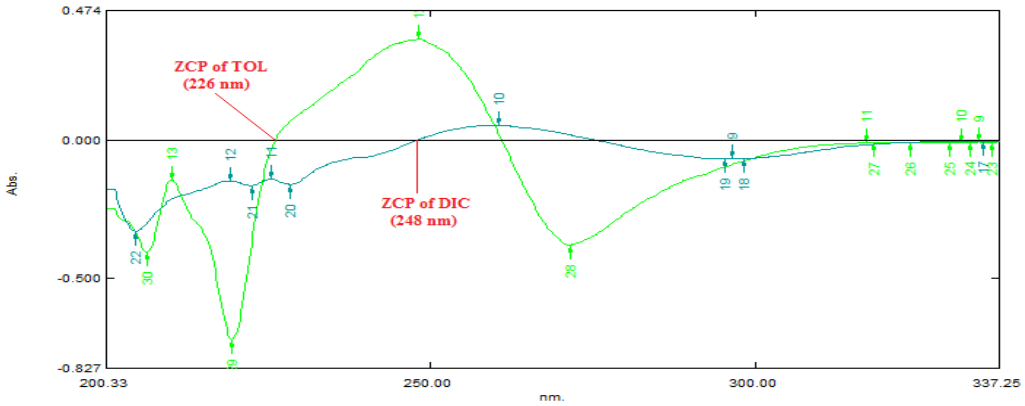

Fig: 3 Overlain first order derivative absorption spectra of tablet DIC (5 µg/ml) and TOL (15 µg/ml) in distilled water

VALIDATION OF THE DERIVATIVE SPECTROSCOPY METHOD

Linearity

The Beer's law was obeyed. Linear correlation was obtained between D_1 absorbance and concentration of DIC (2-10 µg/ml) and TOL (5-25 µg/ml). 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 3.

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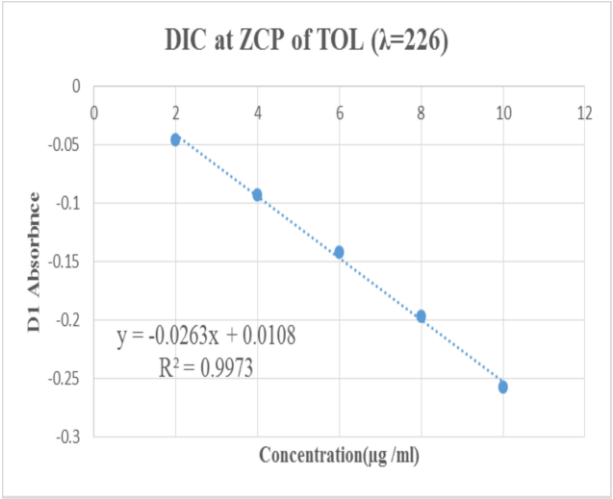

Fig: 4 Calibration Curve of DIC at 226 nm

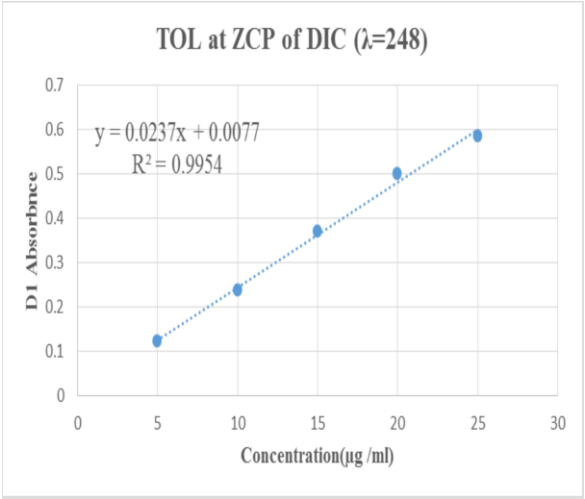

Fig: 5 Calibration Curve of TOL at 248 nm

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

Table 4 : Repeatability Data (n=6)

Sr. No.	Concentration (µg/ml)		D_1 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			-0.1428	0.369
SD			0.0021	0.0033
%RSD			1.8618	0.8999

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Table 5: Intraday precision data for DIC and TOL

DIC			TOL		
Conc (µg/ml)	D_1 Abs Mean ± S.D. (n=3)	% R.S.D	Conc (µg/ml)	D_1 Abs Mean ± S.D. (n=3)	% R.S.D
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 6: Interday precision data for DIC and TOL

DIC		
Conc (µg/ml)	D_1 Abs Mean ± S.D. (n=3)	% R.S.D
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 7: 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 8: 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			

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ISSN: 2347-7881

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 6

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

TOLPERITAS-D®	**Label claim (mg)**		**Amount found (mg)**		**% Label claim (mg) (n = 3)**	
	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
3	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

RESULT AND DISCUSSION

Table 10: Trials for the selection of different mobile phase

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)

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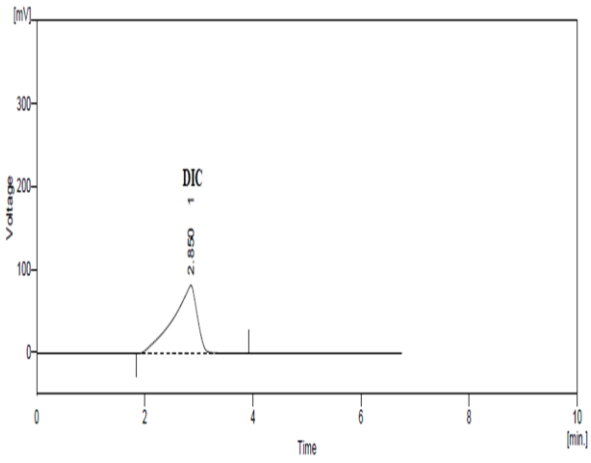

Fig 6: Chromatogram of DIC Mobile Phase – Water: Methanol (50: 50 v/v)

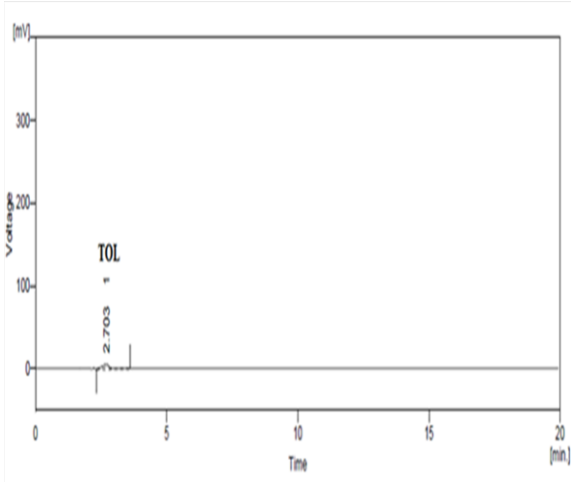

Fig 7: Chromatogram of TOL Mobile Phase – Water: Methanol (50: 50 v/v)

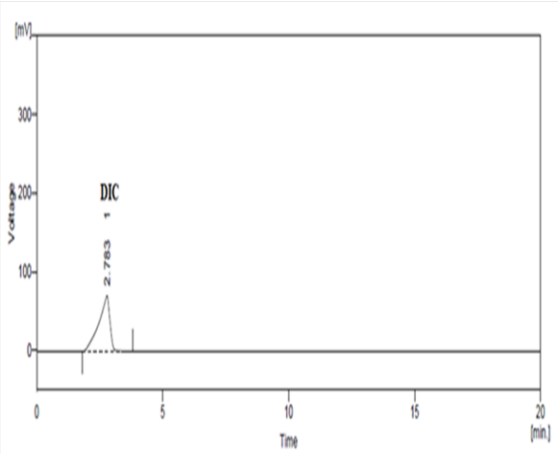

Fig 8: Chromatogram of DIC – TOL Mobile Phase – Water: Methanol (30: 70 v/v)

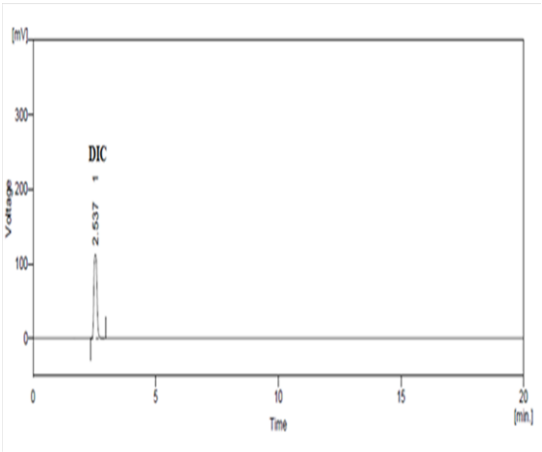

Fig 9: Chromatogram of DIC – TOL Mobile Phase – Water: ACN (30: 70 v/v)

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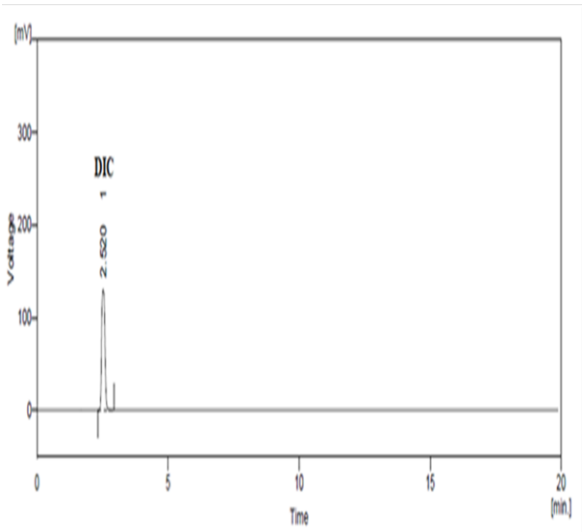

Fig 10: Chromatogram of DIC – TOL Mobile Phase – Water: ACN (15: 85 v/v)

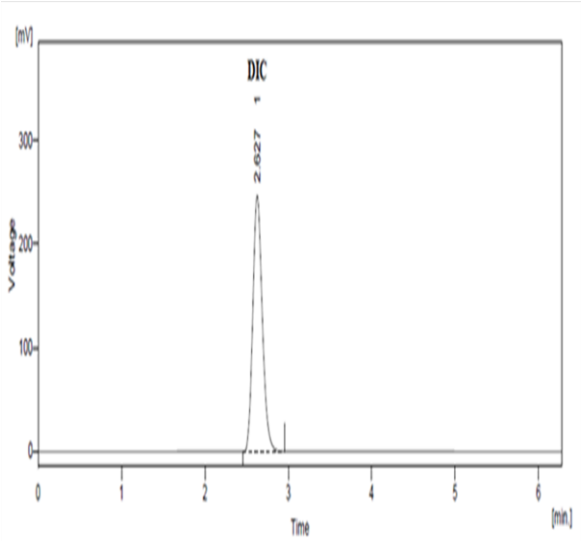

Fig 11: Chromatogram of TOL Mobile Phase – 20 mM Phosphate Buffer (pH 4.5): ACN (30: 70 v/v)

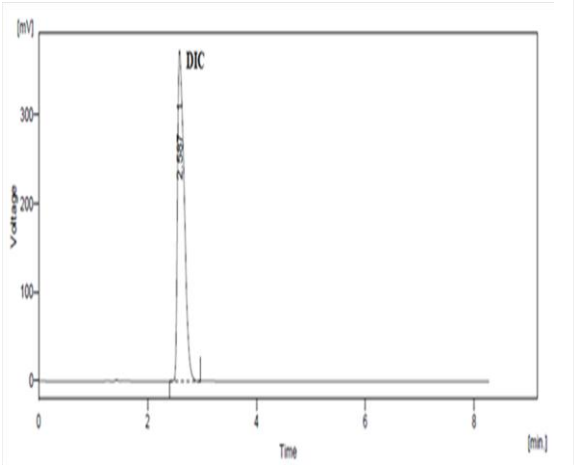

Fig 12: Chromatogram of DIC – TOL Mobile Phase – 20 mM Phosphate Buffer (pH 4.5): ACN (30: 70 v/v)

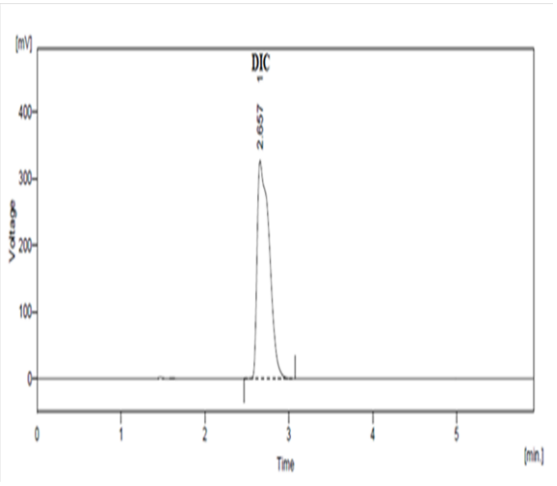

Fig 13: Chromatogram of DIC – TOL Mobile Phase – 20 mM Phosphate Buffer (pH 4.5): ACN (40: 60 v/v)

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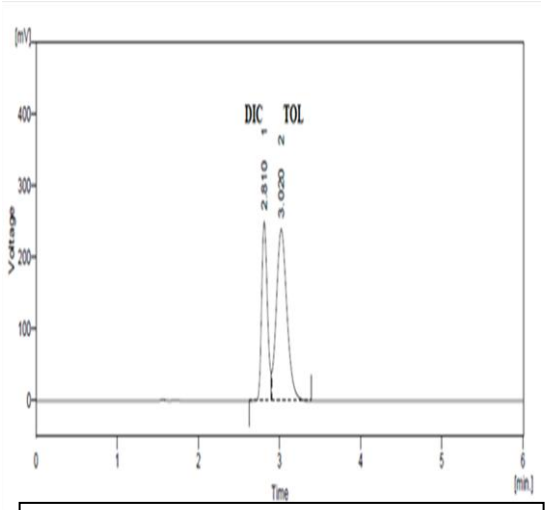

Fig 14: Chromatogram of DIC – TOL Mobile Phase – 20 mM Phosphate Buffer (pH 4.5): ACN (50: 50 v/v)

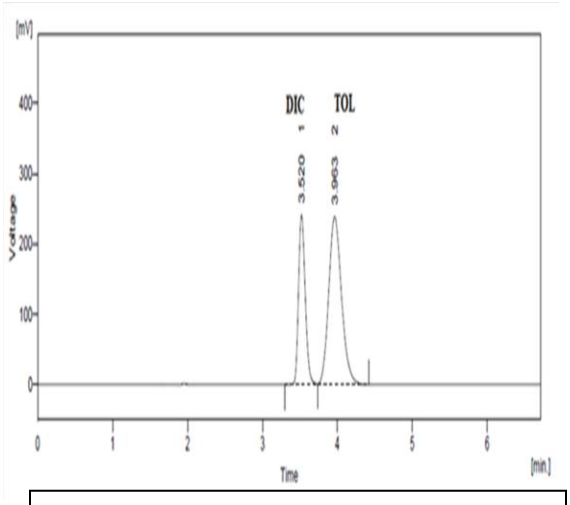

Fig 15: Chromatogram of DIC – TOL Mobile Phase – 20 mM Phosphate Buffer (pH 4.5): ACN (60: 40 v/v)

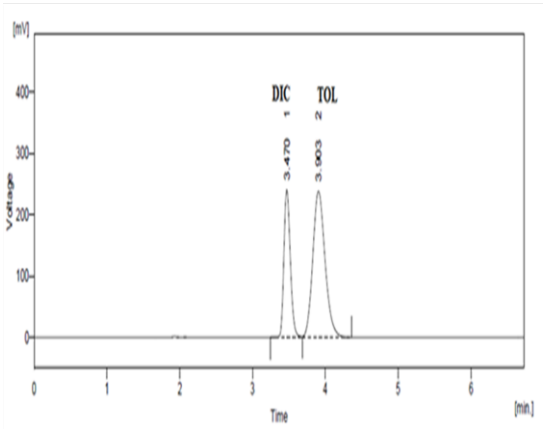

Fig 16: Chromatogram of DIC – TOL Mobile Phase – 20 mM Phosphate Buffer (pH 5.0): ACN (60: 40 v/v)

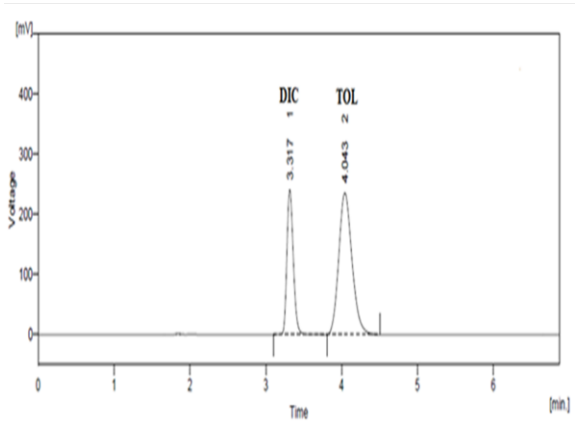

Fig 17: Chromatogram of DIC – TOL Mobile Phase – 20 mM Phosphate Buffer (pH 4.0): ACN (60: 40 v/v)

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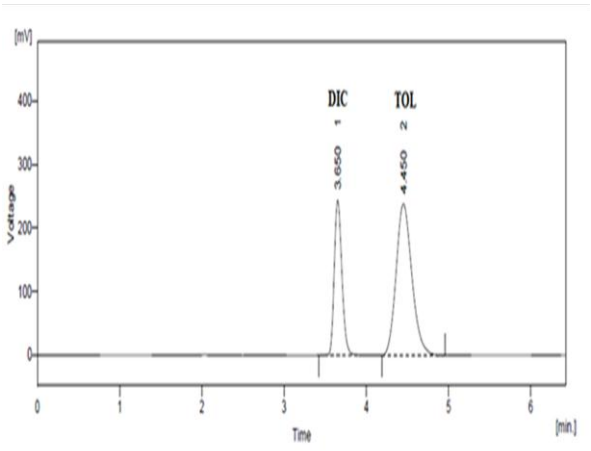

Fig 18: Chromatogram of DIC – TOL Mobile Phase – 20 mM Phosphate Buffer (pH 4.0): ACN (70: 30 v/v)

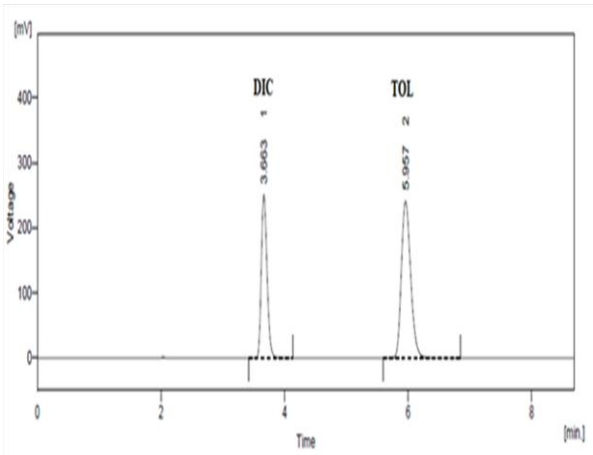

Fig 19: Chromatogram of DIC – TOL Mobile Phase – 20 mM Phosphate Buffer (pH 3.5): ACN (60: 40 v/v)

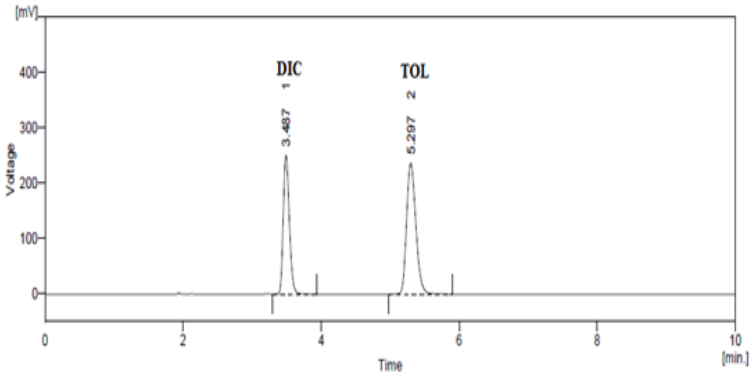

Fig 20: Chromatogram of DIC – TOL Mobile Phase – 20 mM Phosphate Buffer (pH 3.5): ACN (50: 50 v/v)

METHOD DEVELOPMENT AND OPTIMIZATION

Table 11: Optimized chromatographic conditions

Parameter	Chromatographic Conditions
Stationary phase	BDS hypersil C_{18}, (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

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METHOD DEVELOPMENT:

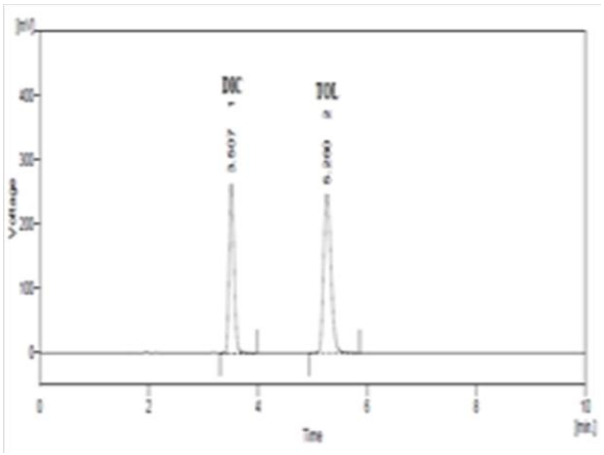

Fig 21: Chromatogram of TOL and DIC standard solution in 20 mM Phosphate Buffer (pH 3.5): ACN (50:50 v/v)

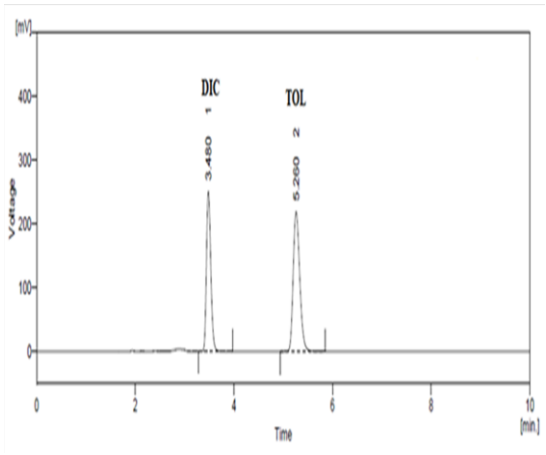

Fig 22: Chromatogram of TOL and DIC sample solution in 20 mM Phosphate Buffer (pH 3.5): ACN (50:50 v/v)

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, 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 13.

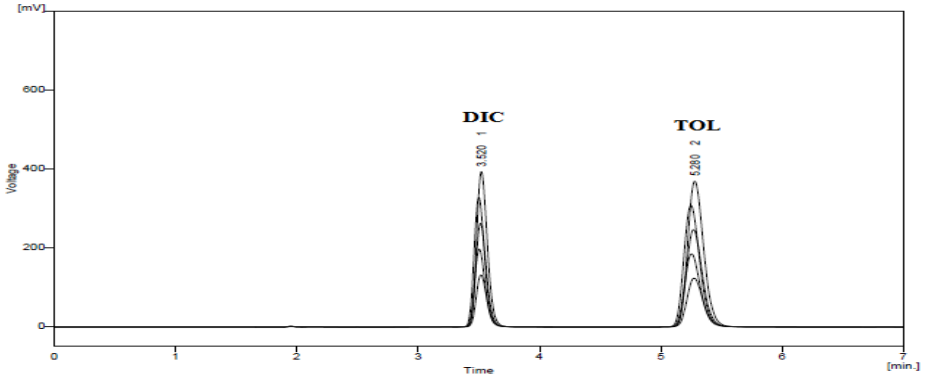

Fig 23: Overlain Chromatograms of DIC (2-10 µg /ml) and TOL (6-30 µg /ml)

Table 12: Linearity data for DIC

Sr. No.	Conc. (µg /ml)	Area Mean ± S.D. (n=6)	% R.SD
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

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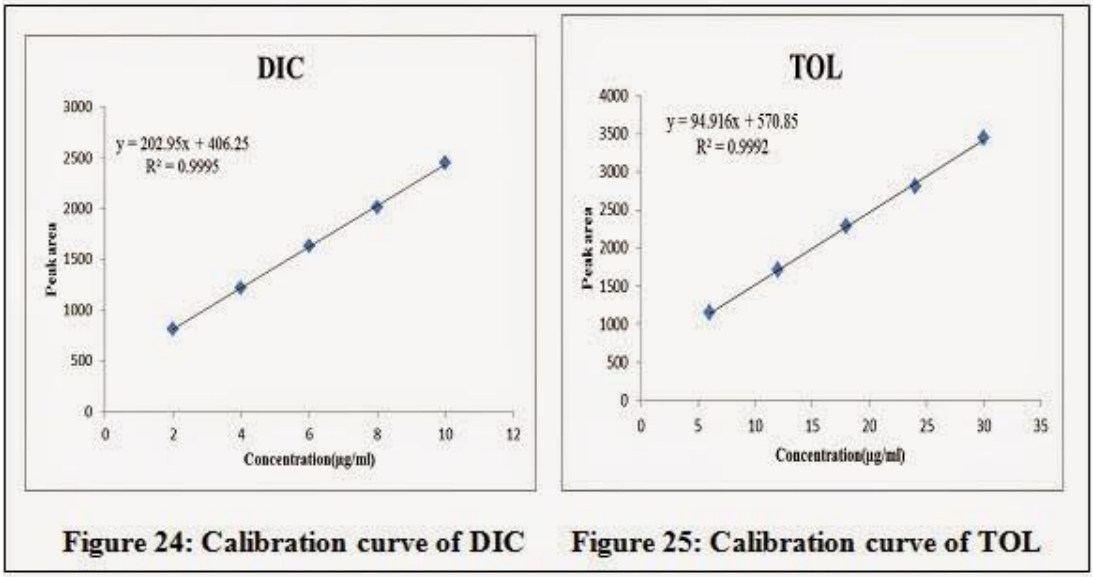

Figure 24: Calibration curve of DIC **Figure 25: Calibration curve of TOL**

Table 13: Linearity data for TOL

Sr. No.	Conc. (µg /ml)	Area Mean ± S.D. (n=6)	% R.SD
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 14: 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:
Following parameters were calculated for system suitability of RP-HPLC method.

Table 15: 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	

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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 6.7

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 17 and 18

Table 16: Repeatability data for DIC and TOL

Sr. no.	Concentration (µg/ml)		D_1 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 17: Intraday precision data for DIC and TOL

DIC			TOL		
Conc (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
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 18: Inter-day precision data for DIC and TOL

DIC			TOL		
Conc (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
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 19 and 20

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Table 19: 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 20: 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 21: 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

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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. The results are shown in Table 14.

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 [15]. 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 15.

Table 22: Assay data of pharmaceutical formulation (n=3)

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

CONCLUSION

A HPLC method has been developed and validated for the determination of DIC and TOL in tablet dosage form. The method was found to be specific as there was no interference of any excipients and impurities. The proposed method was found to be simple, accurate, precise and robust. Hence, it can be used successfully for the routine analysis of DIC and TOL in pharmaceutical dosage forms.

- Statistical Comparison of The Developed Methods

Comparison of Developed Methods by Statistical *t* - TEST

Table 23: Comparison of UV and HPLC method for determination of DIC and TOL

Parameters	DIC		TOL	
	UV	HPLC	UV	HPLC
Drug ± SD, % (n=3)	99.67 ± 1.63	100.49 ± 0.54	100.4 ± 1.33	99.75 ± 0.48
Tabulated *t*- Value	2.131		2.131	
Calculated *t*- Value	0.317		0.714	

The assay results for DIC and TOL in tablet dosage form, obtained using UV and HPLC methods were compared statistically by applying the two tail paired *t*-test. The calculated *t*- value for DIC (0.317) and TOL (0.714) is less than the tabulated *t*- value (2.131) at the 95% confidence interval.

$$t_{calculated} < t_{tabulated}$$

P - Value for the DIC and TOL were found to be 0.38 and 0.25 respectively. *P* - Value should be more than 0.05.

Therefore no significant difference was found in the content of DIC and TOL determined by the proposed UV and HPLC methods.

A UV spectrophotometric method has been developed and validated for the determination of DIC and TOL in tablet dosage form. The method was found to be specific as there was no interference of any excipients and impurities. Distilled water was used as a solvent. Hence, proposed method is a cost effective. The proposed method was found to be simple, accurate, precise and robust. Hence, it can be

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used successfully for the routine analysis of DIC and TOL in pharmaceutical dosage forms. A HPLC method has been developed and validated for the determination of DIC and TOL in tablet dosage form. The method was found to be specific as there was no interference of any excipients and impurities. The proposed method was found to be simple, accurate, precise and robust. Hence, it can be used successfully for the routine analysis of DIC and TOL in pharmaceutical dosage forms.

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* Both the methods were validated as per ICH Q2R1 guideline.
* Spectrophotometric and RP-HPLC methods were compared by statistical t - Test.