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DEVELOPMENT AND VALIDATION FOR DETECTION OF DIC AND IHC IN ELTROMBOPAG OLAMINE TABLET BY RP-HPLC METHOD

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ABSTRACT

An accurate and precise HPLC method was developed for the determination of DIC and IHC in Eltrombopag Olamine tablet. Separation of the drug was achieved on a Develosil ODS-MG-3100×4.6 mm 3µ column using a mobile phase consisting of 10mM Phosphate buffer (pH 6.0): Acetonitrile: water. The flow rate was 6.0 mL/min and the detection wavelength was 254 nm. The linearity was observed in the range of 15-150 ppm for DIC and IHC with a correlation coefficient of 0.997and 0.996 respectively. The proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of DIC and IHC in Eltrombopag Olamine tablet.

KEYWORDS: HPLC method, Eltrombopag Olamine tablet, Method Validation, DIC (Dimethyl Isopropyl Carboxylic acid) and IHC (Isopropyl Hydroxy Carboxylate).

1. INTRODUCTION

High performance liquid chromatography (HPLC)

The technique of High performance liquid chromatography is so called because of its improved performance in terms of rapidity, specificity, sensitivity, accuracy, convenience, ease of automation and the cost of analysis when compared to classical column chromatography. Advances in column technology, high pressure pumping system and sensitive detectors have transformed liquid column chromatography into a high speed, efficient, accurate and highly resolved method of separation.[1-4]

Estimation of multicomponent dosage forms by HPLC[5]

Most of the drugs in multicomponent dosage forms can be analysed by HPLC method because of several advantages like rapidity, specificity, accuracy, precision, ease of automation and eliminates tedious extraction and isolation procedures. Some of the advantages are,

- Speed (analysis can be accomplished in 20 minutes or less),
- Greater sensitivity (various detectors can be employed),
- Improved resolution (wide variety of stationary phases),
- Reusable columns (expensive columns but can be used for many samples),
- Ideal for the substances of low volatility,
- Easy sample recovery, handling and maintenance,
- Instrumentation lends itself to automation and quantitation (less time and less labour),
- Precise and reproducible,
- Calculations are done by integrator itself and
- Suitable for preparative liquid chromatography on a much larger scale.

Separation Principles of HPLC[6-10]

Adsorption chromatography employs high-surface area particles that absorb the solute molecules. Usually a polar solid such as a silica gel, alumina or porous glass beads and a non-polar mobile phase such as heptane, octane or chloroform are used in adsorption chromatography. In adsorption chromatography, adsorption process is described by competition model and solvent interaction model. Competition model assumes that entire surface of the stationary phase is covered by mobile phase molecules as result of competition for absorption site. In solvent interaction model the retention results from the interaction of solute molecule with the second layer of adsorbed mobile phase molecules. The differences in affinity of solutes for the surface of the stationary phase account for the separation achieved.

In partition chromatography, the solid support is coated with a liquid stationary phase. The relative distribution of solutes between the two liquid phases determines the separation. The stationary phase can either be polar or non polar. If the stationary phase is polar and the mobile phase is non polar, it is called normal phase partition chromatography. If the opposite case holds, it is called reversed-phase partition chromatography. In normal phase mode, the polar molecule partition preferentially in to the stationary phase and are retained longer than

non-polar compounds. In reverse phase partition chromatography, the opposite behavior is observed.[7]

TYPES OF HPLC TECHNIQUES[10-15]

1) Based on Modes of Chromatography

a) Normal phase chromatography

b) Reverse Phase Chromatography

2) Based on principles of Separation

a) Adsorption chromatography

b) Ion-exchange chromatography

c) Affinity chromatography

d) Chiral chromatography

3) Based on the Elution Technique

a) Isocratic separation

b) Gradient separation

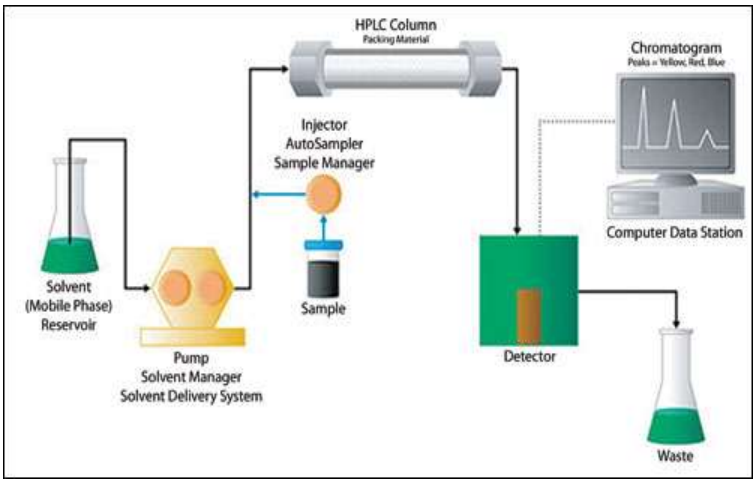

Fig. 1: Block diagram of HPLC.

2. DRUG PROFILE

ELTROMBOPAG OLAMINE[17-21]

Eltrombopag olamine is a biphenyl hydrazone.

Eltrombopag is a medication that has been developed for certain conditions that lead to thrombocytopenia (abnormally low platelet counts). It is a small molecule agonist of the c-mpl (TpoR) receptor, which is the physiological target of the hormone thrombopoietin.

Eltrombopag was discovered as a result of research collaboration between GlaxoSmithKline and Ligand Pharmaceuticals. Designated an orphan drug in the United States and European Union, it is being manufactured and marketed by GlaxoSmithKline under the trade name **Promacta** in the USA and is marketed as **Revolade** in the EU[20]

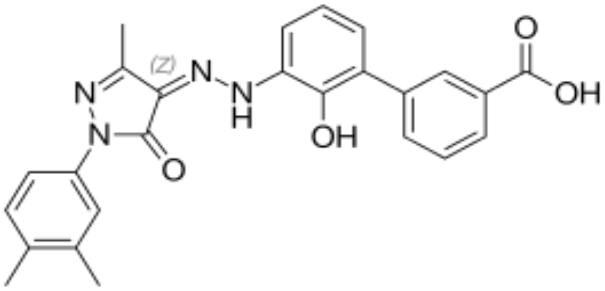

Fig. 2: Structure of Eletrombopag Olamine.

PHYSICOCHEMICAL PROPERTIES[21]

IUPAC name : 3'-{(2*Z*)-2-[1-(3,4-dimethylphenyl)-3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene]hydrazino}-2'-hydroxy-3-biphenylcarboxylic acid

Formula **:** $C_{25}H_{22}N_4O_4$

Molecular Mass **:** 442.467 g/mol

Category : Aplastic anemia

Physical state **:** white solid crystals

Solubility **:** Soluble in Water and insoluble in aqueous buffer**.**

3. INSTRUMENT AND CHEMICALS USED

Instruments used

- HPLC : Empower AD-LC-95
- Weighing balance : Single pan balance (Sartorius)
- Analytical Balance : Accurate 0.1 µg (Mettler Toledo)
- Solvent filtration unit :Millipore (Rankem)
- Syringe filters :PVDF filtes (Zodiac Life Sciences, India)
- Water bath :(Lab companion)
- Sonicator : Bandelin Sonorex
- PH meter : Polomon –LP139SA

Reagents used

- DIC : Dimethyl Isopropyl Carboxylic acid
- IHC : Isopropyl Hydroxy Carboxylate
- HPLC grade water :Milli `Q'
- HPLC grade Acetonitrile : Rankem
- Ortho phosphoric acid(88%) : Rankem
- HPLC grade Triethyl amine :Rankem
- HPLC grade Acetonitrile : Rankem

Drug sample

- Batch Number : EL0041114
- Month of Manufacture :March 2016
- Month of Expiry : February 2018
- Manufactured By : HETERO Drugs. Ltd.
- Label Claim : Each vial contains 10 mg/ml.
- **Working Reference Standards**
- Eltrombopag Olamine : HETERO Laboratories Ltd.
- Percentage Purity :99.46%

4. METHOD DEVELOPMENT

TRIAL I: CHROMATOGRAPHIC CONDITIONS

Column : Inertsil CN-3 5µm (4.6x250mm)
Mobile phase : Acetonitrile and water(50:50 v/v)
Flow rate : 0.6 ml/min
Mode : Gradient
Wavelength : 254nm
Column temperature : 30^0C
Injection volume : 10 µL
Run time : 45 min

Table-Gradient program for trail 1.

Time	Solvent-A	Solvent-B
0	50	50
8	50	50
20	30	70

35	30	70
40	50	50
45	50	50

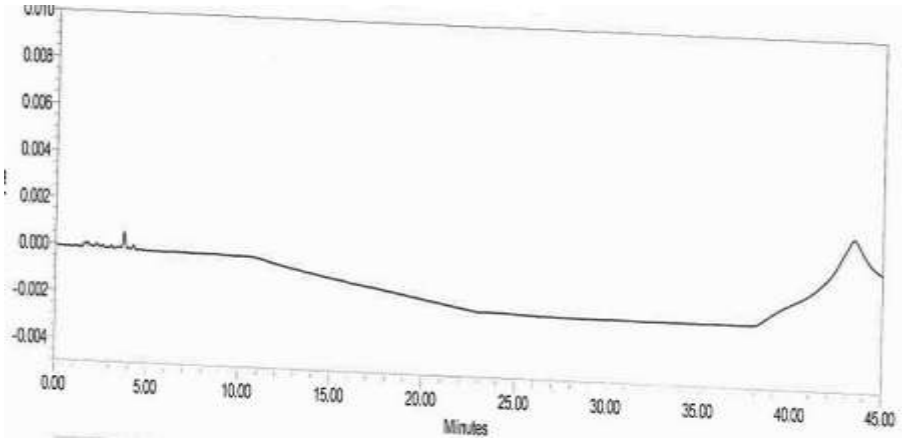

Fig: 3. Chromatogram of Trail I.

OBSERVATION

No response was observed.

TRIAL II: CHROMATOGRAPHIC CONDITIONS

Column : Develosil ODS-MG-3100×4.6 mm 3µ (or) Equivalent

Mobile phase : Acetonitrile :Water (50:50V/V)

Flow rate : 0.6 ml/min

Mode : Gradient

Wavelength : 254 nm

Column temperature : 30^0C

Injection volume : 10 µL

Run time : 45min

Table Gradient Program for Trail 2.

Time	Solvent-A	Solvent-B
0	50	50
8	50	50
20	30	70
35	30	70
40	50	50
45	50	50

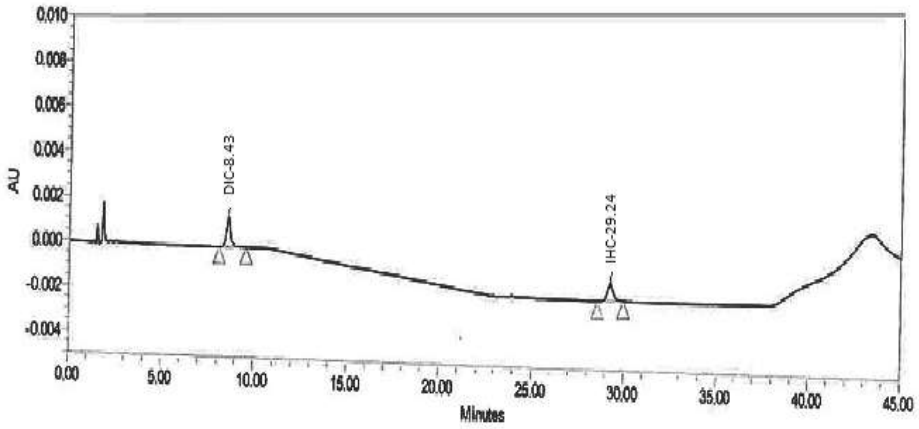

Fig: 4. Chromatogram of Trail II.

OBSERVATION

low. response peaks were observed.

6.2.3 TRIAL III: CHROMATOGRAPHIC CONDITIONS.

Column : Develosil ODS-MG-3100×4.6 mm 3µ (or) Equivalent

Mobile phase : Acetonitrile :Water (50:50 V/V)

Flow rate : 0.6 ml/min

Wavelength : 254µm

Column temperature : 50 ^{0}C

Injection volume : 10 µL

Run time : 45 min

Table-Gradient Program for Trail 3.

Time	Solvent-A	Solvent-B
0	40	60
8	40	60
20	30	70
35	30	70
40	40	60
45	40	60

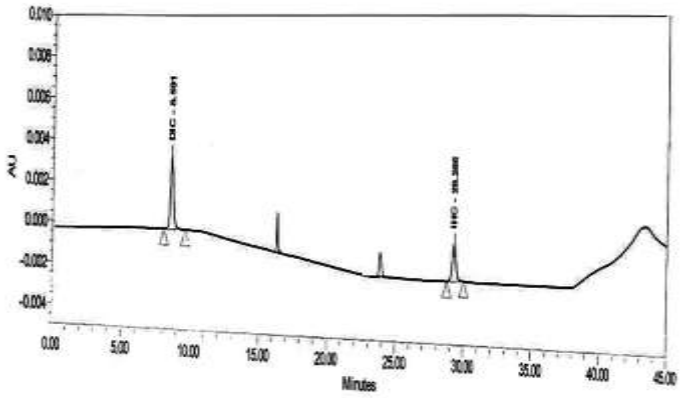

Fig: 5. Chromatogram of Trail III.

Observation

Unwanted peaks were observed.

TRIAL IV: CHROMATOGRAPHIC CONDITIONS

Column	: Develosil ODS-MG-3100×4.6 mm 3µ (or) Equivalent
Mobile phase	: Acetonitrile :Water(50:50 v/v)
Mode	: Gradient
Flow rate	:0.6 ml/min
Wavelength	: 254 nm
Column temperature	: 50^0C
Injection volume	: 10 µL
Run time	: 45 min

Gradient Program for Trail. 4.

Time	Solvent-A	Solvent-B
0	30	70
8	30	70
20	20	80
35	20	80
40	30	70
45	30	70

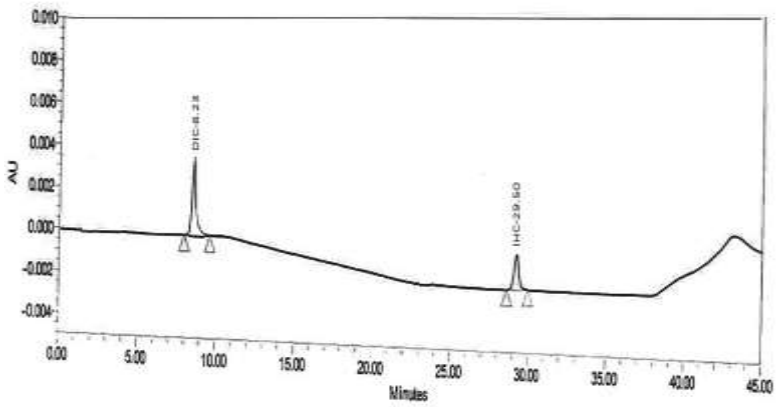

Fig: 6. Chromatogram of Trail IV.

Observation

Tailing was observed.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Column : Develosil ODS-MG-3100×4.6 mm 3µ (or) Equivalent

Mobile phase : Acetonitrile: Water (50:50 v/v)

Mode : Gradient

Flow rate : 0.6 ml/min

Wavelength : 254 nm

Column temperature : 50^0C

Injection volume : 10 µL

Run time : 45 min

Gradient Program for Standard

Time(min)	Solvent-A	Solvent-B
0	30	70
8	30	70
20	5	95
35	5	95
40	30	70
45	30	70

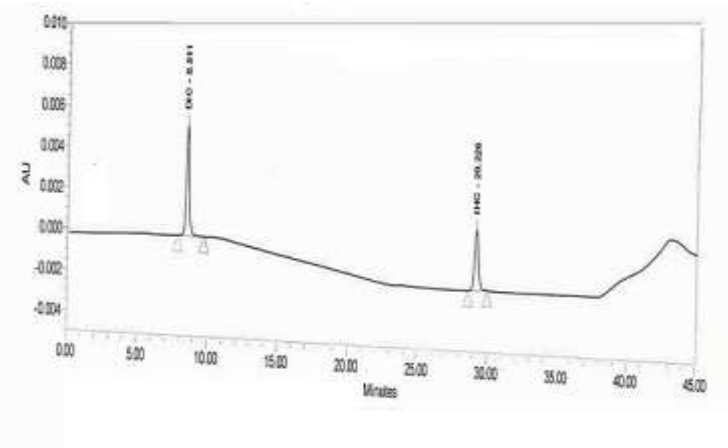

Fig: 7. Final chromatogram of standard.

Observation

Finally peak was observed with good response.

Table: 13. Results of Optimized method for Standard.

Name of Peak	Retention time(min)	Area	USP Tailing	Injection Volume
DIC	8.511	80523	1.08	10µl
IHC	29.226	55917	1.10	10µl

6.2.6 Final chromatogram for sample.

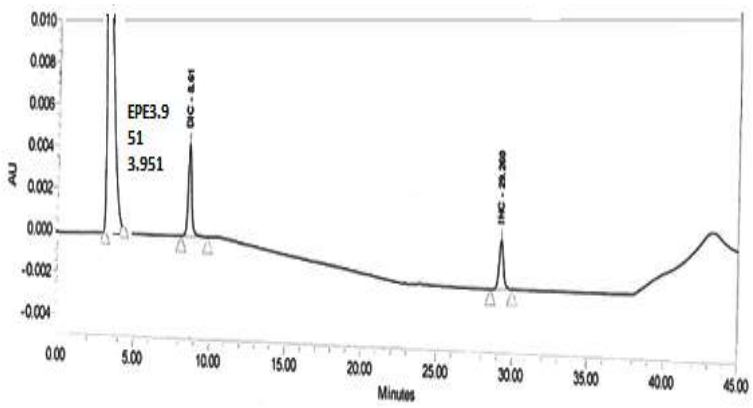

Fig: 8 Final chromatogram of sample.

Observation

finally peak was observed with good response.

Table: 14. Results of Optimized method for sample.

Name of Peak	Retention time(min)	Area	USP Tailing	Injection Volume
DIC	8.61	26348	1.02	10µl
IHC	29.26	14009	1.10	10µl

Table: 15. Results of %Assay of DIC and IHC.

Name	As	At	Wt. equivalent taken (mg)	%Assay
DIC	80.523	26348	5.0	0.09
IHC	55917	14009	15.0	0.07

CONCLUSION

%Assay of DIC and IHC in tablet is found to be 0.09, 0.07% and which are within acceptance criteria 95-105%.

5. ANALYTICAL METHOD VALIDATION RESULTS

Table 16: Results of %RSD of DIC and IHC.

S.NO	Injection Number	Peak area for DIC	Peak area for IHC	Acceptance criteria
1	01	53294	31527	The % RSD of peak areas of DIC &IHC should not be more than 10.0
2	02	54076	32429	
3	03	54388	32534	
4	04	53127	31297	
5	05	53798	31787	
6	06	52791	31049	
Mean		53579	31770	
%RSD		1.14	1.9	

Table 17: Results from system suitability studies of DIC and IHC.

System suitability parameters	Observed value		Acceptance criteria
The Tailing for DIC &IHC in standard solution	1.08	1.	NMT 2.0
Theoretical plates for DIC and IHC in standard solution	8000	8526	NLT 2000

OBSERVATION

The %Relative standard deviation of individual area response of six replicate injections for DIC AND IHC was found to be 1.12 and 1.09. The %Relative standard deviation of areas of six replicate injections for DIC and IHC standard were found to be within limits. The tailing factor for DIC and IHC peaks was found to be 1.12 and 1.09. The tailing factor for DIC and IHC peaks was found to be within limits. The number of theoretical plates for DIC and IHC were found to be 8000 and 8526.

Table 18: Table of results of Linearity of Detector Response.

Standard concentration (µg/ml)	Peak Area	
	DIC	IHC
50	26342	14189
75	40140	24245
100	53447	34465
125	67102	44602
150	80336	56024
Correlation	1.0000	0.9950

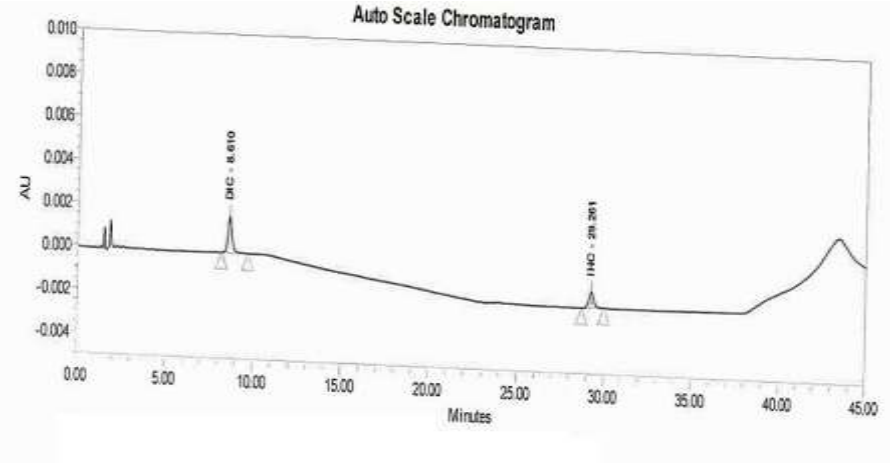

Fig. 9: Chromatogram of Linearity 1 (50 mcg/ml).

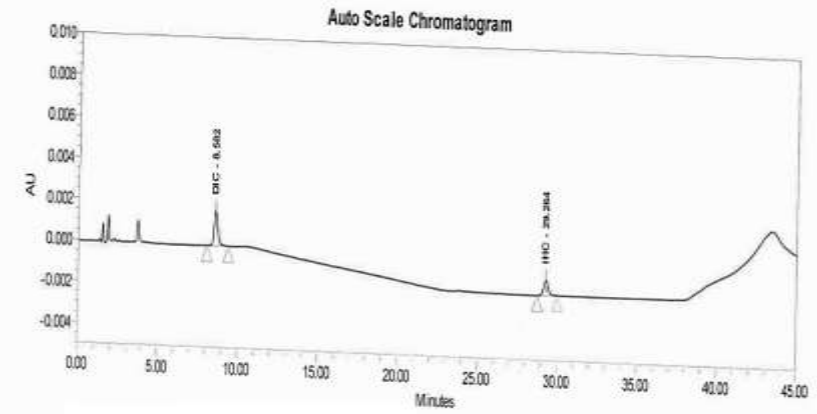

Fig: 10 Chromatogram of Linearity 2 (75 mcg/ml).

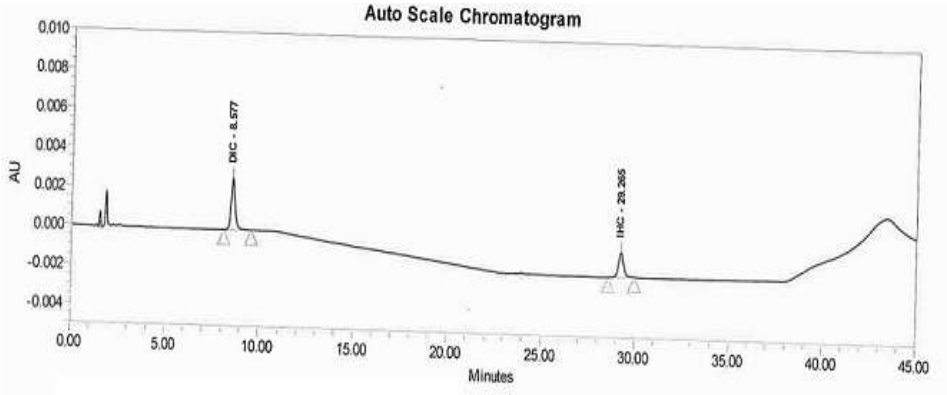

Fig: 11. Chromatogram of Linearity 3 (100 mcg/ml).

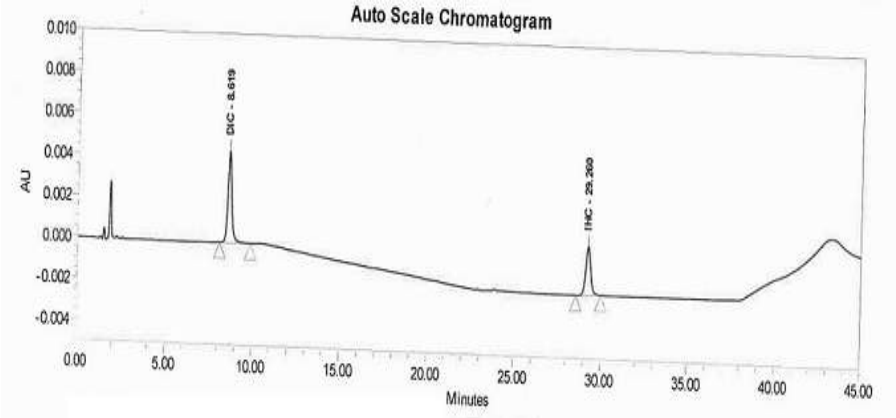

Fig: 12 Chromatogram of Linearity 4 (125mcg/ml).

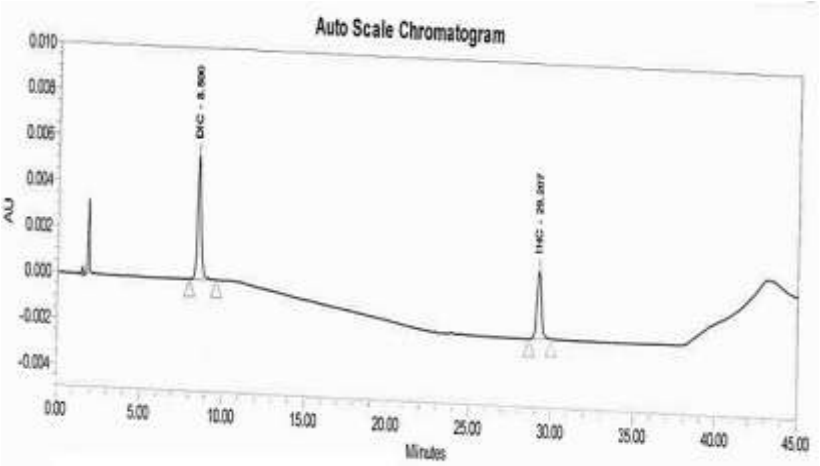

Fig: 13. Chromatogram of Linearity 5 (150 mcg/ml).

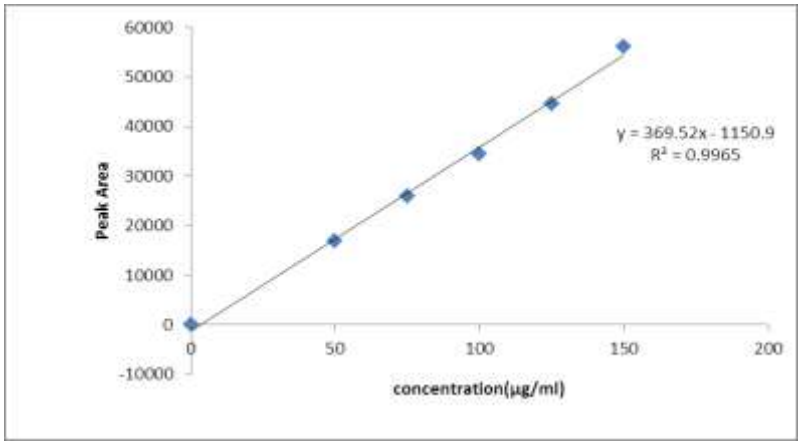

Fig: 14. Linearity Plot of DIC.

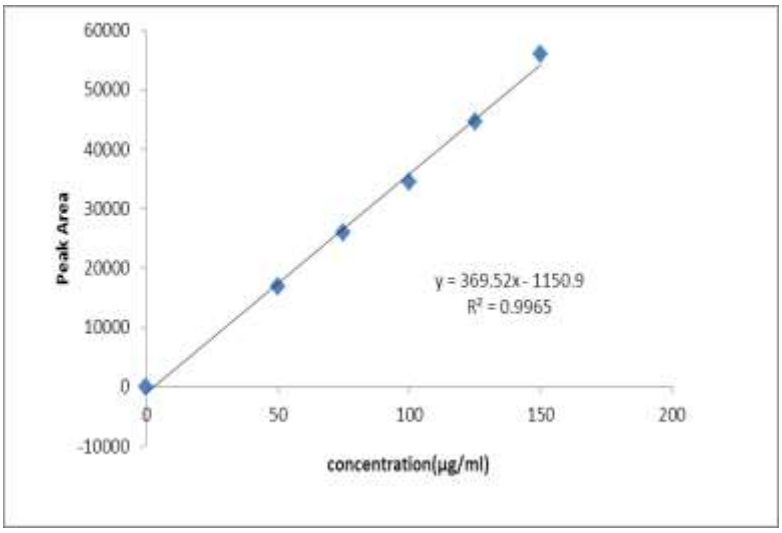

Fig: 15. Linearity Plot of IHC.

Table 19: Precision for DIC and IHC.

S.NO	Injection Number	Peak area for DIC	Peak area for IHC
1	Standard 1	40550	24453
2	Standard 2	40656	24386
3	Standard 3	40687	24358
4	Standard 4	40635	24482
5	Standard 5	40655	24568
6	Standard 6	40672	24676
Mean		40642.5	24487.1
%RSD		0.18	0.25

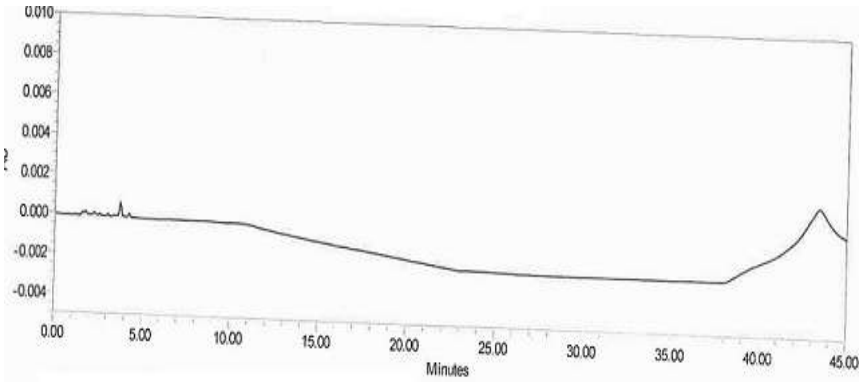

Fig: 16 Chromatogram of Blank.

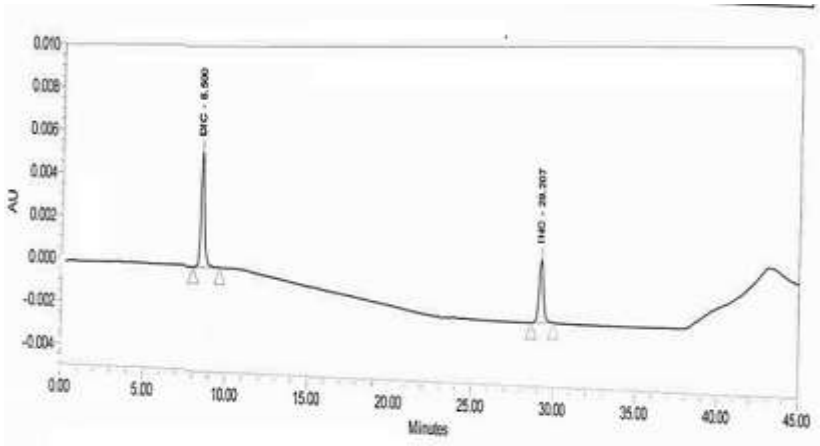

Fig: 17 Chromatogram of standard.

Table 20. Accuracy for DIC and IHC.

% Spiked	Weight added (mg)		Weight Recoverd(mg)		% Recovery	
	DIC	IHC	DIC	IHC	DIC	IHC
50	2.50	6.26	2.46	6.33	98.40	101.12
	2.50	6.38	2.48	6.29	99.20	98.59
	2.52	6.23	2.49	6.46	98.81	103.69
100	5.08	12.54	4.88	12.47	96.06	99.44
	5.12	12.56	4.98	12.79	97.26	101.83
	5.06	12.58	4.76	12.29	97.07	97.69
150	7.57	18.89	7.39	18.79	97.62	99.47
	7.73	18.73	7.68	18.83	99.35	100.53
	7.58	18.71	7.47	18.66	98.55	99.73

Table 21: Results of Robustness – DIC.

S.No.	Parameter	Condition	System suitability results		
			% RSD	USP tailing	USP Plate Count
1	Flow rate by ± 10%	0.5 ml	0.63	1.27	6172
		0.6 ml	0.39	1.10	10356
		0.7 ml	0.66	1.16	7864
2	Column Oven temperature by ± 5°C	35°C	0.69	1.11	5679
		40°C	0.24	1.12	9845
		45°C	0.70	1.19	5661
3	pH of Buffer solution by ± 0.2 units	6.2	1.42	1.56	5072
		6.0	0.16	1.10	8854
		5.8	0.39	1.32	6095
4	Wavelength of analysis ± 5nm	249 nm	1.46	1.70	5965
		254 nm	0.39	1.02	8847
		259 nm	0.60	1.68	7258

Table 22: Results of Robustness – IHC.

S.No.	Parameter	Condition	System suitability results		
			% RSD	USP tailing	USP Plate Count
1	Flow rate by ± 10%	0.5 ml	0.54	1.46	5345
		0.6 ml	0.27	1.08	9732
		0.7 ml	0.52	1.08	8652
2	Column Oven temperature by ± 5°C	35°C	0.56	1.21	6565
		40°C	0.29	1.20	9792
		45°C	0.71	1.27	8190
3	pH of Buffer solution by ± 0.2 units	6.2	0.33	1.28	4562
		6.0	0.25	1.10	9769
		5.8	0.24	1.10	9025
4	Wavelength of analysis ± 5nm	249 nm	0.56	1.07	4451
		254 nm	0.28	1.05	9712
		259 nm	0.65	1.11	6788

Table: 23. Results of LOD and LOQ.

Sample	LOD(µg/ml)	LOQ(µg/ml)
DIC	5	14.9
IHC	3.1	10.5

6. SUMMARY

Table 22: Summary of validation parameters by HPLC method.

Parameters	DIC	IHC
Tailing factor	1.08	1
%RSD	1.14	1.9
Theoretical plates	8000	8526
Correlation coefficient	1.0000	0.9950

%RSD	0.18	0.25
Mean % recovery for 50, 100, 150% respectively	NLT 95.0% NMT 105.0%	NLT 95.0% NMT 105.0%
Interference	No interference	No interference
Flow rate by ± 10%	All the All the system suitability parameters are within the limit for all the variable parameters, for all the three drugs	
Column Oven temperature by ± 5°C		
pH of Buffer solution by ± 0.2 units		
Wavelength of analysis ± 5nm		
Organic composition of mobile phase by ± 5%		
Standard deviation method	5.0 µg/ml	14.9 µg/ml
	3.1 µg/ml	10.5 µg/ml

7. CONCLUSION

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost RP-HPLC method. It is successfully applied for the determination of DIC and IHC in pharmaceutical preparations without the interferences of other constituent in the formulations.

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with 10mM Phosphate buffer (pH 6.0): Acetonitrile:water (90:10 v/v) with 0.6 ml/min flow rate is quite robust.

The optimum wavelength for detection was 254 nm at which better detector response for drug was obtained. The average retention time for DIC and, IHC were found to be 8.511, 29.226 respectively. The linearity was observed in the range of 15-150 ppm for the drugs with a correlation coefficient of 1.000, 0.999 respectively. The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found in the range of NLT 95.0 – NMT 105.0%.

Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of three days. These results show the accuracy and reproducibility of the assay.

From the above experimental data and results, the developed HPLC method is having the following advantages:

- The standard and sample preparation requires less time.
- No tedious extraction procedure was involved in the analysis of formulation.
- Suitable for the analysis of raw materials, applicable to dissolution studies and can be used for the content uniformity studies.

Hence, the chromatographic method developed for DIC and IHC is said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

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