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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF DICYCLOMINE HYDROCHLORIDE AND DEXKETOPROFEN IN ITS PHARMACUTICAL DOSAGE FROM

DIVYESH S PATEL, SMITA JOSHI, VIDHI KOTADIYA

K. B. Raval College of Pharmacy, Kasturinagar, Shertha, Gandhinagar-38242

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Abstract: A simple stability indicating high Performance Liquid Chromatographic method was developed and validated for simultaneous estimation of Dexketoprofen and Dicyclomine Hydrochloride in bulk and combined pharmaceutical dosage form. Reverse phase High Performance Liquid Chromatography was performed on Young Lin 9101, using a mixture of phosphate buffer (pH-5): acetonitrile (60:40 v/v) as mobile phase with flow rate 1.0 ml/min. Detecting wavelength was selected 212nm. The retention time for Dexketoprofen and Dicyclomine Hydrochloride was found to be 3.550 min. and 5.187 min, respectively. The linearity of Dexketoprofen and Dicyclomine Hydrochloride were found in range of 12.5-37.5 µg/ml and 10-30 µg/ml, respectively. The Dexketoprofen and Dicyclomine Hydrochloride were subjected to stress condition including acidic, alkaline, oxidation, thermal and sunlight degradation. The method was validated as per ICH guidelines.

Keywords: RP-HPLC, Stability indicating method, Dexketoprofen (DEX), Dicyclomine Hydrochloride (DIC).

Corresponding Author: MR. DIVYESH S PATEL

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INTRODUCTION

Chemically, Dexketoprofen (DEX) (Figure 1) {2-[(3-benzoylphenyl propanoic acid)]} is a water-soluble salt of the dextrorotatory enantiomer of the nonsteroidal anti-inflammatory drug (NSAID) ketoprofen. The enantiomer is a relatively new oral NSAID with analgesic, anti-inflammatory and anti-pyretic properties and is one of the most potent in vitro inhibitors of prostaglandin synthesis. Literature survey reveals that DEX can be estimated by spectrophotometry, HPLC and by HPTLC methods individually or in combination with other drugs. Chemically, Dicyclomine Hydrochloride (DIC) (Figure 2) is {2-diethylaminoethyl-bicyclohexyl-1-carboxylatehydrochloride} official in IP. Dicyclomine Hydrochloride is antispasmodic agent. Literature survey reveals that DIC can be estimated by HPTLC and spectrophotometric method. No reports were found for simultaneous estimation of Dexketoprofen and Dicyclomine Hydrochloride by Stability Indicating RP-HPLC method. The objective of work was to develop and validate simple, accurate and reproducible procedure of Dexketoprofen and Dicyclomine Hydrochloride as the bulk drug and in tablet dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.

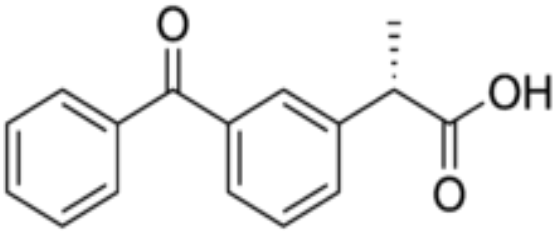

Fig. 1 Dexketoporfen

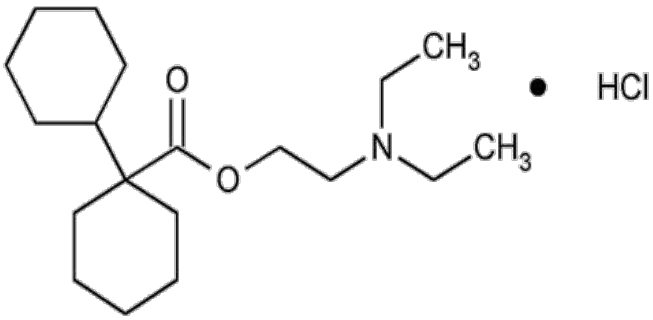

Fig. 2 Dicyclomine Hydrochloride

METHOD AND MATERIALS

Chemicals and Reagents

DEX and DIC were kindly given as a gratis sample by Gitar laboratory, Ahmedabad respectively. The market formulation INFEN SPAS (DEX 25 mg and DIC 20 mg) was procured from local market which is manufactured by Emcure pharmaceuticals Ltd., India. Acetonitrile (HPLC Grade) were obtained from Finar Ltd. and Water (HPLC Grade) and O- phosphoric acid were obtained from Merck specialties pvt, Ltd., Mumbai.

RP-HPLC Instrumentation and Conditions:

The chromatographic separation achieved by using BDS Hypersil C_{18} (25cm x 0.46 cm) column and phosphate buffer (pH-5): acetonitrile (60:40 v/v) as mobile phase at flow rate of 1.0 ml/min with detection wavelength 212 nm.

Preparation of mobile phase

Mobile phase was prepared by mixing 600ml of acetonitrile and 400ml of phosphate buffer (pH-5.0), filtered through 0.45µm Whatmann filter and sonicated for 10 min.

Preparation of Stock and Standard Solution

For Dexketoprofen:

Dexketoprofen stock solution (1000 µg/ml): Accurately weighed 25mg of Dexketoprofen was taken in 10ml volumetric flask and diluted with methanol up to the mark.

Dexketoprofen standard solution (250µg/ml): prepared by transferring 2.5 ml from stock solution, and diluted up to the mark with methanol in 10ml volumetric flask

For Dicyclomine Hydrochloride:

Dicyclomine Hydrochloride standard stock solution (1000 µg/ml): Accurately weighed 20 mg of Dicyclomine Hydrochloride was taken in 10ml volumetric flask and diluted with methanol up to the mark.

Dicyclomine Hydrochloride working standard stock solution (200µg/ml): prepared by transferring 2 ml from stock solution, and diluted up to the mark with methanol in 10ml volumetric flask.

Preparation of standard solution of binary mixtures of Dexketoprofen (25 µg/ml) and Dicyclomine Hydrochloride (20 µg/ml)

Take 1 mL from the Dexketoprofen stock solution and 1ml from Dicyclomine Hydrochloride stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Preparation Sample solution

Sample Stock Solution (Dexketoprofen 250 µg/ml, and Dicyclomine Hydrochloride 200 µg/ml):Take powdered dosage form 30mg (equivalent to 25 mg of Dexketoprofen, and 20 mg of Dicyclomine Hydrochloride) was transferred to a 100 ml volumetric flask containing 60 ml methanol and Shake for 15 min and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

Standard Sample Preparation (Dexketoprofen 25 µg/ml, and Dicyclomine Hydrochloride 20 µg/mL): Take 1 mL from standard stock solution and transferred to 10 ml volumetric flask and made up volume up to the mark with the mobile phase.

Optimization of RP-HPLC method:

The pure drug solution of Dexketoprofen (25µg/ml) and Dicyclomine Hydrochloride (20µg/ml) were injected individually in to HPLC system and allow to run in different mobile phase like Methanol, Water: Methanol, Water: Acetonitrile, Phosphate Buffer: Methanol, Phosphate Buffer: Acetonitrile were tried in order to find the optimum condition for the separation of Dexketoprofen and Dicyclomine Hydrochloride. It was found that mobile phase containing Phosphate Buffer (pH=5): Acetonitrile(60:40 v/v) at a flow rate of 1 ml/min with detecting wavelength 212nm gave satisfactory result with sharp, well defined and resolving peak with minimum tailing as compared to other mobile phases. Under these condition the retention time were typically 3.550 min. for Dexketoprofen and 5.187 min. for Dicyclomine Hydrochloride and optimized chromatographic condition.

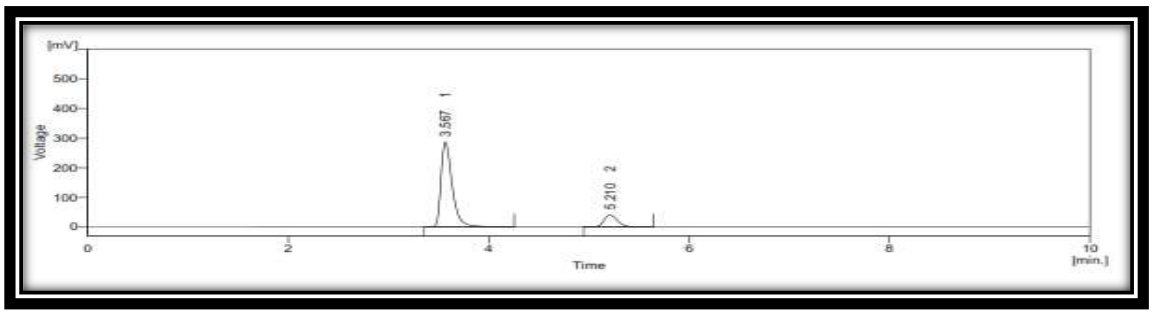

Fig. 3 Chromatogram of standard solution containing 25µg/ml of Dexketoprofen and 20µg/ml of Dicyclomine Hydrochloride using phosphate buffer (pH-5.0): Acetonitrile (60:40 v/v) as mobile phase.

Table: 1 Optimized chromatographic conditions for simultaneous estimation of DEX and DIC.

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	Buffer(pH 5.0) : Acetonitrile (60:40)
column	C18 (25cm x 0.46 cm) Hypersil BDS
Flow rate	1ml/min
Runtime	8 min
Injection volume	20 µL
Detection wavelength	212 nm

HPLC method validation

The developed HPLC method was validated as per ICH guidelines for following parameters Linearity, Limit of Detection, Limit of Quantification, accuracy, Precision, Robustness.

Forced degradation studies

The study was intended to ensure the effective separation of DEX, DIC and its degradation peaks of formulation ingredients at the retention time of DEX and DIC. Forced degradation study was performed to evaluate the stability indicating properties and specificity of the method.

Acid degradation

1 ml standard stock solution of DEX, DIC and mixture of standard solution were taken. 2 ml of 0.1N HCL was added and kept for 3hr at room temperature in 10 ml volumetric flask. After 3 hour the solution was neutralized with 2 ml 0.1 N NaOH to stop the degradation further. Now make up volume with mobile phase [Buffer (pH-5.0)-Acetonitrile 60:40 v/v]. The forced

degradation was performed in the dark to exclude the possible degradation effect of light and control the respective solution was made at each stage of degradation study to eliminate possible changes due to heat and light. The resulting solution (25µg/ml of DEX and 20µg/ml of DIC and combined standard mixture of 25µg/ml of DEX and 20µg/ml of DIC) were injected in HPLC system and the chromatograms were recorded. Chromatograms are shown in figure 4.

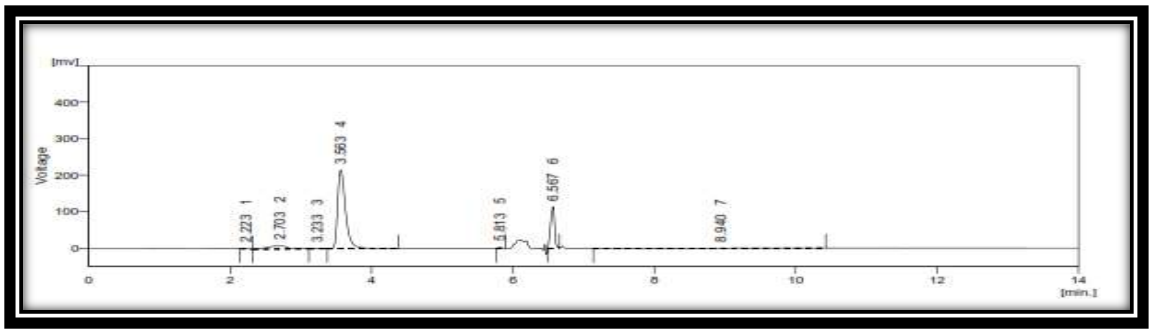

Fig.4 Chromatogram of combined DEX and DIC in acid degradation (0.1 N HCL, 3 hour).

Basic degradation

1 ml standard stock solution of DEX, DIC and mixture of standard solution were taken. 2 ml of 0.1 N NaOH was added and kept for 3hr at room temperature in 10 ml volumetric flask. After 3 hour the solution was neutralized with 2 ml 0.1 N HCL to stop the degradation further. Now make up volume with mobile phase [Buffer (pH-5.0)-Acetonitrile 60:40 v/v]. The forced degradation was performed in the dark to exclude the possible degradation effect of light and control the respective solution was made at each stage of degradation study to eliminate possible changes due to heat and light. The resulting solution (25µg/ml of DEX and 20µg/ml of DIC and combined standard mixture of 25µg/ml of DEX and 20µg/ml of DIC) were injected in HPLC system and the chromatograms were recorded. Chromatograms are shown in figure 5.

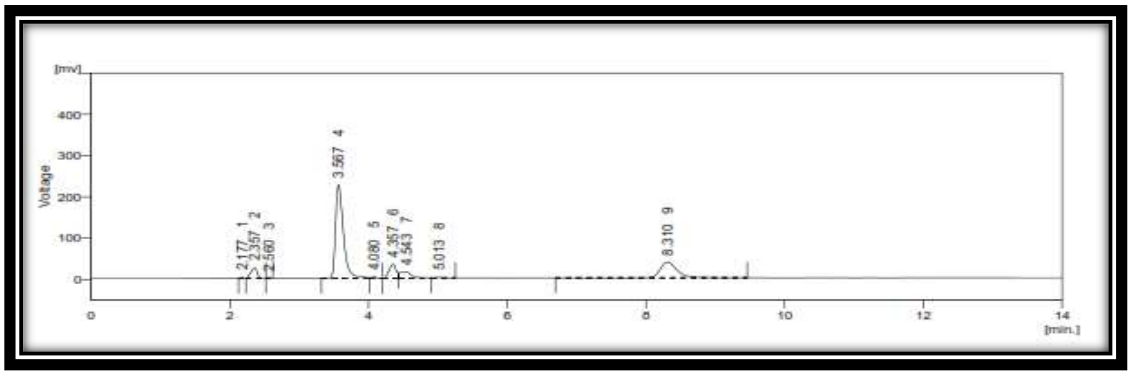

Fig. 5 Chromatogram of combined DEX and DIC in basic degradation (0.1 N NaOH, 3 hour).

Oxidation degradation

1 ml standard stock solution of DEX, DIC and mixture of standard solution were taken in 10 ml volumetric flask, respectively. 2 ml of Hydrogen peroxide (3%) was added in 10 ml volumetric flask, respectively and kept for 3 hour in room temperature. After 3 hour the solution was make up with mobile phase [Buffer (pH-5.0)-Acetonitrile 60:40 v/v]. The forced degradation was performed in the dark to exclude the possible degradation effect of light and control the respective solution was made at each stage of degradation study to eliminate possible changes due to heat and light. The resulting solution (25µg/ml of DEX and 20µg/ml of DIC and combined standard mixture of 25µg/ml of DEX and 20µg/ml of DIC) were injected in HPLC system and the chromatograms were recorded. Chromatograms are shown in figure 6.

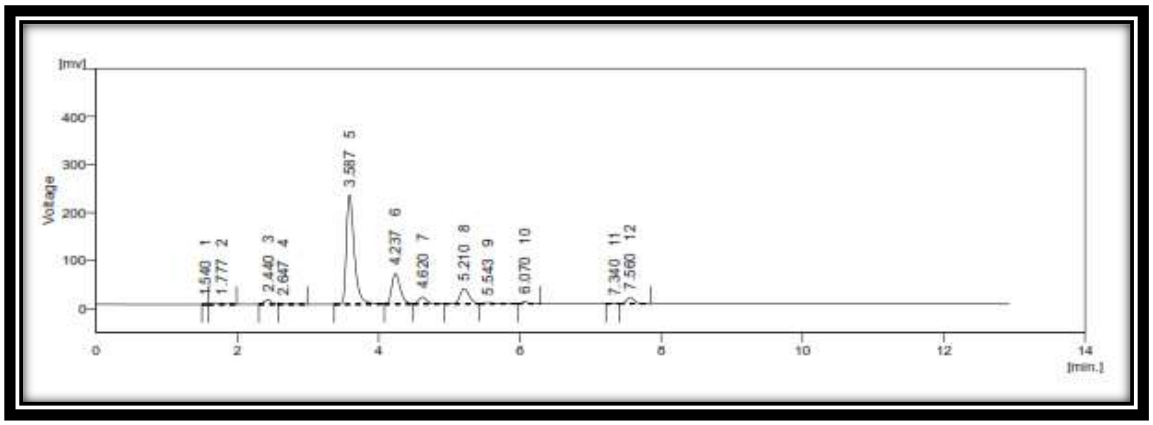

Fig.6 Chromatogram of combined DEX and DIC in oxidative degradation (3% H_2O_2, 3 hour).

Thermal degradation

1 ml standard stock solution of DEX, DIC and mixture sample solution were taken. Solutions were exposed to temperature of 105 ^{0}C for 2 hour in oven. After 2 hour, solution were diluted with mobile phase [Buffer (pH-5.0)-Acetonitrile 60:40 v/v] up to 10 ml. The resulting solution (25µg/ml of DEX and 20µg/ml of DIC and combined standard mixture of 25µg/ml of DEX and 20µg/ml of DIC) were injected in HPLC system and the chromatograms were recorded. Chromatograms are shown in figure 7.

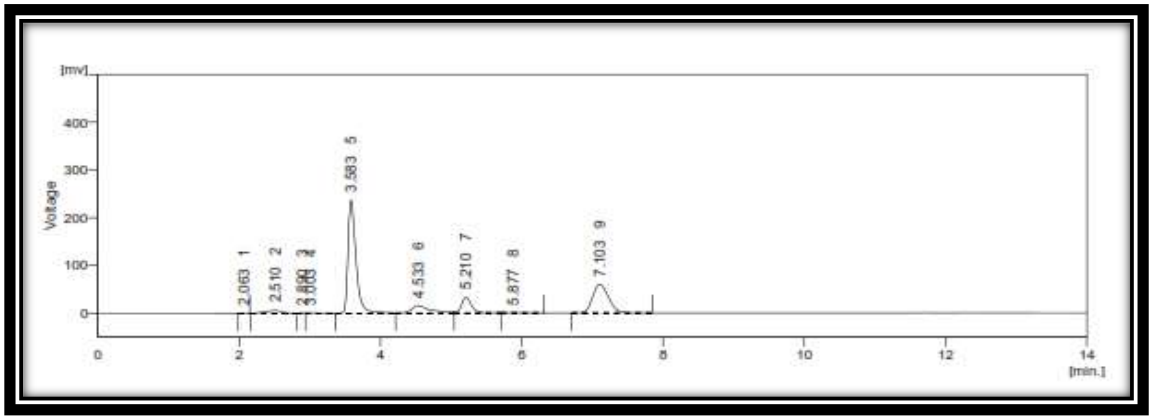

Fig.7 Chromatogram of combined DEX and DIC in thermal degradation (105°C, 2 min.).

Sunlight degradation

1 ml standard stock solution of DEX, DIC and mixture sample solution were taken and exposed to sun light for 6 hour. After 6 hour solution were diluted with mobile phase [Buffer (pH-5.0)-Acetonitrile 60:40 v/v] up to 10 ml. The resulting solution (25µg/ml of DEX and 20µg/ml of DIC and combined standard mixture of 25µg/ml of DEX and 20µg/ml of DIC) were injected in HPLC system and the chromatograms were recorded. Chromatograms are shown in figure 8.

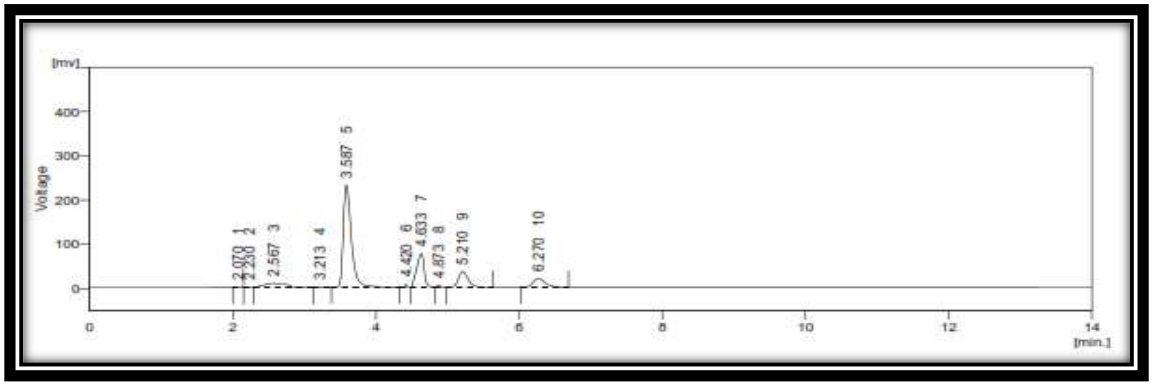

Fig. 8 Chromatogram of combined DEX and DIC in photo degradation (6 hr.).

RESULTS AND DISCUSSION

Linearity:

The linearity of analytical procedure is it ability (within given range) to obtain test result which are directly proportional to concentration of analyte in sample. The drug response was linear (R^2 = 0.9995 for Dexketoprofen and 0.9995 for Dicyclomine Hydrochloride) over the concentration range between 10-40 µg/ml for Dexketoprofen and 10-35 µm/ml for Dicyclomine

Hydrochloride. The linear equation for the calibration plots were y=88.783x-23.816 for Dexketoprofen and y=18.414x-5.2992 for Dicyclomine Hydrochloride.

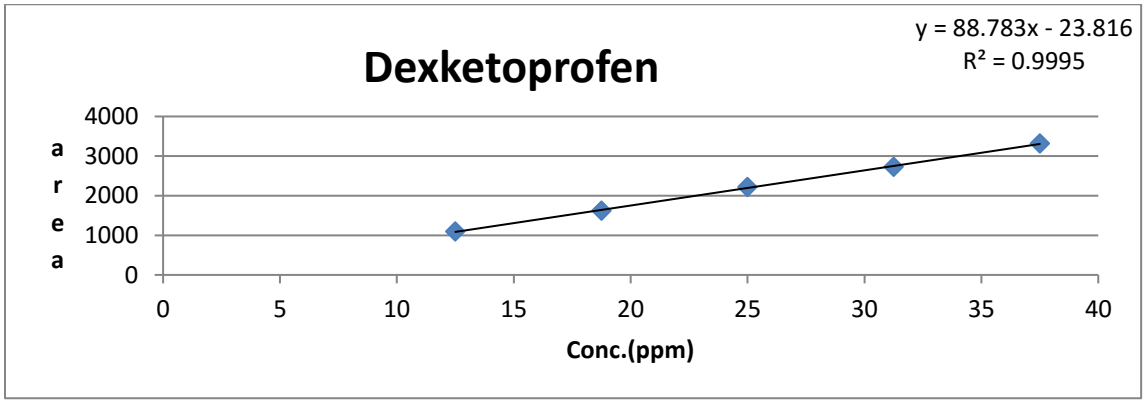

Fig.9 calibration curve of Dexketoprofen

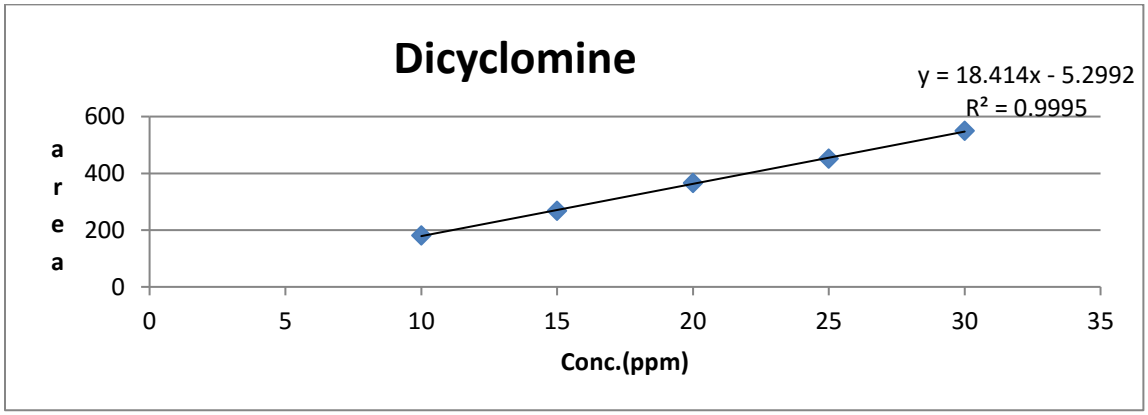

Fig.10 calibration curve of Dicyclomine Hydrochloride

Table: 2 Calibration curve data for Dexketoprofen and Dicyclomine Hydrochloride.

DEX		DIC	
Concentration(µm/ml)	**Mean Peak Area(n=3)**	**Concentration (µg/ml)**	**Mean Peak Area (n=3)**
12.5	1097.524	10	180.811
18.75	1620.277	15	267.38
25	2215.167	20	366.325
31.25	2727.406	25	451.203
37.5	3118.43	30	549.241
Correlation coefficient	0.99973	**Correlation coefficient**	0.99972
Intercept	23.3837	**Intercept**	3.9259
Slope	88.78	**Slope**	18.41

Regression equation	y=88.783x-23.816	**Regression equation**	y=18.414x-5.299
LOD (µg/ml)	0.869	**LOD (µg/ml)**	0.703
LOQ (µg/ml)	2.633	**LOQ (µg/ml)**	2.132

Sensitivity:

The detection limit of an individual analytical procedure is the lowest amount of analyte in sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analyte procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ were calculated by respective equations. The LOD value were found to be 0.869µg/ml and 0.7037µg/ml for Dexketoprofen and Dicyclomine Hydrochloride respectively. The LOQ value were found to be 2.633 µg/ml and 2.1324 µg/ml for Dexketoprofen and Dicyclomine Hydrochloride, respectively.

Precision:

The precision of analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple sampling of same homogenous sample under the reproducibility. Precision may be considered at three level: repeatability, intermediate precision and reproducibility. The results of the repeatability, intra-day precision and inter-day precision experiments are shown respectively as given in (Table: 3) and (Table: 4).the developed method was found to be precise as the RSD values for repeatability of intra-day and inter-day precision study were < 2%.

Table: 3 Repeatability study of Dexketoprofen an. Dicyclomine Hydrochloride.

concentration	DEX (25µg/ml)	DIC (20µg/ml)
Area	2206.292	364.76
	2125.424	365.521
	2215.186	357.26
	2219.618	366.98
	2208.505	365.127
	2212.907	365.888
Mean	2197.988667	364.256
±SD	35.86447396	3.511139
%RSD	1.631695127	0.963921

Table: 4 intra-day and inter-day precision of DEX and DIC.

Drug	Intra-day precision			Inter-day precision		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
DEX	12.5	1082.969667±18.616369	1.719012129	12.5	1082.969667±18.616369	1.719012129
	25	2183.3463±41.801341	1.945538777	25	2183.3463±41.801341	1.945538777
	37.5	3275.647333±54.182858	1.654111488	37.5	3275.647333±54.182858	1.654111488
DIC	10	178.738666±2.2793	1.2752	10	178.738666±2.2793	1.2752
	20	363.29433±2.399	0.66038	20	363.29433±2.399	0.66038
	30	543.004±6.7259	1.23866	30	543.004±6.7259	1.23866

Accuracy:

The accuracy of an analytical procedure express 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. As show in (table: 5) and (table: 6), good recovery of the Dexketoprofen and Dicyclomine Hydrochloride in the range from 99.1 to 100.3 were obtained at various added concentrations.

For Dexketoprofen

12.5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 212 nm. The amount of Dexketoprofen was calculated at each level and % recoveries were computed.

Table: 5 Recovery data for Dexketoprofen.

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	12.5	10	9.87226	98.722	99.266 ± 0.7933
2		12.5	10	9.89072	98.907	
3		12.5	10	10.0169	100.169	
4	100 %	12.5	12.5	12.4070	99.024	99.30± 0.3032
5		12.5	12.5	12.4070	99.256	
6		12.5	12.5	12.4527	99.622	
7	120 %	12.5	15	14.9081	99.387	99.40± 0.259
8		12.5	15	14.8739	99.159	
9		12.5	15	14.9510	99.673	

For Dicyclomine Hydrochloride.

10 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 212 nm. The amount of Dicyclomine Hydrochloride was calculated at each level and % recoveries were computed.

Table: 6 Recovery data for Dicyclomine Hydrochloride.

Sr. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	10	8	7.95315	99.41446	99.885 ±1.126
2		10	8	8.12983	101.6229	
3		10	8	8.0695	100.8695	
4	100 %	10	10	9.97018	99.70188	99.598± 0.649
5		10	10	10.0989	100.9894	
6		10	10	10.0305	100.3055	
7	120 %	10	12	12.0982	100.8187	99.601± 0.490

8		10	12	11.9786	99.82215	
9		5	6	5.9771	99.619	

Robustness:

The standard deviation of the area was calculated for each parameter and the %RSD was found to be less than 2%. Result show low value of %RSD as show in (table: 7) and signify the robustness of the method**.**

Table: 7 Robustness data of Dexketoprofen and Dicyclomine.

Parameter	Normal condition	Change in condition	Drug	Conc.(µg/ml)	Mean area (n=3)±SD	%RSD
Mobile phase ratio (Phosphate Buffer: Acetonitrile)	60:40 V/V	62:42	**DEX**	25	2145.788±38.422	1.790
			DIC	20	355.496±3.927	1.104
		58:38	**DEX**	25	2254.986±35.164	1.559
			DIC	20	373.072±4.783	1.282
Change in flow rate	1 ml/min	1.2 ml/min	**DEX**	25	2150.019±32.490	1.511
			DIC	20	356.0287±3.1113	0.8744
		0.8 ml/min	**DEX**	25	2278.453±40.416	1.773
			DIC	20	378.983±1.9240	0.5076
Change in pH	5.0	5.2	**DEX**	0.92556	0.92556	0.92556
			DIC	25	0.92556	0.92556
		4.8	**DEX**	25	2258.099±37.301	1.65188
			DIC			

				20	375.316±1.9573	0.5215

Forced degradation study

The results of the forced degradation study of DEX and DIC combined dosage form are summarized in table 8 & 9.

Table 8: Results of forced degradation study of DEX in combined dosage form

Stress condition	Time (hr.)	Retention time (min)	Area	% degradants
Acidic (0.1N HCL)	3	3.577	1719.328	21.54
Basic (0.1 N NaOH)	3	3.573	1812.079	17.31
Oxidative (3% H_2O_2)	3	3.587	1779.582	18.79
Thermal (105 °C)	2	3.583	1866.841	14.84
Sunlight exposure	6	3.587	1828.084	16.59

Table 9: Results of forced degradation study of DIC in combined dosage form

Stress condition	Time (hr.)	Retention time (min)	Area	% degradants
Acidic (0.1N HCL)	3	5.210	299.296	21.40
Basic (0.1 N NaOH)	3	5.210	332.223	12.76
Oxidative (3% H_2O_2)	3	5.210	300.953	20.98
Thermal (105 °C)	2	5.210	321.986	15.45
Sunlight exposure	6	5.740	323.930	14.94

CONCLUSION

The proposed stability-indicating HPLC method was validated as per ICH guideline and applied for the determination of Dexketoprofen and Dicyclomine Hydrochloride in combined pharmaceutical dosage forms. It can also be successfully applied to perform long-term and accelerated stability studies of combined dosage formulation of Dexketoprofen and Dicyclomine Hydrochloride.

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