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Simultaneous estimation of dexketoprofen and dicyclomine in bulk and in tablet dosage form using reversed-phase high performance liquid chromatography

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ABSTRACT

The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of Dexketoprofen and Dicyclomine in bulk and in pharmaceutical dosage form. Chromatographic separations was achieved on Waters Younglin system C-18 (5µm, 250×4.6 mm) HPLC column within a short runtime of 10 min. HPLC system having isocratic mode, with mobile phase containing methanol: Potassium Dihydrogen Phosphate buffer 20 mM (pH 3) (70:30% v/v) and flow rate maintained at 1.0 mL/min was used. Effluents were monitored at 234 nm. Retention time of Dexketoprofen and Dicyclomine was found to be 5.0 and 6.9 min respectively. Linearity was studied in the concentration range of 10 µg/mL to 60µg/mL for both drugs respectively, with a correlation coefficient of 0.999 and 0.999 respectively.

Keywords: Reverse Phase-HPLC**,** Simultaneous Estimation**,** Dexketoprofen, Dicyclomine

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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 [1]. Literature survey reveals that DEX can be estimated by spectrophotometry [2], HPLC [3- 4] and by HPTLC [5] methods individually or in combination with other drugs. Chemically, Dicyclomine Hydrochloride (DIC) (Figure 2) is {2-diethylaminoethyl-bicyclohexyl-1-carboxylate hydrochloride} official in IP [6]. Dicyclomine Hydrochloride is antispasmodic agent. Literature survey reveals that DIC can be estimated by HPTLC [5] and spectrophotometric method [7]. No reports were found for simultaneous estimation of Dexketoprofen and Dicyclomine Hydrochloride by HPLC method. The objective of work was to develop and validate simple, accurate and reproducible procedure for the simultaneous HPLC analysis 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 [8].

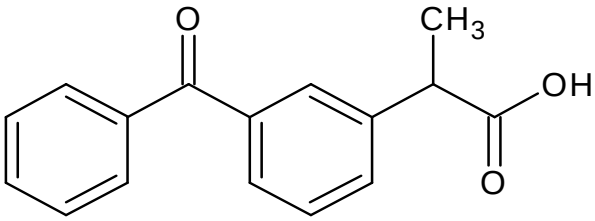

Figure1. Chemical structure of Dexketoprofen

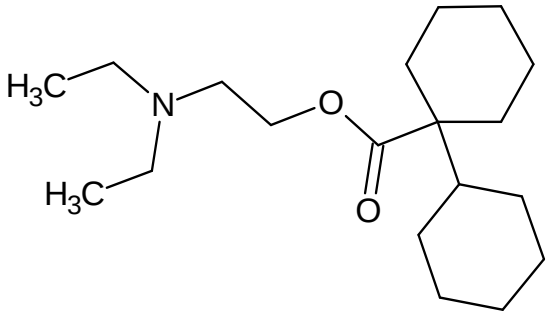

Figure2. Chemical structure of Dicyclomine

MATERIALS AND METHODS

Materials and Reagents

Dexketoprofen (99.26 %) and Dicyclomine (99.89 %) were obtained as gift samples from Emcure Pharmaceutical Ltd. Pune (India). All other reagents and solvents utilized were of analytical (AR) grade and are obtained from Merck Chemicals Ltd, Mumbai (India).

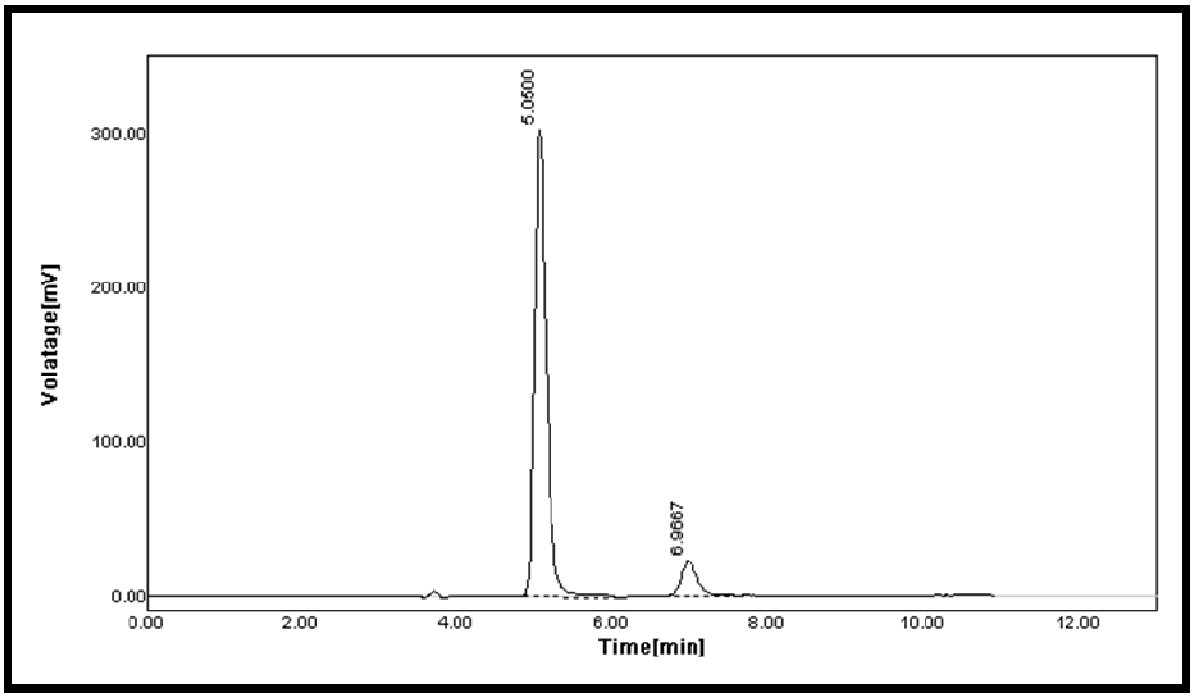

Figure 3: Typical chromatogram of Dexketoprofen and Dicyclomine showing RT 5.0 and 6.9 respectively

Instrumentation and chromatographic condition

Analysis was performed on Waters Younglin HPLC separation module within built UV- detector on analytical column Phenomenex Gemini C 18 (5 µm, 250 mm X 4.6 mm). Chromatographic software Empower 2 was used for data collection and processing. The samples were introduced through a Rheodyne injection valve with 20 µL sample loop. Simultaneous separation and quantification of DEX and DIC were performed by use of an isocratic mobile phase prepared from 70:30 (*v/v*) methanol: Potassium dihydrogen phosphate buffer, pH 3 (adjusted with ortho phosphoric acid) giving well resolved, sharp peak for DEX and DIC with a retention time (RT) 5.0 and 6.9 respectively (Figure 3).The flow rate was maintained at 1.0 mL/min, UV detection was performed at 234 nm and ambient temperature (25^0 C) for column oven was found to be the best for analysis.

Preparation of standard and sample solutions

Independent stock solution of 100 µg/mL of each Dexketoprofen and Dicyclomine were prepared in methanol.

VALIDATION

The method was validated by establishing linearity, accuracy, inter - day and intra - day precision of measurement of sample application. The limit of detection and limit of quantification were also determined.

Linearity

For constructing calibration plots, a series of five dilutions in the concentration range 10-60 µg/mL for both drugs **Table 1.**

Table 1. Linearity of DEX and DIC for proposed method (n=5)

Parameters	DEX	DIC
Linear range (µg mL^{-1})	10 – 60	10 – 60
Slope	75.52	7.56
Intercept	104	50.55
Correlation Coefficient (r^2)	0.999	0.999

(DEX: Dexketoprofen and DIC: Dicyclomine)

Precision

In order to validate and prove the applicability of the method, a laboratory mixture of DEX and DIC was prepared from the stock solutions in the ratio corresponding to amounts in the dosage form. For quantitative estimation of the mixture, three series (20, 30, 40 µg/mL for both drugs) were prepared, with three solutions for each concentration **Table 2.**

Table 2 Results of Precision Study

Drugs	Conc. [µg/mL]	Intraday Amount found [µg]		Interday Amount found [µg]	
		Mean ± S.D.	% R.S.D*	Mean ± S.D.	% R.S.D*
DEX	20	19.91 ± 15.13	1.05	20.13 ± 18.73	1.28
	30	29.92 ± 23.81	1.07	30.10 ± 29.20	1.30
	40	40.04 ± 31.71	1.05	40.28 ± 30.64	1.01
DIC	20	19.99 ± 1.51	1.51	20.26 ± 1.15	1.12
	30	30.05 ± 2.08	1.17	30.49 ± 1.0	0.55
	40	40.19 ± 4.04	1.59	40.54 ± 3.0	1.17

**mean of three determinations*

Recovery

The recovery experiments were performed by adding known amounts to tablet. The recovery was performed at three levels, 80, 100 and 120% of DEX and DIC standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated by using formula **Table 3.**

Table 3 Results of Recovery studies

Drugs	Initial Amount [µg/mL]	Amount added [µg/mL]	Amount recovered* ± S.D. [µg/mL]	% Recovered	% R.S.D.
DEX	25	20	40.10± 29.14	100.25	0.96
	25	25	44.57± 49.89	101.30	1.48
	25	30	56.01± 33.40	101.83	0.78
DIC	20	16	36.22± 1.52	100.61	0.68
	20	20	40.36± 1.52	100.91	0.59
	20	24	44.46± 3.05	101.06	1.06

**mean of three determinations*

Tablet Assay

Twenty tablets (Infen-Spas) (each contained 25 mg DEX and 20 mg DIC) were accurately weighed and finely powdered. A quantity of the powder containing weight equivalent to 25 mg DEX and 20 mg DIC was transferred to a 25 mL volumetric flask and methanol (15 mL) was added followed by ultrasonication for 10 min. The solution was then diluted to volume with the same solvent and filtered. From that six solutions were prepared affording final concentrations of 25 µg/mL for DEX and 20 µg/mL for DIC and were used for further analysis **Table 4.**

Table 4 Results of Tablet Assay

Drugs	Label claim [mg/Tab]	% label claim*	% R.S.D.
DEX	25.0	100.79	0.62
DIC	20.0	100.98	1.44

**mean of six determinations*

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × N/B and LOQ = 10 × N/B where 'N' is the standard deviation of the peak areas of the drug (n=3), taken as the measure of the noise, and 'B' is the slope of the corresponding calibration plot. The signal to noise ratio was determined. The LOD was regarded as the amount for which the signal to noise ratio was 3:1 and LOQ regarded as the amount for which the signal to noise ratio was 10:1 Results are shown in **Table 5.**

Table 5 LOD and LOQ of the method

Drugs	LOD	LOQ
DEX	1.02	3.06
DIC	1.07	3.21

Robustness

By introducing small but deliberate changes in the mobile phase composition (± 2 ml, detection and flow rate (± 0.9 - 1.1mL/min) robustness of the described method was studied **Table 6.**

Table 6 Robustness of the method

Conc. [µg/ml]	Retention time (R_T)		Tailing factor (Tf)	
	DEX	DIC	DEX	DIC
A: Flow Rate (ml/min)				
0.90	5.4	7.1	0.86	1.13
1.00	5.0	6.9	1.22	1.09
1.10	4.7	6.7	1.26	1.14
Mean	5.03	6.9	1.22	1.1
B: Percentage methanol in mobile phase (v/v) (± 2%)	4.8	6.7	1.57	1.50
	5.0	6.9	1.39	1.33
	5.4	7.1	1.51	1.54
Mean	5.06	6.9	1.49	1.45

RESULTS AND DISCUSSION

A RP-HPLC method was optimized with a view to develop an accurate and reproducible method so as to resolve drugs. Isocratic elution is simple, requires only one pump and flat baseline separation for easy and reproducible results. Optimization of method was done by altering almost all chromatographic conditions and the effect on

retention and peak shape were monitored for DEX and DIC. The final chromatographic conditions are set for stationary phase giving satisfactory resolution and run time with reversed phase Waters Younglin system C_{18} (5µm, 250×4.6 mm) column. A series of mobile phases varying the pH and volume fractions of acetonitrile and methanol are also tested and the best results were obtained by use of mobile phase consisting of methanol: Potassium dihydrogen phosphate buffer, pH 3 (adjusted with ortho phosphoric acid) in 70:30 giving well resolved, sharp peak for DEX and DIC with a retention time (RT) of 5.0 and 6.9 min respectively. The flow rate of 1.0 mL/min at 234 nm and 25^0 C temperature for column oven was found to be the best for analysis. % RSD was less than 2 in intraday, interday precision and in each parameter of robustness. So the proposed method is more precise and robust. The results of specificity studies indicated no interference from excipients, impurities.

Table 7 Final Chromatographic Conditions

Chromatographic Mode	Chromatographic Condition
Standard solution (µg/mL)	100 µg/mL of DEX and DIC in methanol
HPLC System	Younglin 1100 Series HPLC system
Pump	Gradient pump
Detector	UV Detector
Degasser	G1322A
Data processor	Ezechrome Elite Chromatographic data system
Weighing Balance	Shimandzu AUX 120
Digital pH Meter	Systronics µ pH System 362
Ultrasonicator	ENERTECH Electronics Pvt. Ltd.
Stationary phase	Phenomenex Gemini C18 (5 µm, 250 mm X 4.6 mm i.d.)
Mobile phase	methanol: Potassium dihydrogen phosphate buffer, pH 3 (70:30% *v/v*)
Detection wavelength	234 nm
Flow rate	1 mL/min
Sample size	20 µL

Table 8 System Suitability parameters

System suitability parameters	Proposed Method (OM)	Proposed Method (MS)
Retention Time (T_R)	5.0	6.9
Theoretical Plate (N)	5057.7	5651.3
Tailing Factor (T)	1.25	1.20

CONCLUSION

The modalities adopted in experiment were successfully validated as per ICH guidelines analytical procedures laid down in routine analysis. The proposed method was validated by preliminary analysis of standard sample and by recovery studies. The percentage of average recoveries for DEX and DIC was obtained 101.12 and 100.86 respectively. A validated RP-HPLC method has been developed for the determination of DEX and DIC in bulk and in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its number of theoretical plates was also calculated. It was observed that all the values are within the limits. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Dexketoprofen and Dicyclomine in tablet formulation.

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