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Validated HPTLC Method for Simultaneous Estimation of Diclofenac Potassium and Dicyclomine Hydrochloride in Tablet Formulation

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ABSTRACT

This article describes HPTLC method for the simultaneous determination of diclofenac potassium and dicyclomine HCl from tablet dosage form. The method employs a precoated silica gel 60 F_{254} (0.2 mm thickness) on aluminium sheets and a mobile phase toluene: acetone: methanol: conc. ammonia in the ratio of 5.0: 2.0: 1.0: 0.02 (v/v/v/v), having chamber saturation for 30 min at room temperature. The mobile phase was run upto 8 cm. The R_f values were found to be 0.51 ± 0.01 and 0.74 ± 0.01 for diclofenac potassium and dicyclomine HCl respectively. The plate was scanned and quantified at 215 nm for determination of diclofenac potassium then, the dipping agent sprayed on plate for determination of dicyclomine hydrochloride and plate scan at 523 nm. The linear detector response was observed between 1800 ng/spot to 9000 ng/spot and 1300 ng/spot to 6500 ng/spot for diclofenac potassium and dicylomine HCl respectively. The method so developed was validated for its accuracy and precision. The LOD and LOQ were found to be 40 ng/spot and 100 ng/spot for diclofenac potassium and 250 ng/spot and 800 ng/spot for dicylomine HCl respectively. The recovery was carried out by standard addition method. The Average recovery was found to be 99.92% ± 0.546 and 99.1± 0.847 for diclofenac potassium and dicyclomine HCl respectively. Statistical analysis showed that the method was repeatable and selective for the quantitation of the drug in injection dosage form and for routine quality control of raw materials of the drug.

Keywords: diclofenac potassium (DCH), dicyclomine hydrochloride (DCL), HPTLC, (ICH), NSAID, pharmaceutical formulation.

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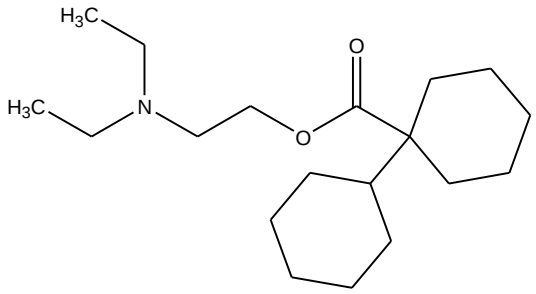
INTRODUCTION

A diclofenac potassium (DCL) is used as an analgesic, antipyretic, and antacid for the treatment of fever, pain, and acidity. A dicyclomine hydrchloride (DCH) is use as an antispasmodic agent. Diclofenac, as the potassium salt, is a benzeneacetic acid derivative, designated chemically as 2-[(2, 6-dichlorophenyl) amino] benzene acetic acid, monopotassium salt. The mechanism of action of DCL, like that of other nonsteroidal anti-inflammatory drugs, is not completely understood but may be related to prostaglandin synthesize inhibition. Dicyclomine hydrochloride **(**DCH) is chemically2-diethylaminoethyl- bicyclohexyl-1-carboxylate hydrochloride, dicyclomine Hydrochloride is antispasmodic agent [1-3].



HCL

Figure 1 - Chemical structure of dicyclomine HCl.

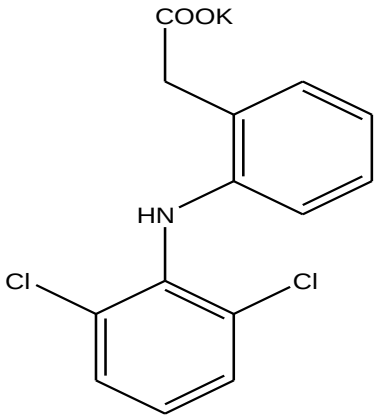


Figure 2 - Chemical structure of diclofenac potassium.

Literature review reveals that few spectroscopic method, HPLC and HPTLC have been reported for estimation of diclofenac Potassium individually and in combination with other drugs and capillary gas—liquid chromatography is reported on dicyclomine Hydrochloride. No reports were found for simultaneous estimation of diclofenac Potassium and dicyclomine Hydrochloride by HPTLC method. The objective of work was to develop and validate simple, accurate, and reproducible procedure for the simultaneous HPTLC analysis of diclofenac potassium 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. A sensitive, simple, selective, precise, and accurate HPTLC method of analysis for diclofenac potassium and dicyclomine hydrochloride both as a bulk drug and in tablet formulation was developed and validated. The method used HPTLC aluminium plates precoated with silica gel $60F_{254}$ as the stationary phase, and the mobile phase consisted of toluene–acetone–methanol–conc. ammonium (5: 2: 1: 0.02, v/v/v/v). Densitometric evaluation of the separated zones was performed at 215 nm for diclofenac potassium then dipping agent sprayed on plate for determination of dicyclomine Hydrochloride and plate again scan at 523nm. The retention factor for diclofenac Potassium and dicyclomine hydrochloride were found to be 0.51 ± 0.02 and 0.72 ± 0.03. The linear regression analysis data for the calibration plots showed a good linear 5 relationship over the concentration range of 1800-9000 ng/spot for diclofenac potassium, and 1300-6500 ng/spot for dicyclomine Hydrochloride. The method was validated for precision, robustness, and recovery according to International Conference on Harmonization guidelines [5-12]. No chromatographic interference from the tablet excipients was found.

Statistical analysis showed that the method was repeatable and selective for the simultaneous quantitation of the two drugs in tablet formulation and for routine quality control of raw materials of the drugs [13-21].

MATERIALS AND METHODS

Analytically pure samples of diclofenac potassium (Batch no. DFK/10090046) and dicyclomine hydrochloride (Batch no. DC/1008028) working standards were obtained as generous gifts from Zim laboratories and Palam Pharmaceutical India, respectively. Fixed-dose combination tablets (Cataspa) containing 50 mg diclofenac Potassium and 20 mg dicyclomine Hydrochloride were procured from USV limited, India. All chemicals and reagents were of analytical-grade and were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

The HPTLC system consisted of a Camag Linomat 5 semi-automatic spotting device (Camag, Muttenz, Switzerland), a Camag twin-trough chamber (10 cm × 10 cm), camag Wincats software 1.4.4.6337 and a 100 µl Hamilton syringe. Sample application was done on precoated silica gel 60 F_{254} TLC plates (10 cm × 10 cm). TLC plates were pre-washed with methanol and activated at 80°C for 5 min prior to the sample application. Densitometric analysis was carried out utilizing Camag TLC scanner 3.

Preparation of Standard Stock Solutions

Dicyclomine hydrochloride [DCL] (20 mg) and diclofenac potassium [DCH] (50 mg) were weighed separately, transferred to separate 10 ml volumetric flasks and dissolved in 10 ml of methanol. Stock solutions obtained of concentration 2000 ng/spot for dicyclomine

Hydrochloride and 5000 ng/spot for diclofenac Potassium. The Solution was prepared as per formulation in pharmaceutical formulation. The stock solution was stored at 2–8°C protected from light. For analysis of the pharmaceutical formulation, twenty tablets were weighed and their average weight was calculated.

HPTLC method and chromatographic Conditions

The HPTLC procedure was optimized to develop a simultaneous assay method for DCL and DCH. The mixed standard stock solution (20 mg/ml DCL, and 50 mg/ml DCH) was spotted onto HPTLC plate and developed in different mobile phases. Development of a Simultaneous assay method for DCL and DCH was very critical because the sensitivity of DCH is very less to UV detection while scanning, it require derivatization by using dipping agent. Initially, different mobile phase were tried such as a toluene—methanol (6 + 2 v/v/v) was tried, but in this system DCL moved and but DCH did not moved. Hence, in order to move DCH, n propanol- ethyl acetate- n-hexane- water- glacial acetic acid(5+2+3+1+0.1 v/v/v), But effective separation was not observed. Finally, a mobile phase consisting of toluene–acetone–methanol–conc. ammonia (5 + 2 + 1 + 0.02, v/v/v/v) was found to be optimum. After chamber saturation, the plates were developed to a distance of 80 mm and then dried in hot air. Densitometric analysis was carried out using a camag TLC Scanner 3 (camag) in the absorbance mode at 215 nm for DCL and 523nm for DCH. The slit dimension was kept at 5.0 mm × 0.45 mm and a scanning speed of 20 mm/s was employed. The chromatograms were integrated using wincats evaluation software (Version 1.1.3.0).

Validation of the Method

Validation of the optimized HPTLC method was carried out with respect to the following parameters:

Linearity

The mixed different standard stock solution was prepared to obtain concentrations of 1.8 mg/ml, 3.6 mg/ml, 5.4 mg/ml, 7.2 mg/ml, 9.0 mg/ml for DCL and 1.3 mg/ml, 2.6 mg/ml, 3.9 mg/ml, 5.2 mg/ml, 6.5 mg/ml for DCH From mixed standard stock solution, 1µL of each different concentration were spotted on the HPTLC plate to obtain final concentrations of 1800 ng/spot, 3600 ng/spot, 5400 ng/spot, 7200 ng/spot, 9000 ng/spot for DCL and 1300 ng/spot, 2600 ng/spot, 3900 ng/spot , 5200 ng/spot, 6500 ng/spot for DCH. Each different concentration was applied six times on the HPTLC plate. The plate was then developed using the optimum mobile phase, and the peak areas were plotted against the corresponding concentrations to obtain the calibration plots.

Limit of Detection and Quantification

The limits of detection (LOD) and quantification (LOQ) were calculated from the slope (s) of the calibration plot and the standard deviation of the response (SD).

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (1800 ng/spot, 5400 ng/spot and 9000 ng/spot for DCL; 1300 ng/spot, 3900 ng/spot and6500 ng/spot for DCH three times on the same day. The intermediate precision of the method was checked by repeating the studies on 3 different days.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for DCL and DCH in sample was confirmed by comparing the R_f and spectra of the spots with that of standards. The peak purity of DCL and DCH were assessed by comparing the spectra at three different levels, i.e. peak start, peak apex and peak end positions of the spot.

Robustness

For determining robustness introduction of small changes in the mobile phase composition (±0.1 ml for each component) was done and the effects on the results were examined.

Mobile phases having different compositions, e.g. toluene–acetone–methanol–conc. ammonia (5.1: 2: 2: 0.01, v/v/v/v), (4.9: 2: 2: 0.01, v/v/v/v), (5: 2.1: 2: 0.01, v/v/v/v), and (5: 1.9: 2: 0.01, v/v/v/v), were used to develop chromatograms. The amount of mobile phase was varied over the range of ±5%. The plates were prewashed with methanol and activated at 110°C for 2, 5, and 7 min prior to chromatography. The time from spotting to chromatography and from chromatography to scanning was varied by ±10min.

The robustness of the method was determined at three different concentration levels: 1800, 5400 and 9000 ng/spot for DCL; and 1300, 3900, and 6500 ng/spot for DCH.

Accuracy

Accuracy was measured by applying the method to preanalyzed drug sample (DCL, and DCH combination tablet) to which known amounts of DCL and DCH standard powder corresponding to 80, 100, and120% of the label claim had been added (standard addition method). The drug sample and spike were mixed, and the powder was extracted and analyzed by the optimized method.

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Analysis of a Marketed Formulation

To determine the content of DCL and DCH in a pharmaceutical tablet (brand name: CATASPA); label claim: 50 mg DCL, and 20 mg DCH tablet), 20 tablets were weighed, their mean weight was determined, and they were finely powdered. The weight of the tablet triturate equivalent to 50 mg DCL, and 20 mg DCH was transferred into a 25 ml volumetric flask containing 20 ml methanol. The solution was sonicated for 45 min, and diluted to 25 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min .Then above filtered solution was produced a concentration of 2000, and 800 µg/ml for DCL and DCH, respectively, and 1 ml of this solution (4000 and 1600 ng/spot for DCL, and DCH, respectively) was applied to an HPTLC plate that was developed in the optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

RESULTS AND DISCUSSION

The results of validation studies on the simultaneous HPTLC determination method developed for DCL and DCH with toluene–acetone–methanol–conc. Ammonia (5 + 2 + 1 + 0.02, v/v/v/v) as the mobile phase are given below.

Linearity

The drug response was linear over the concentration range between 1800–9000 ng/spot for DCL and 1300–6500 ng/spot for DCH in table 1.

Table 1. Linear regression data for calibration plots

Parameter	Diclofenac potassium	Dicyclomine Hydrochloride
Linearity range ng/spot	1800-9000 ng/spot	1300-6500 ng/spot
r^2	0.999	0.998
Slope	1.967	0.1246
Intercept	406.1	453.37

[a] n = 6, r^2 -coefficient of correlation.

Precision

The results of the repeatability and intermediate precision experiments are shown in table. The developed method was found to be precise, with RSD values for repeatability and intermediate precision studies below 2% as recommended by ICH guidelines.

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Table 2. Precision studies

Concentration, ng/spot	Repeatability Measured concentration SD	RSD %	Intermediate Precision Measured concentration SD	RSD %
		DCL		
1800	1.80±0.56	1.3	36.25±4.6	0.5
5400	360.93±2.5	1.72	31.40±5.7	0.14
9000	178.77±1.2	0.6	4.64±0.58	0.02
		DCH		
1300	2.55±0.4	0.27	9.54±0.12	0.99
3900	30.13±1.5	0.95	7.35±0.71	0.23
6500	31.95±2.3	0.81	3.55±0.5	0.09

[a] n = 6

Table 3. Robustness testing:-

Parameter	SD of peak area of DCL	%RSD	SD of peak area for DCH	%RSD
Mobile phase composition(±0.1)	31.72	0.44	5.33	0.58
Amount of mobile phase (±5 %)	98.23	1.35	8.21	0.88
Temperature	93.45	1.13	11.57	0.92
Relative Humidity	82.12	0.99	13.41	0.96
Plate pre-treatment	89.10	1.03	9.45	0.98
Time from spotting to chromatography(±10 min)	112.45	1.23	12.45	1.05
Time from chromatography to scanning (±10min)	56.45	0.86	15.64	1.41

[a] n = 6

Table 4.Recovery studies

Drug	Label claim mg/tablet	Amount added %	Total amount mg	Amount recovered, mg RSD %	Recovery %
DCL	50	80	90	89.87±0.52	99.86
		100	100	99.36±0.23	99.36
		120	110	109.75±0.3	99.78
DCH	20	80	36	35.9±0.3	99.73
		100	40	39.6±0.86	99.23
		120	44	43.59±0.12	99.08

[a] n = 6

Table: 5 Applicability of the HPTLC method for the analysis of the pharmaceutical formulations

Sample	Label claim (mg)	Drug Content (%)	% R.S.D.
DCL	50mg	99.52	1.18
DCH	20mg	99.92	1.42

LOD and LOQ

S/N of 3:1 and 10:1 were obtained for the LOD and LOQ, respectively. The LOD and LOQ were found to be 40 and100 ng/spot for DCL 250 and 800 ng/spot for DCH respectively.

Robustness

The RSD of peak areas was calculated for each parameter and was found to be less than 2%. The low values of the RSD, as shown in table indicated robustness of the method.

Specificity

The peak purity of DCL and DCH was assessed by comparing their respective spectra at the peak start (S), peak apex (M), and peak end (E) positions of the spot. It was found that r (S, M) = 0.999 and r (M, E) = 0.999. A good correlation (r = 0.999) was obtained between the standard and sample spectra of DCL and DCH.

Recovery studies

As shown from the data in table good recoveries of the DCL and DCH in the range from 98 to 99%were obtained for the various added concentrations.

Analysis of a formulation

Experimental results of the amount of DCL and DCH in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets. Fixed dose combination tablets were analyzed using the proposed procedures [Table 5].

Table: 6 The data of summary of validation parameters are listed

Parameter	DCL	DCH
Linearity range (ng/spot)	1800- 9000	1300- 6500
Correlation coefficient	0.999 ± 0.05	0.9987 ± 0.03
Limit of detection (ng/spot)	40.0	250
Limit of quantitation (ng/spot)	100	800
Recovery (n = 6) (80 %) (100%) (120%)	 99.86 99.36 99.78	 99.73 99.23 99.08
Precision (% R.S.D.) repeatability Inter day	 1.3 1.42	 0.95 0.84
Robustness	Robust	Robust
Specificity	0.9992	0.9987

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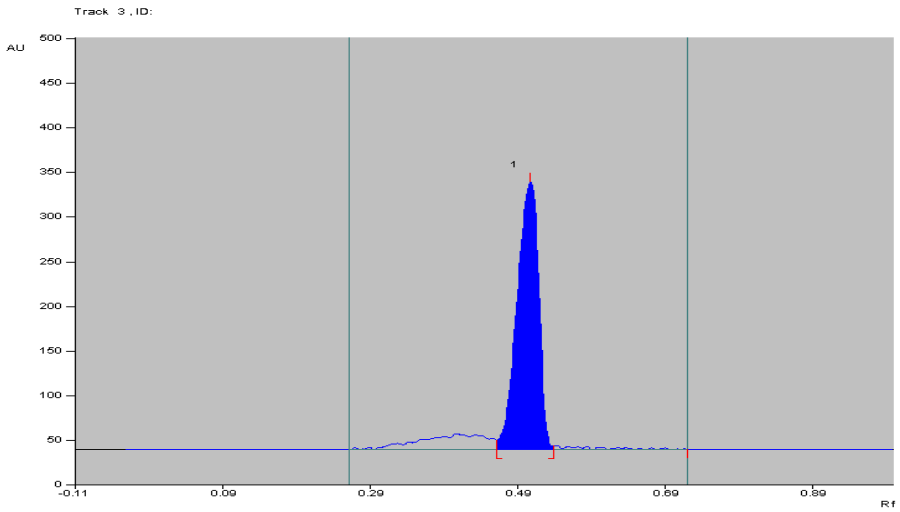

Figure 3-Densitogram of diclofenac potassium (R_f = 0.51) of formulation (CATASAPA) showing no interference of excipients in analysis when scan at 215nm.

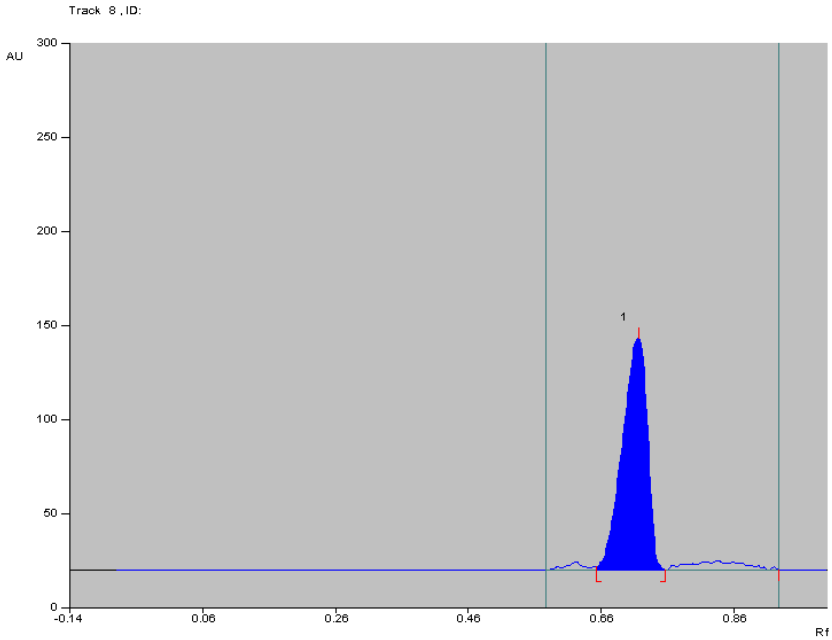

Figure 4- Densitogram of dicyclomine hydrochloride (R_f = 0.74) of formulation (CATASAPA) showing no interference of excipients in analysis when scan at 523nm after spreading of dipping agent show blue spot against pink background.

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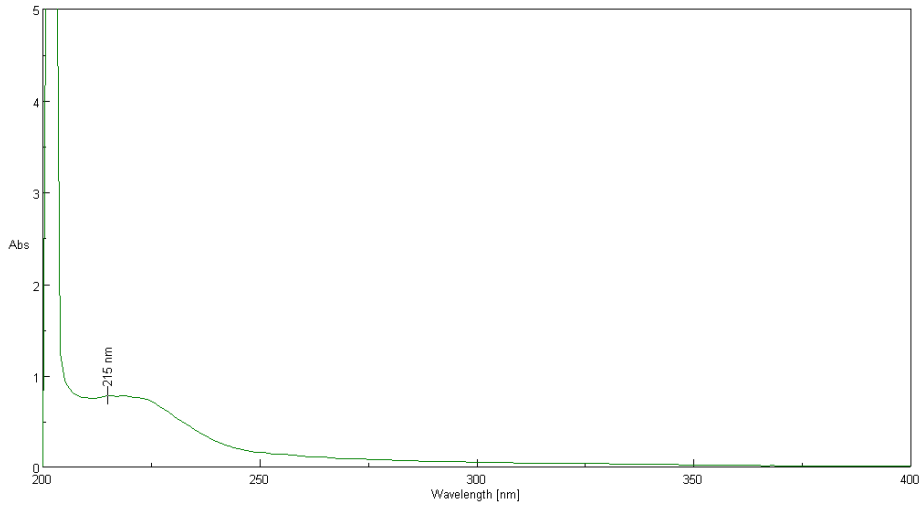

Figure 5 - In situ spectra of diclofenac potassium

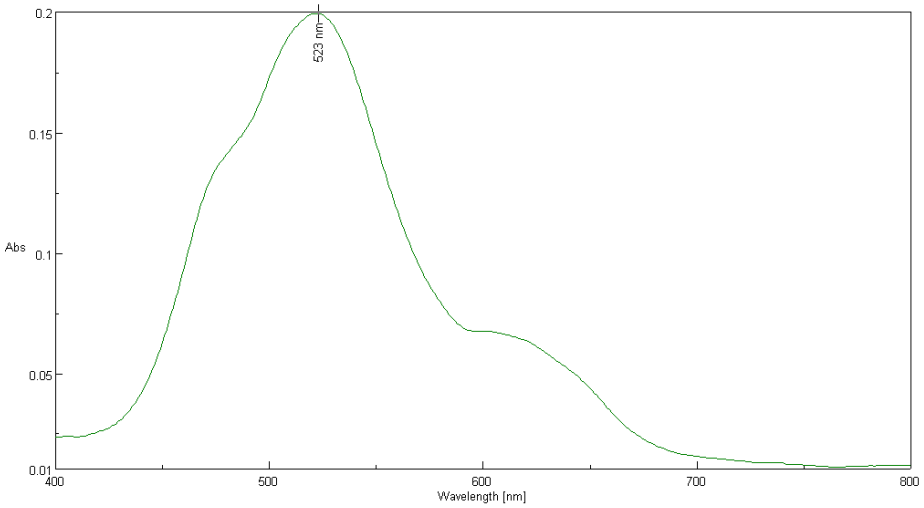

Figure 6 - In situ Spectra of dicyclomine hydrochloride after derivatization.

CONCLUSIONS

Introducing HPTLC into pharmaceutical analysis represents a major step in terms of quality assurance. Today, HPTLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput, and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC; thus reducing the analysis time and cost per analysis. The developed HPTLC technique is precise, specific, and accurate. Statistical analysis proves that the method is suitable for the analysis of DCL and DCH as a bulk drug and in

pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of DCL and DCH and also for its estimation in plasma and other biological fluids.

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