MULTISCIA
JOURNALS PUBLISHERS

FRONTIERS IN PHARMACEUTICAL ANALYSIS

ISSN: (3065- 1352)

https://multisciajournals.com/journals/index.php/fpa

editor.fpa1@gmail.com

Frontiers in Pharmaceutical Analysis
Volume 1 Issue 3 2025

Creation and Verification of a Stability Indicating Assay Method for Identifying a Model Drug in Pharmaceutical Dosage Form and Bulk Using HPLC

B Krishnamurti, KS Rani
Department of **Pharmaceutical Analysis**

Article Info

Received: 30-05-2025 Revised:06-07-2025 Accepted:18-07-2025 Published:28-07-2025

ABSTRACT

Developing an RP-HPLC method for determining a model drug in pharmaceutical dose form and bulk was the goal of the current effort. A Hypersil BDS C18 column (250 x 4.60 x 5µm) with a mobile phase consisting of 7.5 Phosphate buffer: Acetonitrile in a ratio of 300:700 was used to optimize the Agilent Chromatographic system. Using UV detection at 215 nm, the flow rate was set to 2.0 ml/min. The retention durations for DCL elution were 9.30±0.3 min. For DCL, Beer's Lambert's Law was followed within the concentration ranges of 27.97–51.94 mcg/ml. The method's applicability for quantitative drug analysis in a liquid dosage form is confirmed by the high recovery and low coefficients of variation. The method's sensitivity and significance for analyzing DCL in pharmaceutical dosage form and pure form without excipient interference are demonstrated by statistical analysis. According to ICH criteria, the approach was validated.

Keywords: The stability suggesting study, HPLC, and dicyclomine hydrochloride.

INTRODUCTION

2- (diethylamino) ethylbicyclohexyl-1-carboxylate hydrochloride is the chemical name for dicyclomine hydrochloride (DCL) [1]. Compared to M2 and M4 receptors, it binds to M1 and M3 receptors more firmly. It has almost twice the musculotropic activity of papaverine and one-eighth the neurotropic activity of atropine. It is used to treat a variety of smooth muscle spasms, especially those related to the gastrointestinal tract, because of its spasmolytic properties. Additionally, it helps with biliary dysfunction, pylorospam, and dysmenorrhea [2]. It is used to treat irritable bowel syndrome, a particular kind of digestive issue. It lessens intestinal and stomach cramping sensations. This drug relaxes the muscles in the stomach and intestines and slows the normal movements of the gut.

MATERIALS AND METHODS

Chemicals and reagents

Alkem Research Centre Taloja (Mumbai), India, provided the reference standard of DCL, which was used just as is without any additional purification. A Milli-QRO water purifying system produced Agilent HPLC grade. Every chemical and reagent utilized was of analytical reagent grade.

Chromatography

An HPLC system with a waters pump, a UV-visible detector, and a Hypersil BDS C18 column (250 x 4.60 x 5 µm) for separation was employed for the analysis. Mobile Phase 7.5 Phosphate Buffer: 0.45 □ filter paper filtered with acetonitrile (300:700). After that, a sonicator was used to degas it. For analysis, a flow rate of 2.0 milliliters per minute was used. Following appropriate dilutions, the sample was scanned in the UV region, and the maximum absorbance was discovered at λ max 215 nm.

Standard solution Preparation

In a 100 ml volumetric flask, 40 mg of dicyclomine hydrochloride were dissolved. Pour in 40 ml of diluent 1. Sonicate to dissolve (8.5 ml hydrochloric acid in 1000 ml methanol), then add diluent 1 to make up the volume. Use dialuent 2 to further dialute 5 ml of this solution into a 50 ml volumetric flask. (80:20 methanol to water)

Preparation of calibration curve Using a standard solution, the linearity of dicyclomine hydrochloride was assessed in the range of 27.97 mcg/ml to 51.94 mcg/ml, or 70%–130% of the test concentration. System Suitability test

System compatibility tests are an essential component of the LC Method in the process of optimizing the circumstances of the suggested Method, according to the Indian Pharmacopoeia 2007. In order to demonstrate the system suitability, the chromatographic parameters for DCL were assessed after the system suitability test solution was injected.

Table 1 System Suitability Parameters

System Suitability Parameters	DCL*
Retention Time	9.30 + 0.3 min
Area Under Curve	35922
Theoretical Plates	5176
Tailing Factor	1.80

(* Results are average of 3 readings)

Validation

The linearity, selectivity, accuracy, precision, and robustness of the approach were all validated in accordance with ICH recommendations. Using a drug sample and a combination of standards, selectivity was examined to maximize separation and detection. By using the approach to analyze a standard drug solution in the chosen concentration range for both drugs, linearity of the method was achieved. A recovery study using standard medications was conducted to assess the correctness of the suggested approach. Three varying concentrations of standard chemicals were added to the samples. The samples underwent duplicate analysis by previously created ideal circumstances. The spike recoveries were computed using the average contents of the target compounds that were collected. Experiments on repeatability, interday reproducibility, and intraday reproducibility were used to assess precision. Six injections of a normal medication solution were administered. Each constituent's mean quantity and standard deviation value were computed.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

After using a reverse phase C18 column with a different mobile phase, the best chromatographic conditions were achieved. Acetonitrile was chosen as the mobile phase instead of potassium dihydrogen phosphate (7.5 Ph buffer solution: Acetonitrile (300:700)). To attain the best peak separation, several mobile phase trials were attempted. An adequate response was obtained when 215 nm was chosen as the detecting wavelength. During the analysis, the column's temperature was kept at 30 °C. Elution was performed using mobile phase at a flow rate of 2.0 ml/min.

Quantification of drugs present in marketed formulation

The chromatograms demonstrated that the substance was completely separated. Additionally, the ingredients were measured in relation to the standard. Table 2 and the HPLC Chromatogram for DCL Fig. 1 display the results.

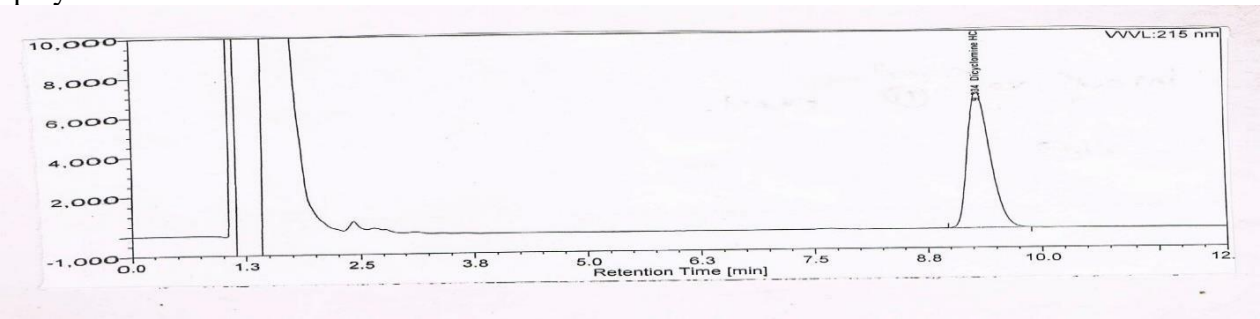

Fig 1: HPLC Chromatogram of standard

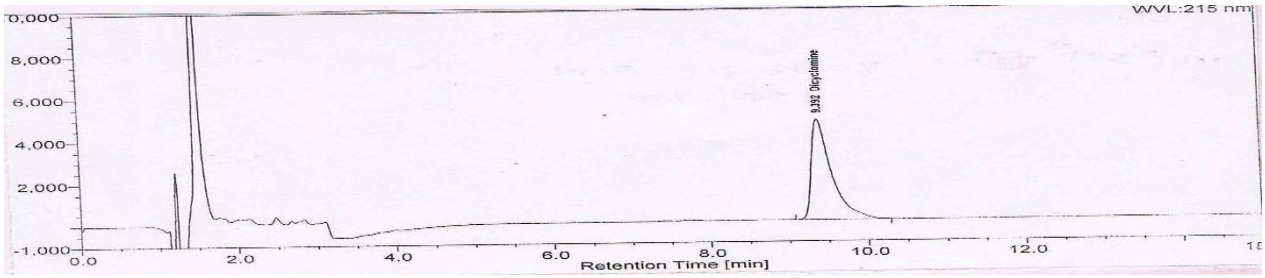

Fig 2: HPLC Chromatogram of test

Table 2: Analysis of DCL in Liquid Formulation

Std. Conc. mcg/ml	**DCL**
1	101.59
2	98.48
3	99.30
Mean	99.84
%found*	99.84
SD	1.467
%RSD	1.47

*Each reading is mean reading of three batch of formulation

Method validation for HPLC

By specifying the selectivity, linearity, accuracy, precision, and robustness, the HPLC method was validated. The precision of the retention time and the selectivity of the medicines eluted were considered when evaluating the procedure for qualitative purposes. Even at high concentrations, a high reproducibility in the retention time was achieved for both medicines and standards. Linearity, accuracy, precision, and robustness were assessed for quantitative purposes. Peak area and drug concentration within respective ranges were shown to be linearly correlated. Regression coefficient (r2) values for DCL greater than 0.99, confirming the approaches' linearity (Table 4). A reasonable level of accuracy was suggested by the high recovery values (99.50% - 98.13%).

Table 3: Recovery Studies of Formulation

Level of Recovery (%)	**70**	**100**	**130**
	DCL	**DCL**	**DCL**

Amount present (mg)	10	10	10
Amount of Std. added (mg)	7.12	10.24	13.75
Amount recovered (mg)	7.26	10.05	13.64
% Recovery	101.59	98.48	99.32

For the degree of repeatability of the devised approach, the relative standard deviation of all the parameters was less than 3.5% (Table 4). The method's precision was demonstrated by the low intraday and interday precision coefficient

REFERENCES:

1. Chandran M., Mohamed Halith S., Mohamed P. U., and Wahab S. U. creation of the RP-HPLC technique for the simultaneous measurement of the combination tablet dosage form of drotaverine HCl and mefenamic acid. 2011; 3(1); 115-117; International Journal of Pharmaceutical Sciences.
2. Principles of Pharmacology, 2nd Ed., Paras Medical Publisher, 2007: P. 368 Sharma H.L., Sharma K.K.
3. Hasumati Raj and Dipal Prajapati. Mefenamic acid and dicyclomine hydrochloride are simultaneously estimated using a spectroscopic technique. 2012; 3(10); 3766-3776; IJPSR.
4 USP 30, NF25, Rockville, MD, United States Pharmacopoeia, 2007.
5. Meeta Baldania, Vaishali Karkhanis, Ronak Dedania, Zarna Dedania, and Vidya Sagar. Omeprazole and ondansetron in combination dose forms can be simultaneously estimated using the RP-HPLC technique. 108–111 in Asian J. Research Chem. 2009; 2(2).
6. Arumughan C., Sarin Kumar K., and Rajit Arimboor. RP-HPLC with DAD for the simultaneous measurement of phenolic acids in sea buckthorn. 2008; 47; 31–38; Journal of Pharmaceutical and Biomedical Analysis.
7. Panjagala Satyanarayana, Chandra Mohan Rao G, Sudarshan Reddy P, and Shanmugasundaram P. Aceclofenac and zinc carnosine can be measured simultaneously in tablet and bulk dose forms using a validated HPLC technique. 109–114 in Int. J. Pharm & Ind. Res. 2011; 1(2).
8. Godge R.K., Pattan S.R., Jamdar S.G., Dighe N.S., Daithankar A.V., Nirmal S. A., and Pai M.G. Paracetamol and etoricoxib can be simultaneously estimated from bulk and tablets using the RP-HPLC technique. 2009; 1(1); 329-335; Journal of Chemical and Pharmaceutical Research.
9. Development and validation of a reversed-phase HPLC technique for the simultaneous quantification of azithromycin and ambroxol hydrochloride in tablet dosage form by Shaikh K.A., Patil S.D., and Devkhile A.B. 2008; 48, 1481–1484, Journal of Pharmaceutical and Biomedical Analysis.
10. Kanungo S.K., Dewani A.P., Barik B.B., Chipade V.D., Bakal R.L., and Chandewar A.V. Phenylepherine, paracetamol, caffeine, and chlorpheniramine in bulk and commercial formulations can be determined using the RP-HPLC-DAD technique. Arabian Journal of Chemistry, online revised proof, in press, July 28, 2012.
14. Gopala Swamy T., Nagaraju K.,

Lakshmana Rao, A. RP-HPLC method for pharmaceutical dosage form simultaneous estimation of hydrochlorothiazide and telmisartan. Drug Development & Res. Int. J. 2011; 3(4); 362- 368.