

REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DICYCLOMINE HYDROCHLORIDE, PARACETAMOL AND MEFENAMIC ACID IN BULK AND TABLET DOSAGE FORM

ESHA KHADGE¹, SAVITA YADAV², JAHNAVI RAO^{2*}¹Department of Quality Assurance Techniques, Bharati Vidyapeeth University, Poona College of Pharmacy, Erandwane, Pune, India.²Department of Pharmaceutical Chemistry, Bharati Vidyapeeth University, Poona College of Pharmacy, Erandwane, Pune, India.

Email: raojanhavi@rediffmail.com

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ABSTRACT

Objective: The objective of this study was to develop and validate a simple, precise and accurate reverse phase high performance liquid chromatographic method for simultaneous estimation of dicyclomine hydrochloride, paracetamol, and mefenamic acid from multicomponent tablet dosage form.

Methods: The optimized chromatographic separation was achieved using a stationary phase of hypersil gold ODS- C18 (250 × 4.6 mm i.d, 5 µm particle size) column and mobile phase of acetonitrile: Methanol: Potassium dihydrogen phosphate buffer, pH adjusted to 4.5 with orthophosphoric acid (OPA) in the ratio of 50: 30: 20 (v/v/v) with a flow rate of 1 mL/minute. The ultraviolet detection was carried out at 260 nm.

Results: The retention times of paracetamol, dicyclomine hydrochloride and MEF were found to be 4.2, 6.1 and 3.4 minutes, respectively. The method was validated for linearity, precision, accuracy, and robustness. The developed method provided linear responses within the concentration ranges 2-12 µg/mL for dicyclomine hydrochloride, 50-300 µg/mL for paracetamol, and 25-150 µg/mL for MEF. The % recovery obtained were found to be 99.09-99.44% for dicyclomine hydrochloride, 99.22-99.51% for paracetamol, and 99.49-99.60 for MEF.

Conclusion: The proposed method enables rapid quantification and simultaneous analysis of all the drugs from commercial formulations without any interference of excipients. Hence, the developed method can be successfully applied for routine analysis of dicyclomine hydrochloride, paracetamol, and MEF in their combined tablet formulation.

Keywords: Dicyclomine hydrochloride, Paracetamol, Mefenamic acid, High performance liquid chromatography.

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INTRODUCTION

Combination of dicyclomine hydrochloride (DCM), paracetamol (PARA), and mefenamic acid (MEF) are used for the treatment of periodic spasms, pain in abdomen, pain in stomach, intestinal cramps, irritable bowel syndrome, inability to control urine and other conditions. These drugs work by reducing muscle contractions and pain; increasing the pain threshold and increases the blood flow across the skin, heat loss, and sweating. Dicyclomine hydrochloride is a muscarinic antagonist used as an antispasmodic and in urinary incontinence. Chemically, dicyclomine hydrochloride is known as 2-(diethylamino) ethyl 1-cyclohexylcyclohexane-1-carboxylate; hydrochloride. Paracetamol is used to treat mild to moderate pain (from headaches, menstrual periods, toothaches, backaches, osteoarthritis, or cold/flu aches and pains) and to reduce fever. Paracetamol chemically is 4-acetaminophenol. MEF is nonsteroidal anti-inflammatory drugs used as antipyretics, analgesics, and anti-inflammatory agents. Chemically, MEF is 2-(2, 3-dimethylanilino) benzoic acid (Fig. 1a-c.) [1-4].

Literature survey reveals that many spectrophotometric methods [5-10], high performance liquid chromatography (HPLC) [11-24], high performance thin layer chromatography [25-29], liquid chromatography-mass spectrometry [30], and GC [31] based methods have been reported for estimation of these drugs alone as well as in combination with other drugs in pharmaceutical dosage forms. Reversed phase (RP-HPLC) method offer significant economic advantages over the techniques cited above. Therefore, this work was

aimed to develop and validate a new RP-HPLC method for simultaneous estimation of dicyclomine hydrochloride, paracetamol, and MEF in pharmaceutical dosage form.

METHODS

Chemicals and reagents

Blue Cross Laboratories Ltd., Goa kindly supplied gift samples of dicyclomine hydrochloride and MEF. Paracetamol gift sample was obtained from Molychem Manufacturers and Importers of Laboratory Reagents and Fine Chemicals, Mumbai. All chemicals and reagents used were of analytical grade and purchased from Merck Chemicals, India. CYCLOPAM PLUS tablet formulation containing dicyclomine hydrochloride 20 mg, paracetamol 500 mg, and MEF 250 mg was procured from local market.

Instrumentation

Chromatographic separation was achieved using HPLC system consisted of Intelligent HPLC pump model (Jasco PU 2080 plus), an autosampler and ultraviolet-visible (UV/VIS) (Jasco UV 2075 plus) detector. The output signal was monitored and processed using Jasco Borwin version 1.5, LC-Net II/ADC software. Hypersil gold ODS- C18 (250 mm, 4.6 mm id, 5 µm particle size) was used as the stationary phase. Mobile phase consisting acetonitrile: Methanol: Potassium dihydrogen phosphate buffer, pH adjusted to 4.5 with orthophosphoric acid (OPA) in the ratio of 50: 30: 20 (v/v/v) was delivered at a flow rate of 1 mL/minute. The detector was set at the wavelength of 260 nm. Injection volume was kept 20 µL.

Selection of wavelength

A UV spectrum of dicyclomine hydrochloride, paracetamol, and MEF in methanol was recorded by scanning the solution in the range of 200 nm to 400 nm. Dicyclomine hydrochloride, paracetamol, and MEF were showing significant absorption at wavelength 260 nm. Hence that was selected as the wavelength for analysis.

Preparation of standard stock solutions

A standard mixed stock solution of dicyclomine hydrochloride, paracetamol, and MEF was prepared by accurately weighing dicyclomine hydrochloride (20 mg), paracetamol (500 mg) and MEF (250 mg) into a 100 mL volumetric flask. The drugs were dissolved in acetone and the solution was diluted to volume. Stock solution was filtered through a 0.45 µm membrane filter. The stock solution was further diluted with mobile phase to obtain a solution of DCM (2 µg/mL), PARA (50 µg/mL) and MEF (25 µg/mL), respectively.

Preparation of sample solution

About 20 tablets of the pharmaceutical formulation CYCLOPAM PLUS (containing 20 mg of DCM, 500 mg of PARA and 250 mg of MEF) were assayed. They were crushed to a fine powder, and an amount of the powder equivalent to 20 mg of DCM, 500 mg of PARA and 250 mg of MEF was weighed and transfer in a 100 mL volumetric flask. The powder obtained was dissolved in acetone. The volumetric flask was sonicated for 15 min to effect complete dissolution of the DCM, PARA and MEF; the solution was then made up to volume with mobile phase. The solution was filtered through Whatman filter paper. The aliquot portion of the filtrate was further diluted to get a final concentration of 2 µg/mL of DCM 50 µg/mL of PARA and 25 µg/mL of MEF.

Method validation

The proposed method was validated for linearity, limit of detection (LOD) and limit of quantitation (LOQ), precision, accuracy and robustness as per International Conference on Harmonization (ICH) guideline [32].

Linearity

DCM (20 µg/mL), PARA (500 µg/mL) and MEF (250 µg/mL) stock solution was prepared, and it was diluted to prepare working standard solutions of 2-12 µg/mL of DCM, 50-300 µg/mL of PARA and 25-150 µg/mL of MEF, respectively. These solutions were injected in triplicate into the column, and the chromatograms were recorded with the optimized chromatographic conditions. The calibration curves of the area versus concentration were recorded for the three drugs.

LOD and LOQ

The method is said to be sensitive if it detects very low concentration of the analyte. It is based on the LOD and LOQ values. ICH guideline specifies three different types of methods to determine the LOD and LOQ values which include visual evaluation signal to noise ratio, and the

standard deviation of response and the slope of the calibration curve. In the current study, the LOD and LOQ were based on the second approach and were calculated according to S/N 3:1 and 10:1, respectively.

Precision

The precision of the method was determined by three independent injections of three different concentrations of each drug (4, 8, 12 µg/mL for DCM, 100, 200, 300 µg/mL for PARA and 50, 100, 150 µg/mL for MEF), which were injected on the same day (intra-day precision or repeatability), and the values of relative standard deviation (% RSD) were calculated. The same concentration solutions were injected on different days to determine interday precision.

Recovery studies

The accuracy of the method, expressed in terms of recovery studies, was performed by standard addition method by adding known amount of drug to the real samples. Samples were suitably diluted and injected at each concentration levels in triplicate.

Robustness of method

The robustness of the developed method was determined by altering the experimental conditions such as flow rate, pH and percentage of methanol in mobile phase so as to check the deviation from the optimized chromatographic condition.

RESULT AND DISCUSSION

Optimized chromatographic conditions

The method development was finally optimized with the following conditions: Mobile phase consisting of acetonitrile: Methanol: Potassium dihydrogen phosphate buffer, pH adjusted to 4.5 with OPA in the ratio of 50: 30: 20 (v/v/v) and hypersil gold ODS C-18 (250 mm × 4.6 mm; 5 µm particle size) column as stationary phase. The analysis was carried out in an isocratic elution mode using a flow rate of 1.0 mL/min, injection volume of 20 µL at room temperature, and the detection of analyte was recorded at 260 nm. The mobile phase solvents were filtered through 0.45 µm member filter before delivering into the HPLC system. The selectivity of the method was performed by injecting the diluents and the mobile phase for any coeluting peaks, at retention times (Rt) of PARA, DCM and MEF were found to be 4.2, 6.1 and 3.4 minutes, respectively. The peak shape of all the drugs was satisfactory and suitable. Typical chromatograms are shown in Fig. 2 performance liquid chromatography chromatogram of dicyclomine, Paracetamol and mefenamic acid.

System suitability

The system suitability test plays an important role in the development of chromatographic method; it was used to insure that, the system is working correctly. There are system suitability parameters such as Rt, peak area, theoretical plates (N), and tailing factor were checked. This test was performed during development of the method. This test was performed by injecting the standard mixture in 5 replicates (Table 1).

Validation of the developed hplc method

Linearity

The linearity range was established at 2-12 µg/mL for DCM, 50-300 µg/mL for PARA and 25-150 µg/mL for MEF. The linear regression equations were $Y = 11203X + 90.78$ ($r^2 = 0.998$) for DCM,

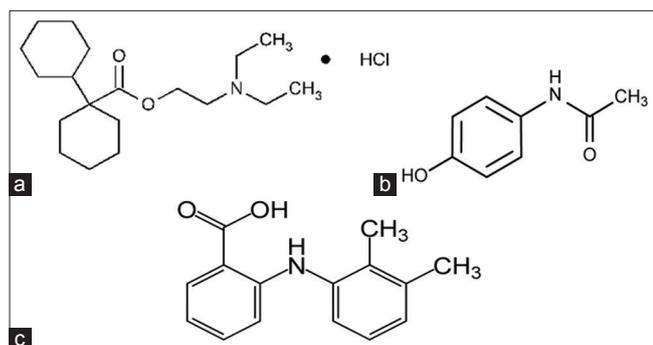


Fig. 1: (a) Chemical structure of dicyclomine hydrochloride, (b) Chemical structure of paracetamol, (c) Chemical structure of mefenamic acid

Table 1: System suitability studies

Parameters	DCM HCl	PARA	MEF
Rt (minute)	4.2	3.4	6.1
Asymmetry	1.156	1.173	1.345
Plates	4543	17445	4600
Area	113589	435140	115018
Tailing factor	1.4	1.2	1.7

Rt: Retention times, DCM: Dicyclomine, MEF: Mefenamic acid, PARA: Paracetamol DCM: Dicyclomine, MEF: Mefenamic acid

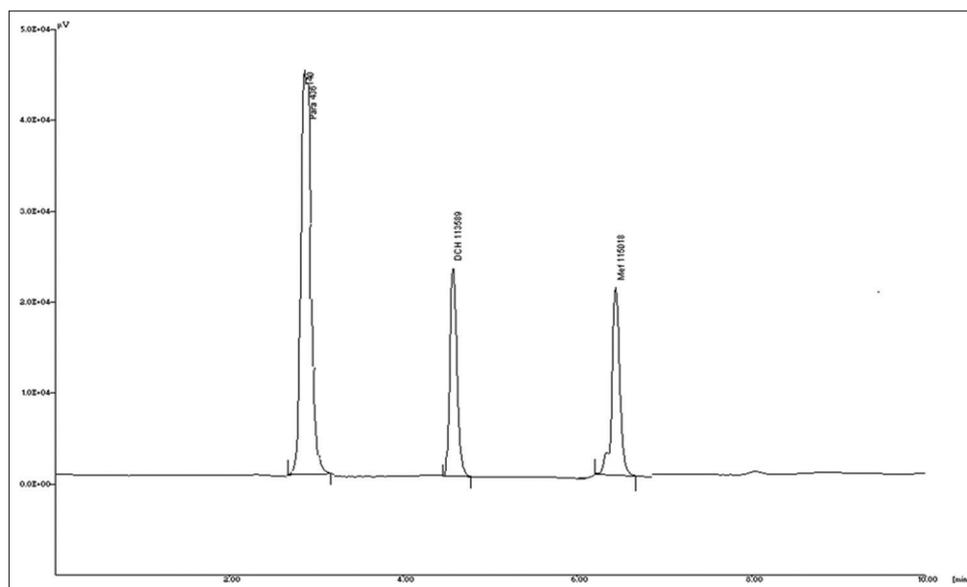


Fig. 2: High performance liquid chromatography chromatogram of dicyclimine, PARA and mefenamic acid

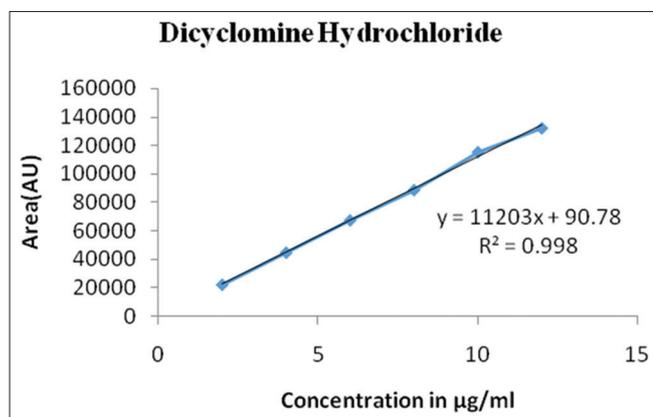


Fig. 3: Calibration curve of dicyclimine

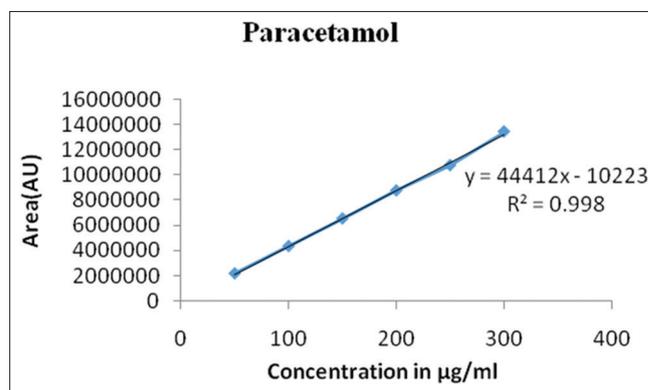


Fig. 4: Calibration curve of Paracetamol

$Y = 44412X - 10223$ ($r^2 = 0.998$) for PARA and $Y = 11478X - 11071$ ($r^2 = 0.997$) for MEF. The plots obtained from linear regression are given in Figs. 3-5 for DCM, PARA and MEF, respectively.

LOD and LOQ

The LOD and LOQ were found to be 0.4 µg/mL and 1 µg/mL for DCM, 15 µg/mL and 48 µg/mL for PARA and 5 µg/mL and 16 µg/mL for MEF, respectively, which indicate that the method was sensitive.

Precision

The intra- and inter-day precision studies showed a % RSD for DCM, PARA and MEF was <2%, respectively, which proved the method was sufficiently precise (Table 2).

Accuracy

Accuracy of the method was determined by applying the proposed method to synthetic mixture containing known amount of each drug to 80%, 100%, and 120% of the label claim. The % recovery obtained was found to be in the range of 99.09-99.44% for DCM, 99.22-99.51% for PARA, and 99.49-99.60 for MEF. The results were summarized in Table 3.

Robustness

Robustness studies were conducted by adjusting flow rate (± 0.1 mL/minute), percentage of methanol in mobile phase ($\pm 1\%$),

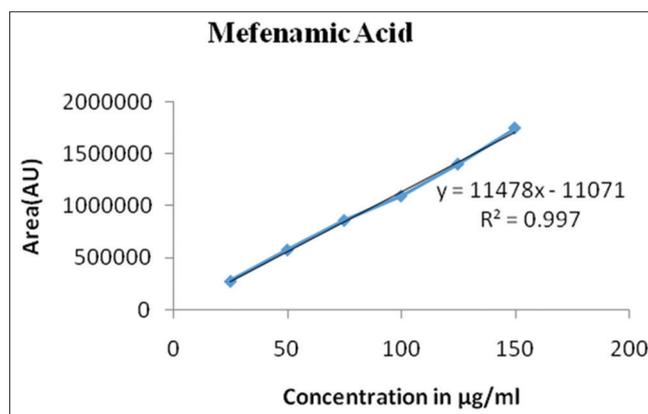


Fig. 5: Calibration curve of mefenamic acid

and pH of buffer (± 0.1). On changing these chromatographic conditions, the method was proven to be robust as there was no greater deviation in the R_t and peak shape (Table 4).

Analysis of the marketed formulation (assay)

The developed method was applied for the determination of DCM, PARA and MEF in tablet dosage form. The obtained result for DCM, PARA and MEF was comparable with a corresponding label claim. The % content

Table 2: Precision studies

Concentration ($\mu\text{g/mL}$)	Intra-day precision (n=3)			Inter-day precision (n=3)		
	Measured concentration	(%) RSD	Recovery (%)	Measured concentration	(%) RSD	Recovery (%)
DCM						
4	3.970	1.08	99.25	3.969	1.14	99.23
8	7.96	1.10	99.50	7.97	1.10	99.63
12	11.92	1.07	99.33	11.90	1.11	99.17
PARA						
100	99.09	1.10	99.09	99.05	1.10	99.05
200	198.8	1.09	99.40	198.5	1.12	99.25
300	297.5	1.03	99.17	297.2	1.04	99.07
MEF						
50	49.8	1.08	99.60	49.5	1.14	99.00
100	99.07	1.01	99.07	99.02	1.15	99.02
150	148.9	1.19	99.27	148.7	1.07	99.13

DCM: Dicyclomine, MEF: Mefenamic acid, PARA: Paracetamol

Table 3: Accuracy (n=3)

Drug	Level of recovery (%)	Amount present (mg)	Amount of drug added (mg)	Total amount (mg)	Amount recovered	% recovery
DCM	80	20	16	36	35.8	99.44
	100		20	40	39.7	99.25
	120		24	44	43.6	99.09
PARA	80	500	400	900	893.0	99.22
	100		500	1000	995.1	99.51
	120		600	1100	1092.0	99.27
MEF	80	250	200	450	447.7	99.49
	100		250	500	498.0	99.60
	120		300	550	547.3	99.51

DCM: Dicyclomine, MEF: Mefenamic acid, Rt: Retention times, PARA: Paracetamol

Table 4: Robustness of method

Chromatographic factors	Level	Rt (minute)		
		DCM	PARA	MEF
Flow rate (mL/min)				
0.9	-0.1	4.31	3.52	6.31
1	0.0	4.2	3.4	6.1
1.1	+0.1	4.06	3.31	6.03
% of methanol				
29	-1	4.38	3.58	6.17
30	0.0	4.2	3.4	6.1
31	+1	4.1	3.35	6.06
pH of buffer				
4.4	-0.1	4.12	3.37	6.06
4.5	0.0	4.2	3.4	6.1
4.6	+0.1	4.32	3.44	6.18

DCM: Dicyclomine, MEF: Mefenamic acid, Rt: Retention times, PARA: Paracetamol

was calculated and found to be 99.57%, 99.71%, and 99.64% for DCM, PARA, and MEF, respectively.

CONCLUSION

A simple, accurate, and specific RP-HPLC method has been developed for quantitative determination of dicyclomine hydrochloride, paracetamol and MEF in bulk and pharmaceutical dosage form. The method was duly validated using required statistical parameters. From these results, it can be concluded that the proposed method is quite precise and accurate. The absence of additional peaks in the chromatogram indicated that there is no interference of the common excipients used in the tablets. The proposed HPLC method is sensitive and repeatable for the analysis of dicyclomine hydrochloride, paracetamol, and MEF in tablet dosage form.

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