RESEARCH ARTICLE

Development and Validation of HPLC Method for Estimation of Some Active Analytes in Combined Dosage form an Application to *In-vitro* Dissolution Studies

Mayuri N. Deshmukh[1*], Vaibhav P. Uplanchiwar[1], Vinod M. Thakare[1], Namrata S. Mane[1], Sanjana N. Gaikwad[1], Narendra R. Dighade[1], Hina D. Mehta[1], Ravi Bakal[2], Prashant Umate[3]

[1]*Nagpur College of Pharmacy, Wanadongri, Hingna Road, Nagpur, Maharashtra, India.*
[2]*P. Wadhawani College of Pharmacy, Yavatmal, Maharashtra, India.*
[3]*Department of Dravyaguna, Datta Meghe Ayurvedic Medical College, Hospital and Research Centre, Nagpur, Maharashtra, India.*

Received: 25th December, 2022; Revised: 10th February, 2023; Accepted: 30th April, 2023; Available Online: 25th June, 2023

ABSTRACT

This paper describes a new, simple, accurate and economical method of development and validation of HPLC method for the assessment of dicyclomine hydrochloride with omeprazol magnesium combine dosage form. The method development trial was carried out initially using C8 (100×0.46×3.5 µm) and C18 GraceSmart (250×4.6×5 µm) as stationary phase and acetate buffer (pH 4.5) and methanol as mobile phase in proportion 60:40v/v. The optimized conditions of factors were sample size 50 µL and wavelength (λ_{max}) 215 nm. The developed method was then applied to *in-vitro* dissolution studies for the dicyclomine hydrochloride and omeprazole magnesium. As per ICH guidelines, stability testing was done. The estimated method can be used to analyze the pharma industries' products.

Keywords: Dicyclomine hydrochloride, Omeprazol magnesium, Stability indicating RP-HPLC Method, Validation, *In-vitro* dissolution.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.2.16

How to cite this article: Deshmukh MN, Uplanchiwar VP, Thakare VM, Mane NS, Gaikwad SN, Dighade NR, Mehta HD, Bakal R, Umate P. Development and Validation of HPLC Method for Estimation of Some Active Analytes in Combined Dosage form an Application to In-vitro Dissolution Studies. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):334-339.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The field of research known as "analytical chemistry" focuses on determining the chemical composition of various substances. Anything we use or eat is made up of chemicals. Therefore understanding their chemical makeup is crucial. Analytical chemistry is crucial in all fields of chemistry, including agriculture, medicine, the environment, forensics, industry, metalworking, and pharmaceuticals.[1]

The pharmaceutical analysis focuses on quantitative and qualitative drug analysis in bulk, dosage forms, and biological samples. Qualitative analyses reveal details about the material, such as the functional group to which the molecules or atoms belong. Comparatively, quantitative analysis pinpoints the sample's precise number of analyte molecules. Samples are analyzed qualitatively and quantitatively. Analytical chemist applies a scientific approach consisting of a series of steps.[2]

- Understanding and defining the goal of the analysis
- Nature of the sample
- Literature search
- Plan of action and execution

Drug Profile

Irritable bowel syndrome symptoms are treated with dicyclomine. Dicyclomine belongs to the group of drugs known as anticholinergics. By preventing the activity of a specific natural chemical in the body, it calms down intestinal muscular spasms (Figure 1). Stustomach acid production.

While omeprazole is prescribed to individuals who suffer from regular heartburn (heartburn occurring two or more times per week). Proton-pump inhibitors, like omeprazole, are a type of drug which help by neutralising stomach acid production (Figure 2).

MATERIAL AND METHOD

Instruments and Equipments

W515 Autosmpler- HPLC Waters 600- 996 PDA Empower, UV-visible spectrophotometer-Shimadzu 1601-Thermo

**Author for Correspondence: vmthakre@gmail.com*

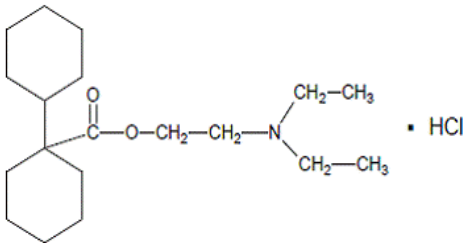

Figure 1: Structure dicyclomine hydrochloride

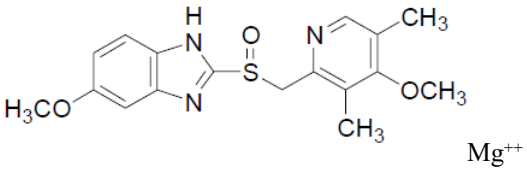

Figure 2: Structure omeprazole magnesium

Lab-Visionpro, Dissolution Apparatus- DBK instruments, Weighing balance- Citizen, pH meter- Digital pH Meter, Vaccum filter- Sartorius, Oven- Bio-Technics.

Chemicals and Reagents

HPLC Grades solvents, AR Grade-Sodium acetate, orthophosphoric acid, potassium dihydrogen phosphate, hydrochloric acid, disodium hydrogen phosphate were used.

Determination of Wavelength Maxima using UV-vis Spectrophotometer

Determination of wavelength maxima for dicyclomine HCl

Dicyclomine hydrochloride was quantitatively estimated using the single point standardisation method. The medication was dissolved both in bulk and in its dose form using a 0.1N HCl solvent system. The UV absorbance was calculated at 213 nm (DIC maximum) (Figure 3).

Determination of Wavelength Maxima for Omeprazole mg

Omeprazole hydrochloride was quantitatively estimated using the single point standardisation method. The medication was dissolved both in bulk and in its dose form using a 0.1N HCl solvent system. The UV absorbance was calculated at 301 nm. (Figure 4).

HPLC Method Estimation of Dicyclomine HCl and Omeprazole mg

Selection of stationary phase

C18 Grace Smart (250 × 4.6 × 5 µm) column- stationary phase.

Selection of mobile phase

Acetate buffer (pH 4.5) : Methanol (60 : 40 v/v) - mobile phase.

Reagents and Chemicals

Acetate buffer (pH 4.5)

Solution A1: 6.0 g (5.8 mL) glacial acetic acid dissolved in water makeup volume 1000 mL in volumetric flask (0.1 M acetic acid).

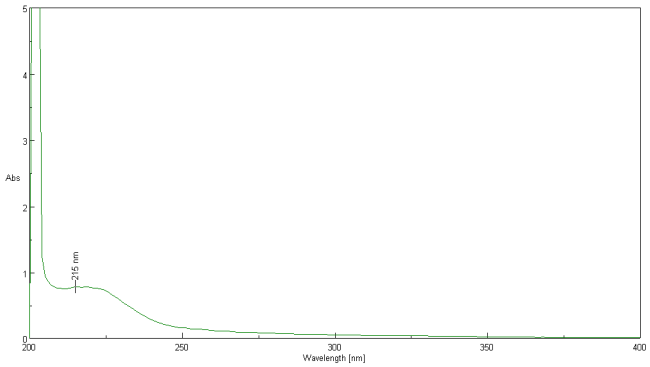

Figure 3: Wavelength maxima for dicyclomine HCl

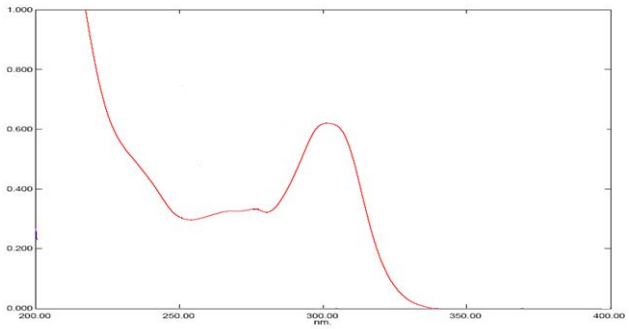

Figure 4: Wavelength maxima for omeprazole mg

Solution A2: 8.2 g of sodium acetate dissolve in water adjust volume 1000 mL (0.1 M sodium acetate)

Mix 510 mL volume of A1 and 490 mL volume of A2 to produce 1000 mL and pH was adjust with A1.

Diluent

Methanol was used as diluent.

Analysis of Active Pharmaceutical Ingredients

Stock solutions (standard)

- *Stock solution of Dicyclomine HCl (Standard-100 µg/mL) (Solution A)*

Fine powder, precisely measured dicyclomine HCl (10 mg) was mixed in methanol (100 mL), stir well. Then, add methanol to bring the volume of the new, 100 mL volumetric flask upto 10 mL.

- *Stock solution of omeprazole Mg (Standard-100 µg/mL) (Solution B)*

Fine powder, precisely measured, mix the 10 mg of magnesium omeprazole with 100 mL of methanol. Then, add methanol to bring the volume of the new, 100 mL volumetric flask upto 10 mL.

- *Combine working standard solution (10 µg/mL of Dicyclomine HCl and 10 µg/mL of Omeprazole mg) (Solution C)*

Withdraw 10 mL of the A and B standard stock solution and place it in a 100 mL volumetric flask; then, using methanol as the diluent, dilute the solution to the mark.[3,4]

Table 1: Optimization of chromatographic conditions

Chromatographic mode	*Chromatographic condition*
Stationary phase	C18 GraceSmart (250 × 4.6 × 5 µm)
Mobile phase	Acetate buffer (pH 4.5) : Methanol (60 : 40 v/v)
Wavelength	215 nm
Flow rate	1.2 mL/min
Sample size	50 µL
Pump mode	Isocratic
Diluent	Methanol

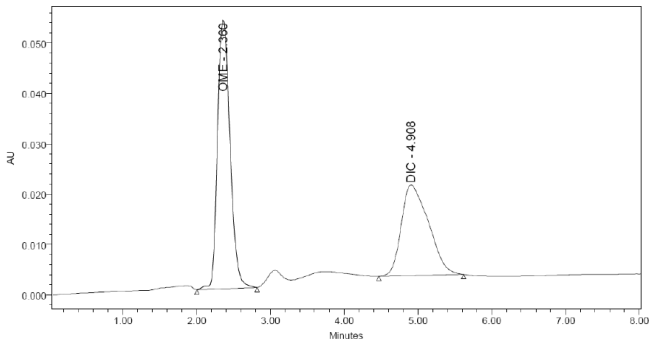

Figure 5: Dicyclomine HCl and omeprazole mg chromatogram

Optimization of Chromatographic Setting

Optimized chromatographic conditions used for analysis of dicyclomine HCl and omeprazole mg are shown in Table 1.

The mix working standard solution was analyzed using the optimized chromatographic conditions as mentioned in Table 2. The HPLC chromatogram obtained is as shown in Figure 5.

System Suitability Parameter

Testing the system's appropriateness is crucial for guaranteeing the chromatographic system's quality performance. For system suitability testing, chromatographic conditions were applied to the mix working standard solution C. Table 2 displays the findings.

Linearity and Range Study

Aliquots of 5, 8, 10, 12, and 15 mL were drawn from the stock standard solutions A and B and placed in an assortment of 100 mL volumetric flasks. Solutions were diluted to mark by methanol to obtain a final concentration in range of 50 to 150 µg/mL for dicyclomine HCl and omeprazole mg i.e. 50, 80, 100, 120 and 150% of analyte in the dosage form. Three separate sets of measurements were taken at every concentration. Samples were analyzed by optimized chromatographic conditions as mentioned in Table 3. The data for the calibration curve for dicyclomine HCl and omeprazole mg are expressed in Table 4.

Table 2: System suitability factor

S. No.	*Peak area*		*Retention time*	
	Dicyclomine HCl	*Omeprazole mg*	*Dicyclomine HCl*	*Omeprazole mg*
1	46107	63161	4.91	2.36
2	46105	63164	4.90	2.38
3	46104	63161	4.89	2.35
Mean	46105.33	63162	4.9	2.36
± S.D.	1.527525	1.732051	0.01	0.015275
%RSD	0.003313	0.002742	0.204082	0.646344

S.No.	*Asymmetry*		*Theoretical plates*	
	Dicyclomine HCl	*Omeprazole mg*	*Dicyclomine HCl*	*Omeprazole mg*
1	1.51	1.36	8468.4	5164.81
2	1.50	1.38	8466.9	5162.24
3	1.54	1.35	8467.7	5168.25
Mean	1.516667	1.363333	8467.667	5168.433
± S.D.	0.020817	0.015275	0.750555	3.718391
%RSD	1.372527	1.120434	0.008864	0.071944

Method Validation as Per ICH Q2 (R1) Guidelines

Validation

A process has been validated if written evidence shows that it consistently produces a product satisfying its intended standards and quality attributes, such as in pharmaceutical dosage form manufacturing.[5,6]

The suggested method was tested according to the ICH Q2 (R1) recommendations.[7]

Table 3: Linearity and range study

S.No.	*Addition level range labeled claim*	*Dicyclomine HCl*			*Omeprazole Mg*		
		Amt. µg/mL (n=3)	Mean peak area response	Amt. recovered	Am.t (µg/mL) (n=3)	Peak area response	Amt. recovered
1	50%	5	235305	50.43	5	326004	50.59
2	80%	8	367853	79.28	8	505293	79.40
3	100%	10	461075	99.57	10	631616	99.70
4	120%	12	559245	120.94	12	757451	119.92
5	150%	15	691651	149.76	15	947022	150.39
Correlation Coefficient		0.999			0.999		
Slope		4594			6223		
Intercept		3626			11166		

Accuracy

It was carried out with a recovery investigation employing standard addition method at 80, 100, and 120% levels. A measured quantity of standard dicyclomine HCl and omeprazole mg was added to a sample that had already been analyzed, and the sample was put through the suggested HPLC method.

Standard stock solution of Dicyclomine HCl (100 µg/mL) (Drug solution A)

Powder that was weighed correctly in 100 mL of methanol, 10 mg of dicyclomine HCl was dissolved. Mix it well. Then, put 10 mL of solution from above into the new 100 mL volumetric flask and fill it with methanol.

Standard stock solution of Omeprazole mg (100 µg/mL) (Drug solution B)

Powder that was weighed correctly in 100 mL of methanol, 10 mgof omeprazole mg was dissolved. Mix it up well. Then, put 10 mL of solution from above into a new 100 mL volumetric flask and fill it up with methanol.

Procedure

Standard and sample solution were analyzed as per optimized chromatographic conditions as shown in Table 1 and 2. Samples were analyzed for three times.[8]

Calculation

Their individual linearity curves were used to figure out how much of each drug was present. Formulas 4 and 5 were used to figure out how much drug was in a given amount, and formula 6 was used to figure out how much drug was in a given amount.

$$Y = mx + c \quad (4)$$

$$x = \frac{(Y-c)}{m} \quad (5)$$

Where,
Y= Peak area of sample solution,
x= Found concentration

$$\% \text{ Recovery} = \frac{S}{A+B} \times 100 \quad (6)$$

Where,
S = Recovered conc. (µg/mL)
A = Actual conc. (µg/mL)
B = Added concentration in µg/mL

Precision study

Dicyclomine HCl and omeprazole mg reproducibility was evaluated by repeatedly injecting a 10 µg/mL homogeneous sample under the same conditions for a brief period of time. Table 4 displays the findings of the repeatability investigation.

Intermediate precision was precise by multiple injections of a 10 µg/mL sample of dicyclomine HCl and omeprazole Mg within-laboratories variations such as different days and different analysts.

Linearity and range study

Linearity study was performed by using five different concentration of drug solution. To generate final concentration of 50–150 µg/mL of dicyclomine HCl and omeprazole mg, aliquot portions (5, 8, 10, 12, 15 mL) of solution A and B were transferred to series of 100 mL volumetric flasks and diluted up to mark using diluents. Three separate sets of measurements were taken at each concentration. Dicyclomine HCl and omeprazole mg calibration curves were generated by relating peak area to medication concentration. Table 5 displays the linearity results.

Placebo study

The placebo solution was made by not weighing quantity of drug in sample for the assay preparation, diluting it according to test method, and then injecting it into the HPLC system. At the time that dicyclomine HCl and omeprazole mg stayed in the body, there were no peaks from the placebo.

Robustness

Studying how the factors change at the same time can help you figure out how stable a method is. Robustness testing was done to find out about the peak area and retention time, two of the most important factors affecting reaction.

They looked at the flow rate in mL/min (x1), the pH (x2), and the wavelength in nm (x3). The research domain of the factors that were chosen. The ranges that were looked at were small changes from the best chromatographic conditions. The answers that were taken into account were peak area (R1), theoretical plates (R2), tailing factor (R3), and retention time (R4). The subsequent chromatographic conditions and ranges were examined during testing for stability (Figure 6 and 7).[9]

Application of Proposed Method to *In-vitro* Dissolution Study

Selection of dissolution apparatus

Finalized dosage form was a delayed-release tablet. So, by default, USP Apparatus II i.e. paddle apparatus, was chosen.

Table 4: System precision showing repeatability

Injection No.	*Area Response*	
	Dicyclomine HCl	*Omeprazole mg*
1	46107	63161
2	46105	63164
3	46104	63161
Average	46105.33	63162
SD	1.527525	1.732051
% RSD	0.003313	0.002742

Table 5: Results of linearity analysis

Parameters	*Dicyclomine HCl*	*Omeprazole mg*
Beers law limit	50–150 µg/mL	50–150 µg/mL
Regression equation	y = 4594x + 3626	y = 6223x + 11166
Slope	4594	6223
Intercept	3626	11166
Correlation coefficient	0.999	0.999

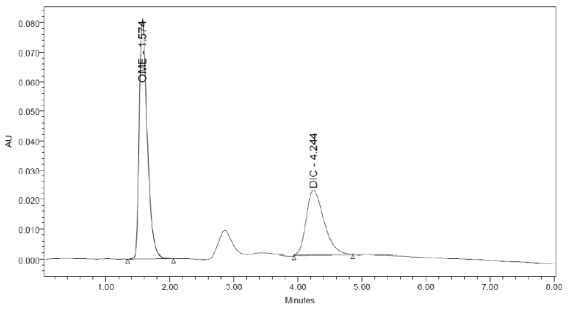

Figure 6: Change in flow rate of 1.1

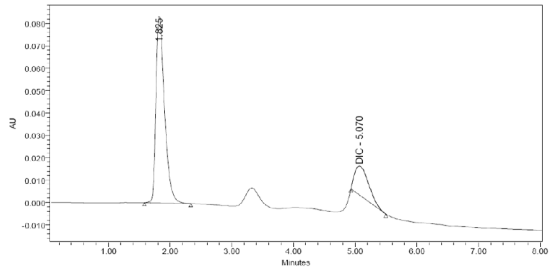

Figure 7: Change in flow rate of 1.3

Table 6: Optimized dissolution parameters

Apparatus	*USP- II (paddle)*
Solvent	0.1 N HCl for first two hours and a phosphate buffer with a pH of 6.8 for last 45 minutes.
Time intervals	For first two hours, there are 15 minute breaks, and for next 45 minutes, there are 5 minute breaks.
Dissolution medium volume	900 mL
Paddle rotation speed	100 rpm
Temperature	37 ± 0.5˚C
Sampling time	2 hours 45 minutes

Selection of dissolution medium

Considering the release target of dosage form, 0.1 N HCl and phosphate buffer pH 6.8 was chosen as the dissolution media for determining the drug release of dicyclomine HCl and omeprazole mg. As the tablet is enteric coated, it needed to keep it in both mediums for the described time period as per USP. Hence, the optimized dissolution medium was 0.1 N HCl and phosphate buffer pH 6.8 for both components.

Selection of time points

Formulation was a delayed-release tablet. Hence it showed delayed release. The time profile for first 2 hours in 0.1 N HCl was at intervals of 15 minutes i.e. 15, 30, 45, 60, 75, 90, 105 and 120 minutes. Subsequently, the 0.1 N HCl medium was replaced by phosphate buffer pH 6.8. The samples were withdrawn with an interval of 5 minutes. for further 45 minutes. i.e. 5, 10, 15, 20, 25, 30, 35, 40 and 45 minutes. After studying the release pattern, 2 hours 45 minutes was selected as the single-time sampling point Table 7.[10]

CONCLUSION

This research set out to find a reliable, quick, and low-cost method of determining how well dicyclomine HCl and omeprazole mg dissolve together in a pharmaceutical formulation. To determine the concentrations of dicyclomine HCl and omeprazole mg in synthetic combinations in the absence of interference from the excipients, the suggested HPLC approach provides a simple, accurate, and repeatable option. It's a quick and easy examination technique that requires less than 8 minutes of your time. The HPLC approach proposed for determining the concentrations of dicyclomine HCl and omeprazole mg concentrations in the body is more selective than alternative techniques.

The technique was tested and used to successfully quantify active medicinal ingredients in pharmaceutical formulations. The excellent accuracy and precision of the suggested method is reflected in low relative standard deviation and high recovery%. In addition, the method is straightforward, can be used with a large concentration range, takes less time than alternative approaches, and uses readily available reagents, making it a practical and acceptable choice for routine detection of both pure drug substances and pharmaceutical formulations.

The present study also described a highly precise, correct and reproducible dissolution method for the determination of %drug release of dicyclomine HCl and omeprazole mg from marketed tablet preparation (Ranispas®). The dissolution method with HPLC analysis for a combination of dicyclomine HCl and omeprazole mg (10 + 10 mg) tablet was applied successfully.

ACKNOWLEDGMENT

Authors would like to communicate their appreciation to the principals and faculty of P. Wadhawani College of Pharmacy in Yavatmal and Nagpur College of Pharmacy in Nagpur for their assistance during the writing process.

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