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Research Article

APPLICATION OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF OMEPRAZOLE AND DICYCLOMINE HYDROCHLORIDE IN BULK DRUG AND TABLET FORMULATION

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Abstract

Aims: The paper describes an HPTLC method for the simultaneous determination of Omeprazole and Dicyclomine Hydrochloride from tablet dosage forms. **Method:** This employs a precoated silica gel 60 F_{254} (0.2 mm thickness) plate on aluminium sheets and a mobile phase toluene: acetone: methanol: ammonia in the ratio of (7: 1.5: 1: 0.1) (v/v/v/v), having chamber saturation for 40 min at room temperature. The mobile phase was run upto 8 cm. The plate was scanned and quantified at 345 nm for both Omeprazole and Dicyclomine Hydrochloride. **Result:** The R_F values were found to be 0.34 ± 0.02 and 0.76 ± 0.02 for Omeprazole and Dicyclomine Hydrochloride, respectively. The linear detector response was observed between 400-2400 ng/spot and 500-3000 ng/spot for Omeprazole and Dicyclomine Hydrochloride, respectively. The developed method was validated for accuracy (99.45 ± 0.54 % and 99.86 ± 0.44 %) for Omeprazole and Dicyclomine Hydrochloride, respectively, Precision (intra-day RSD 0.56–1.13 % and inter-day RSD 0.76–1.10 % for Omeprazole and intra-day RSD 1.08–1.21 % and inter-day RSD 1.15–1.19 % for Dicyclomine Hydrochloride, respectively). The recovery was carried out by standard addition method. The average recovery was found to be 100.27 % and 99.91 % for Omeprazole and Dicyclomine Hydrochloride, respectively. **Conclusion:** The proposed HPTLC method is less expensive, simpler, rapid and is more flexible than the reported methods.

Keywords: Densitometry, Dicyclomine Hydrochloride, Omeprazole, quantification, thin layer chromatography, validation.

1. Introduction

Omeprazole and Dicyclomine Hydrochloride are available in the tablet dosage form in the market in the ratio of 1:1. Omeprazole, 5-methoxy-2-[(4-methoxy-3,5-dimethyl-pyridinyl)methyl]sulfinyl]-1H-benzimidazole, is a substituted benzimidazole that inhibits gastric secretion by altering the activity of H^+/K^+ ATPase, which is the final common step of acid secretion in parietal cells[1]. **(Figure 1).**

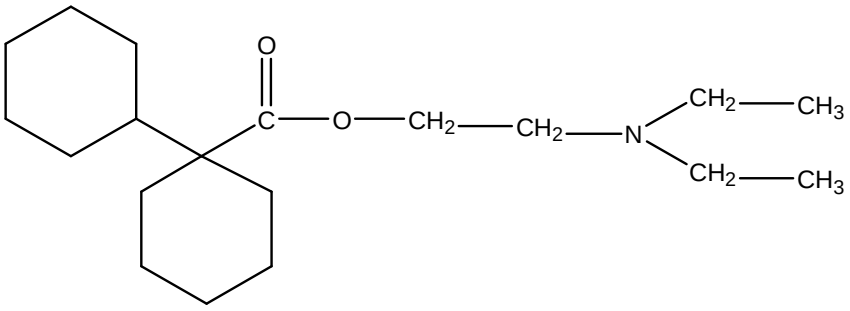

Figure 1: Chemical structure of Omeprazole

Dicyclomine, also known as Dicycloverine, is chemically 2-(diethyl amino)ethyl-bi (cyclohexane)-1-carboxylate[2]. Dicyclomine is used to treat intestinal hyper motility, the symptoms of Irritable Bowel Syndrome (IBS) (also known as spastic colon). It relieves muscle spasms and cramping in the gastrointestinal tract by blocking the activity of acetylcholine on cholinergic (or muscarinic) receptors on the surface of muscle cells. It is a smooth muscle relaxant and it has 72 % of the antimuscarinic power of atropine[3]. **(Figure 2)**

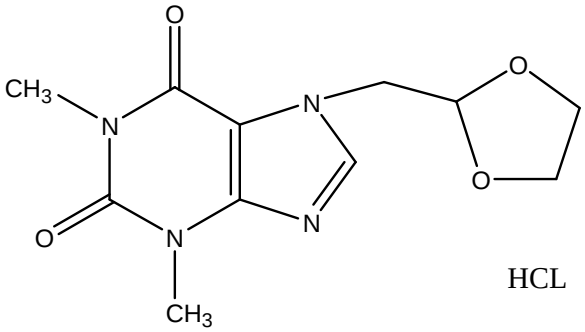

Figure 2: Chemical structure of Dicyclomine Hydrochloride.

A number of methods have been reported in the literature for the estimation of Omeprazole both individually as well as in combination with other drugs. Simultaneous estimation of Omeprazole by HPTLC method from bulk and dosage forms [4, 5, 6, 7, 8], simultaneous spectrophotometric estimation of Omeprazole [9].

Reported methods for Dicyclomine Hydrochloride includes simultaneous determination and validation of Dicyclomine Hydrochloride by HPTLC in bulk and pharmaceutical preparations [10, 11, 12, 13], use of pi-acceptors for spectrophotometric determination of Dicyclomine Hydrochloride [14].

To our knowledge, no article related to HPTLC determination of Omeprazole and Dicyclomine Hydrochloride in fixed dose combination has been reported in literature. The objective of the present work was to develop an accurate, specific and reproducible method for the simultaneous determination of Omeprazole and Dicyclomine Hydrochloride in pharmaceutical formulations by HPTLC. The proposed method has been optimized and validated as per the International Conference on Harmonization (ICH) guidelines [15, 16, 17].

2. Experimental

2.1. Materials

Akriti Pharmaceuticals Pvt. Ltd. Jejuri (Pune), India, kindly supplied pure drug samples of Omeprazole and Dicyclomine Hydrochloride as a gift. They were used without further purification and certified to contain 99.94 % (w/w) and 100.24 % (w/w) for Omeprazole and Dicyclomine Hydrochloride on dried basis, respectively. Fixed dose tablets of Ranispas (Batch No-RS10-013) containing 10 mg each of Omeprazole and Dicyclomine Hydrochloride were purchased from local market made by Mankind limited. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

2.2. Instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60 F_{254} plates, [20 cm × 10 cm with 250 μm thickness; E. Merck, Darmstadt, Germany)] using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110 ºC for 5 min prior to chromatography. A constant application rate of 0.1 μL/s was used and the space between two bands was 6 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 mm/s. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase consisted of toluene: acetone: methanol: ammonia in the ratio of (7: 1.5: 1: 0.1) (v/v/v/v) and 9.6 mL of mobile phase was used per chromatography run. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30

min at room temperature (25 ^{o}C ± 2) at relative humidity of 60 % ± 5. Each chromatogram was developed over a distance of 8 cm following the development, the HPTLC plates were dried in a stream of air with the help of an air dryer in a wooden chamber with adequate ventilation. The direction of flow of air in the laboratory was maintained unidirectional (laminar flow, towards the exhaust). As Dicyclomine hydrochloride is non UV absorbing compound, it could not be scanned under UV detector. After the TLC plate was developed in mobile phase, derivatizing of the plate was carried out as explained in section 2.5. The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample amounts.

2.3. Preparation of Standard Stock Solutions

Standard stock solution of concentration 1000 µg/mL for both Omeprazole and Dicyclomine Hydrochloride were prepared separately using methanol. From the standard stock solution, the mixed standard solutions were prepared using methanol to contain 100 µg/mL of Omeprazole and Dicyclomine Hydrochloride, respectively. The stock solution was stored at 2-8 °C protected from light.

2.4. Preparation of derivatizing agent

Potassium thiocyanate (6.06 g), Cobalt chloride (5 g) and Sodium acetate (3.4 g) were dissolved in sufficient water, 2.5 mL of 1 N HCl was added and volume was made up to 25 mL with water. From this solution 20 mL was further diluted to 50 mL with methanol, filtered and stored at room temperature [18].

2.5. HPTLC method Optimization and chromatographic conditions

The TLC procedure was optimized for simultaneous estimation of Omeprazole and Dicyclomine Hydrochloride. The mixed standard stock solution (100 µg/mL of Omeprazole and 100 µg/mL of Dicyclomine Hydrochloride) were taken and 10 µL samples were spotted on to TLC plates and run in different solvent systems. Initially, toluene, acetone and methanol were tried in different ratios but sharp peaks were not obtained. Hence, ammonia was tried along with above mobile phase. Finally for effective separation of Omeprazole and Dicyclomine Hydrochloride, the mobile phase containing a mixture of toluene: acetone: methanol: ammonia (7: 1.5: 1: 0.1 v/v/v/v) was found to be optimum **(Figure 3)**. The above mobile phase improved the peak shape and gave suitable R_f value for both the drugs. In order to reduce the neckless effect TLC chamber was saturated for 30 min. The plates

were developed for a distance of 80 mm and then dried in hot air, which takes approximately 20 min for complete development of the TLC plate. As Dicyclomine Hydrochloride is non UV absorbing compound, it could not be scanned under UV detector. After the TLC plate was developed in mobile phase, derivatizing agent was poured on the plate and dried. Blue spots against light pink background were scanned at 345 nm within 20 min as later background starts getting darker. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode and operated by CATS software (V 3.15, Camag). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample amounts.

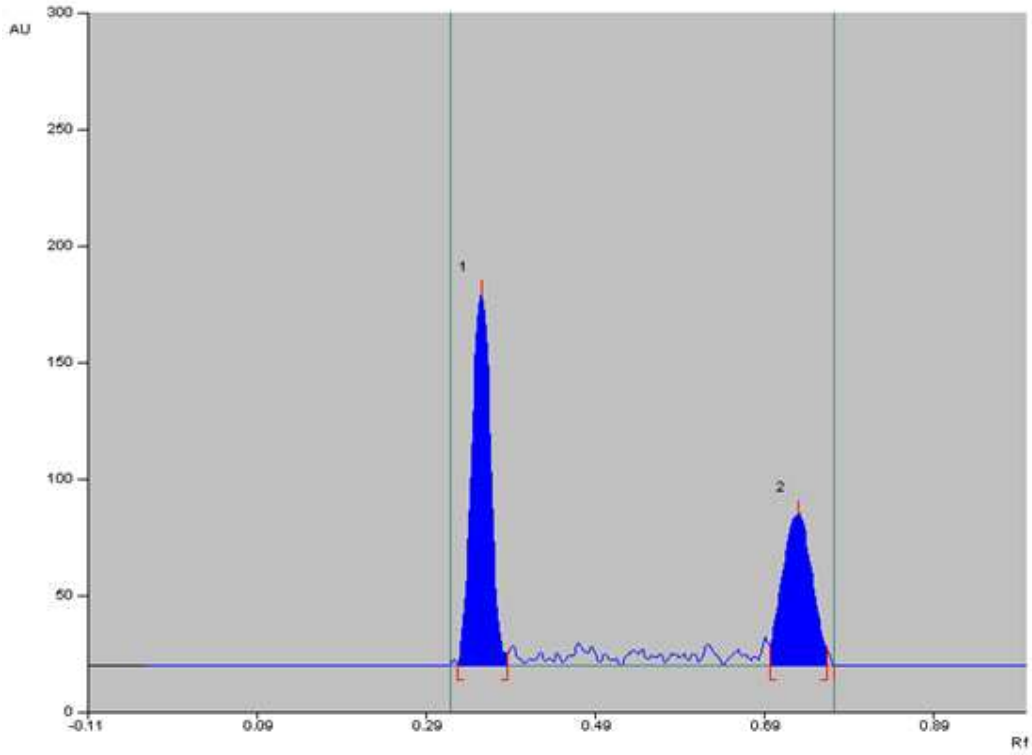

Figure 3: Densitogram of Omeprazole R_F (0.34) and Dicyclomine Hydrochloride R_F (0.76)

Mobile phase: toluene: acetone: methanol: ammonia (7: 1.5: 1: 0.1) (v/v/v/v)

Concentration of drugs: 100 µg/mL of Omeprazole and 100 µg/mL of Dicyclomine Hydrochloride

Application volume: 10 µL

Wavelength: 345 nm

2.6. Validation of the Method

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

2.6.1. Linearity and range

From the mixed standard stock solution 400 µg/mL of Omeprazole and 500 µg/mL of Dicyclomine Hydrochloride, 1 to 6 µL solutions were spotted on TLC plate to obtain final concentration of 400-2400 ng/spot for Omeprazole and 500-3000 ng/spot for Dicyclomine Hydrochloride. Linearity of the method was studied by spotting six concentrations of the drug, each concentration was applied three times to the TLC plates. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

2.6.2. Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (400, 1200 and 2000 ng/spot for Omeprazole and 500, 1500, 2500 ng/spot for Dicyclomine Hydrochloride) of the drugs six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

2.6.3. Limit of Detection and Quantitation

The limits of detection (LOD) and quantification (LOQ) were determined as the amount of the analyte for which the signal-to-noise ratios (S/N) were 3 and 10, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background (N), by spotting a blank, then calculating S/N for different amount of Omeprazole and Dicyclomine Hydrochloride. The LOD was found to be 3:1 and the LOQ was 10:1. The LOD and LOQ were experimentally verified by diluting known concentrations of a standard solution of Omeprazole and Dicyclomine Hydrochloride until the average responses were approximately 3 or 10 times the standard deviation of the responses for the six replicate determinations.

2.6.4. Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different compositions like toluene: acetone: methanol: ammonia (7: 1.4: 1: 0.1 v/v/v/v), (7: 1.6: 1: 0.1 v/v/v/v), (7.1: 1.5: 1: 0.1 v/v/v/v), (6.9: 1.5: 1: 0.1 v/v/v/v), were tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of ± 5 %. The time from

spotting to chromatography and from chromatography to scanning was varied from 10 min. The robustness of the method was determined at three different concentration levels 400, 1200 and 2000 ng/spot and 500, 1500 and 2500 ng/spot for Omeprazole and Dicyclomine Hydrochloride, respectively.

2.6.5. Specificity: The specificity of the method was determined by analyzing standard drug and test samples. The spot for Omeprazole and Dicyclomine Hydrochloride in the samples were confirmed by comparing the R_f and spectrum of the spot with that of a standard. The peak purity of Omeprazole and Dicyclomine Hydrochloride was determined by comparing the spectrum at three different levels of the spot i.e. peak start (S), peak apex (M) and peak end (E).

2.6.6. Accuracy*:* Accuracy of the method was determined by applying the method to drug sample (Omeprazole and Dicyclomine Hydrochloride combination tablets) to which known amount of Omeprazole and Dicyclomine Hydrochloride standard powder corresponding to 80, 100 and 120 % of label claim had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

2.6.7. Analysis of marketed formulation

The marketed formulation was assayed to determine the content of Omeprazole and Dicyclomine Hydrochloride in conventional tablet (Brand name: Ranispas, Label claim: 10 mg Omeprazole and 10 mg Dicyclomine Hydrochloride per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 10 mg of Omeprazole and 10 mg of Dicyclomine Hydrochloride was transferred into a 50 mL volumetric flask containing 30 mL methanol, sonicated for 30 min and diluted up to 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (200 and 200 µg/mL for Omeprazole and Dicyclomine Hydrochloride, respectively). Then 5 µL of this solution (1000 ng/spot and 1000 ng/spot for Omeprazole and Dicyclomine Hydrochloride, respectively) was applied to a HPTLC plate which was developed in optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipients interference with the analysis was examined.

3. Results and Discussion

The results of validation studies on the simultaneous estimation method developed for Omeprazole and Dicyclomine Hydrochloride in the current study are being discussed below:

3.1. Linearity

Linear relationships were observed by plotting drug concentrations against peak areas for each compound. Omeprazole showed linear response in the concentration range of 400, 800, 1200, 1600, 2000, 2400 ng/spot and Dicyclomine Hydrochloride showed linear response in the concentration range of 500, 1000, 1500, 2000, 2500, 3000 ng/spot, respectively. The corresponding linear regression equation was y = 0.5344x + 2237.7 for Omeprazole and y = 0.4578x + 1533.7 for Dicyclomine Hydrochloride with square of correlation coefficient (R^2) of 0.9987 for Omeprazole and 0.9987 for Dicyclomine Hydrochloride, respectively.

3.2. Precision: The results of the repeatability and intermediate precision experiments are shown in **Table 1**. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2%, respectively as recommended by ICH guidelines.

Table-1: Precision studies

Concentration (ng/spot)	Repeatability[a]			Intermediate precision[a]		
	Measured conc. ± SD	(%) RSD	Recovery (%)	Measured conc. ± SD	(%) RSD	Recovery (%)
Omeprazole						
400	393.32 ± 4.1	1.03	98.33	391.56 ± 4.3	1.08	97.89
1200	1191.67 ± 8.4	0.67	99.30	1194.38 ± 8.6	0.49	99.53
2000	1995.19 ± 10.7	0.91	99.75	2001.23 ± 9.9	1.18	100.06
Dicyclomine Hydrochloride						
500	497.92 ± 3.9	1.28	99.58	501.34 ± 3.7	1.14	100.26
1500	1498.12 ± 6.9	0.94	99.87	1492.59 ± 6.7	1.24	99.50
2500	2501.11 ± 9.8	1.58	100.04	2497.54 ± 9.6	1.83	99.90

[a] n = 6

3.3. LOD and LOQ: Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ, respectively. The LOD and LOQ were found to be 350 ng/spot and 400 ng/spot for Omeprazole and 450 ng/spot and 500 ng/spot for Dicyclomine Hydrochloride, respectively.

3.4. Robustness of the method

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

Table-2: Robustness testing.

Parameter	SD of peak area for **Omeprazole**	% RSD	SD of peak area for **Dicyclomine Hydrochloride**	% RSD
Mobile phase composition (± 0.1 ml)	6.72	1.13	6.96	1.18
Amount of mobile phase (± 5 %)	4.43	1.20	4.55	1.14
Time from spotting to chromatography (10 min)	3.99	0.91	4.01	0.98
Time from chromatography to scanning (10 min)	3.76	1.02	3.98	1.43

[a] n = 6

3.5. Specificity: The peak purity of Omeprazole and Dicyclomine Hydrochloride was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9981 and r (M, E) = 0.9997. A good correlation (r = 0.9992) was also obtained between the standard and sample spectra of Omeprazole and Dicyclomine Hydrochloride, respectively.

3.6. Recovery Studies

As shown from the data in **Table 3** good recoveries of the Omeprazole and Dicyclomine Hydrochloride in the range from 99.50 to 101.77 % were obtained at various added concentrations.

Table-3: Recovery studies[a]

Label claim (mg/tablet)	Amount added (mg)	Total amount (mg)	Amount Recovered (mg) ± % RSD	% Recovery
Omeprazole				
10	8 (80%)	18	18.32 ± 0.97	101.77
10	10 (100%)	20	19.91 ± 1.03	99.55
10	12 (120%)	22	21.89 ± 1.00	99.50
Dicyclomine Hydrochloride				
10	8 (80%)	18	17.93 ± 1.21	99.61
10	10 (100%)	20	20.12 ± 1.07	100.60
10	12 (120%)	22	21.90 ± 0.98	99.54

[a] n = 6

3.7. Analysis of formulation

Experimental results of the amount of Omeprazole and Dicyclomine Hydrochloride 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. Two lots of Omeprazole and Dicyclomine Hydrochloride combination tablets were analyzed using the proposed procedures and the results are summarized in **Table 4**.

Table-4: Analysis of commercial formulation.

Omeprazole	Omeprazole found (mg per tablet)	
(10 mg)	Mean ± SD (n= 6)	Recovery (%)
1st Lot	10.02 ± 0.63	100.02
2nd Lot	9.96 ± 0.97	99.60
Dicyclomine Hydrochloride	Dicyclomine Hydrochloride found (mg per tablet)	
(10 mg)	Mean ± SD (n= 6)	Recovery (%)

1st Lot	9.99 ± 0.99	99.90
2nd Lot	9.88 ± 1.01	98.80

4. Conclusion

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 Omeprazole and Dicyclomine Hydrochloride as a bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Omeprazole and Dicyclomine Hydrochloride and also for its estimation in plasma and other biological fluids

5. Acknowledgement

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