HPTLC Determination of Rabeprazole and Domperidone in Capsules and its Validation

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Abstract

This paper describes a validated high-performance thin-layer chromatography (HPTLC) method for simultaneous estimation of rabeprazole (RA) and domperidone (DO) in pure powder and in capsule formulations. An HPTLC method separation is achieved on an aluminum sheet of silica gel $60F_{254}$ using ethyl acetate–methanol–benzene–acetonitrile (30:20:30:20 v/v) as mobile phase. Quantitation is achieved with UV detection at 287 nm over a concentration range of 400–1200 ng/spot and 600–1800 ng/spot with mean recovery of 99.82 ± 0.74 and 99.43 ± 0.68 for RA and DO, respectively, in the HPTLC method. This method is simple, precise, and sensitive, and it is applicable for the simultaneous determination of RA and DO in pure powder and in capsule formulation.

Introduction

Rabeprazole (RA), 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl]-1H-benzimidazole, is a proton pump inhibitors that suppress gastric acid secretion by specific inhibition of the gastric H+, K+ ATPase enzyme system at the secretory surface of the gastric parietal cell.

The chemical name of Domperidone (DO) is 5-chloro-1-[1-[3-(2,3-dihydro-2-oxo-1H-benzimidazole-1-yl)propyl]-4-piperidinyl]-1-3-dihydro-2H-benzimidazole-2-one. It is a peripheral dopamine–2-receptor antagonist. It is a unique gastrokinetic and anti-emetic drug (1,2).

Several techniques, for example spectrophotometric, liquid chromatography tandem mass spectrometry (LC–MS–MS), high-performance thin-layer chromatography (HPTLC), and high-performance liquid chromatography (HPLC), have been reported for the determination of RA in pharmaceuticals and biological samples (3–11). As far as DO is concerned, many reports are available for its estimation either in pharmaceutical formulations or in biological samples using LC–MS, HPLC, spectrophotometry, HPTLC, or voltametry (12–22). Hence, no official method is available for estimating RA and DO in formulations by HPTLC.

This paper describes the development and validation of an HPTLC method for the assay of RA and DO in raw materials and capsules.

Experimental

Apparatus

HPTLC was performed with Camag HPTLC equipment (Muttinz, Switzerland) comprising Linomat V auto sample applicator, Camag Scanner-III, Camag flat bottom and twin trough developing chamber, and UV cabinet with dual wavelength UV lamp. In this method, $60F_{254}$ TLC plates used were silica gel with fluorescent indicator 254 nm, layer thickness (0.2 mm) 20 × 10 cm aluminum (E-Merck-KgaA).

Reagents and materials

RA and DO pure powder were kindly gifted by Torrent Pharmaceutical (Ahmedabad, India) with 99.94% and 99.92% purity, respectively. Ethyl acetate, ammonium acetate, benzene, acetic acid, and ammonia were procured from S.D. Fine chemical (Ahmedabad, India) and were of analytical grade.

Chromatographic conditions

Solutions of the RA and DO were applied to silica gel $60F_{254}$ TLC plates (20 × 10 cm) by means of Linomat V automatic spotter equipped with a 100-µL syringe. The plate was developed in a developing chamber previously saturated for 30 min with a mobile phase of ethyl acetate–methanol–benzene–acetonitrile (30:20:30:20 v/v) to 8 cm. The spots were scanned with a Camag Scanner III at 287 nm.

Preparation of RA and DO standard stock and working solutions

Accurately weighed RA (200 mg) and DO (300 mg) were transferred to a 100-mL amber-colored volumetric flask, dissolved and diluted to the mark with methanol to obtain a working standard

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solution having concentration of RA (2000 µg/mL) and DO (3000 µg/mL) for HPTLC method. One mL of this solution was further diluted to 10 mL with methanol to obtain working standard solution with RA (200 µg/mL) and DO (300 µg/mL) for HPTLC method.

Preparation of sample solutions

Powder (pellets) of each of 10 capsules (2 brands) were weighed and analyzed as follows. A mass of pellets (powder), equivalent to powder of one capsule, was weighed and transferred to a 100-mL amber-colored volumetric flask, and methanol (80 mL) was added. It was sonicated for 15 min, and the final volume was made to the mark with methanol to get solution with RA (200 µg/mL) and DO (300 µg/mL). The mixture was then filtered through a nylon 0.20 µm–47 mm membrane filter.

Method validation

Calibration curve (Linearity)

Analysis was performed on 20 × 10 cm HPTLC silica gel $60F_{254}$ aluminum plate. Calibration curves were plotted over a concentration range 400–1200 ng/spot and 600–1800 ng/spot for RA and DO, respectively. Standard zones were applied to the layer as bands by means of a Camag Linomat V automatic spotter equipped with a 100 µL syringe and operated with the following settings: band length, 6 mm; distance between bands, 8 mm; distance from the plate side edge, 10 mm; and distance from the bottom of the plate, 10 mm. For the calibration curves, accurately prepared standard solution of RA and DO (2.0, 3.0, 4.0, 5.0, 6.0 µL) were applied to the plate. Plate was developed in a developing chamber previously saturated with the mobile phase for 30 min. After development, the plate was air dried, and standard zones were quantitated by linear scanning at 287 nm by a Camag TLC scanner-III with a deuterium source. The calibration curves were constructed by plotting peak areas versus concentrations with the help of win-CATS (one software to manage all TLC steps). Each reading was an average of three determinations.

Accuracy (% Recovery)

The accuracy of the methods was determined by calculating recoveries of RA and DO by the standard additions method. Known amount of standard solution of RA (400, 600, 800 ng/spot) and DO (600, 900, 1200 ng/spot) for the HPTLC method were applied to a prespotted sample solution of capsule dosage forms. The amount of RA (400 ng/spot) and DO (600 ng/spot) was estimated by applying these values to the regression equation of the calibration curve.

Precision

Method precision (Repeatability)

The precision of the instrument was checked by repeated scanning of the same spot (n = 7) of RA (800 ng/spot) and DO (1200 ng/spot) without changing the position of the plate for the HPTLC method.

Intermediate precision (Reproducibility)

The intra- and inter-day precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentration of RA (400, 800, 1200 ng/spot) and DO (600, 1200, 1800 ng/spot) for the HPTLC method. The results are reported in terms of relative standard deviation.

Limit of detection and quantitation

The limit of detection (LOD) and quantitation (LOQ) of the drug were calculated using the following equations as per ICH guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where σ is the standard deviation of the response, and S is the standard deviation of y-intercept of regression lines.

Analysis of RA and DO in combined capsule dosage form

Capsules containing RA (20 mg) and DO (30 mg) of the following two brands (Alkem Lab Ltd., Mumbai, India and Shaimil Laboratory, Baroda, India) were purchased from a local market. The response of capsule dosage forms were measured at 287 nm for quantitation of RA and DO, respectively, by using the HPTLC instrument as described earlier. The amount of RA and DO present in sample solution were determined by fitting the responses into the regression equation for RA and DO.

Results and Discussion

Several mobile phases were tried to accomplish good separation of RA and DO. Using the mobile phase of ethyl acetate–methanol–benzene–acetonitrile (30:20:30:20 v/v) and 20 × 10 cm HPTLC silica gel $60F_{254}$ aluminum plate, better separation was attained where R_f values were 0.78 for RA and 0.55 for DO. A wavelength of 287 nm was used for the quantitation of the

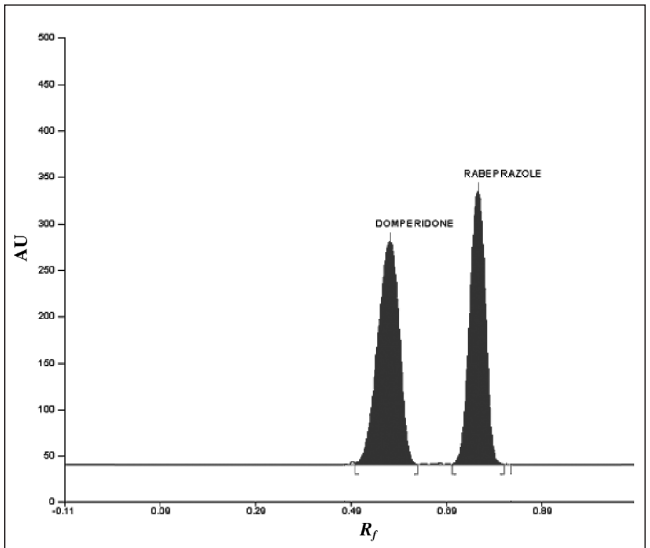

Figure 1. Chromatogram of rabeprazole and domperidone from capsule formulation.

Table I. Regression Analysis of the Calibration Curves for RA and DO by the Proposed HPTLC Method

	HPTLC method	
Parameter	RA	DO
Concentration range (ng/spot)	400–1200	600–1800
Slope	8.38	7.99
Standard deviation of slope	0.05	0.02
Intercept	286.42	883.35
Standard deviation of intercept	1.03	0.63
Correlation coefficient	0.993	0.990

Table II. Summary of Validation Parameters for the Proposed HPTLC Method

	HPTLC method	
Parameter	RA	DO
LOD	131.70 ng/spot	196.98 ng/spot
LOQ	399.09 ng/spot	596.91 ng/spot
Accuracy (%)	99.24–100.67	99.26–100.18
Repeatabilty (% RSD, *n* = 6)	0.379	0.253
Precision (% RSD)		
Interday (*n* = 3)	0.185981–0.512181	0.281866–1.177167
Intraday (*n* = 3)	0.186814–1.537508	0.476814–0.986562

drugs. Better resolution of the peaks with clear base line separation was found (Figure 1).

Validation of the proposed methods

Linearity

The least squares method was used for calculation of slope, intercept, and correlation coefficient. Linear correlation was obtained between peak areas and concentrations of RA and DO in range of 400–1200 ng/spot and 600–1800 ng/spot, respectively, for HPTLC method. The linearity of the calibration graphs was validated by the high value of correlation coefficients of the regression (Table I).

Accuracy

The recovery experiments were carried out by standard addition method. The percent recoveries obtained were 99.82 ± 0.74 and 99.43 ± 0.68 for RA and DO, respectively (Table I). The low value of SD indicates that both the methods are accurate.

Precision

Method precision

For RA and DO in combined solution, the relative standard deviation (RSD) was found to be 0.379% and 0.253%, respectively, for this method (Table II). The lower value of % RSD indicated that the proposed method is repeatable.

Table III. Assay Results of Combined Dosage Form Using The Proposed HPTLC Method

Formulation	RA ± SD* (*n* = 5)	DO ± SD (*n* = 5)
A	99.49% ± 0.23	99.31% ± 0.52
B	99.12% ± 0.72	99.59% ± 0.93

* SD = Standard deviation.

Intermediate precision

For RA and DO, the % RSD of the intra- and inter-day study was found to be in the range of 0.185981–1.537508 and 0.281866–1.177167, respectively, for the HPTLC method. The low % RSD values of intra-day and inter-day variations reveal that the proposed method is robust (Table II).

LOD and LOQ

The LOD and the LOQ of the drugs were calculated as in the text. LOD for RA and DO were found to be 131.70 ng/spot and 196.98 ng/spot, respectively, by HPTLC method. LOQ for RA and DO were found to be 399.09 ng/spot and 596.91 ng/spot, respectively, by this method (Table II). These data show that the method is sensitive for the determination of both RA and DO.

Assay of the capsule dosage form (RA: 20 mg and DO: 30 mg per capsule)

The proposed validated methods were successfully applied to determine RA and DO in their combined capsule dosage form (Capsule A and B). The results obtained for RA and DO were comparable with the corresponding labeled amounts (Table III).

Conclusion

The results of the analysis of pharmaceutical dosage forms by the proposed method is highly reproducible, reliable, precise, sensitive, accurate, and are in good agreement with the label claim of the drug, meaning that the method can be used as a standard pharmacopoeial method for the determination of RA and DO in capsules using the HPTLC system. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with RA and DO. The methods can be used for the routine simultaneous analysis of the RA and DO in pharmaceutical preparations. The advantages of the proposed methods involve simple procedure for sample preparation and relatively short time of analysis. This proposed method can be used in routine pharmaceutical analysis.

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