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Development of High Pressure Thin Layer Chromatographic Method for Simultaneous Estimation of Ranitidine HCl and Domperidone in Their Combined Dosage Form

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An accurate and precise high pressure thin layer chromatographic method for simultaneous estimation of ranitidine HCl and domperidone in their combined dosage form has been developed. The study employs a silica gel $60GF_{254}$ on aluminium foil and a mobile phase comprising methanol : 1,4-dioxane (4 : 6 v/v). The detection was carried out at 282 nm. The linear detector response for ranitidine HCl was observed between 3.0 to 50 μg/mL while for domperidone 0.2 to 3.5 μg/mL. The recovery study was carried out by standard addition method. The recovery was found to be 100.25 ± 0.859, 100.78 ± 0.592 for ranitidine HCl and 100.29 ± 0.394, 99.54 ± 0.436 for domperidone.

Key Words: Ranitidine HCl, Domperidone, HPTLC, Validation.

INTRODUCTION

Ranitidine HCl (RAN) is a white pale yellow crystalline powder, sensitive to light and moisture. Chemically, it is 1,1-ethenediamine, N-[2[[[5(dimethylamino)-methyl]-2-furanyl]methyl]thio]ethyl]-N′-methyl-2-nitro monohydrochloride. It is used as H_2 receptor antagonist and also used in management of ulceration[1]. It is official in I.P.[2] and U.S.P.[3]. Domperidone (DOM) is a white or almost white powder; chemically, it is 5-chloro-1-[1-[3-(2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)propyl]-4-piperidinyl]-1,3-dihydro-2H-benzimidazol-2-one. It is used as a dopamine antagonist and antiemetic drug and is official in B.P.[4]

Literature survey revealed that there are many methods like HPLC[5–13], UV-spectrophotometric[14–16] HPTLC[17, 18] NMR[19] for determination of ranitidine HCl. However, methods like HPLC[20–26], spectrophotometric[27, 28], HPTLC[29, 30] have been reported for estimation of domperidone. The only method reported for simultaneous estimation of ranitidine HCl and domperidone in their combined dosage form is by HPLC, therefore, an attempt has been made to develop a more accurate, precise, reproducible and economic HPTLC method over the available HPLC method.

EXPERIMENTAL

All chemicals and reagents were of AR/HPLC grade. The instrument used in the present study was CAMAG-HPTLC system comprising CAMAG LINOMAT IV automatic sample applicator, CAMAG TLC SCANNER III with CATS 4 software, CAMAG twin trough glass chamber were used.

Experimental Chromatographic Conditions

Standard experimental conditions were followed during the present experimental study. Stationary phase: silica gel 60 GF_{254} TLC precoated aluminium foiled plates, Mobile phase: methanol : 1,4-dioxane (4 : 6 v/v); saturation time: 10 min; thickness of plate: 200 μm; sample application: 6 mm band; separation technique: ascending, temperature: 20 ± 5°C; relative humidity: 50–60%; migration distance: 70 mm; scanning mode: Absorbance/reflectance; detection wavelength: 282 nm; the detection wavelength was selected from overlain spectra of both the drugs in methanol.

Selection of wavelength: The separated bands on HPTLC plates were scanned over the wavelength of 200–400 nm.

Calibration curve response: Standard solution ranging from 2–12 μL was applied on TLC plates by microlitre syringe with the help of automatic sample applicator. The plates were developed, dried and densitometrically scanned at 282 nm. Peak height and areas were recorded for each concentration of drugs and curves (concentration *vs.* peak height/area) were constructed.

Laboratory mixtures: The satandard and sample laboratory mixtures were prepared to get final concentration as that of standard solution. On HPTLC plates, three spots of standard and seven spots of samples were applied, developed and scanned densitometrically at 282 nm.

The per cent estimation of drug in laboratory mixture was calculated by using the formula:

$$\text{Per cent estimated} = \frac{\text{Amount estimated}}{\text{Amount applied}} \times 100 \qquad (1)$$

Assay: Twenty film coated tablets (RANDOM-labelled to contain RAN 150 mg and DOM 10 mg per tablet) were weighed and finely powdered. An accurately weighed quantity of tablet powder equivalent to 25.0 mg of RAN was transferred in 25.0 mL of volumetric flask; near about 23.0 mg of DOM was added to make the ratio of 1 : 1, filtered and then the solution was further diluted to get final concentration of standard solution.

The per cent labelled claim of drug estimated in marketed formulation was calculated by using the formula

$$\% \text{ Labelled claim} = \frac{\text{Amount estimated}}{\text{Amount applied (labelled claim basis)}} \times 100 \qquad (2)$$

Validation of Proposed Method

The proposed method was validated by considering the following parameters:

Accuracy: The accuracy of the proposed method was ascertained by carrying out recovery studies by standard addition method. The recovery study was performed to determine if there is positive or negative interference from excipients present in the formulation. The method was ascertained on the basis of recovery study by applying the standard addition method to the preanalysed sample.

The per cent recovery was calculated by using the following formula:

$$A = \frac{E_W \times 25}{V_S}; \qquad B = \frac{T_E \times T_W}{A_V}$$

$$\text{Per cent recovery} = \frac{A - B}{C} \times 100 \tag{3}$$

where A = total drug estimated in mg, E_W = weight (μg) of drug estimated in V_S, V_S = volume (μL) of sample solution applied on TLC plate, B = weight (mg) of drug contributed by tablet powder, T_E = estimated weight (mg) of drug tablet, T_W = weight (g) of tablet powder, A_V = average weight (g) of tablet, C = amount of pure drug added (mg).

Precision: Precision of an analytical method is expressed as S.D. or R.S.D. of series of measurement. It was ascertained by replicate estimation of drug by proposed method.

Specificity: The specificity is the ability to access unequivocally the analyte for ascertaining the presence of components that may be expected to be present, such as impurities, degradation products and matrix components. The sample solution was prepared to get mixed standard solution and allowed to be stored for 24 h under the following different conditions: room temperature (normal), at 50°C after addition of 1.0 mL of 0.1 N of HCl (acid), at 50°C after addition of 1.0 mL of 0.1 N of NaOH (alkali), at 50°C after addition of 3% of H_2O_2 (oxidation), at 60°C (heat), in UV-cabinet at 265 nm (UV). After 24 h the contents of the flask were shaken with methanol for 15 min and volume was made up to 25.0 mL, filtered, diluted and analyzed as previously described.

Ruggedness: Ruggedness was determined under different conditions, *i.e.*, different days and different analysts.

RESULTS AND DISCUSSION

Various pure solvents of varying polarity, *viz.*, methanol, ethyl acetate, chloroform, toluene etc. and their mixtures in different proportions were tried as mobile phase for development of chromatogram. The mobile phase which was found to be more suitable was methanol : 1,4-dioxane (4 : 6 v/v); it gave the resolution of two components reasonably good with R_f values 0.78 for RAN and 0.33 for DOM. The 282 nm wavelength was selected for densitometric evaluation of chromatogram as both drugs have sufficient and high absorbance and showing better sensitivity (Fig. 1). The concentration response plots of drug show a linearity over the concentration range of 3.0 to 50 μg/mL for RAN and 0.2 to 3.5 μg/mL for DOM with coefficient of correlation values 0.9823, 0.9907 and 0.9987, 0.9948 by peak height and peak area for both drugs respectively. The calibration curves for both the drugs are shown in Figs. 2 and 3.

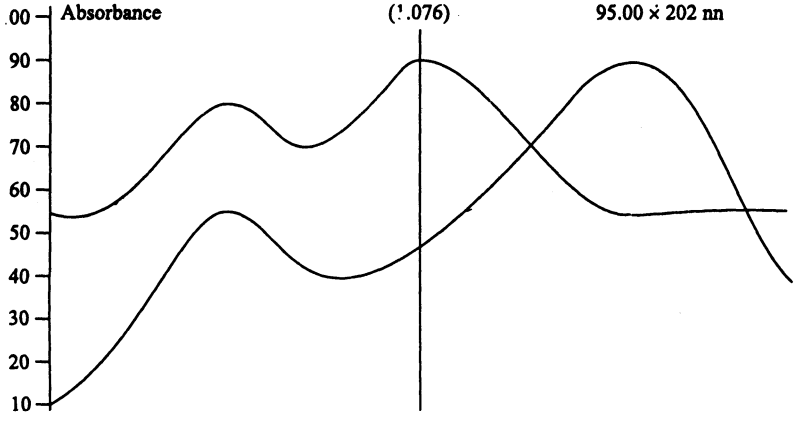

Fig. 1. Selection wavelength for densitometric evaluation of RAN and DOM

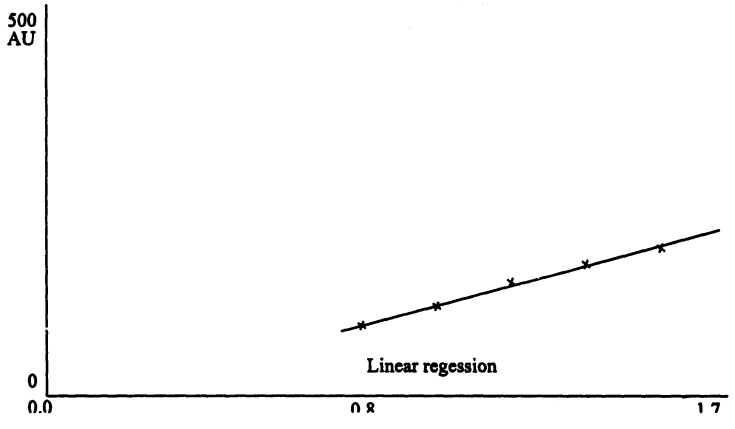

Fig. 2. Linearity reange of RAN by height and area

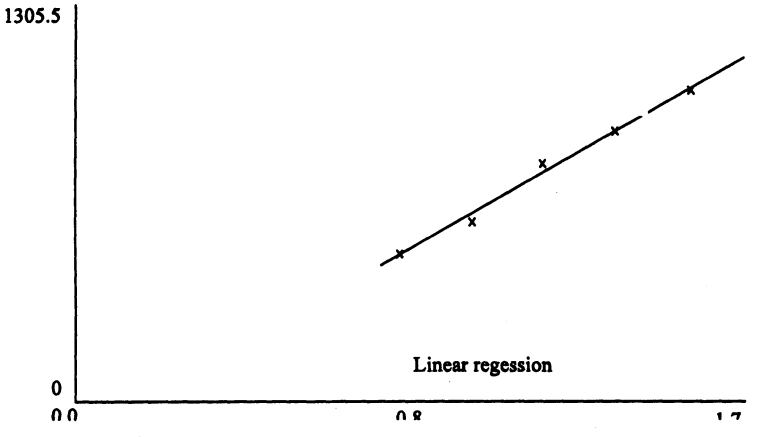

Fig. 3. Linearity range of DOM by height and area

The per cent estimations of drug in laboratory mixture with ±S.D. were found to be 100.18 ± 0.965, 99.81 ± 0.082 and 100.06 ± 0.154, 100.19 ± 1.582 by peak height and peak area for both the drugs and the per cent drug estimation in marketed formulation shows 101.04 ± 0.865, 99.44 ± 0.493 and 99.63 ± 0.654, 9.45 ± 0.713 by peak height and peak area for both the drugs, respectively (Table-1). The chromatograms are shown for laboratory mixture and marketed formulation in Figs. 4 and 5. The results emphasize upon accuracy and precision of the method.

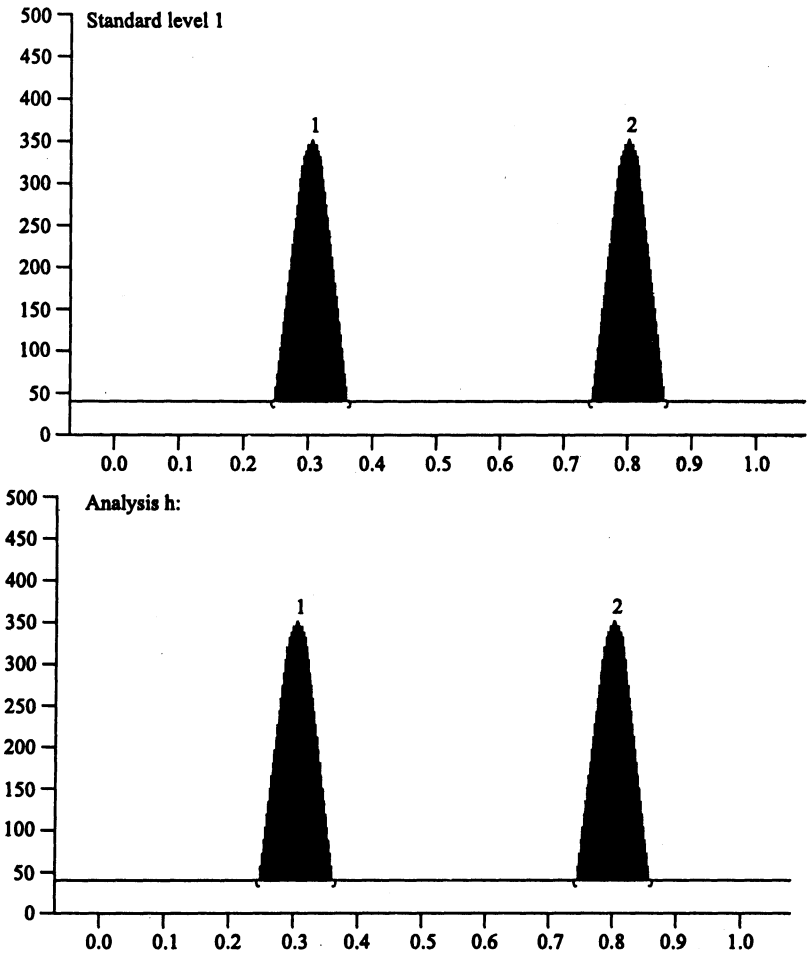

Fig. 4. Densitogram of RAN and DOM for laboratory mixture

The method was validated according to ICH guidelines. The accuracy of the method was evaluated by percentage recovery (by standard addition method) of both the drugs by peak height and peak area. The average recovery was found to be 100.25 ± 0.859, 100.78 ± 0.592 and 100.29 ± 0.394, 99.59 ± 0.436, respectively. The results of the method lying the prescribed limit of 98–102% show that the method is free from interference of excipients (Table-1).

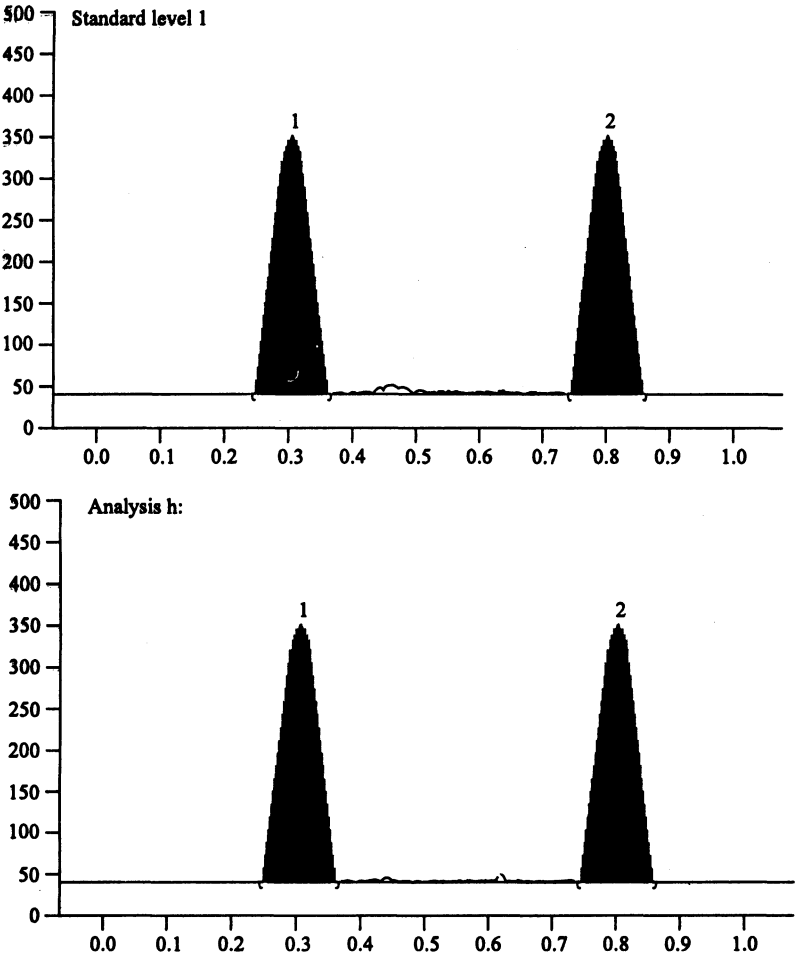

Fig. 5. Densitogram of RAN and DOM for marketed formulation

The replicate estimation of both RAN and DOM in the same batch of tablet as analyzed by the proposed method yielded quite concurrent results indicating the reliability of the method. The values of S.D. or R.S.D. and coefficient of correlation are within the prescribed limit of 2% showing high precision of the method (Table-2).

In specificity study, the sample was allowed to face different stress conditions like acid, alkali, oxide, heat and UV-visible light shows (Table-3), degradation of the drugs under acidic oxide and acidic conditions, but this method is incapable of finding the exact degradation of drugs. The last parameter studied was ruggedness which shows that the result of estimation for the proposed method was reproducible under different conditions like different day and by different analyst (Table-4).

TABLE-1
PER CENT ESTIMATION OF DRUG FROM LABORATORY MIXTURE AND MARKETED FORMULATION

S. No.	Sample	Statistics	% estimation of labelled claim				% Recovery			
			RAN		DOM		RAN		DOM	
			By height	By area	By height	By area	By height	By area	By height	By area
1.	Laboratory Mixture	Mean	100.18	99.81	100.06	100.19	—	—	—	—
		± S.D.	0.965	0.08	0.154	1.582	—	—	—	—
		C.V.	0.966	0.081	0.159	1.578	—	—	—	—
2.	Marketed Preparation	Mean	101.04	99.44	99.63	99.45	100.25	100.78	100.29	99.54
		± S.D.	0.865	0.493	0.645	0.713	0.859	0.592	0.394	0.436
		C.V.	0.712	0.492	0.643	0.712	0.847	0.585	0.385	0.437

Each reading is the mean of four observations

TABLE-2
RESULTS FOR THE PRECISION OF PROPOSED ANALYTICAL METHOD

Weight of tablet powder	% Drug Estimated			
	RAN		DOM	
	Peak height	Peak area	Peak height	Peak area
0.5050	102.65	99.96	100.14	100.24
0.5132	100.14	98.80	98.67	98.26
0.5125	100.85	98.83	98.65	99.59
0.5590	100.26	100.88	100.74	100.72
Mean	101.04	99.42	99.63	99.45
± S.D.	0.865	0.493	0.645	0.713
% C.V.	0.712	0.492	0.643	0.712

Each reading is the mean of four observations.

TABLE-3
RESULTS OF SPECIFICITY STUDY

S. No.	Sample	% Labelled claim			
		RAN		DOM	
		By height	By area	By height	By area
1.	Normal	101.04	99.44	99.63	99.45
2.	Acid	98.23	99.72	96.24	92.70
3.	Alkali	97.73	98.72	91.71	94.50
4.	Oxide	94.72	93.20	87.19	89.62
5.	Heat	98.79	99.24	97.27	98.99
6.	U.V.	92.79	89.92	98.92	99.27

TABLE-4
RESULTS OF RUGGEDNESS STUDY (DIFFERENT DAYS)

Sr. No.	Days	% Labeled claim			
		RAN		DOM	
		By height	By area	By height	By area
1.	Day-1	100.29	99.28	100.09	100.21
2.	Day-4	99.72	99.29	99.92	101.29
3.	Day-7	98.62	98.92	99.02	99.59
	Mean	99.40	99.16	99.67	100.39
	± S.D.	0.782	0.979	0.729	0.812
	C.V.	0.787	0.987	0.726	0.815

The above evaluated parameters in the proposed method revealed that the study signifies a simple, accurate, fast, precise and reproducible HPTLC method for simultaneous estimation of RAN and DOM in their combined dosage forms and can be used for routine analysis of both the drugs in commercially available marketed formulation.

TABLES-5
RESULTS OF RUGGEDNESS STUDY (DIFFERENT ANALYSTS)

S. No.	Days	% Labeled claim			
		RAN		DOM	
		By height	By area	By height	By area
1.	Analyst-1	99.92	99.21	100.20	99.97
2.	Analyst-2	99.25	99.13	100.51	100.02
3.	Analyst-3	99.71	99.98	99.25	100.13
	Mean	99.62	99.44	99.98	100.04
	± S.D.	1.029	0.921	0.759	0.821
	C.V.	1.021	0.920	0.742	0.819

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