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Simultaneous Estimation and Validation of Esomeprazole and Domperidone by HPTLC in Pure and Pharmaceutical Dosage Forms

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A simple, rapid, sensitive high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of esomeprazole and domperidone in pure and pharmaceutical dosage form. It was performed on TLC plate pre-coated with silica gel $60F_{254}$ as a stationary phase using mobile phase composing of chloroform:acetonitrile:ammonia (5:10:0.25) and the detection was carried out in absorbance/reflectance mode at 222 nm showing R_f value 0.76 for esomeprazole and 0.89 for domperidone. The percentage estimation of labeled claims of esomeprazole and domperidone from marketed tablet were found to be 99.55 and 99.60, respectively. The method was validated in terms of accuracy, precision, specificity and ruggedness. Linearity was observed between 600-1400 µg/mL for domperidone and 1200-2800 µg/mL for esomeprazole. The percent recovery study was done by standard addition method and were found in the range of 99.73 and 99.59, respectively. The proposed method is precise, accurate and can be used for routine analysis of esomeprazole and domperidone in tablets.

Key Words: HPTLC, Esomeprazole, Domperidone.

INTRODUCTION

Esomeprazole[1-4] (ESO) belongs to gastrointestinal drugs category to suppress the gastric acidity treatment of peptic ulcer by inhibiting the proton pump. Chemically, it is known as 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole (m.w. 713.13). Esomeprazole is cost effective in the treatment of gastric oesophageal reflux diseases. Esomeprazole is (S-isomer of omeprazole), the first single optical isomer proton pump inhibitor generally provides better acid control than racemic proton pump inhibitors.

Domperidone[1-4] (DOM) is a unique gastro kinetic and anti-emetic drug. It is a peripheral dopamine D2-receptor antagonist, regulates the motility of gastric and small intestinal smooth muscle and has been shown to have

some effects on the motor function of the oesophagus. It increases the duration of antral and duodenal contractions and also LES resting pressure, thus stimulating gastric emptying both in animals and in man and is also effective in relief of symptoms of reflux oesophagitis. Domperidone 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) (m.w. 425.92). Domperidone has been analyzed by RIA[4-7], HPLC with UV[8,9], HPTLC[13,14], mass spectrophotometry[11,12] and in plasma[16].

The literature survey[4-16] indicates that ESO and DOM has been determined individually by RIA, HPTLC, HPLC with UV in pure and pharmaceutical dosage forms. No method has been developed so far for the simultaneous estimation of ESO and DOM. Hence, an attempt has been made to develop a simple, precise, accurate and economical method using high performance thin layer chromatography method for the simultaneous estimation of ESO and DOM in pure and pharmaceutical dosage forms.

EXPERIMENTAL

All chemicals and reagents used were of AR/HPLC grade. Silica gel $60F_{254}$ pre-coated aluminum plates with thickness 200 µm, E-Merck, Germany were used as a stationary phase, the instrument used was CAMAG-HPTLC system comprising of CAMAG LINOMAT-IV automatic sample applicator, CAMAG TLC Scanner III with CAT S 4 software, CAMAG-UV cabinet and CAMAG twin trough glass chamber with stainless steel lids. The source of radiation was deuterium lamp emitting a continuous UV spectrum between 190-400 nm. Pure standards of ESO and DOM were obtained as a gift samples from Maral laboratories.

Preparation of standard solutions: Accurately weighed quantity of 200 mg of ESO (RS) and 100 mg of DOM (RS) was dissolved in methanol and chloroform (1:1) and made up to 10 mL to obtain a stock solution of 20000 µg/mL of ESO and 10000mg/mL of DOM.

Chromatographic conditions: Optimized standard chromatographic conditions required were, stationary phase comprising of TLC aluminum foiled plates pre-coated with silica gel $60F_{254}$ with thickness of 200 µm. Chloroform:acetonitrile:ammonia in the ratio of 5:10:0.25 (v/v) solution was used as a mobile phase and the chamber was saturated for 10 min. Sample was applied at a constant rate of 0.16 µL/s having scan speed 10 mm/s with 16 mm band width the samples were separated by ascending technique. The chamber was maintained at 20 ± 5°C and 50-60 % relative humidity. The scanning was carried out by absorbance/reflectance mode with slit dimension 5 × 0.45 mm. The detection was carried out at 222 nm.

Calibration curve: ESO and DOM solutions ranging from 1200-2800 and 600-1400 µg/mL were applied on TLC plate with the help of auto-

matic sample applicator. The plates were developed, dried and densitometrically scanned at 222 nm (Fig. 1). Peak height and area were recorded for each concentration and curves (concentration/peak height/area) were constructed.

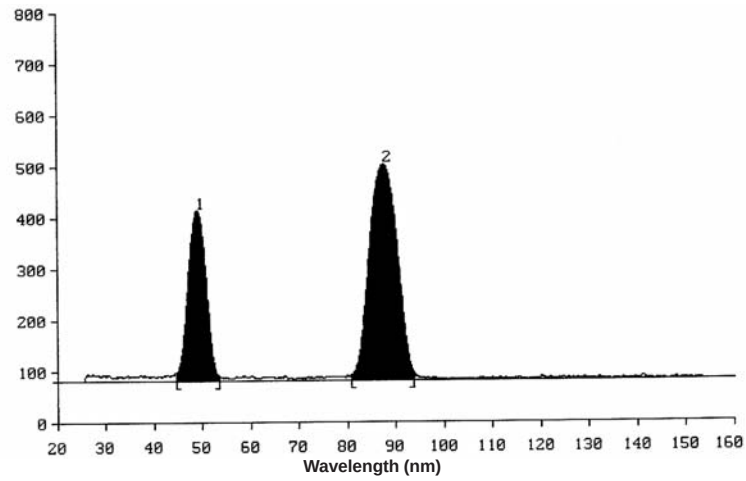

Fig. 1. Assay densitogram of domperidone (1) and esomeprazole (2)

System suitability test: The system suitability test was performed by repeated application, each 10 µL of mixed standard solution and development for chromatogram. The mean standard deviation and coefficient of variance of peak area were calculated.

Standard laboratory mixtures: Different laboratory mixtures were prepared in same manner as that of standard solution to get the final concentration of about 2000 µg/mL of ESO and 1000 µg/mL of DOM. 10 µL of mixed standard solution (duplicate) and laboratory mixture (quadruplet) were applied on TLC plates with 16 mm bandwidth. The plates were then developed in pre-saturated twin trough chamber with mobile phase. After development the plates were dried with the help of hot air dryer and evaluated densitometrically at a wavelength of 222 nm.

Assay procedure: 20 Tablets (Neutraflux labeled to contain each 20 mg of ESO and 10 mg of DOM) were weighed and powdered. An accurately weighed quantity of powder equivalent to 20 mg of ESO and 10 mg of DOM was transferred to 10 mL volumetric flask. The contents were dissolved in methanol and volume made up to the mark. The contents were mixed well using ultrasonicator and filtered through Whatman filter paper no 42. This was used as a sample solution after preparation of the sample the same procedure was followed as under laboratory mixture.

The contents of the drugs in average weight of tablet were calculated as follows:

where, WE = weight of drug estimated (µg), WA = weight of drug applied (µg) on the basis of labeled claim

Validation of proposed method: The proposed method is validated for the following parameters.

Accuracy: The accuracy of the proposed method was ascertained by carrying out recovery studies by standard addition method. Accurately known amounts of standard drugs were added to known amount of pre analysed tablet powder and it was analysed by the proposed method to ascertain if there are positive or negative interferences from excipients present in formulation. The percent recovery was calculated by using following formula.

$$\% \text{ Recovery} = \frac{A - B}{C} \times 100$$

where, A = Total drug estimated in mg, B = Amount of drug contributed by tablet powder (as per proposed method), C = Amount of pure drug added.

Precision: Replicate estimations of drugs in sample were carried out by proposed method and standard deviation/relative standard deviation value was calculated as a measure of precision.

$$\% \text{ Labeled claim} = \frac{WE}{WA} \times 100$$

Ruggedness: Ruggedness was tested under different conditions, *i.e.*, analyzing the samples on different days and by different analysts.

RESULTS AND DISCUSSION

Various pure solvents of varying polarity *viz.*, acetonitrile, chloroform, toluene and diethyl ether and their mixtures in different proportions were tried as a mobile phase for development of chromatogram. The mobile phase was found to be more suitable was chloroform:acetonitrile:ammonia in the ratio 5:10:0.25 (v/v), it gave the good resolution of two components reasonably good with R_f values of 0.76 of ESO and 0.89 DOM. The 222 nm wavelengths were selected for densitometric evaluation of chromatogram as both drugs have sufficient and high absorbance and showing better sensitivity.The percent estimations of drugs in the laboratory mixture with the ± SD were found to be 99.82 and 100.08 % by peak area for both the drugs and the percent drug estimation in marketed formulation shows 99.82 and 99.90 % by peak areas for both drugs, respectively.

The concentration response plots of drugs show linearity over the concentration range of 1200-2800 µg/mL for ESO and 600-1400 µg/mL for DOM with coefficient of correlation values 0.9981, 0.9971, respectively and the linearity values are given in Table-1.

TABLE-1
CONCENTRATION *VERSUS* PEAK AREA OF ESOMEPRAZOLE AND DOMPERIDONE

Drug	Drug concentration (µg/mL)	Peak area (n=5)*	Coefficient of variance (%)
Esomeprazole	1200	23875.1	0.54
	1600	31098.2	0.12
	2000	40241.2	0.14
	2400	48452.1	0.24
	2800	54012.2	0.35
Domperidone	600	9533.1	036
	800	12623.2	0.54
	1000	15868.2	0.24
	1200	19012.1	0.12
	1400	23401.2	0.13

*Mean of five values.

The intra-day and inter-day variations of the method were determined using five replicate injections of three different concentrations, which were prepared and analyzed on the same day and three different days over a period of two weeks, a low coefficient of variation was observed and results are given in Table-2.

TABLE-2
PRECISION OF METHOD

Drug	Concentration (µg/mL)	Observed concentration (n=5)*			
		Intraday	CV (%)	Interday	CV (%)
Esomeprazole	200	200.106	0.18	200.006	0.11
	400	400.138	0.22	400.142	0.13
	600	600.182	0.14	600.112	0.18
Domperidone	100	100.013	0.16	100.011	0.21
	200	200.124	0.28	200.021	0.13
	300	300.218	0.17	300.130	0.12

*Mean of five values.

TABLE-3
RECOVERY STUDIES

Drug	Amount added	Amount recovered	Mean amount found (n=5)*	Mean recovery (%)
Esomeprazole	2	2.013	2.014	100.32
	4	4.035	4.204	100.68
	6	6.120	6.230	100.21
Domperidone	1	0.980	0.990	100.27
	2	2.016	2.018	99.64
	3	2.984	2.990	100.21

*Mean of five values.

To ensure the reliability and accuracy of the proposed method recovery studies were carried out by mixing a known quantity of drug with pre-analyzed sample and contents were reanalyzed by the proposed method and was found to 100.15 and 99.98 %, respectively. The values were given in Table-3.

The drug content in the tablet was quantified using the proposed analytical method. The system suitability parameters are given in Table-4.

TABLE-4
SYSTEM SUITABILITY PARAMETERS

Parameter	Esomeprazole	Domperidone
Resolution factor	2.16	1.86
Tailing factor	1.7	1.64
Linearity range (µg/mL)	1200-2800	600-1400
Limit of detection (µg/mL)	0.063	0.043
Limit of quantitation (µg/mL)	0.210	0.144
Relative standard deviation (%)	2.057	2.367

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