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Rapid densitometric method for simultaneous analysis of famotidine and domperidone in commercial formulations using HPTLC

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ABSTRACT

A rapid and simple high performance thin layer chromatography (HPTLC) method was developed for simultaneous determination of famotidine and domperidone from combined dosage form and validated as per ICH guidelines. Separation was performed on silica gel precoated aluminum plate 60 F_{254}, [(20 × 10 cm) as stationary phase and using a mobile phase comprising of ethyl acetate: methanol: water (8.0: 1.5: 0.3 v/v/v). After development, plates were observed under UV light at 288 nm. The Rf values found to be 0.42±0.02 and 0.67±0.02 for famotidine and domperidone respectively. Validation parameters of the proposed HPTLC method were in compliance with the ICH guidelines. The LOD and LOQ were found to 200 ng/spot and 300 ng/spot for famotidine and 100 ng/spot and 150 ng/spot for domperidone respectively. The percentage average recovery was found to be 98.88 % and 98.26 % for famotidine and domperidone respectively. Famotidine and domperidone were quantified and found to be 97.11±0.27 % and 97.78±0 .35 % in combined dosage form respectively. The described method has the advantage of being rapid and easy. Hence it can be applied for routine quality control analysis of Famotidine and domperidone from combined dosage forms.

Key Words: Famotidine, Domperidone, simultaneous, Validation, HPTLC.

INTRODUCTION

Famotidine (N^1-(amino sulfonyl) – 3- {[[2[(diamino methylene) – amino]-4 – thiazolyl] methyl] thio} propanamidine)(Fig 1 A) is a competitive inhibitor of histamine H2-receptors used as a ant-ulcerative agent. Famotidine inhibits histamine H2-receptors and reduces the gastric juice secretion. It also reduces the acid and pepsin content, as well as the volume, of basal, nocturnal, and stimulated gastric secretion [1, 2]

Analytical methods are available for the quantitative estimation of famotidine including high performance liquid chromatography [3-12], spectrophotometery [13-14] etc.

Domperidone (5-chloro-1-[-1-[3-(2,3-dihydro-2-oxo-1H-benzimadozol-1-yl)propyl]-4-piperi -dinyl]-1,3 dihydro-2H-benzimidazol-2-one) (Fig 1 B) is a inhibitor of dopamine receptor and used as a antinauseant. The antiemetic properties of domperidone are related to its dopamine receptor blocking activity at both the chemoreceptor trigger zone and at the gastric level. Domperidone also facilitates gastric emptying and decreases small bowel transit time by increasing esophageal and gastric peristalsis and by lowering esophageal sphincter pressure [1,2].

Analytical methods are available for the quantitative estimation of domperidone including high performance liquid chromatography [15-21], high performance thin layer chromatography [22-24], spectrophotometery [25-27] etc.

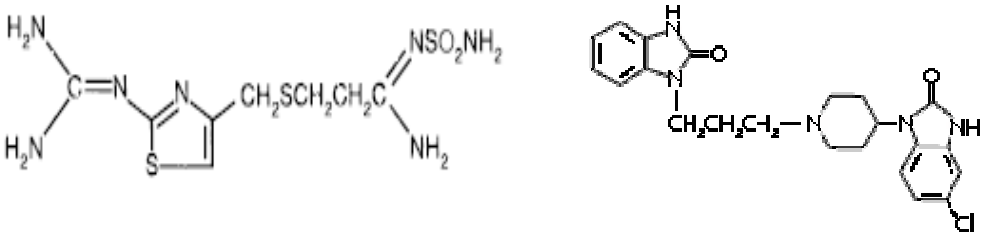

Fig.1: Chemical structure of Famotidine (A) and Domperidone (B)

Moreover the literature survey revealed that so far, only spectrophotometric method [28] and HPTLC method [29] has been reported for estimation of famotidine and domperidone in combined dosage form. In the HPTLC method mobile phase reported is butanol: water (7:1) but as due to high viscosity of butanol this method is time consuming. Also the method used absorption wavelength as 220 nm whereas the maximum absorbance isobestic point was 280 nm, which leads to inaccuracy and less sensitivity.

Hence an attempt was made to develop rapid, simple, precise and accurate HPTLC method for the simultaneous estimation famotidine and domperidone in tablets.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals used in the experiments were of analytical grade. The reference standard famotidine and domperidone were received as a gift sample from Glenmark Pharmaceuticals Limited, Nashik (Maharashtra, India). The commercial formulation Famodon (Famodon, Ozone pharmaceutical Ltd., Indore Label claim- Famotidine-20 mg and Domperidone-10 mg), was purchased from local market of Pune, Maharashtra, India.

HPTLC Instrumentation and Chromatographic conditions:

The samples were spotted in the form of bands 6 mm width with a Camag 100 microlitre sample syringe (Hamilton, Bonaduz, Switzerland) on silica gel precoated aluminum plate 60 F_{254}, [(20 × 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 ^{o}C for 5 min prior to chromatography. A constant application rate of 0.1µLs^{-1} was employed and space between two bands was 5 mm. The slit dimension was kept at 5mm × 0.45 mm and 10 mm s^{-1} scanning speed was employed. The monochromator bandwidth was set at 20 nm, each track was scanned thrice and baseline correction was used. A solvent system of ethyl acetate: methanol: water (8.0 : 1.5 : 0.3 v/v/v) was used as the mobile phase and 15mL of mobile phase was used per chromatography. Linear ascending development was

carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 ^{o}C ± 2) at relative humidity of 60% ± 5. The length of chromatogram run was 8 cm. After development, the plate was dried in air and scanned at 288 nm using absorbance reflectance mode for famotidine and domperidone. Densitometry scanning was performed on Camag TLC Scanner III and operated using 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 diffused light. Evaluation was via peak areas with linear regression.

Preparation sample solutions

Twenty tablets of commercial formulation (Famodon Tablets) were weighed (each containing 20 mg of famotidine and 10mg of domperidone) and their average weight was calculated. Then tablets were finely powdered and powder equivalent to 20 mg of famotidine and 10 mg of domperidone was accurately weighed and dissolved in 50 ml of methanol. The solution was centrifuged for 15 min at 600 rpm. The solution was filtered through Whatman filter paper no. 41 and the residue was washed with methanol and volume was adjusted to 50 ml with the same solvent to obtain the final concentrations of 400 µg/ml of famotidine and 100 µg/ml of domperidone. This solution was subjected to HPTLC for simultaneous estimation of famotidine and domperidone.

Preparation of Standard Solution

Standard solution of famotidine A stock solution of famotidine was prepared by dissolving 20 mg of accurately weighed famotidine in methanol and making up the volume to 10 ml with methanol. From this stock solution standard solutions of 120 µg/ml to 280 µg/ml were prepared by transferring aliquots of stock solution to 10 ml volumetric flasks and adjusting the volume with methanol.

Standard solution of domperidone A stock solution of domperidone was prepared by dissolving 10 mg of accurately weighed domperidone in methanol and making up the volume to 10 ml with methanol. From this stock solution standard solutions of 30 µg/ml to 70 µg/ml were prepared by transferring aliquots of stock solution to 10 ml volumetric flasks and adjusting the volume with methanol.

Calibration Curve Standard solution (10 µL) of famotidine (1200–2800 ng band^{-1}) and domperidone (300–700 ng band^{-1}) were applied in triplicate on precoated silica gel 60 F254 HPTLC plates (E. Merck) of uniform thickness of 0.2mm. The plates were developed in a solvent system of ethyl acetate: methanol: water (8.0: 1.5: 0.3 v/v/v) in CAMAG twin trough chamber up to a distance of 8 cm. After development, the plate was dried in air and scanned at 288 nm using absorbance reflectance mode by CAMAG Scanner 3 and WINCATS software for famotidine and domperidone. The peak areas were recorded. Respective calibration curves were prepared by plotting peak area vs. concentration of famotidine and domperidone applied.

Method validation ICH guidelines were followed for the validation of the analytical procedure [CPMP/ICH/381/95; CPMP/ICH/281/95]. The method was validated for linearity, precision, repeatability, and accuracy [30].

Linearity was determined by construction of calibration curve and least-square regression analysis as described above. In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), blank methanol was spotted to determine signal-to-noise ratio. LOD was considered as 3:1 and LOQ as 10:1. LOD and LOQ were experimentally verified by diluting known concentrations of famotidine and domperidone until the average responses were approximately three or ten times the standard deviation of the responses for six replicate determinations.

Precision, as %RSD, of the method was checked by determining repeatability of sample application. The repeatability of the method was affirmed by analyzing 2000 ng/spot of standard solution of famotidine, and 500 ng/spot of standard solution of domperidone after application on the HPTLC plate (n = 6) and was expressed as %RSD.

Variability of the method was studied by intra-day precision and inter-day precision. Intra-day precision and inter-day precision was studied by analyzing aliquots of standard solution of famotidine (1200, 2000, 2800 ng/spot), and domperidone (300, 500, 700 ng/spot) on the same day (intra-day precision) and on different days (inter-day precision) respectively. The results were expressed as %RSD.

Robustness of the method was checked by making intentional changes in the mobile phase composition (± 0.01 ml). The amount of mobile phase and temperature were changed in the range of ± 5%. Time from spotting to chromatography and from chromatography to scanning was varied (0, 30, 60 and 90 min). Robustness was done at three different concentration levels (1200, 2000, 2800 ng $spot^{-1}$ for famotidine and 300, 500, 700 ng $spot^{-1}$ for domperidone).

The accuracy of the method was tested by performing recovery studies at three levels (80, 100, and 120% standard addition). The amount of famotidine and domperidone present in the commercial formulation was determined from the regression equation. Known amount of the standard was added at three levels and recovery was found. The percent recovery as well as the average percent recovery was calculated.

Stability of sample solution was determined by preparing solutions of two different concentrations of famotidine (1200 and 1600 ng/spot) and domperidone (300 and 400 ng/spot). These prepared solutions were stored at room temperature and analyzed after 0.5, 1.0, 2.0, 4.0 and 24 hrs. To determine band stability, two-dimensional chromatography using same mobile phase was used to reveal any decomposition occurring during application and development.

Quantification of famotidine and domperidone from commercial formulation 10 µl of sample solution was applied in triplicate on precoated silica gel 60 F254 HPTLC plate (E. Merck) (0.2 mm thickness). Plate was developed in the solvent system of ethyl acetate: methanol: water (8.0: 1.5: 0.3 v/v/v) and scanned at 288 nm for famotidine and domperidone. The peak areas and absorption spectra were recorded. To check the identity of the bands UV absorption spectrum of each standard was overlayed with the corresponding band in the sample track. To check the purity of the bands in the sample solution the absorption spectra were recorded by overlaying at start, middle and end position of the bands. The amount of famotidine and domperidone samples was calculated using the calibration curve.

RESULTS AND DISCUSSION

Initially, mobile phase was selected on the basis of previous reports of famotidine and domperidone [3-12, 22-24, 29]. Various solvent systems like chloroform: methanol, chloroform: methanol: acetic acid, ethyl acetate: ethanol: formic acid, ethyl acetate: methanol, chloroform: benzene: toluene and chloroform: benzene: toluene: acetic acid were tried to separate and resolve the spots of famotidine and domperidone. After several modifications & trials the final mobile was optimized as ethyl acetate: methanol: water (8.0: 1.5: 0.3 v/v/v) which was found to give desirable Rf value (Fig 3). The optimized mobile phase can able to give symmetrical, well-resolved reproducible peaks with good shape and baseline separation. The Rf values obtained were 0.42±0.02 and 0.67±0.02 [Fig. 2b] for famotidine and domperidone respectively.

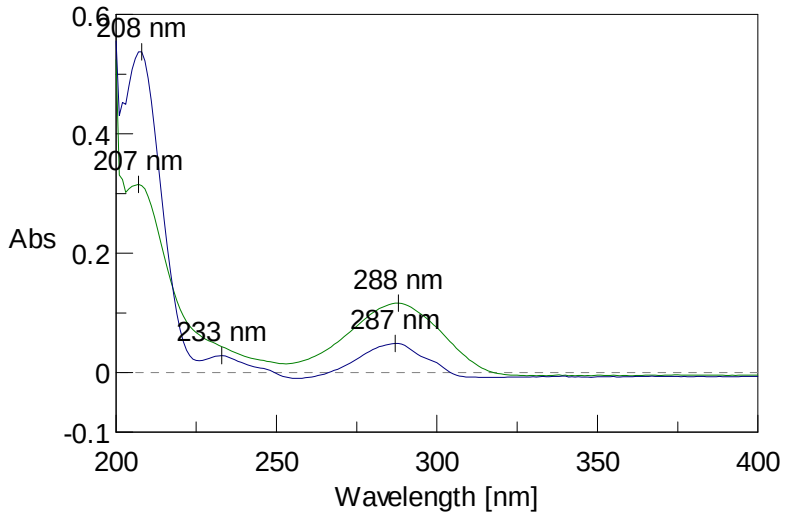

Fig. 2 a: Overlain spectrum for famotidine (λ max- 287nm) and Domperidone (λ max- 288nm) by UV

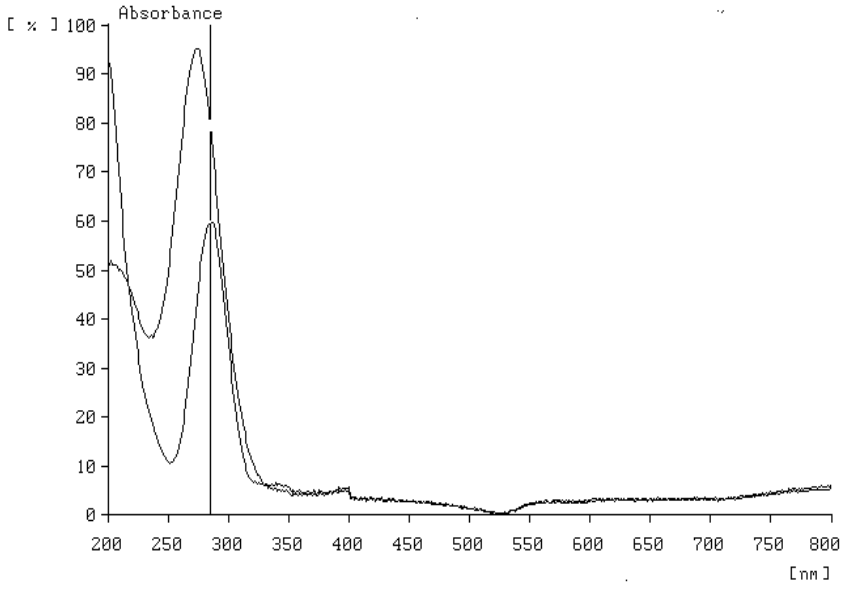

Fig. 2 b: Overlain in situ spectrum for famotidine and domperidone

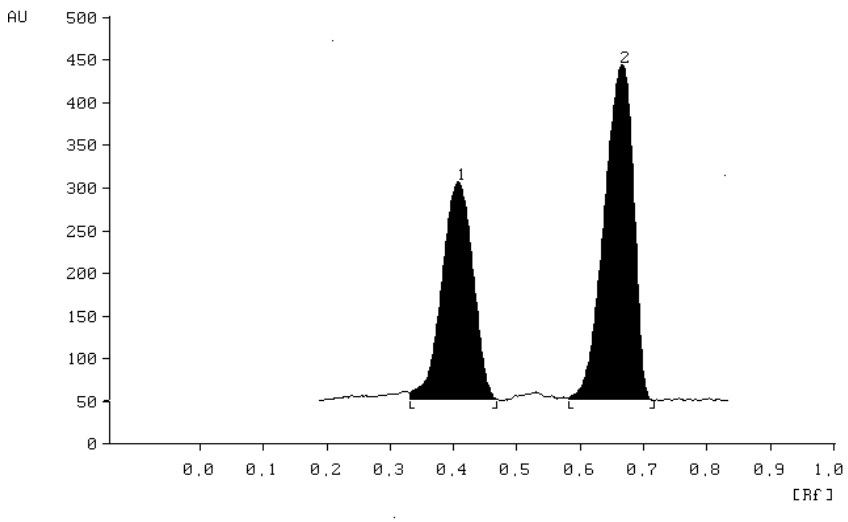

Fig. 3: Densitogram of standard famotidine (R_f, 0.42±0.02) and Domperidone (R_f, 0.67±0.02)

Linearity

A good linearity was achieved in the concentration ranges of 1200–2800 ng band^{-1} for famotidine, and 300–700 ng band^{-1} for domperidone respectively [Table 1]. The regression equations and correlation coefficients for the references were [y =3.777X + 4835] (r^2 = 0.992) for famotidine, and [y = 15.83X + 3124] (r^2 = 0.999) for domperidone.

Instrumental precision and inter-day and intra-day precision

Instrumental precision was checked by analyzing repeatability of the sample application. For repeatability peak areas of famotidine (2000 ng/spot) and domperidone (500 ng/spot) (n=6) were measured. The repeatability of sample application was expressed in terms of % RSD and found to be < 2% for both, alizarin and betulinic acid, as recommended by ICH guidelines.

Standards of famotidine (1200, 2000, 2800 ng/spot), and domperidone (300, 500, 700 ng/spot) were spotted both at intra-day (spotting each concentration five times within 24 h) and inter-day (spotting each concentration four times during 5 days interval separated by at least 24 h) intervals to check the precision. The results are shown in Table 2 and are expressed as % relative standard deviation (%R.S.D.).

Limits of detection (LOD) and limits of quantification (LOQ)

Serial dilutions of famotidine and domperidone were analyzed by HPTLC method. The LOD and LOQ were obtained with the signal-to-noise ratio of 3 and 10. LOD represents the lowest concentrations of famotidine and domperidone that can be detected, whereas the LOQ represents the lowest concentrations of famotidine and domperidone that can be determined with acceptable precision and accuracy. The LOD and LOQ were found to 200 ng/spot and 300 ng/spot for famotidine and 100 ng/spot and 150 ng/spot for domperidone respectively. This indicated that the new method exhibited a good sensitivity for the quantification of famotidine and domperidone. In order to obtain more accurate regression, the lower limit of linearity was adjusted to be higher than LOQ. The concentrations of famotidine and domperidone in sample solutions were within the range of linearity.

Robustness

For robustness analysis, the standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2%. The low values of %RSD as shown in Table 3 indicate the method is robust.

Recovery

The recovery is used to evaluate the accuracy of the method. The percent recovery as well as average percent recovery was calculated. Recovery studies at three different levels were done in commercial formulation by accurately spiked with various concentrations of reference solutions just prior to the extraction. The percentage average recovery at three different levels for famotidine was found to be 98.88 % and domperidone was found to be 98.26 %. The results are shown in Table 4.

Stability Studies

No additional peak was found in the chromatogram showed that the compounds are stable in sample solutions after storage of 24 hours. Analysis by two dimensional chromatography revealed no evidence of decomposition, indicative of band stability.

Quantification of famotidine and domperidone from commercial formulation

All the samples were extracted, as described above and analyzed by HPTLC. The content of each compound was determined by the corresponding regression equation and results are summarized in Table 5. The results indicated that both compounds were detected in commercial formulation. The densitogram of commercial formulations are represented in Fig. 4. The summary of validation parameters is given in Table 6.

Table 1: Linear regression data for calibration curve of famotidine and domperidone

Parameters	Famotidine*	Domperidone*
Linearity range(ng/spot)	1200-2800	300-700
r^2	0.9927	0.9993
Slope ± SD	3.7775 ± 0.059	15.839 ± 0.066
Intercept ± SD	4835.8 ± 1.54	3124.5 ± 0.77

**n = 6*

Table 2: Precision of the method for Famotidine and domperidone

Drug	Conc. (µg/ml)	Intraday		Interday	
		Conc. Found ± SD	RSD (%)	Conc. Found ± SD	RSD (%)
Famotidine	1200	1201.70 ± 7.327	0.609	1203.85 ± 2.548	0.210
	2000	2055.84 ± 6.397	0.311	2057.08 ± 6.381	0.310
	2800	2790.05 ± 8.677	0.310	2795.18 ± 1.726	0.061
Domperidone	300	296.13 ± 0.381	0.128	296.37 ± 0.694	0.234
	500	501.73 ± 4.329	0.863	502.64 ± 4.607	0.916
	700	695.66 ± 0.478	0.068	697.60 ± 0.698	0.010

Table 3: Robustness of Famotidine and Domperidone

Parameter	% RSD	
	Famotidine	Domperidone
Mobile phase composition(±0.1ml)	1.65	0.87
Amount of mobile phase (±5%)	1.13	1.03
Time from spotting to chromatography	1.02	0.94
Time from chromatography to scanning	1.03	1.05

**n=3.*

Table 4: Recovery Studies

Drug	Label Claim (mg)	Amount added (%)	Amount found* (mg)	Recovery (%)	Average (%)
Famotidine	20	80	35.55 ± 0.354	98.10 ±0.116	98.88
		100	39.67 ± 0.071	99.13 ±0.116	
		120	43.73 ± 0.072	99.43 ±0.151	
Domperidone	10	80	17.47 ± 0.212	98.10 ± 1.078	98.26
		100	19.67 ± 0.071	98.27 ± 1.078	
		120	21.65 ± 0.212	98.42 ± 1.123	

**Mean ± Standard deviation (n=3)*

Table 5: Estimation of Drug Content in Sample

Samples	Drug Content* (% w/w)	
	Famotidine	**Domperidone**
Commercial Formulation	97.11±0.27	97.78±0.35

Table 6: Summary Table of the validation parameter

Parameters	Famotidine	Domperidone
Linearity range (ng /spot)	1200-2800	300-700
Correlation coefficient	0.9927	0.9993
LOD (ng /spot)	200	100
LOQ (ng /spot)	300	150
Recovery	98.88	98.26
Repeatability of sample application	0.27	0.28
Repeatability of sample measurement	0.22	0.33
Specificity	0.993	0.999
Robustness	Robust	Robust

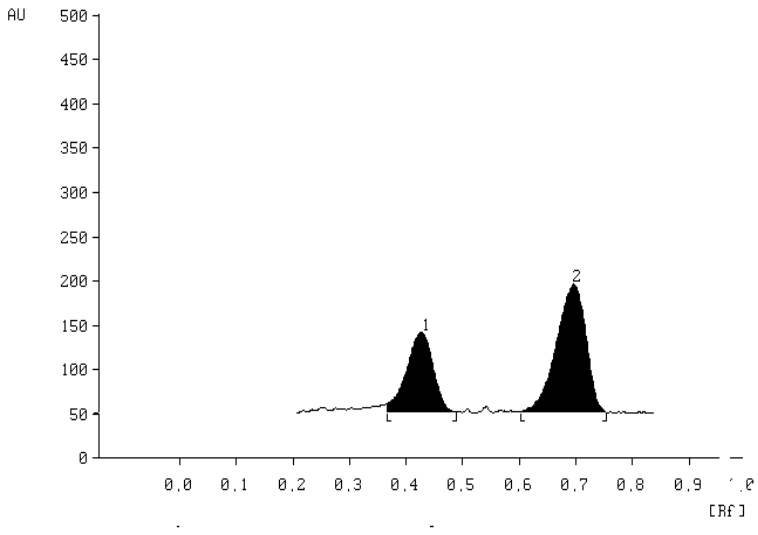

Fig. 4: Densitogram of marketed formulation containing famotidine (Rf 0.42±0.02) and domperidone (Rf, 0.67±0.02)

CONCLUSION

The present work established an accurate and rapid validated HPTLC method for the simultaneous estimation of famotidine and domperidone. The proposed method was found to

be suitable for estimation of these compounds in commercial formulations as it is proved to be precise, reproducible, reliable, accurate and robust. Hence this method can be used for as a rapid analytical tool in routine analysis to monitor loss or variation of the content in various commercial formulations.

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