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Research Article

VALIDATED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF LAFUTIDINE AND DOMPERIDONE IN BULK DRUG AND FORMULATION

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ABSTRACT

This paper describes a new, simple, precise, and accurate HPTLC method for simultaneous estimation of lafutidine and domperidone as the bulk drug and in tablet dosage forms. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F_{254} as the stationary phase and the solvent system consisted of ethyl acetate: methanol : water (8:1:0.3) (v/v/v). The separated zones were densitometrically evaluated at 223 nm. The two drugs were satisfactorily resolved with R_f values 0.34 ± 0.02 and 0.64 ± 0.02 for lafutidine and domperidone, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (200-1000ng/spot for lafutidine and 600-3600 ng/spot for domperidone). The developed method is simple, sensitive and precise; it can be used for the routine testing of formulations in the quality control departments.

Keyword: Thin layer chromatography, Densitometry, Validation and quantification, Lafutidine and Domperidone.

INTRODUCTION

Lafutidine (LFT), N-(4-(4-piperidinylmethyl) pyridyl-2-oxy) butenyl-2(furfurylsulfinyl) acetamide **(Figure 1)** is a histamine H_2-antagonist with gastro protective activity. It is used in the treatment of gastric ulcers and before general anaesthesia to reduce the incidence of aspiration pneumonia.

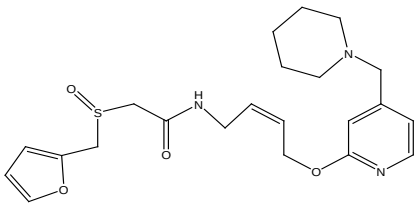

Fig. 1: Structure of lafutidine

Domperidone (DOM), 5-chloro-1-(1-[3-(2-oxo-2, 3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)propyl]piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2(3H)-one **(Figure 2)** is a peripheral dopamine-receptor blocker. It, lower oesophageal sphincter pressure, gastric motility and peristalsis, thus facilitating gastric emptying and decreasing small bowel transit time, generally to suppress nausea and vomiting. It has also been used to stimulate lactation in women, and could be used for the purpose of breast enlargement.

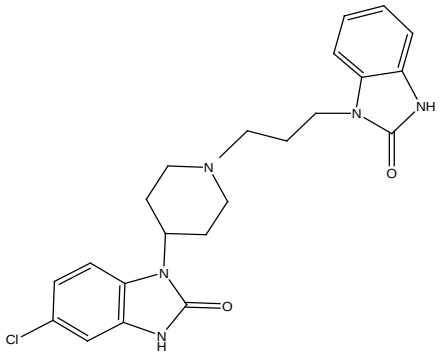

Fig. 2: Structure of Domperidone

Over the past decade HPTLC has been successfully used in the analysis of pharmaceuticals, plant constituents, and biomacromolecules. It also facilitates automatic application and scanning in situ.

Literature reports several methods for the estimation of LFT [1-2] and DOM [3] individually and in combination with other drugs [4-12].

In view of this, high performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. A major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase, thereby reducing the time and cost of analysis [13]. HPTLC can be used for the analysis of large as well as small samples . Furthermore for such methods extraction procedure is not always required and could be used for analyzing drug without any interference from excipients.

To our knowledge, no article related to HPTLC determination of LFT and DOM 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 LFT and DOM in pharmaceutical formulations by HPTLC.

The proposed method was validated as per ICH guidelines and its updated international convention.

MATERIALS AND METHODS

Materials

Pharmaceutical grade LFT (batch no. VA/125/698/110) and DOM (batch no. DOM/07/0331) working standards were obtained as generous gifts from Emcure Pvt. Ltd. Pune, India respectively. Fixed-dose combination tablets Lafaxid-D (batch no.AIA-11003) containing 10 mg of LFT and 30 mg DOM were purchased from local market, All chemicals and reagents were of analytical-grade and were purchased from Merck Chemicals, Mumbai, India.

INSTRUMENTATION

The samples were spotted in the form of bands of width 6 mm with a Camag 100 µL 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 °C for 5 min prior to sample application. 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 ethyl acetate: methanol: water (8:2:0.3) (v/v/v) and 11.3 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 °C ± 2) at relative humidity of 60 % ± 5. Each chromatogram was developed over a distance of 8 cm. Following the development the TLC plates were dried in a stream of air with the help of an air dryer in a wooden chamber with adequate ventilation. The flow rate in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 223 nm 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.

Preparation of Standard Stock Solutions

Standard stock solution of concentration 1000 µg/mL of LFT and 1000 µg/mL of DOM was prepared using methanol. From the standard stock solution, the mixed standard solutions were prepared using methanol to contain 200-1000 µg/mL of LFT and 600-3000 µg/mL of DOM. The stock solution was stored at 2-8 °C protected from light.

Optimization of the HPTLC method

The method was optimized with a view to develop a simultaneous assay method for LFT and DOM. The mixed standard stock solution (200 µg/mL of LFT and 600 µg/mL of DOM) were taken and 10 µL samples were spotted on to TLC plates and run in different solvent systems. Initially, ethyl acetate, water and methanol were tried in different ratios. Finally, the mobile phase consisting of ethyl acetate: methanol: water (8:2:0.3) (v/v/v) was found optimum **(Figure 3)**. The mobile phase was run upto a distance of 8 cm; which takes approximately 20 min.

Validation of the method

Validation of the optimized TLC method was carried out with respect to the following parameters as per ICH guidelines.

Linearity and range

From the mixed standard stock solution, five different concentrations (200- 1000 ng/spot of LFT and 600-3600 ng/spot of DOM) were spotted on the TLC plate. Each concentration was applied six times on the TLC plate. 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.

Precision The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (200, 600 and 1000 ng/spot for LFT and 600, 2400, 3600 ng/spot for DOM) of the drugs six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and limit of quantitaiton

The limits of detection (LOD) and quantification (LOQ) were calculated from the slope (s) of the calibration plot and the standard deviation of the response (SD).

Robustness of the method

The effects following the introduction of small changes in the mobile phase composition were examined on the results. Mobile phases having different compositions, e.g. ethyl acetate: methanol: water (7.5:1.0:0.3 v/v/v), (8:0.5:0.3 v/v/v), (8:1:0.2 v/v/v), were tried and chromatograms were run. The amount of mobile phase was varied in the range of ±5 %. The plates were prewashed by methanol and activated at 60 °C ± 5 for 2, 5, 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and 60 minutes. Robustness of the method was done at three different concentration levels 200, 600, 1000 ng/spot and 600, 2400, 3600 ng/spot for LFT and DOM respectively.

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The spot for LFT and DOM in the samples were confirmed by comparing the R_F and spectrum of the spot with that of a standard. The peak purity of LFT and DOM was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

Accuracy

Accuracy of the method was checked by applying the method to preanalyzed drug sample (LFT and DOM combination tablet) to which known amount of LFT and DOM corresponding to 80, 100 and 120 % of label claim has been added (standard addition method), mixed and the sample was extracted and analyzed by running chromatogram in optimized mobile phase.

Analysis of a marketed formulation

To determine the content of LFT and DOM in the marketed tablet (Brand name: Lafaxid-D, Label claim: 10 mg LFT and 30 mg DOM per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 10 mg of LFT and 30 mg DOM was transferred in 25 mL volumetric flask, 20 mL of methanol was then added, and sonication was done for 45 min with swirling. After sonication, the volume was made up to mark with the methanol, and mixed well. The solution was filtered through Whatman filter paper 41. Then fixed volumes of solutions (1 µL) were spotted on plates and analyzed for LFT and DOM. The analysis was repeated in triplicate. This was done to confirm no interference of the excipients in the analysis.

RESULTS AND DISCUSSION

The results of validation studies on the simultaneous estimation method developed for LFT and DOM in the current study involving ethyl acetate: methanol: water in the ratio of 8.0: 1: 0.3 (v/v/v) as the mobile phase shows highest resolution, minimum tailing and R_f values of 0.34 and 0.64 for LFT and DOM, respectively (Fig 3). UV scanning at 200-400 nm for both LFT and DOM show that 223 nm is the suitable wavelength for detection of drugs (Fig 4).

Linearity

The LFT and DOM showed a good correlation coefficient (r^2 = 0.998 for LFT and 0.998 for DOM) in the concentration range of 200–1000 ng/spot for LFT and 600–3600 ng/spot for DOM (table 1).

Precision

The results of the repeatability and intermediate precision experiments are shown in table 2. 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.

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 13 ng/spot and 40 ng/spot for LFT and 12 ng/spot and 36 ng/spot for DOM respectively.

Robustness of the method

The standard deviation of the areas of the peaks was calculated for each parameter and the % RSD were found to be less than 2%. The low values of the % RSD indicated robustness of the method Table 3.

Specificity

The peak purity of LFT and DOM was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9986 and r (M, E) = 0.9994 for LFT and r (S, M) = 0.9985 and r (M, E) = 0.9991 for DOM. A good correlation (r = 0.9935 and r =0.9993 for LFT and DOM respectively) was also obtained between the standard and sample spectra of LFT and DOM respectively.

Recovery Studies

Good recoveries of the LFT and DOM were obtained at various added concentrations as shown in Table 4.

The results obtained after applying the HPTLC method for the analysis of the pharmaceutical formulations are indicated in Table 5

The summary of validation parameters is given in Table 6.

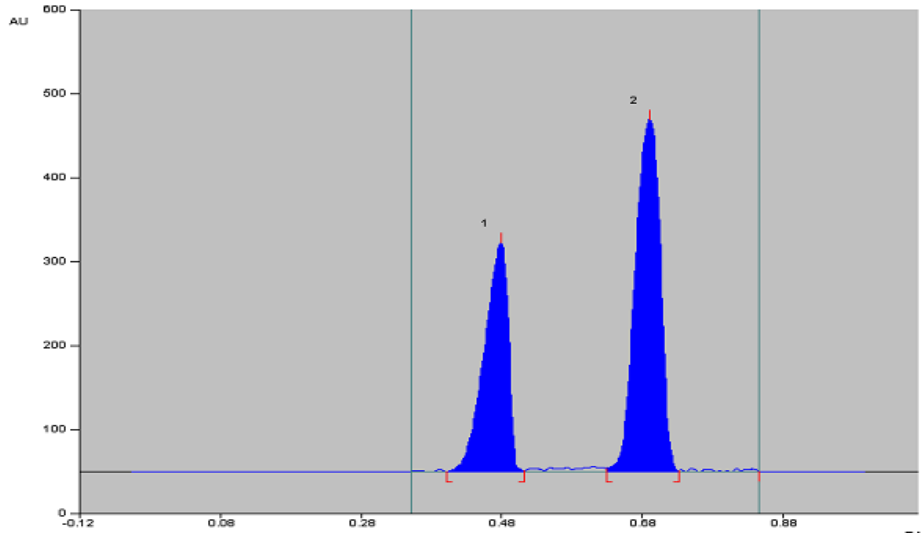

Fig. 3: Densitogram of lafutidine (2000 ng/spot); peak 1 (standard) (R_F : 0. 34, domperidone (6000 ng/spot); peak 2 (standard) (R_F: 0. 64). Ethyl acetate: Methanol: water (8:1:0.3) (v/v/v).

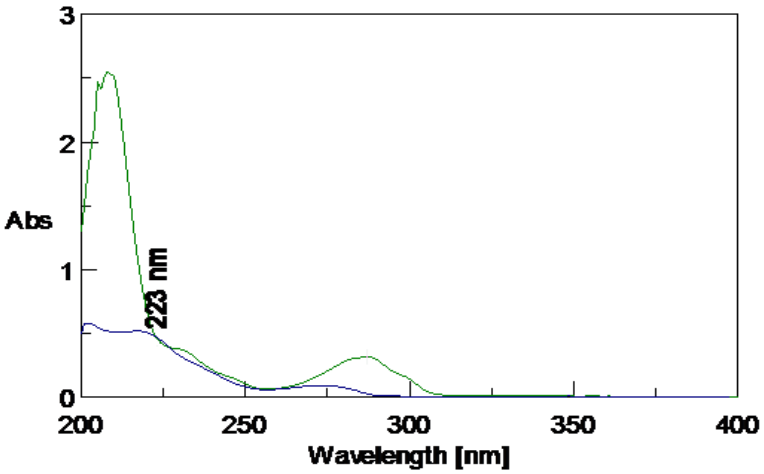

Fig. 4: Overlain UV spectrum of lafutidine and domperidone measured from 200 to 400 nm.

Table 1: Linear regression data for the calibration curves[a]

Compound	Linearity (ng.spot^{-1})	y = A + Bx		r^2
		A	B	
LFT	200-1000	19.25	3.865	0.998
DOM	600-3000	1432	4.674	0. 998

[a] n = 6; r^2, coefficient of correlation

Table 2: Repeatability and intermediate precision of HPTLC method[a]

Compound	Repeatability		Intermediate precision	
	S.D of areas.	% R.S.D.	S.D of areas.	% R.S.D.
LFT	15.89	1.49	20.34	1.30
DOM	60.39	1.19	64.78	1.84

[a] n = 6

Table 3: Robustness testing[a]

Parameter	LFT		DOM	
	SD of peak area	% RSD	SD of peak area	% RSD
Mobile phase composition	18.90	0.92	800.23	0.93
Amount of mobile phase	15.78	1.12	916.45	1.02
Plate pretreatment	20.38	0.02	780.45	0.90
Time from spotting to chromatography	19.33	0.91	901.36	0.88
Time from chromatography to scanning	19.73	0.85	950.01	0.60

Table 4: Recovery Studies

Label claim	Amount of drug added (%)	Total amount of drug present (ng/spot)	Amount found (ng/spot)	% Recovery
LFT	80	720	715.75	99.41
10 mg	100	800	797.6	99.70
	120	880	873.4	99.25
DOM	80	2160	2152	99.65
30 mg	100	2400	2400.96	100.04
	120	2640	2626	99.48

Table 5: Analysis of the pharmaceutical formulations

Sample	Label claim (mg)	Drug Content (%)	% R.S.D.
LFT	10	103.31	1.09
DOM	30	100.91	0.53

Table 6: summary of validation parameters

Parameter	LFT	DOM
Linearity range ($ng.mL^{-1}$)	200- 1000	600- 3600
Correlation coefficient	0.998 ± 0.05	0.998 ± 0.06
Limit of detection ($ng.mL^{-1}$)	13	12
Limit of quantitation ($ng.mL^{-1}$)	40	36
Recovery (n = 6)	99.45	99.72
Precision (% R.S.D.)	1.49	1.30
Repeatability	1.19	1.84
Intermediate		
Robustness	Robust	Robust
Specificity	0. 9935	0. 9993

Analysis of a Formutation

Experimental results of the amount of LFT and DOM 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 (**Table 5**).

CONCULSION

The developed TLC method is precise, specific and accurate. It is concluded that the developed method offered several advantages such as rapid, cost effective, simple mobile phase and easy sample preparation steps and improved sensitivity made it specific, reliable and easily reproducible in any quality control set-up providing all the parameters are followed accurately for its intended use. It may be extended to study the degradation kinetics of lafutidine and domperidone and also for its estimation in plasma and other biological fluids. The proposed TLC method is less expensive, simpler, rapid, and more flexible than HPLC

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