

VALIDATED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF LAFUTIDINE AND DOMPERIDONE IN A TABLET DOSAGE FORM

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ABSTRACT

A reliable simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for estimation of lafutidine (LF) and domperidone (DP) in combined tablet dosage forms. The analysis was performed on 10×20 cm aluminium-precoated plates coated with 0.2 mm layers of silica gel 60 F₂₅₄ (E-Merck, Germany) with ethyl acetate: methanol: toluene: acetone: glacial acetic acid (1.0: 1.5: 4.0: 2.0: 0.2 v/v/v/v/v) as mobile phase. Camag TLC Scanner III was used for the UV densitometric scanning at 285 nm. This system was found to give a compact spot of LF and DP at retention factor (R_f) value of 0.32±0.03 and 0.65±0.02 respectively. Linear regression analysis data for the calibration curve showed good relationship with respect to peak area in the concentration 100-500ng per spot for both LF and DP with (r²= 0.9993) and (r²= 0.9985) for LF and DP respectively. Limit of detection (15.51 ng/spot for LF and 23.04 ng/spot for DP), limit of quantification (47.03 ng/spot for LF and 69.83 ng/spot for DP) were found. The proposed HPTLC method has potential applications in determination of lafutidine and domperidone in tablet formulations.

Key words: Lafutidine; Domperidone; HPTLC; tablet formulation.

INTRODUCTION

Lafutidine¹ is chemically 2-[(2-Furanylmethyl)-sulfinyl]-N-[(2Z)4-[[4-(1-piperidinylmethyl)-2-pyridinyl]oxy]-2-butenyl]-acetamide (Fig 1). It is used as H₂ antagonist. For estimating LF, LC-ESI-MS method² has been reported in bioequivalence study, LC-tandem mass spectrometry method³ for the simultaneous determination of four H₂ antagonists in human plasma, UV simultaneous method⁴ and derivative spectroscopy method⁵, RP- HPLC⁶ method in tablet formulation, for combined dosage form with rabeprazole sodium. Domperidone⁷ is chemically, 5-Chloro-1-[1-[3-[2,3-dihydro-2-oxo-1H-benzimidazol-1-yl]propyl]-4-piperidinyl]-1,3-dihydro-2H-benzimidazol-2-one (Fig 2). It is used as peripheral dopamine antagonist. Literature survey reveals that RP-HPLC method⁸, HPTLC method^{9,10} and simultaneous estimation by spectrophotometric methods¹¹⁻¹⁴ have been reported for combined dosage forms with rabeprazole sodium, paracetamol, tramadol HCl and pantoprazole except lafutidine. Perusal of literature surveyed, shows that there is no HPTLC assay method for simultaneous estimation of LF and DP in combined dosage form. The aim of the present study is to develop and validate a simple, rapid, accurate, economical and reproducible method for the analysis of LF and DP in pharmaceutical formulation using HPTLC method. The proposed method was validated using International Conference on Harmonization Guidelines (ICH Guideline 1996)¹⁵.

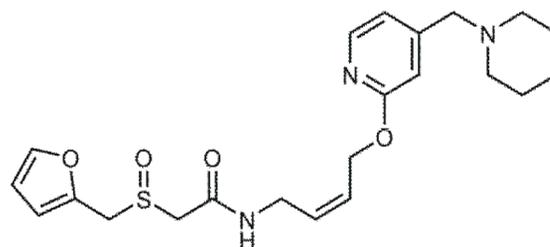


Fig. 1: Chemical structure of Lafutidine

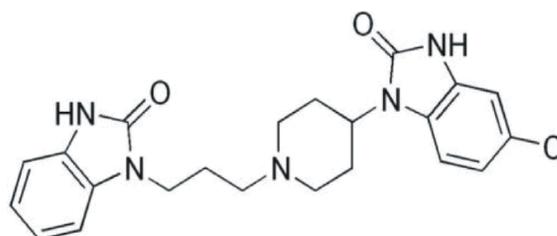


Fig. 2: Chemical structure of Domperidone

MATERIALS AND METHODS

Materials

Pharmaceutical grade LF and DP (gift samples) were obtained from Madras Pharmaceuticals Ltd., Chennai, India certified to contain LF (99.68%) and DP (99.74%) (w/w) on dried basis. Commercially available LAFAXID-D (Alkem) tablets claimed to contain 10mg of lafutidine, 30mg of domperidone were utilized in the present work.

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All the chemicals and reagents used in the study were of HPLC grade and were purchased from Merck Chemicals, Mumbai, India.

HPTLC instrumentation and conditions

HPTLC densitometric analysis was performed on 10×20 cm aluminum-packed plates coated with 0.2 mm layers of silica gel 60 F₂₅₄ (E-Merck, Germany). The plates were prewashed with methanol and activated at 110°C for 5 min, prior to the chromatography. Samples were applied to the TLC plates as 6 mm bands using a Camag Linomet 5 Automatic TLC sample applicator fitted with a Camag microlitre syringe. A constant application rate of 100 nl/s was used. Linear ascending development of the plates to a distance of 80 mm was performed with ethyl acetate: methanol: toluene: acetone: glacial acetic acid (1.0: 1.5: 4.0: 2.0: 0.2 v/v/v/v/v) as mobile phase in a Camag Automatic Developing Chamber previously saturated with mobile phase vapors for 30 min at the temperature (25±2°C) and relative humidity (60±5%).

Densitometrical scanning

The plate was scanned at 285nm, using a Camag TLC scanner III equipped with win CATS version 1.3.0 in absorbance mode and the deuterium lamp. The slit dimensions were 5.00×0.45 mm and the scanning speed was 20 mm/s.

Preparation of standard solution

LF and DP stock solution was prepared by taking 10mg in methanol and sonicated for 10 min to obtain standard stock solution concentration of 1000µg/ml. For assay, from the stock solution 0.5ml of LF and 1.5 ml of DP were transferred to 10 ml flask and diluted to volume with methanol to obtain the final concentration contains 50 mcg/ml for LF and 150 mcg/ml for DP. The standard working solutions of LF and DP were prepared by dissolving 1ml of each drug in 10 ml methanol separately to obtain final concentration of 100µg/ml of both LF and DP.

Preparation of Sample Solution

Twenty tablets (LAFAXID-D; Label claim 10mg of LF and 30mg of DP) were weighed and average weight was calculated. An amount of tablet powder equivalent to 10 mg of LF and 30 mg DP was accurately weighed, transferred into a 10ml volumetric flask and dissolved in methanol by sonication for 30 min and diluted up to the mark with methanol to get the stock solution. From this the assay solution containing 50µg/ml of LF and 150µg/ml of DP was prepared. The analysis was repeated six times.

METHOD VALIDATION

Validation of the optimized HPTLC method was carried out according to ICH norms using following parameters.

Linearity and range

From the standard working solutions 1 to 5 µl of LF and DP were spotted on the TLC plate to obtain the final concentration 100-500 ng/spot for both LF and DP. The plate was then developed using the previously described mobile phase and peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Precision

The precision of the method was verified by repeatability. The intra-day precision (RSD %) assessed by analyzing three different concentrations 200, 300 and 500 ng/spot for both LF and DP standard drug solutions within the calibration range of six replicates on the same day. Inter-day precision (RSD %) was assessed by analyzing drug solutions within the calibration range (200, 300 and 500 ng/spot for both LF and DP, from the drug solution, six times) on three different days over a period of a week.

Limit of detection and limit of quantitation

Limits of detection (LOD) and quantitation (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratio of 3 for LOD and 10 for LOQ respectively. LOD and LOQ were determined by standard deviation (SD) method. They were determined from the slope of the calibration (S) curve and SD of the Y-intercept of regression line using following equations: LOD = 3.3 X SD/S, LOQ = 10 X SD/S.

Robustness of the method

Robustness of the proposed HPTLC densitometric method was determined to evaluate the influence of small deliberate changes in the chromatographic conditions during determination of LF and DP. Robustness was determined by changing in detection wavelength and with different analyst.

Recovery

Accuracy of the method was carried out by applying the proposed method to the test sample (LF and DP combination tablet). To which a known amount of LF and DP sample corresponding to 80%, 100% and 120% of label claim (spiked method) and analyzed by running chromatogram in an optimized mobile phase. This was done to check the recovery of the drug at different levels in the formulation.

Specificity

The specificity of the method was ascertained by the analysis of drug standards and samples. Peak purity for LF and DP was assessed by comparing the spectra of standards with those acquired at three different points of spectra obtained from the test sample, i.e. the peak start (S), peak apex (M) and peak end (E) positions.

Analysis of a marketed formulation

The working sample and standard solution containing 50µg/ml of LF and 150µg/ml of DP was prepared. Two µl of the working solution was spotted (containing 100 ng/spot for LF and 300 ng/spot for DP) and developed in an optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined. The area of the peak corresponding to the R_f value of LF and DP standard was recorded and the amount present was calculated.

RESULTS AND DISCUSSION

Method development

The mobile phase composition was optimized to establish a simultaneous determination of LF and DP. The mobile phase ethyl acetate: methanol: toluene:

acetone: acetic acid (1.0: 1.5: 4.0: 2.0: 0.2 v/v/v/v/v) resulted in good resolution, sharp and symmetrical peaks LF for R_f 0.31 and DP for R_f 0.60. Densitometric scanning of the bands showed maximum absorbance at approximately 285 nm. The HPTLC chromatograms of standard and sample (LF and DP in LAFAXID-D (Alkem) tablets) LF and DP were recorded. The HPTLC chromatograms of LF and DP in standard and sample are shown in Fig. 3 and 4. The 3D spectrum is given in Fig. 5.

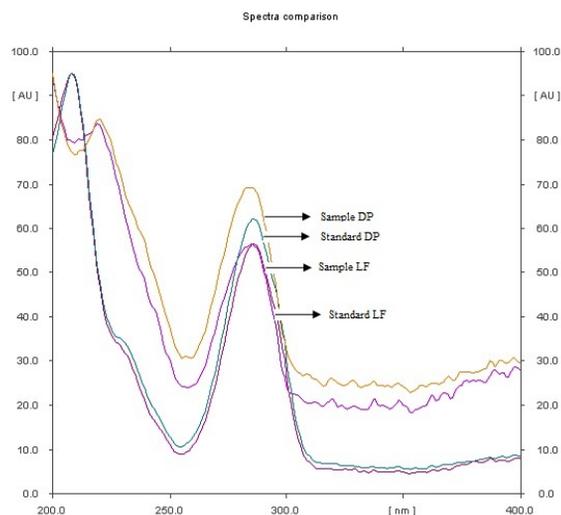


Fig. 3: Overlain spectra of standard and samples

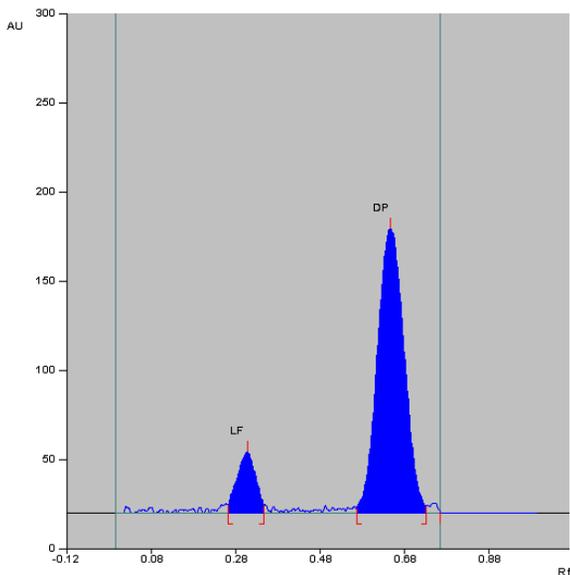


Fig. 4: Typical HPTLC chromatogram of LF and DP

Method Validation

The developed method was validated using different parameters such as the specificity, calibration curve, precision, recovery, robustness, LOD, LOQ and accuracy according to ICH norms.

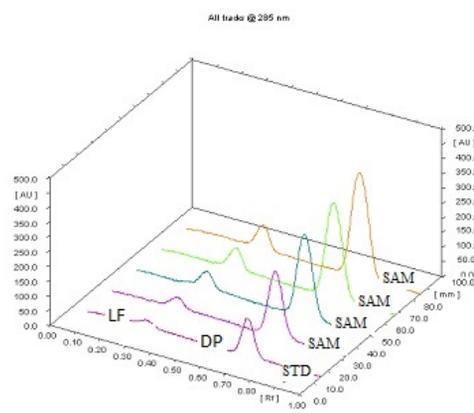


Fig. 5: 3D spectra of standard and samples

Linearity and range

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the range 100-500 ng/spot for both LF and DP (Fig 6 and 7). The linear regression equations were $Y=10.274X+160.21$ ($r^2=0.9993$) for LF and $Y=18.83X+321.15$ ($r^2=0.9985$) for DP. The Linear regression data is shown in Table: 1.

Linearity of lafutidine

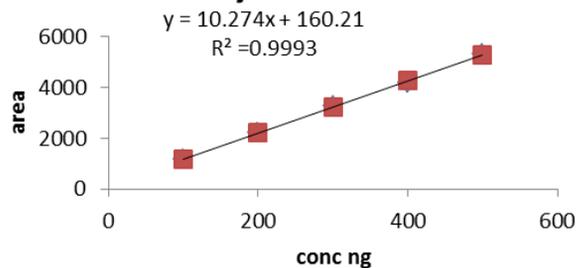


Fig. 6: Linearity curve of lafutidine

linearity of domperidone

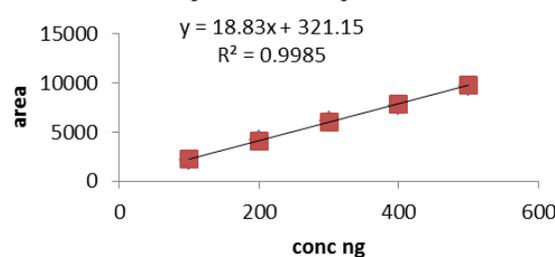


Fig.7: Linearity curve of domperidone

Table 1: Method validation parameters of proposed method

Validation parameters	Lafutidine	Domperidone
Linearity range ng/spot	100-500	100-500
Regression e equation	$Y=10.274x+160.21$	$Y= 18.83x+ 321.15$
Slope	10.27	18.83
Intercept	160.21	321.15
Correlation coefficient	0.9993	0.9985
LOD	15.51	47.03
LOQ	23.04	69.83

Precision

Results from determination of intraday and interday precision were expressed as SD and relative standard deviation (RSD %) which are shown in Table: 2. In intraday precision RSD was in the range 0.20, 0.41 and 0.20 for LF and 0.06, 0.05 and 0.06 for DP. In interday precision RSD was in the range 0.23, 0.33, 0.12 for LF and 0.05, 0.33, 0.02 for DP. These low values of % RSD indicate that the proposed method was precise and accurate.

Table 2: Precision of proposed method

Drugs	Conc. (ng/spot)	Intra-day amount found (ng)		Inter-day amount found (ng)	
		Mean SD N=6	%RSD	Mean SD N=6	% RSD
LF	200	199.98±0.419	0.20	200.01±0.48	0.23
	300	300.82±1.25	0.41	300.11±1.02	0.33
	500	500.42±0.83	0.20	499.48±0.50	0.12
DP	200	199.93±0.62	0.06	199.90±0.48	0.05
	300	300.73±0.63	0.05	299.26±0.40	0.03
	500	500.99±0.39	0.06	500.30±0.30	0.02

Limit of detection and limit of quantitation

The LOD and LOQ were found to be 15.51ng/spot and 47.03 ng/spot for LF and 23.04 ng/spot and 69.83 ng/spot for DP. This indicates the method is sufficiently sensitive.

Robustness of the method

The results of robustness are shown in Table: 3. Low values of relative standard deviation of peak areas were less than 2%. This % RSD indicates the robustness of the method.

Table 3: Robustness of the method

Parameters	Lafutidine peak area	SD/%RSD	Domperidone peak area	SD/%RSD
Nanometer change +2 nm	1344.62	2.92/0.21	5356.5	2.50/0.04
Nanometer change - 2 nm	2425.5	4.43/0.18	8046.72	2.17/0.027
Analyst I	99.30	0.50/1.34	98.49	0.06/0.26
Analyst II	99.50	0.41/0.83	99.33	0.18/0.84

Recovery

In the recovery study a known amount of LF and DP from the pharmaceutical dosage forms applied with 80%, 100% and 120% of label claim (i.e., spiked drug) for LF 80,100 and 120 ng/spot and for DP 240,300 and 360 ng/spot were spotted and developed in an optimized mobile phase. The results of recovery study were 99.10-100.50% for LF and 97.51-99.38 % for DP. The results of recovery study as listed in Table: 4.

Table 4: Recovery of proposed method

Drug mg/tablet	% amount added	Total amount mg	Amount recovered (mg)	% Recovery ±SD	%RSD
LF-10	80	8	7.93	100.62±0.34	0.43
	100	10	9.95	99.10±0.41	0.50
	120	12	11.9	100.50±0.10	0.35
DP-30	80	24	23.40	97.51±0.06	0.08
	100	30	29.80	99.38±0.66	0.06
	120	36	35s. 70	99.25±0.10	0.09

Specificity

The mobile phase resolved both the drugs very efficiently as shown in Fig 4 and 5. Typical overlain absorption spectra of LF and DP are shown in Figure 3; the peak purity of LF and DP was assessed by comparing their respective spectra at the peak start, apex, and end position of the spot, $r^2 = 0.9993$ and $r^2 =$

0.9985. A good correlation was also obtained between the standard and sample spectra of LF and DP respectively. Excipients from formulation did not interfere with the assay.

Analysis of marketed formulation

Experimental results for the amount of LF and DP in tablets, expressed in percentage of label claims were in good agreement and thereby suggesting that there is no interference from any of the excipients which are normally present in tablets. The drug content was found to be 98.49-99.30 for LF and 99.50-99.33 for DP and the result is tabulated in Table: 5.

Table 5: Analysis of the tablet Formulation

Brand name/Label claim and analyst	Lafutidine present at each tablet	SD/%RSD	Domperidone at each tablet	SD/%RSD
Lafaxid-D/LF 10mg, DP 30mg Analyst I	99.30	0.50/1.34	98.49	0.06/0.26
Analyst II	99.50	0.41/0.83	99.33	0.18/0.84

CONCLUSION

The HPTLC method established for simultaneous determination of LF and DP is simple, accurate, reproducible and sensitive. The results of proposed HPTLC method were highly reproducible, reliable and in good agreement with the labeled claim of the drug. It can be used for standardization and quality control of bulk and commercial formulations.

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