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Research Article

VALIDATED NORMAL PHASE HPTLC METHOD FOR SIMULTANEOUS QUANTIFICATION OF LEVOSULPIRIDE AND ESOMEPRAZOLE IN CAPSULE DOSAGE FORM

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ABSTRACT

Objective: Present research work was undertaken to develop a simple, rapid and precise high performance thin layer chromatographic (HPTLC) method for the simultaneous analysis of Levosulpiride (LSP) and Esomeprazole (ESP) in capsule dosage form.

Methods: Separation of Levosulpiride and Esomeprazole was achieved on precoated aluminium plates with silica gel 60 F_{254}. Solvent system used for separation was ethyl acetate: methanol: ammonia (9: 1: 0.5, v/v/v). Detection wavelength selected for the scanning in reflectance absorbance mode was 216 nm.

Results: The retardation factor (*Rf*) for LSP and ESP were found to be 0.30 ± 0.02 and 0.64 ± 0.02, respectively. The method was validated as per the ICH Q2 (R1) guidelines. Linearity range was found to be 100-1000 ng band^{-1} for both Levosulpiride and Esomeprazole.

Conclusion: The validated densitometric method can be used for the concurrent quantification of Levosulpiride and Esomeprazole in combined capsule dosage form.

Keywords: Levosulpiride, Esomeprazole, HPTLC, Validation.

INTRODUCTION

Levo enantiomer of sulpiride is Levosulpiride (LSP). Chemically it is N - [[(2S) - 1 -ethylpyrrolidin-2-yl] methyl] - 2 - methoxy - 5 – sulfamoylbenzamide [1]. LSP is an atypical antipsychotic and a prokinetic agent. It is used in several indications like depression, psychosis, somatoform disorders, emesis and dyspepsia [2]. The S-enantiomer of Omeprazole is Esomeprazole (ESP) and is official in IP, USP and EP.

This is the first optically pure enantiomer in the category of proton pump inhibitors and is mainly used to decrease the gastric acid secretion. Chemically it is 5 - methoxy -2- [(S) - [(4-methoxy-3, 5-dimethyl -2- pyridinyl) methyl] sulfinyl] -1H-benzimidazole-1-yl [3]. It is used in the treatment of gastroesophageal reflux disease (GERD) and to reduce the risk of gastric ulcers induced by non steroidal anti-inflammatory drugs.

ESP irreversibly inhibits the gastric parietal H^+/K ATPase which is mainly involved in hydrochloric acid production in stomach [4, 5].

Literature survey revealed that UV [6-10], HPLC [11-17] and HPTLC [18] methods are reported for the estimation of LSP and ESP either alone or in combination with other drugs. However no HPTLC method has been reported for the simultaneous estimation of LSP and ESP. The present work is an attempt to develop and validate a simple and accurate method for simultaneous estimation of LSP and ESP by densitometric method.

MATERIALS AND METHODS

Materials and chemicals

Levosulpiride was obtained as a gift sample from Wanbury Ltd, Mumbai. Esomeprazole was obtained as a gift sample from Cipla Ltd, Kurkumbh. The pharmaceutical dosage forms used for the assay study were Nexpro L (Torrent Pharmaceuticals Ltd.) and Sompraz L (Sun Pharmaceutical Industries Ltd.) purchased from local market. Both contain Levosulpiride 75 mg and Esomeprazole 40 mg per capsule.

All chemicals used were of analytical grade and purchased from Merck specialities Pvt. Ltd. (Mumbai, India). Water used in the study was double distilled. Precoated silica gel aluminium HPTLC plates 60 F_{254} were purchased from E. Merck, Mumbai, India.

Instrumentation and chromatographic conditions

HPTLC plates used for the chromatography were 20 cm × 10 cm in dimensions. Application of the sample spot on the plate was carried out by CAMAG (Muttenz, Switzerland) Linomat V sample applicator with a 100 µL (CAMAG) syringe. The spots applied were 6 mm wide and 5 mm apart. Chromatographic development was carried out in 20 cm × 10 cm twin trough glass chamber previously saturated with mobile phase for 10 min. at room temperature (25 ± 2 °C). The solvent front was 80 mm and 20 mL mobile phase was used per development. Plates were dried in the current of air. Densitometric scanning was performed in reflectance-absorbance mode at 216 nm using Camag TLC scanner III operated by winCATS software version 1.4.4.

Preparation of standard solution for linearity

A standard stock solution of LSP and ESP was prepared separately by dissolving 10 mg of standard drug in 10 mL methanol and 1 mL of the resulting solution was further diluted to 10 mL with methanol to get final concentration of 100 µg/mL.

Preparation of standard solution for recovery study

Standard solution was prepared by dissolving 8 mg of standard ESP and 15 mg of standard LSP in methanol in a 10 mL volumetric flask and finally diluted up to the mark with methanol. The final concentration of LSP and ESP in the solution was 1500 and 800 µg/mL, respectively.

Selection of detection wavelength

After chromatographic development bands were scanned in the range of 200 to 400 nm and spectra were overlain. LSP and ESP showed considerable absorbance at 216 nm and hence was selected for densitometric analysis.

Preparation of sample solution

Content of twenty capsules were weighed accurately; the average weight was calculated and finely powdered. Powder equivalent to 40 mg of ESP and 75 mg of LSP was weighed and transferred into 50 mL volumetric flask, sonicated for 15 min. and diluted up to mark with methanol to obtain the final concentration 800 and 1500 µg/mL of ESP and LSP, respectively. The solution was filtered

through Whatman filter paper no.41 and first few drops of filtrate were discarded.

Method validation

The developed method was validated for linearity, range, precision, accuracy, specificity, LOD and LOQ as per ICH Q2 (R1) guidelines [19].

Linearity and range

Linearity was evaluated by applying five different concentrations six times to the HPTLC plate in the range of 100 - 1000 ng band^{-1} for both LSP and ESP. Calibration curve of peak area versus concentration was plotted and data was subjected to least square linear regression analysis and the slope, intercept and correlation coefficient for the calibration curve were estimated.

Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) were calculated to determine sensitivity as 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the linearity plot.

Specificity

In specificity studies, LSP and ESP standard solutions and the marketed sample solutions were applied on a HPTLC plate. The plate was developed in the mobile phase and scanned. The peak purity of LSP and ESP were assessed by comparing the UV spectra of drugs at peak start, peak apex and peak end positions of the band i.e., r (start, middle) and r (middle, end).

Precision

Precision of the method was analyzed by intra and inter-day variation studies. To study intra-day variation, sets of three different drug sample concentrations of LSP and ESP in triplicates (400, 600 and 800 ng band-1) were spotted and analyzed on the same day. To study inter-day variation study, triplicates of above mentioned three different drug concentration were analyzed on three consecutive days.

Accuracy

The accuracy of the method was evaluated by standard addition method. Samples of LSP and ESP were spiked with 80, 100 and 120 % of standard LSP and ESP.

Robustness: Robustness was studied by carrying out small but deliberate changes in the analytical conditions. The analytical conditions varied were mobile phase combination (± 0.1 mL), amount of mobile phase (± 5 %), time from band application to chromatographic development and time from chromatography to scanning (+ 10 min). One factor was varied at a time to study the effect. The robustness of the densitometric method was studied six times at concentration of 600 ng band^{-1} for both LSP and ESP. The standard deviation of peak areas and % relative standard deviation (% RSD) were calculated for each variable factor.

Solution stability

Solution stability of LSP and ESP standard solutions (100 ng band^{-1}) was studied at an interval of 6 hrs up to 48 hrs when stored at room temperature and estimated by comparing peak areas at each time interval against freshly prepared standard solution.

RESULTS AND DISCUSSION

Optimization of HPTLC method

To obtain the desired *Rf* value range (0.2 - 0.8), minimum resolution (Rs ≥ 1.5), different mobile phases containing various ratios of toluene, dichloromethane, n-hexane, ethanol, methanol, water, ethyl acetate, and acetone were tried. Finally, the mobile phase consisting of ethyl acetate: methanol: ammonia (9: 1: 0.5, v/v/v) was selected as it gave well resolved peaks. The optimum wavelength for detection and quantitation used was 216 nm. The retardation factor (*Rf*) for LSP and ESP were found to be 0.30 ± 0.02 and 0.64 ± 0.02, respectively (Figure 2).

Table 1: Linear regression data for the calibration curves (n = 6).

Parameters	Levosulpiride	Esomeprazole
Linearity range[a]	100 - 1000	100 - 1000
r^2	0.9994	0.9994
Slope	6.1366	7.6853
Intercept	331.24	50.921
LOD[a]	31.363	30.631
LOQ[a]	95.042	92.822
Sy.x	58.323	70.594

n - no of replicates, [a] Concentration in ng band^{-1}, r^2 - Square of correlation coefficient, LOD - Limit of detection, LOQ - Limit of quantitation, Sy.x - Standard deviation.

Table 2: Intra and inter day precision (n = 3).

Standard drugs	Concentration Taken [a]	Concentration obtained [a]		Precision obtained [b]	
		Intra day	Inter day	Intra day	Inter day
Levosulpiride	400	399.485	412.575	0.957	0.825
	600	616.869	609.807	0.822	0.943
	800	812.688	840.011	0.774	0.876
Esomeprazole	400	393.272	398.52	0.982	0.937
	600	604.584	604.281	1.068	0.874
	800	796.639	807.396	1.166	0.802

n - no of replicates, [a] Concentration in ng band^{-1}, [b] Precision as % RSD, RSD - Relative standard deviation.

Table 3: Results of recovery studies (n = 6)

Parameter	LSP			ESP		
Amount Taken[a]	375	375	375	200	200	200
Amount Added[a] (%)	300 (80)	375 (100)	450 (120)	160 (80)	200 (100)	240 (120)
Amound Found[a]	665.661	753.011	809.611	358.686	392.357	448.199
% Recovery	98.616	100.401	98.135	99.635	98.089	101.863
SD	70.193	63.921	31.139	49.131	39.513	55.608
%RSD	1.589	1.291	0.588	1.750	1.289	1.591

n - no of replicates, [a] Concentration in ng band^{-1}, SD - Standard deviation, RSD - Relative standard deviation.

HPTLC method validation

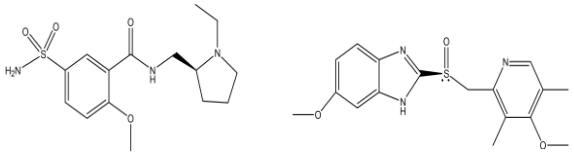
Linearity and range

Calibration curves of standard drugs concentration and peak areas found to be linear over a range of 100 to 1000 ng band^{-1} for both LSP and ESP (Table 1).

Sensitivity

The LOD and LOQ for LSP and ESP were found to be 31.363 and 95.042 ng band^{-1} and 30.631 and 92.822 ng band^{-1}, respectively.

Specificity: The peak purity for LSP and ESP was assessed by comparing visible spectra acquired at the start (S), apex (M), and end (E) of the peak obtained from the scanning of band, that is, r (S, M) = 0.999, 0.998 and r (M, E) = 0.999, 0.998, respectively. Peak purity data showed that peaks obtained for LSP and ESP were pure.



1a 1b

Fig. 1: Chemical structures of Levosulpiride (1a) and Esomeprazole (1b).

Table 4: Robustness testing (n = 6, 600 ng band^{-1})

Parameter	SD of concentration found		% RSD	
	Levosulpiride	Esomeprazole	Levosulpiride	Esomeprazole
Mobile phase (ethyl acetate) composition (± 0.1 mL)	6.43	11.76	0.23	0.37
Amount of mobile phase (± 5 %)	14.4	9.97	0.54	0.33
Time from band application to chromatography (+ 10 min)	6.06	12.09	0.22	0.39
Time from chromatography to scanning (+ 10 min)	9.5	9.07	0.35	0.29

n - no of replicates, SD - Standard deviation, RSD - Relative standard deviation **Figure legends**

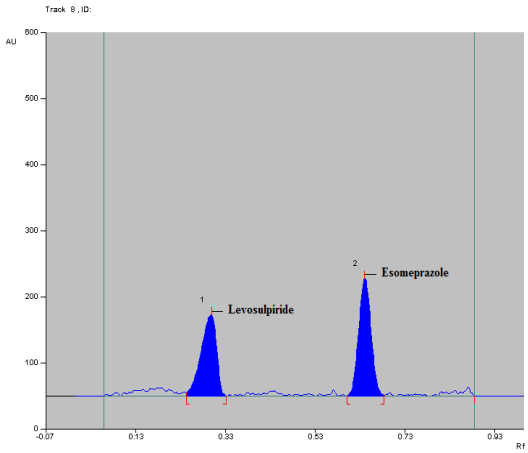


Fig. 2: Densitogram obtained from mixed standard solution of Levosulpiride and Esomeprazole scanned at 216 nm Precision

Intra-day precision, as % RSD was found to be 0.774 – 0.957 % for LSP and 0.982 -1.166 % for ESP. Inter-day variation, as % RSD was found to be 0.825 – 0.943 % for LSP and 0.802 – 0.937 % for ESP. As recommended by ICH guidelines, both intra and inter-day precision studies showed % RSD < 2, indicating good precision (Table 2).

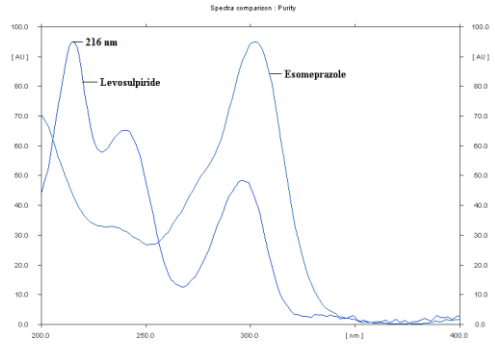


Fig. 3: Ultra violet spectrum of Levosulpiride and Esomeprazole standards

Accuracy

Recovery for LSP and ESP was found to be 98.13 - 100.4 % w/w and 98.08 - 101.86 % w/w, respectively indicating reliability of the method for simultaneous estimation of LSP and ESP in the marketed formulation used in the study (Table 3).

Robustness studies

Robustness of the densitometric method was checked after deliberate alterations of the analytical parameters (Table 4). It showed that peak areas of interest remained unaffected by small changes of the operational parameters (% RSD < 2) which indicate that the method is robust.

Solution stability

Stability of standard solutions of LSP and ESP were assessed at room temperature for 48 hrs. The % RSD was found less than 2 indicate that the solutions were stable for 48 hrs. at room temperature.

Analysis of marketed formulation

Developed densitometric method was applied to the selected marketed formulation. Nexpro L was found to contain 98.65 ± 1.08 and 101.23 ± 1.59 % w/w of LSP and ESP, respectively and Sompraz L was found to contain 99.31 ± 0.99 and 100.47 ± 1.91 % w/w of LSP and ESP, respectively.

CONCLUSION

The developed and validated densitometric method is rapid, simple, precise and accurate. Statistical results prove repeatability and selectivity of the method which can be easily applied for simultaneous quantification of LSP and ESP in pharmaceutical formulations.

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