



Original Article

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Levosulpiride and Pantoprazole in Combined Pharmaceutical Dosage Form

K Mamatha *, VVLN Prasad, M Akiful Haque, Vasudha Bakshi

Department of pharmaceutical analysis and quality assurance, School of Pharmacy, Anurag group of institutions, Venkatapur, Rangareddy district, Telangana, India

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ABSTRACT

The objective of present work was to develop and validate a simple, accurate, precise HPLC method for the estimation of levosulpiride and pantoprazole. The chromatographic separation was achieved on a Hypersil BDSC18 column (4.6x250 mm, 5µm particle size). Different mobile phase systems in different proportions were tried. For HPLC method a mobile phase consisting of K₂HPO₄ and acetonitrile (70:30) produced symmetric peak shape with good resolution for both the drugs. Next, the drugs were chromatographed under different flow rates from which a flow rate of 1.2 ml/min was selected. The retention times of Levosulpiride and Pantoprazole were found to be 8.77 min and 2.77 min, respectively. The proposed method was found to have excellent linearity in the concentration range of 1-7 µg/ml with correlation coefficient $r^2=0.999$ and 0.999 for Levosulpiride and Pantoprazole respectively. The method was validated for linearity, precision, LOD, LOQ and robustness. The proposed method optimized and validated as per ICH guidelines.

Keywords: levosulpiride, pantoprazole, precision.

Corresponding author *

K Mamatha

School of Pharmacy, Anurag group of institutions, Venkatapur,
Rangareddy district, Telangana, India
E Mail: mamatha.kokkula820@gmail.com

1. INTRODUCTION

Levosulpiride and Pantoprazole both are proton pump inhibitors. Levosulpiride is chemically C₁₉H₁₉N₂ 8-chloro-6, 11-dihydro-11 (4piperdinylidene)-5H benzo [5,6] cyclohepta [1,2-b] pyridine. Levosulpiride is a

Histamine H₂ Antagonist. It belongs to Phenylpropens. It is freely soluble in water, Methanol. Sparingly soluble in ethanol. Pantoprazole belongs to the class of substituted benzimidazol. It is very soluble in Ethanol, Water.

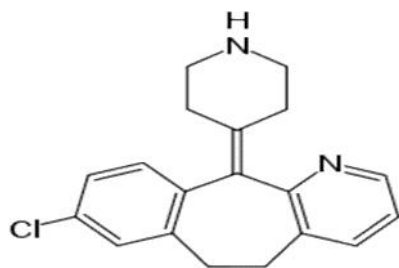


Fig 1: Levosulpiride

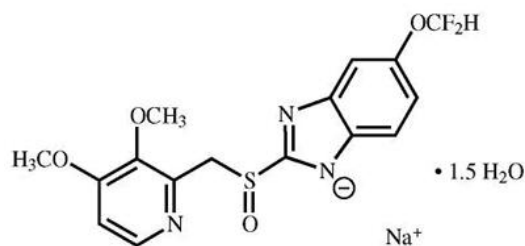


Fig 2: Pantoprazol

Literature survey revealed that several methods were reported for Levosulpiride and Pantoprazole individually and in combinations S.Malathi,RN Dubey,R.Venkatanarayanan et al 2009;Darshan shah,Smitha talaviy,Mandev patel 2012,Siddilingaiah swamy et al, Shah,2006;2010; Krzysztof, 2001; Dhoka, 2010;. Tim reyns,2006;prabhu.s, 2010; Raj k, 2010;Rathinavel,2008; ,Shah,j,2010;s.low, 1989;s.s.Zade,2013; B.thomas, 2010; Khaja, 2010; Deshpande et al., 2010; Nanda, 2009). Therefore, the main objective of this study was to attempt to develop a simple and rapid analytical method for simultaneous estimation of levosulpiride and pantoprazole in a combined dosage form and validate the proposed assay.¹⁻³

2. MATERIALS AND METHODS

Apparatus: The HPLC waters 2690/5 liquid chromatograph equipped with a PDA detector, the software installed was Empower, with 20 μ l loop, Hypersil-BDS C18 column (250mmx4.6mm,5 μ l). The

other instrument included are(SARTORIOUS) electronic balance and a sonicator (Fast clean).

Chemicals and reagents

Active pharma ingredient of levosulpiride and pantoprazole was obtained as a gift sample from Arch pharma ltd, purified water HPLC grade was prepared by triple glass distillation and filtered through a 0.45 μ membrane filter. K₂HPO₄ and Acetonitrile HPLC was run at a flow rate of 1.2ml/min, 20 μ l of the sample was injected in the chromatographic system. Mobile phase comprising of K₂HPO₄ Acetonitrile at the ratio (70:30). The column temperature was ambient with a detection wavelength of 259.

Preparation of standard solution

Stock solutions were prepared by dissolving 10mg of levosulpiride and 10mg of pantoprazole in mobile phase separately. Aliquots of standard solution of levosulpiride and pantoprazole were transferred into 10ml volumetric flasks and solutions were made up to the volume to yield concentrations of levosulpiride and pantoprazole.

Pharmaceutical formulation

Formulation Pantocid-L, manufactured by Sun pharmaceutical Ltd was purchased from the local pharmacy in Hyderabad.

Preparation of sample solution

For analysis of commercial formulation, 20 tablets of oratil cv of levosulpiride 125 mg and pantoprazole 200mg were weighed the average weight was calculated and powdered. A quantity equivalent to 125mg of levosulpiride and 200mg of pantoprazole was weighed and transferred to a 100ml volumetric flask which contain mobile phase and then shake it for 10mins and sonicate it for 20mins. The solution was allowed to stand at a room temperature for 20-30mins and filtered it through a whatmann filter paper. Then suitable aliquots of formulation solution were prepared

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and injected into HPLC to obtain concentration in linearity range.⁴⁻⁶

Validation of analytical method

ACCURACY: Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. Recovery studies of the drug were carried out for determining accuracy parameter. Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out in 50% 100% and 150% levels.^{7,8}

PRECISION: The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The Precision expressed as standard deviation or relative standard deviation.

a. System precision: Standard solution prepared as per test method and injected five times.⁹

b. Method precision: Prepare five sample preparations individually using the single as per test method and injected each solution

LINEARITY: The linearity of analytical method is the ability to elicit test results that are directly proportional to the concentration of analyte in the sample within the given range. The linearity was performed by seven different concentrations, which were injected and calibration curve were plotted. The linearity of levosulpiride and pantoprazole was found to be in the range of 1-7 μ g/ml respectively. The chromatograms of the resulting solutions were recorded. The plot showing linearity and range study for levosulpiride and pantoprazole is shown in figure.

Plot of linearity and range study for levosulpiride

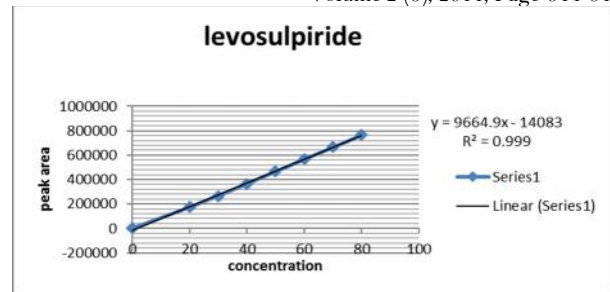


Fig 3: Plot of linearity and range study for levosulpiride

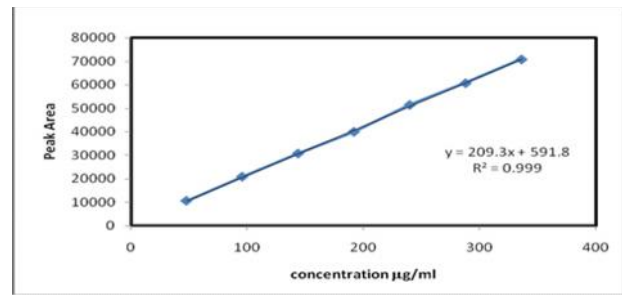
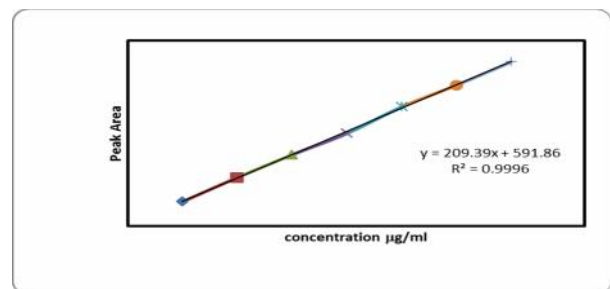


Fig 4: Plot of linearity and range study for pantoprazole

Ruggedness:

a) System to System variability: System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. A comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variables.^{10,11}

Robustness: The robustness of an analytical procedure are a measure of its capacity to remain unaffected by small, but deliberate changes in the method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions



including changes of combination of mobile phase and flow rate. % RSD of assay was calculated.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD of an analytical procedure is the lowest concentration of an analyte in a sample which can be detected but not necessarily quantitated as an exact value where as LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

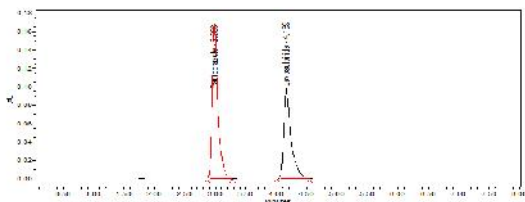


Fig 5: Optimized chromatogram

Table 1: Results of validation parameters of RP-HPLC

Sl. no	Validation Parameter	Levosulpiride	Pantoprazole	Acceptance Criteria
1.	Linearity (in µg)	1-7	48-336	Correlation coefficient
2.	Regression Line Equation	$y = 1585x + 764.2$	$y = 209.3x + 591.8$	($R^2 = 0.999$)
3.	R^2 Value	0.999	0.999	
4.	Precision			
	System Precision (%RSD)	1.57	0.14	RSD < 2%
	Method Precision (%RSD)	1.28	1.53	
5.	LOD	0.0673	3.16	
6.	LOQ	0.202	9.488	-
7.	Assay of marketed formulation	99.25%	98.52%	95-105%
8.	% Recovery	99.07-99.36	99.67-100.6	95-105%
9.	Ruggedness	0.4	0.34	RSD < 2%

3. RESULTS AND DISCUSSION

➤ The slope, intercept and correlation coefficient values were found to be 9664, 14083 and 0.999 and 10675, 14731 and 0.999 for levosulpiride and pantoprazole respectively.

- The LOD of levosulpiride and pantoprazole were found to be 0.0673 µg/ml and 3.16 µg/ml respectively. The LOQ of clavulanic acid and cefpodoxime proxetil found to be 0.202 µg/ml and 9.488 µg/ml respectively.
- Precision of the developed method was studied. Low % RSD values indicate that the method is precise.

4. CONCLUSION

The proposed RP-HPLC method for the estimation of the levosulpiride and pantoprazole in the pharmaceutical dosage form were simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification. The recoveries achieved was good by RP-HPLC method. The methods can be recommended for routine and quality control analysis of these drugs in the pharmaceutical dosage forms. In this proposed method symmetrical peaks with good resolution were obtained.

Table 2: Summary of analysis of levosulpiride and pantoprazole by RP-HPLC METHOD

Drugs	Labeled amount, mg/ tablet	Estimated Amount mg/tablet	% Label claim	% *RSD
LEVO	200	197.56	98.52	1.73
PANTO	125	124.5	99.25	1.13

5. ACKNOWLEDGEMENT

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