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METHOD DEVELOPMENT AND VALIDATION OF LEVOSULPIRIDE AND ESOMEPRAZOLE BY USING RP-HPLC METHOD

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ABSTRACT

A simple Reverse phase high performance liquid chromatographic method has been developed and subsequently validated for Levosulpiride and Esomeprazole Capsules. The separation was carried out by using a acetonitrile and phosphate buffer (pH) 6.8 (50:50 v/v). The detection was carried out at 281 nm. The column was Zorbax ODS C_{18} (250 x 4.6mm). The peak areas corresponding to the concentration range of Levosulpiride 1.5 -10.5 µg/mL and Esomeprazole 20-140 µg/mL prepared in triplicate were plotted against the respective concentrations. The calibration curves were linear in the range studied for Levosulpiride and Esomeprazole, respectively, with mean correlation coefficients (n = 3) of 0.999 . Accuracy of the method was examined by performing recovery studies by standard addition method for drug product. The recovery of the added standard to the drug product sample was calculated and it was found to be 100.64 %w/w and 100.29%w/w for Levosulpiride and Esomeprazole respectively and the % RSD was less than 2 for both the drugs which indicates a good accuracy of the method.

KEYWORDS: Method development, Validation, Levosulpiride, Esomeprazole.

INTRODUCTION

Levosulpiride is an atypical antipsychotic drug primarily used for gastrointestinal disorders, though it is also used to treat certain psychiatric conditions.

Esomeprazole (brand name Nexium, among others) is a proton pump inhibitor (ppi) used to reduce the amount of acid produced in the stomach. It is available by prescription in higher doses and over-the-counter in lower doses for frequent heartburn.

METHOD DEVELOPMENT

Selection of Mobile Phase

The pure drugs Levosulpiride and Esomeprazole were injected in combination in the ratio of their contents in the capsule formulation to the chromatographic system, and run in different mobile phase compositions. Different mobile phases containing different compositions of methanol: water, acetonitrile: water, and acetonitrile: Phosphate buffer were tried for the selection of optimum conditions for the simultaneous determination of Levosulpiride and Esomeprazole. It was found that optimal separation of the two components was achieved with acetonitrile and phosphate buffer (KH_2PO_4 buffer), compared to other mobile phases. Different ratios of the selected mobile phase were tried with varying flow rates and pH. Finally, the mobile phase composition selected for the chromatographic separation of Levosulpiride and Esomeprazole was acetonitrile and phosphate buffer of pH 6.8 in the ration of 50:50 v/v.

Preparation of Mobile Phase

Phosphate buffer pH 6.8: 50mL of 0.2M KH_2PO_4 and 22.4mL of 0.2M NaOH were dissolved in 100mL of distilled water.

Mobile phase was prepared by mixing 500 mL of acetonitrile with 500 mL of phosphate buffer and its pH adjusted to 6.8. The mobile phase was sonicated for 15 min and filtered through a 0.45 µm membrane filter paper.

Preparation of Standard f Stock f Solutions

25 mg each of standard Levosulpiride and Esomeprazole were weighed accurately and transferred in to two separate 25mL flasks, and dissolved in 10mL of solvent, the volume was made up to the mark with solvent to obtain a solution of concentration of 1000 µg/mL of each Levosulpiride

and Esomeprazole (standard A2 respectively). From the above stock solution A1 and A2 respectively 1.5mL and 20 mL aliquots were pipetted in to a 50mL volumetric flask and dissolved in 25mL of the solvent and made up to the mark with the solvent to obtain a final concentration of 400 and 30µg/mL of Esomeprazole and Levosulpiride respectively (working stock solution). The stock solution were filtered through a 0.45µm membrane filter and sonicated for 15min in an ultrasonic bath sonicator.

Preparation of Standard Solution

Transfer 5 mL of standard stock solution into a 20 mL volumetric flask, and dilute with diluent.

Selection of Analytical Wavelength

UV Spectrophotometric determination of Levosulpiride and Esomeprazole of concentration 20µg/mL individually, in overlay mode shows that both the drugs absorb appreciably at 281nm, hence 281nm was selected as the detection wavelength.

MATERIALS USED FOR METHOD DEVELOPMENT AND VALIDATION

INSTRUMENT USED

HPLC: Agilent 1260 series

Software : Open lab

pH Meter : Range from 0 -14.Deep vision

Analytical balance : Accurate to 0.001g- Mettler

Solvent filtration Unit : Milli pore

Syringe filters : Nylon 0.22µ filter, PVDF 0.45 µ filters

Centrifuge : Remi

Sonicator : 1.5LH ultrasonic bath.

Specification	
Column	C18 (Zorbax ODS Column 250mmX 4.6mm), 5µ
Detector	DAD
Temperature	Ambient
Wavelength	281 nm

Analysis of Capsule Formulation

Preparation of Sample Stock Solution

The contents of twenty marketed LIS todac capsules were weighed accurately and their average weight was determined. A mass equivalent to 40 mg Esomeprazole and 3mg Levosulpiride from the contents of the capsule were taken in a 100mL volumetric flask and dissolved in 50mL of the solvent. The solution was kept for sonication 15min. The solution was made up to the mark with the solvent and filtered through a 0.45 µ membrane filter paper sample stock solution 'A'.

Preparation of Sample Solution

Transfer 5.0 mL aliquot of the working sample stock solution A was diluted to 20 mL to obtain a concentration of 7.5 and 100µg/mL of Levosulpiride and Esomeprazole respectively.

Six replicates solutions of this concentration were prepared. A 20µL volume of these solutions were injected to the chromatographic system and their respective chromatograms were recorded at 281nm. From the peak areas the amount of drug present in each sample was determined.

The objective of this experiment was to optimize the assay method for estimation of Levosulpiride and Esomeprazole based on the literature survey and the trails made. The trials mentioned below describes how the optimization was done.

OPTIMIZED HPLC METHOD

After several trials with the different combinations and ratios of solvents, chromatographic parameters of trial-3 were optimized.

Preparation of Mobile phase:

Acetonitrile and Phosphate buffer (pH 6.8) were mixed in the ratio of 50:50 and sonicated to degas.

Chromatographic f condition

Column	:	Zorbax f ODS f (250 f x f 4.6mm, f 5µ)
Column f Temperature	:	Ambient
Flow f rate	:	1.0ml/min
Injection f volume	:	20µl
Detector f wave f length	:	281nm
Run f time	:	10 f min

CONCULSION

Good f separation f and f resolution f between f Levosulpiride f and f Esomeprazole peaks f was f observed.

METHOD VALIDATION

Preparation of Standard Stock Solution

25 f mg f each f of f standard f Levosulpiride f and f Esomeprazole f were f weighed f accurately f and f transferred f in f to f two f separate f 25mL f flasks, f and f dissolved f in f 10mL f of f solvent, f the f volume f was f made f up f to f the f mark f with f solvent f to f obtain f a f solution f of f concentration f of f 1000 f µg/mL f of f each f Levosulpiride f and f Esomeprazole f (standard f stock f solutions f A1 f and f A2 f respectively). f From f the f above f stock f solution f A1 f and f A2 f respectively f 1.5mL f and f 20 f mL f aliquots f were f pipetted f in f to f a f 50mL f volumetric f flasks f and f dissolved f in f 25mL f of f the f solvent f and f made f up f to f the f mark f with f the f solvent f to f obtain f a f final f concentration f of f 400 f and f 30µg/mL f of f Esomeprazole f and f Levosulpiride f respectively f (working f stock f solution). f The f stock f solutions f were f filtered f through f a f 0.45µm f membrane f filter f and f sonicated f for f 15min f in f an f ultrasonic f bath f sonicator.

Table 1: System suitability parameters

Parameters	LIS f	ESM f
Retention Time (min)	4.535	5.400
Tailing	1.1	1.3
Resolution	-	2.79
Theoretical f Plates	13127	14273
%RSD	1.09	0.78

Observation

From the system suitability studies it was observed that all the parameters are within limit, hence it is concluded that the Instrument, Reagents and Column are suitable to perform Assay.

Acceptance criteria

The % RSD of Levosulpirideand Esomeprazole peak areas should be NMT 2%.

The number of theoretical plates (N) for the Levosulpirideand Esomeprazole peaks is NLT 2000.

The Tailing factor (T) for the Levosulpirideand Esomeprazole peaks is NMT 2.0.

LINEARITY

Aliquots of 0.5, 1, 1.5, 2, 2.5, 3, 3.5 mL of working stock solution was serially diluted to 10mL in separate volumetric flasks to obtain a solution of concentrations in the range 1.5-10.5µg/mL of Levosulpiride and 20-140µg/mL of Esomeprazole.

Table 2: Statistical Linearity Validation Data of Levosulpiride and Esomeprazole.

Parameter	Levosulpiride	Esomeprazole
Linearity (µg/mL)	1.5-10.5	20-140
Correlation coefficient	0.9997	0.9993
Slope	754116.2	670332
y intercept	45173.75	-1839124
Limit of detection (µg/mL)	0.144	0.996
Limit of quantitation (µg/mL)	0.431	2.987

ACCURACY

Procedure for the Determination of Accuracy

Recovery studies were performed by applying standard addition method. To a known amount of the pre-analysed drug sample an 80%, 100%, and 120% of standard drug substance was added and suitably diluted. The peak areas of the resultant solutions were measured at 281nm. The amount recovered at each recovery level was determined by substituting the peak response values

in the regression equation.

In 80% recovery level concentration the amount of standard added was 2.4mg Levosulpiride and 32mg Esomeprazole (80% addition). In 100% recovery level concentration the amount of standard added was 3mg Levosulpiride and 40mg Esomeprazole (100% addition). In 120% recovery level concentration the amount of standard added is 3.6mg Levosulpiride and 48mg Esomeprazole (120% addition). To each of the above three recovery levels a sample concentration equivalent to 0.6mg of Levosulpiride and 8mg of Esomeprazole of the capsule dosage form was added.

Table 3: Statistical Validation Data of Accuracy.

	Mean		**Standard deviation**		**% RSD**	
Level of recovery	**LIS**	**ESM**	**LIS**	**ESM**	**LIS**	**ESM**
80 %	3.018	39.698	0.0258	0.3504	0.856	0.882
100 %	3.608	48.339	0.0372	0.3070	1.032	0.635
120%	4.245	56.551	0.0368	0.3787	0.867	0.669

PRECISION

Procedure for the Determination of Precision

The precision of the analytical method was determined a minimum of 6 determinations at the 100% test concentrations. An amount equivalent to 3mg Levosulpiride and 40mg of Esomeprazole was weighed accurately and transferred to a 100mL volumetric flasks and dissolved in a small quantity of solvent and the content was kept in a sonicator for 10min. finally the volume was made up to the mark with the solvent. The solution was filtered through 0.45µ Nylon filter. The above sample solution was suitably diluted with the solvent to obtain a solution of concentration 7.5µg/mL Levosulpiride and 100µg/mL Esomeprazole.

Intra-day Precision

In intraday precision six replicate sample matrices containing 7.5µg/mL Levosulpiride and 100µg/mL Esomeprazole were chromatographically analysed at different time intervals on the same day. The variation of the results within the same day was analysed and statistically validated.

Table 4: Statistical validation data of intraday Precision.

Drug component	Mean	Standard deviation	%RSD
Levosulpiride	99.77 %	0.58	0.58
Esomeprazole	99.76 %	0.78	0.78

LIMIT OF DETECTION

Limit of detection was determined based on the standard deviation of y intercepts of the regression line. The standard deviation of y intercepts obtained from the replicate measurements (n=3) was substituted for σ in the equation 3.3σ/S, and S is the mean of slope of the calibration curves.

LIMIT OF QUANTITATION

Limit of quantitation was determined based on the standard deviation of y-intercepts of the regression line. The standard deviation of y intercepts obtained from the replicate measurements (n=3) was substituted for σ in the equation 10σ/S, and s is the mean slopes of the three calibration curves.

TheLODandLOQvalues weredetermined bythe formulae LOD=3.3XS/mandLOQ=10 f S f /m

SUMMARY

Table 5: Analytical method validation report for Levosulpiride and Esomeprazole.

Parameter	Results	
	Levosulpiride	**Esomeprazole**
wavelength (nm)	281	
Rt (min)	4.5	5.4
Regression equation	y=754116.2x+45173.75	y=670332x-1839124
Correlation coefficient	0.9996	0.9992
Accuracy	100.64 %	100.29 %
LOD (µg/ml)	0.14	0.99
LOQ (µg/ml)	0.43	2.98
Assay	100.2 %	99.4 %
Precision (%RSD)		
Intraday precision	0.58	0.78
Inter day precision	0.65	0.53
Robustness (%RSD)		
Flow rate 1.2mL/min	0.74	0.66
Flow rate 0.8mL/min	0.74	0.66

wavelength 280nm	0.66	0.53
wavelength 282nm	0.95	0.87
Ruggedness (%RSD)		
Analyst 1	0.93	0.73
Analyst 2	0.67	0.58

CONCLUSION

A RP-HPLC method for Levosulpiride and Esomeprazole were developed and validated in capsule dosage form as per ICH guidelines. The results are found to be complying with the acceptance criteria for each of the parameter.

Agilent HPLC (Open Lab software with DAD detector) with Zorbax ODS C_{18} (250X 4.6mm, 5µ) Packed Column, Injection volume of 20µL was injected and eluted with the Mobile phase (Acetonitrile : Phosphate buffer pH 6.8, in the ratio of 50:50%v/v) Which was pumped at a flow rate of 1.0 mL at 281nm. The peak of Levosulpiride and Esomeprazole was found well separated at 4.5 min, 5.4 min. The developed method was validated for various parameters as per ICH guidelines like system suitability, linearity, accuracy, precision, specificity, limit of detection, limit of quantitation, ruggedness, and robustness.

Hence it is concluded that the assay method is found to be valid in terms of reliability, precision, accuracy and specificity and hence it is suitable for routine analysis as well as for stability analysis.

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