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Research Article

DEVELOPMENT AND VALIDATION OF A RP- HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ESOMEPRAZOLE AND LEVOSULPIRIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract

To develop new, simple and economic reversed phase high performance liquid chromatography (RP-HPLC) method for simultaneous estimation of Esomeprazole (ESO) and Levosulpiride (LEVO) in bulk and pharmaceutical dosage form and validate as per ICH guidelines. The method involved Jasco HPLC system on Hi-Q Sil C18 (25 cm × 4.6 mm i.e., 5 µm) column and Isocratic mobile phase contains methanol: buffer (pH 3) (70:30% *v/v*) at a flow rate of 1.2 mL/min. The eluant was monitored at 260 nm. Linearity range 20 to 120 µg mL^{-1} and 75 to 450 µg mL^{-1} for esomeprazole and levosulpiride respectively. The retention time of levosulpiride was eluted at 2.2 min and esomeprazole was eluted at 5.3 min with a correlaton coefficient of of 0.9991 and 0.9997 respectively. The esomeprazole and levosulpiride of precision, percentage recovery was found to be within specified limits (i.e. 99-102%). The new developed method was validated for linearity, precision, accuracy, LOD and LOQ as per ICH guidelines and can be used for routine quality control analysis.

Keywords: RP-HPLC, Esomeprazole, Levosulpiride, simultaneous estimation, Validation.

Introduction

Esomeprazole is a proton pump inhibitor which is used in the treatment of dyspepsia, peptic ulcer disease (PUD), gastroesophageal reflux disease(GORD/GERD) and Zollinger-Ellison syndrome. Esomeprazole is the S-enantiomer of omeprazole. Esomeprazole is reduces acid secretion through inhibition of ATPase in gastric parietal cells. By inhibiting the functioning of this enzyme, the drug prevents formation of gastric acid. Levosulpiride is a substituted benzamide anti-psychotic, reported to be a selective antagonist of dopamine D_2 receptors activity on

both central and peripheral levels. It is an atypical neuroleptic and a prokinetic agent. Levosulpiride is also claimed to have mood elevating properties. Levosulpiride is used in the treatment of psychoses, particularly negative symptoms of schizophrenia, anxiety disorders, dysthymia, vertigo, dyspepsia, irritable bowel syndrome and premature ejaculation. The pharmaceutical dosage forms containing combination drugs are very muchuseful in therapies due to patient compliance.Esomeprazole is a proton pump inhibitor. Levosulpiride is substituted benzamide anti-psychotic drug and it is used for the treatment of psychosis and Esomeprazole to overcome adverse reaction of acidity caused by Levosulpiride. Esomeprazole is chemically (S)-5-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-3H-benzoimidazole. Its molecular formula is $C_{17}H_{19}N_3O_3S$ and molecular weight is 367.4g/mol. Levosulpiride is chemically N-{[(2S)-1-Ethylpyrrolidin-2-yl]methyl}-2-methoxy-5-sulfamoylbenzamide and molecular formula is $C_{15}H_{23}N_3O_4S$ and molecular weight is 341.43g/mol.

The literature revealed, a number of analytical methods were reported for estimation of ESO including UV spectrophotometric and HPLC [1-9]. Method have been studied for determination of LEVO and ESO in bulk and in pharmaceutical formulations. In present work, a successful attempt has been made to estimate both drugs simultaneously in capsule dosage form by RP-HPLC method. The chemical structures of both drugs are as shown in (Figures 1, 2).

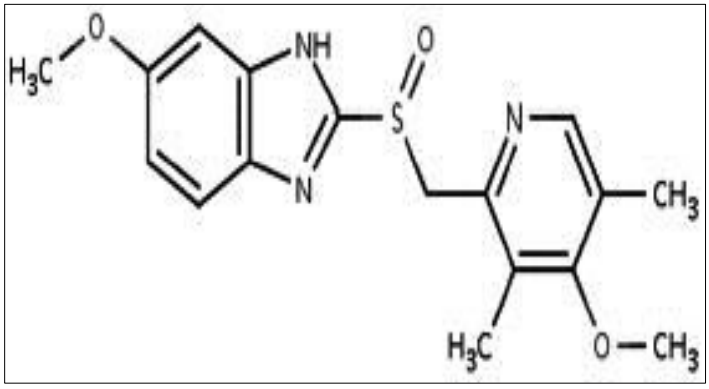


Figure 1: Chemical structure of Esomeprazole (ESO).

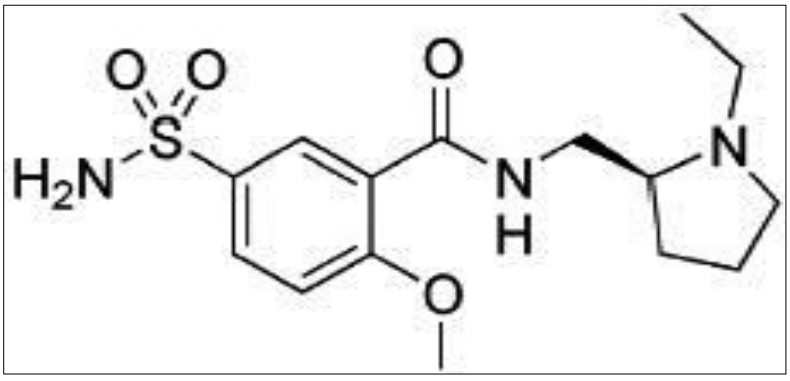


Figure 2: Chemical structure of Levosulpiride (LEVO).

Experimental

Chemicals and Reagents

The pure API samples of Esomeprazole and Levosulpiride were obtained as free gift samples from Mylan Laboratories Limited; Hyderabad and Ajantha Pharma; Mumbai respectively while solvent such as methanol used were of HPLC grade (Thomas Baker), Ortho phosphoric acid and Triethylamine were Merck pvt ltd India and double distilled water used for whole experiment. The marketed combined pharmaceutical dosage form of Esomeprazole (40mg) and Levosulpiride (75mg) i.e. Sompraz–L(capsule) dosage form manufacture by (Sun Pharma, East Sikkim) was purchased from local market.

Instrumentation and Materials

A Jasco HPLC PU-2080 Plus system with UV- VIS detector PU-2075 Plus instrument was use for analysis with Chrome Software. The analytical Column was Hi-Q Sil C-18 (250 mm × 4.6 mm), Particle size : 5 µm

Chromatographic Conditions

Chromatographic separation of ESO and LEVO were performed by use of an isocratic mobile phase prepared from 70:30 (*v/v*) methanol: buffer (10mM, KH_2PO_4), pH 3 (adjusted with ortho phosphoric acid) giving well resolved, sharp peak for LEVO and ESO with a retention time (tR) 2.2 and 5.3 min. (Figure 3). The flow rate was 1.2 mL/min and UV detector was performed at 260 nm and ambient temperature (24^0-26^0 C) for column oven was found to be the best for analysis.

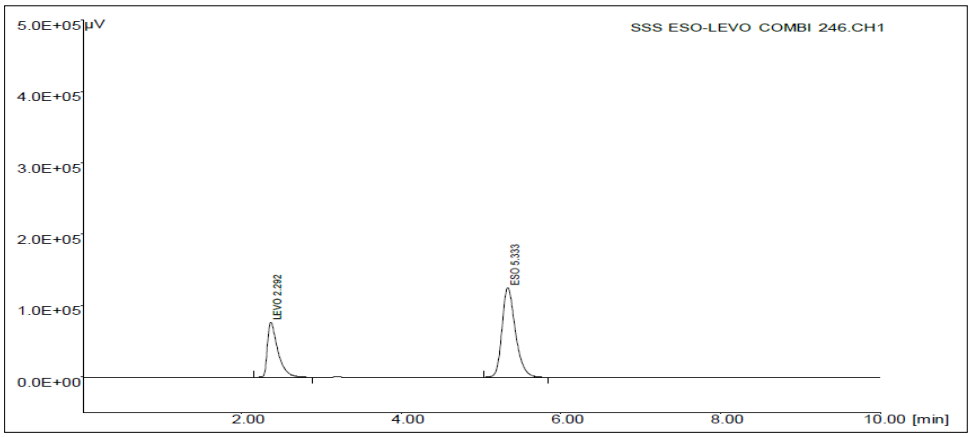


Figure 3: Chromatogram of LEVO(150µg/ml) and ESO (80µg/ml).

Stock Solutions

Weighed accurately of 40 mg ESO and 75 mg LEVO separately dissolved in a 100 mL volumetric flask with mobile phase to get 400 µg/mL and 750 µg/mL solution respectively. Linearity was determined in the range of 20 to 120 µg mL-1 and 75 to 450 µg/ mL for esomeprazole and levosulpiride.

Analysis of the capsule dosage form:

Twenty capsules (Sompraz⁻ L containing 40 mg of ESO and 75 mg of LEVO) were weighed accurately and crushed to form fine powder. Powder weight equivalent to 40 mg of drug containing ESO and it contain 75 mg LEVO were dissolved in a 100 mL volumetric flask with mobile phase. It was sonicated followed by filtration through Whitman filter paper (No. 41). Appropriate volumes of the aliquot were transferred into two set of six different 10 mL volumetric flasks and the volume was made up to the mark with mobile phase to get a concentrations of 40 µg/mL of ESO and 75 ug/mL of LEVO respectively. The solutions were subject to analysis and results obtained as in Table I.

Table I: Assay of ESO and LEVO capsule formulation.

Active Ingredients	Label Claim (mg/tab)	Amount of Drug* Estimated (mg/tab	% Amount of drug found
Esomeprazole	40	39.92	99.51
Levosulpiride	75	76.01	101.35

* Average of Six estimations

Validation Parameters: The developed method was validated as per ICH guidelines in terms of its linearity, accuracy, Limit of detection (LOD), Limit of quantification (LOQ), specificity, intra-day and inter-day precision and repeatability of measurement [11].

Linearity

Appropriate aliquots of standard stock solution were taken in different 10 mL volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 20, 40, 60, 80, 100 and 120 µg/mL of ESO and 75, 150, 225, 300, 375, and 450 µg/mL for LEVO. The solutions were injected using a 20 µL fixed loop system and chromatograms were recorded. ESO follow linearity between 20 to 120 µg/mL and LEVO between 75 and 450 µg/mL. Linearity show in fig.4-7 and results are tabulated in Table II.

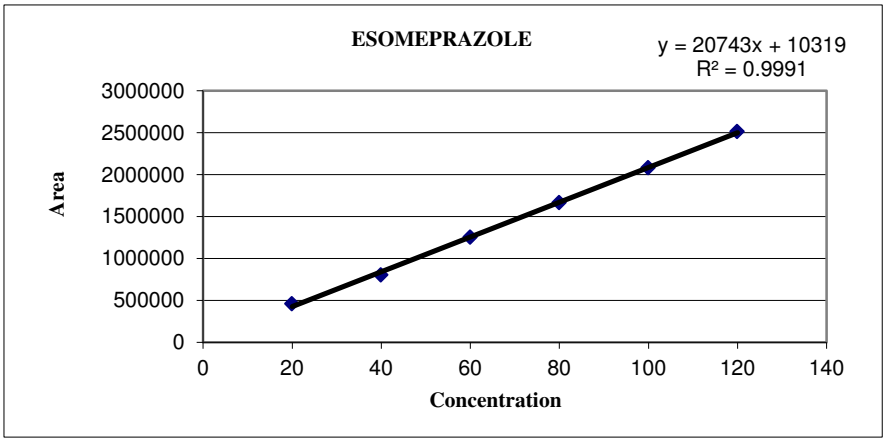


Fig.4: Linearity plot of Esomeprazole.

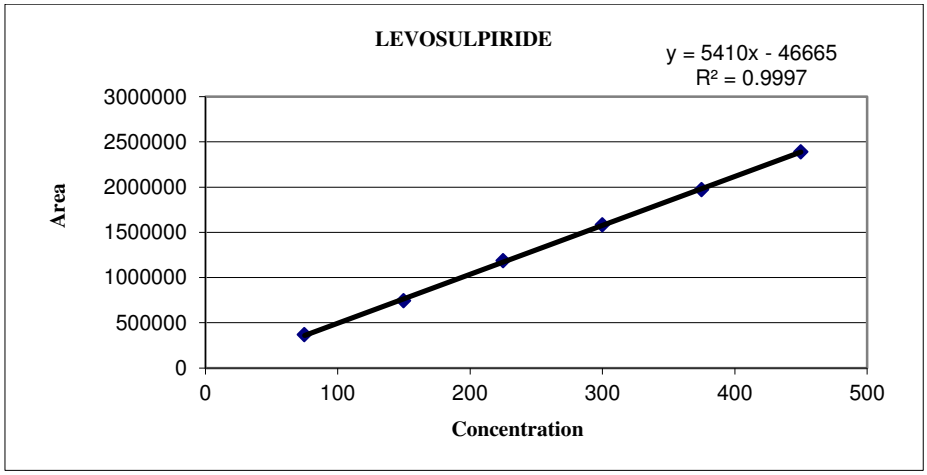
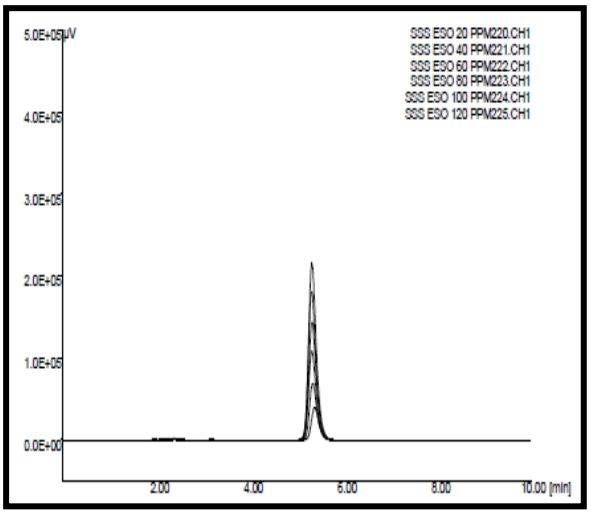


Fig.5: Linearity plot of Levosulpiride.

Table II: Linearity data for ESO and LEVO.

Parameters	Esomeprazole*	Levosulpiride*
Linearity range (µg/ml)	20-120	75-450
Correlation coefficient (r^2)	0.9991	0.9997
Slope	20743	5410
Intercept	10319	46665

*Average of Six estimations.



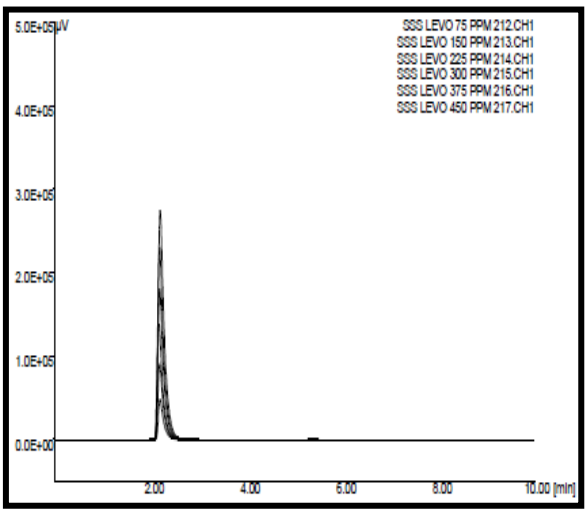


Fig 6: **Overlay Spectrum of Esomerazole** **Fig 7**: **OverlaySpectrum of Levosulpiride**

Accuracy

The accuracy of the method shall be demonstrated through determination on samples in three concentrations from 80% (72 µg/mL for ESO; 135 µg/mL for LEVO), 100% (80µg/mL for ESO; 150 µg/mL for LEVO), and 120% (88 µg/mL for ESO; 165 µg/mL for LEVO), (Three replicates each) of the theoretical concentrations employed as per the usual procedure. Recovery data was given in Table III.

Table III: Accuracy study of ESO and LEVO by RP-HPLC method.

Recovery Level(%)	Drug	Conc. of drug (µg/ml)		% Recovery*	S.D.	%RSD
		Drug taken	Std drug added			
80	ESO	40	32	101.30	0.204	0.201
100		40	40	102.10	0.329	0.322
120		40	48	102.08	0.105	0.103
80	LEVO	75	60	101.29	0.102	0.101
100		75	75	102.03	0.037	0.036
120		75	90	102.61	0.049	0.048

* Average of three determinations

Precision

Intraday and inter-day precision of the assay samples containing ESO having concentrations of 40 µg/mL and LEVO having concentrations of 75 µg/mL were analyzed three times in the same day (intraday) and for three consecutive days (inter-day). Precision was calculated as intra and inter-day coefficient of variation [% C.V. = (S. D. /mean) x 100] as shown in the Table IV.

Table IV: Precision data of ESO and LEVO by RP-HPLC.

Parameter	Drug	Amount of Drug taken(µg/ml)	Mean Amt. Estimated * (µg/ml)	% Mean Amt. Estimated	S. D.	% R. S. D.
Intra- Day	ESO	40	39.87	99.51	0.514	0.517
	LEVO	75	76.12	101.52	0.406	0.400
Inter-Day	ESO	40	40.65	101.17	0.726	0.718
	LEVO	75	76.27	101.70	0.360	0.354

* Average of three determinations

Robustness

Robustness studies are performed by introducing deliberately small changes in, flow rate (±0.2 mL/min^{-1}), the mobile phase composition (± 2 mL) and pH (± 2) Robustness of the proposed method is studied and results tabulated in Table V.

Table V: Robustness Studies.

Factor	Level	Retention time of LEVO (min)	Retention time of ESO (min)
A: Flow Rate (ml/min)			
1.0	-2	2.42	5.52
1.2	0	2.26	5.34
1.4	+2	2.11	5.04
B: Percentage of methanol in the mobile phase (v/v)			
68	-2	2.08	5.16
70	0	2.26	5.34
72	+2	2.41	5.72
C: pH of mobile phase			
7.2	-2	2.16	5.21
7.4	0	2.26	5.30
7.6	+2	2.32	5.41

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

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S)

L.O.Q. = 10(SD/S)

Where,

SD = Standard deviation of the response,

S = Slope of the calibration curve.

Table VI: LOD and LOQ Studies.

Drugs	LOD (µg/ml)	LOQ (µg/ml)
ESO	9.840	19.820
LEVO	2.108	69.594

Results and Discussion

The present method was a sensitive, precise, and accurate HPLC method for the analysis of Esomeprazole (ESO) & Levosulpiride (LEVO). To optimize the mobile phase, various combinations of methanol and buffer were studied on anHi-Q SilC18 column. The mobile phase containing a mixture of Methanol: Phosphate buffer in the ratio of 70:30, (v/v) was carried out and flow rate of 1.2 mL/min found that the resulted peaks with good shape and resolution. For LEVO and ESO with a retention times of 2.2 min and 5.3 min respectively (Figure 3). Detection at 260 nm was chosen for determination of both drugs because it is their isobestic point, having both equal absorbances at that wavelength. The linearity was obeyed in the concentration range of 20-120 µg/mL of ESO and 75-450 µg/mL of LEVO. The number of theoretical plates obtained was 2386 (LEVO) and 5643 (ESO) respectively which indicates the efficient performance of the column. The limit of detection and limit of quantitation were found to be 9.8 µg/mL and 19.8 µg/mL (ESO); 2.1 µg/mL and 69.5µg/mL (LEVO) respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that recipients used in tablet formulations didn't interfere with the estimation of the drugs by the proposed HPLC method.

Conclusion

The new isocratic RP-HPLC method was developed and validated for the simultaneous estimation of Esomeprazole and Levosulpiride**.** The method was validated as per ICH guidelines.The method was found to be simple, accurate, economical, rapid and they can be applied for routine analysis in laboratories and pharmaceutical manufacturers.

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