SIMULTANEOUS ESTIMATION OF ESOMEPRAZOLE AND LEVOSULPIRIDE IN BULK AND IN CAPSULE FORMULATION BY RP-HPLC

RAKESH R. JAIN, PRAVIN O. PATIL, SANJAY B. BARI*

Department of Quality Assurance, H.R. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist: Dhule (M.S.) India 425 405
H. R. Patel Institute of Pharmaceutical Education and Research, Shirpur Dist: Dhule (M.S.) 425405 India
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ABSTRACT

The present work deals with development and validation for simultaneous determination of Esomeprazole and Levosulpiride drugs in pharmaceutical formulations. A rapid, precise and specific reverse phase high performance liquid chromatography (RP-HPLC) method was developed for Esomeprazole and Levosulpiride. Chromatographic separations was achieved on a C-18 (5µm, 250×4.6 mm) HPLC column within a runtime of 10 min. Isocratic mobile phase contain methanol: buffer (pH 3) (65:35% *v/v*) and flow rate was maintained at 1.0 mL/min. Eluate was monitored at 260 nm. Levosulpiride was eluated at 2.7 min and Esomeprazole at 5.7 min. Linearity was studied in the concentratiosn range of 5 to 30 µg mL-1 and 10 to 60 µg/ mL for esomeprazole and levosulpiride respectively, with a correlation coefficient of 0.9995 and 0.9993 respectively. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

Keywords: RP-HPLC, Esomeprazole, Levosulpiride, Capsule Formulation, Validation.

INTRODUCTION

Esomeprazole (ESO) chemically bis (5-methoxy-2-[-(s)-[(4-methaoxy-3,5-dimethyl- 2- pyridinyl) methyl] sulfinyl]-1H-benzimidazole) [1]. The first single optical isomer ESO is a proton pump inhibitor, used for short-term treatment of erosion and ulceratiosn of the esophagus caused by gastro-esophageal reflux disorders. It has a favorable pharmacokinetics profile relative to omeprazole. Levosulpiride (LEVO) is levo-enatiomer of racemic sulpiride, 5- (aminosulfonyl) N-[(1-ethyl- 2 – pyrrolidinyl) methyl] 2 – methoxy benzamide. Levosulpiride is most widely used drug in the treatment of depression, schizophrenia [2]. At low doses, LEVO increases dopaminergic neurotransmission, primarily by the blocking of the dopamine auto receptors, which inhibits the pre-synaptic dopamine synthesis and release of dopamine. Compared with racemic and dextro-forms, the levo-form of sulpiride has greater central antidopaminergic activity, antiemetic and antidyspeptic effects and lower acute toxicity [3].

The literature revealed, a number of analytical methods were reported for estimation of ESO including UV spectrophotometric and HPLC [4-9]. While estimation of LEVO was reported using HPLC and HPTLC [2] and Tandem mass spectrometry [10]. The RP–HPLC [11] and UV-Spectrophotometric [12] 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).

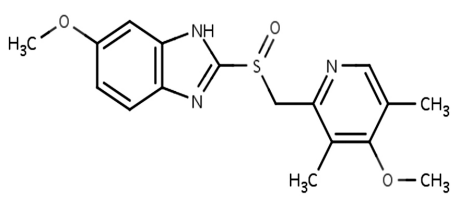

Figure 1: Chemical structure of Esomeprazole (ESO).

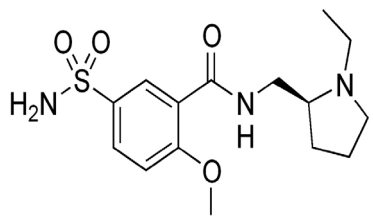

Figure 2: Chemical structure of Levosulpiride (LEVO).

EXPERIMENTAL

Chemicals and Reagents

HPLC grade methanol and analytical grade ortho-phosphoric acid were procured from Merck® India Ltd. (Mumbai). Water was purified with Milli-Q Millipore system. All the solvents and solutions were filtered through a membrane filter (Millipore filter paper, 0.45 µm pore size) and degassed before use. Drug formulation (capsule) Sompraz®L with label claim 40 mg Esomeprazole and 75 mg Levosulpiride was purchased from Indian market and used for estimation.

Instrumentation and Materials

Analysis was performed on Agilent HPLC 1200 series separations module with in-built PDA detector. Chromatographic software Ezechrome Elite was used for data collection and processing. The analytical column was LC-GC Qualisil BDS C18 (5 mm, 250 mm C 4.6 mm).

Chromatographic Conditions

Chromatographic separation and of ESO and LEVO were performed by use of an isocratic mobile phase prepared from 65:35 (*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) 2.7 and 5.7 min. (**Figure 3)**. The flow rate was maintained at 1.0 mL/min, UV detection was performed at 260 nm and ambient temperature (25^0 C) for column oven was found to be the best for analysis.

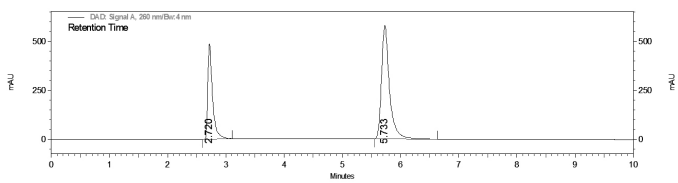

Figure 3: Typical chromatogram of LEVO and ESO.

Stock Solutions

Independent stock solution of 100 µg/mL of each ESO and LEVO were prepared in mobile phase.

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 10 mg of drug containing ESO and LEVO were dissolved in a 100 mL volumetric flask with methanol. It was sonicated followed by filtration through Whatmann 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

e- mail: rxpatilpravin@yahoo.co.in

concentrations of 15 µg/mL of ESO and 30 µg/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.

Amount taken in [µg/mL]	Amount found* [µg/mL] Mean	% Amount Found	% RSD
15	15.08	100.55	0.58
30	30.13	100.44	0.93

*n=6

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 [13].

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 5, 10, 15, 20, 25 and 30 µg/mL of ESO and 10, 20, 30, 40, 50, and 60 µg/mL for LEVO. The solutions were injected using a 20 µL fixed loop system and chromatograms were recorded. ESO follow linearity between 5 to 30 µg/mL and LEVO between 10 and 60 µg/mL. Results are tabulated in **Table II**.

Table II: Linearity data for ESO and LEVO.

Parameters	ESO*	LEVO*
Linear range (µg/mL)	5 - 30	10 - 60
Slope	61262	12896
Intercept	4740	12001
Correlation coefficient (r^2)	0.9996	0.9993

*Mean of six determinations.

Accuracy

Accuracy was found by studying level of recovery using standard addition method. Known amounts of standards of LEVO and ESO was added to pre-analyzed samples at a level from 80% to 120% and then subjected to the proposed RP-HPLC method.

The solutions were then analyzed, and the percentage recoveries were calculated by using formula.

$$\% \text{ Recovery} = \frac{\text{Found analyte mass}}{\text{Added analyte mass}} \times 100$$

Results obtained are tabulated in **Table III.**

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

Level of recovery	Amount of drug added (µg/mL)	% Recovery*	% RSD
80%	12	99.33	1.28
ESO 100%	15	99.38	0.78
120%	18	100.38	1.19
80%	24	100.85	0.49
LEVO 100%	30	101.28	0.87
120%	36	99.47	0.62

*n=6

Precision

Intraday and interday precision of the assay samples containing ESO having concentratiosns of 15, 20, 25 µg/mL and LEVO having concentrations of 30, 40, 50 µg/mL were analyzed three times in the same day (intraday) and for three consecutive days (interday). Precision was calculated as intra and interday 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.

Conc. [µg/mL^{-1}]	Intra-day Amount found [µg/mL]		Inter-day Amount found [µg/mL]	
	Mean	% RSD*	Mean	% RSD*
ESO 15	15.16	1.21	15.08	1.12
20	20.12	1.35	20.24	1.39
25	25.22	1.28	25.16	1.51
LEVO 30	30.13	1.45	30.30	1.27
40	39.85	1.38	39.69	1.09
50	49.85	1.42	50.30	1.25

*n = 3

Robustness

Robustness studies are performed by introducing deliberately small changes in the mobile phase composition (± 5 mL), flow rate (±0.1 mL/min^{-1}). Robustness of the proposed method is studied and results tabulated in **Table V**.

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

The LOD and LOQ were calculated by using the equations LOD = 3.3 × N/B and LOQ = 10 × N/B where 'N' is the standard deviation of the peak areas of the drug (n=3) and 'B' is the slope of the corresponding calibration plot. The signal to noise ratios was determined. The LOD was regarded as the amount for which the signal to noise ratios was 3:1 and LOQ regarded as the amount for which the signal to noise ratios was 10:1.

RESULTS AND DISCUSSION

Optimization of Mobile Phase

A RP-HPLC method was developed for an accurate and reproducible method for esomeprazole and levosulpiride. The method development trials were carried out using different ratios of buffer, acetonitrile and methanol. The method development trial was carried out using Qualisil BDS C_{18} column (250 mm × 4.6mm,5 µ), The mobile phase flow rate 1 mL/min^{-1} was examined. The detection wavelength was 260 nm. Diode array detector was used and from the overlain spectra a wavelength of 260 nm was selected for the estimation of both drugs simultaneously (Figure 2E). With a view to separate both drugs simultaneously (Figure 4), various mobile phases consisting of methanol and buffer, acetonitrile and buffer were tried, but tailing and low resolution of the chromatogram was observed. In trial 1, the mobile phase consisted of acetonitrile and KH_2PO_4 (10mM) buffer in a ratios of (80:20) was tried and chromatogram obtained not showed a better resolution for both drugs. In trial 2 the, mobile phase consisted of methanol and KH_2PO_4 (10 mM) buffer in the ratios of (90:10) was attempted in this chromatogram obtained having the retention time between two separatiosn was very less. While in the trial 3, the mobile phase consisted of methanol and KH_2PO_4 buffer in the ratios of (80:20) was utilized and chromatogram obtained still not showed an improved resolutions for both drugs. In trial 4, the mobile phase consisted of methanol and KH_2PO_4 (10 mM) buffer in the ratios of (65:35) was endeavored, in this mobile phase the chromatogram obtained showed asymmetry within limit as well as retention time within runtime of 10 min (Table VI). In this mobile phase not only separation time between two peak but also theoretical plates was good and so it was used as optimized mobile phase for the estimation of ESO and LEO in capsule formulation. Therefore, mobile phase consisting of Methanol : 10 mM potassium dihydrogen phosphate buffer (pH 3), in 65:35 giving well resolved, sharp peak for LEVO and ESO with a retention times of 2.7 min and 5.7 min respectively (**Figure 3)**. The flow rate of 1.0 mL/min^{-1} at 260 nm and ambient temperature (25^0 C) for column oven was found to be the best for analysis. Methanol was used for separations in the proposed method is cheaper and less toxic as compare to acetonitrile and buffer was used in smaller amount as compare to reported method [11]. Selection of pH is on trail

error basis we have got low tailing and good resolution at pH 3.0 therefore we selected pH 3 for further estimation of ESO and LEO. Detection at 260 nm was chosen for determination of both drugs because it is their isobestic point, having both equal absorbance at that wavelength. This is an advantage in comparison with the detection at 240 nm [11], because at the latter wavelength the molar absorptivity of ESO is less than for LEVO, increasing the uncertainty for the quantification of ESO (Figure 4**).** The amount of both drugs quantified in formulation was better optimized in our method as compare to reported method [11]. The assay % of both drugs obtained was within a pharmacopeial limit, means recovery of drugs from tablet formulation was better and recovery was consistent because series of six results were in good agreement with the label claim. The % RSD less than 2 indicates less error in the showed that proposed method is precise over reported methods. The % RSD was less than 2 in intraday, interday precision and all parameters of robustness are within limit. So the proposed method is more precise, accurate and robust **Table VII.** System suitability parameters were studied by injecting the working standard solution (15µg/ml of ESO and 30µg/ml of LEVO), **Table VIII**.

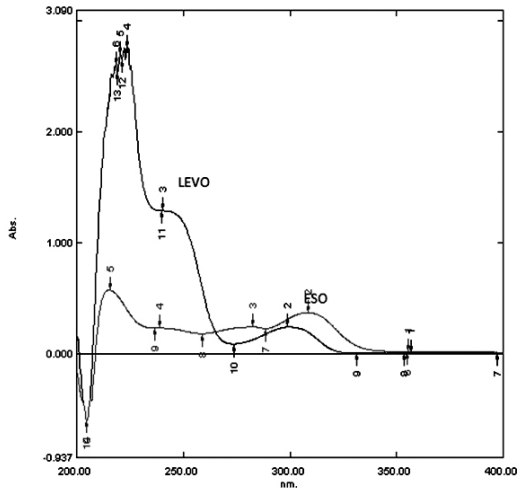

Figure 4 An overlain spectra of ESO and LEVO in methanol.

Table V: Robustness Studies**.**

Chromatographic Conditions	Retention Time tR	Tailing factor	Theoretical Plates
A: Flow rate (mL/min)			
ESO 0.9	6.60	1.50	10021
1.0	5.73	1.40	11121
1.1	5.40	1.54	10896
LEVO 0.9	3.03	1.42	6296
1.0	2.72	1.41	6590
1.1	2.46	1.48	6125
ESO/ LEVO **Mean**	**5.90/2.74**	**1.48/1.44**	**10679/6337**
B: Change in mobile phase composition			
(methanol: buffer 60 : 40 *v/v*) ESO (methanol: buffer 65 : 35 *v/v*) (methanol: buffer 70 : 30 *v/v*)	6.30 5.73 4.90	1.75 1.48 1.54	10240 12256 11248
(methanol: buffer 60 : 40 *v/v*) LEVO (methanol: buffer 65 : 35 *v/v*) (methanol: buffer 70 : 30 *v/v*)	2.71 2.73 2.72	1.64 1.32 1.38	5142 6027 5928
ESO/ LEVO **Mean**	**5.64/2.72**	**1.59/1.44**	**11248/5699**

Table VI: Mobile phase optimization trials.

Trials No.	Mobile phase Compostion	Retention Time tR		Theoretical plates (USP)		Asymmetry (10%)	
		ESO	LEVO	ESO	LEVO	ESO	LEVO
1	Acetonitrile : 25mM KH_2PO_4 (10mM) (pH 3.0) (80:20)	5.98	2.19	10455	5794	1.46	1.24
2	Methanol : 10 mM KH_2PO_4 (10mM) (pH 3.0) (90:10)	3.62	2.68	7424	3729	1.53	1.08
3	Methanol : 10 mM KH_2PO_4 (pH 3.0) (80:20)	4.02	2.66	5358	1575	1.34	0.91
4	Methanol : 10 mM KH_2PO_4 (10mM) (pH 3.0) (65:35)	5.73	2.72	11121	6590	1.40	1.43

Table VII Summary of validation parameters.

Parameters	ESO	LEVO
Linear range (μg/mL^{-1}) [n=6]	5 – 30	10 – 60
Correlation coefficient (r^2)	0.9996	0.9993
Limit of detection (μg/mL^{-1})	0.36	0.45
Limit of quantification (μg/mL^{-1})	1.09	1.39
% Recovery [n=3]	99.33 – 100.38	99.47 -101.28
Precision [%RSD]		
Intra-day [n=3]	1.28	1.42
Inter-day [n=3]	1.34	1.20
Repeatability [n=6]	0.78	0.92
Robustness		Robust

Table VIII: System suitability parameters.

Parameters	ESO	LEVO	Acceptance criteria
Theoretical plate	12483	6007	More than 2000
USP Tailing factor	1.34	1.42	Less than 2
Capacity factor	31.0	66.45	Should be non zero
USP Resolution	16.25	–	More than 2

CONCLUSION

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately rugged and robust and can be used for simultaneous determination of esomeprazole and levosulpiride in capsule formulation. The method was validated as per ICH guidelines.

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