



INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

DEVELOPMENT AND VALIDATION OF SECOND ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF LEVOSULPIRIDE AND ESOMEPRAZOLE IN COMBINED PHARMACEUTICAL FORMULATION

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Accepted Date: 19/04/2014; Published Date: 27/04/2014

Abstract: A new simple, sensitive, rapid, accurate, precise and economical second derivative Spectrophotometric method for the simultaneous estimation of Levosulpiride (LEVO) and Esomeprazole magnesium trihydrate (ESO) in combined pharmaceutical formulation was developed. The derivative Spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The second order derivative spectrums were obtained in methanol and the determinations were made at 260.75 nm (ZCP of ESO) for quantification of LEVO and 302.86 nm (ZCP of LEVO) for quantification of ESO. The linearity was obtained in the concentration range of 10-40 $\mu\text{g/ml}$ for LEVO and 4-28 $\mu\text{g/ml}$ for ESO. The mean recovery was 100.3% –101.7% and 100.2%–101.6% for LEVO and ESO respectively. The results of analysis have been validated statistically as per ICH guidelines.

Keywords: Levosulpiride, Esomeprazole magnesium trihydrate, Second order derivative.



PAPER-QR CODE

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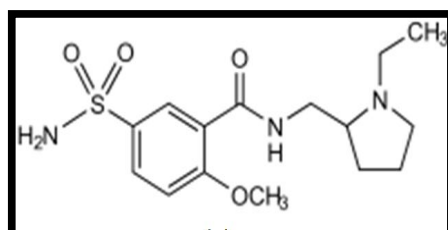
How to Cite This Article:

Rachana Patel, IJPRBS, 2014; Volume 3(2): 785-798

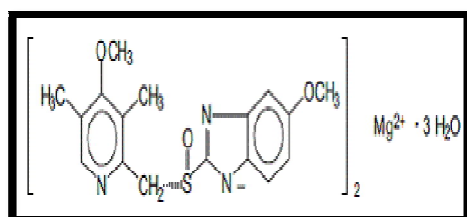
INTRODUCTION

Levosulpiride (LEVO), is chemically N-[(1-ethylpyrrolidin-2-yl) methyl]-2-methoxy-5-sulfamoylbenzamide. It levorotatory enantiomer of sulpiride, a substituted benzamide indicated as an antipsychotic, antidepressant drug¹⁻³[Figure 1a]. LEVO is not official in Pharmacopoeia, but it is listed. It consists of blocking the D₂ dopaminergic receptors, preferentially located on the presynaptic membranes in the dopaminergic pathways of the brain, this means that sulpiride is a selective autoreceptor blocker. Literature survey revealed that various, UV spectroscopy⁴⁻¹⁴, Chromatographic¹⁵⁻²³ methods and LC/MS/MS method²⁴ have been reported for quantitative estimation of LEVO in pharmaceutical formulation and biological fluids individually or in combination with other drugs. Esomeprazole magnesium trihydrate (ESO) is chemically, bis(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole-1-yl) magnesium trihydrate²⁵⁻²⁷ [Figure 1b]. ESO is class of "proton pump inhibitor" that inhibits gastric acid secretion through inhibition of K⁺/H⁺ ATPase in gastric parietal cells. It is S- Enantiomer of Omeprazole . It is used for short term treatment of erosion and ulceration of the esophagus caused by gastro-esophageal reflux disease (GORD), peptic ulcer, NSAID-associated ulceration and Zollinger-Ellison syndrome²⁷. ESO is official in IP (2010)²⁸. The review of literature revealed that various analytical methods involving Spectrophotometry^{11-14, 29-31}, HPLC^{22, 23, 32-44} and HPTLC⁴⁵⁻⁴⁶ have been reported for ESO in pharmaceutical formulation and biological fluids individually or in combination with other drugs.

To the best of our knowledge, there is no published Derivative Spectrophotometric method for this combination. So, the present paper describes a simple, accurate and precise method for simultaneous estimation of LEVO and ESO in combined Pharmaceutical formulation by second order derivative method. The developed method was validated in accordance with ICH Guidelines⁴⁷ and successfully employed for the assay of LEVO and ESO in their combined pharmaceutical formulation.



(a)



(b)

Figure 1: Chemical structure of (a) LEVO and (b) ESO

MATERIALS AND METHODS

Reagents and Chemicals

Analytically pure LEVO and ESO were kindly provided by Intas Pharmaceuticals Ltd. Ahmedabad, Gujarat, India and Torrent Pharmaceutical Ltd as gratis samples. Methanol was used as solvent. Capsule of LEVO and ESO in combined dosage form, Nexpro-L was procured from local market.

INSTRUMENTS

A Shimadzu UV/Vis 1800 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm), 1 cm matched quartz cells and UV probe 2.32 software was used for all the spectral measurements and Shimadzu UV/Vis 1601 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm) and 1 cm matched quartz cells was used for reproducibility study. Calibrated analytical balance K-EA 210 (K-Roy Instrument Pvt. Ltd) was used for weighing purpose.

Spectrophotometric condition

All zero order spectrums (D^0) were converted to second derivative spectrum (D^2) using delta lambda 1.

Preparation of standard stock solutions

An accurately weighed quantity of LEVO (50 mg) and ESO (50 mg) were transferred to a separate 100 ml volumetric flask and 50 ml methanol was added to both volumetric flask and sonicated for 5 minutes. Volume was adjusted up to the mark with methanol to obtain standard solution having concentration of LEVO (500 $\mu\text{g}/\text{ml}$) and ESO (500 $\mu\text{g}/\text{ml}$). 20 ml solutions of LEVO (500 $\mu\text{g}/\text{ml}$) and 20 ml ESO (500 $\mu\text{g}/\text{ml}$) were transferred to a separate 100 ml volumetric flask. Volume was made up to the mark to give a solution containing 100 $\mu\text{g}/\text{ml}$ of LEVO and ESO. From above stock various aliquots were prepared.

Method validation

The standard solutions of LEVO (10- 40 $\mu\text{g}/\text{ml}$) and ESO (4 -28 $\mu\text{g}/\text{ml}$) were scanned separately in the UV range of 200-400 nm. The zero-order spectra obtained was converted to second-derivative spectra. All spectra were overlain and zero crossing point (ZCP) of LEVO was found to be 302.86 nm, and ZCP of ESO was found to be 260.75 nm. The ZCP of LEVO (302.86 nm) is selected for quantification of ESO, whereas the ZCP of ESO (260.75 nm) is selected for quantification of LEVO. Hence 260.75 nm and 302.86 nm were selected as analytical wavelengths for determination of LEVO and ESO respectively [Figure-2]. These two wavelengths can be employed for the determination of LEVO and ESO without any interference from the other drug in their combined dosage form.

Linearity

Appropriate volume of aliquot from LEVO and ESO standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with methanol to give solutions containing 10-40 $\mu\text{g}/\text{ml}$ LEVO and 4-28 $\mu\text{g}/\text{ml}$ ESO. All D^0 and D^2 Spectrum were recorded using above Spectrophotometric condition. D^2 absorbance at 260.75 nm and 302.86 nm were recorded for LEVO and ESO respectively (n=6). Calibration curves were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre-quantified placebo preparation at 3 different concentration levels 50, 100 and 150 %, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured.

Precision

The repeatability was evaluated by assaying 6 times of sample solution prepared for assay determination. The intraday and interday precision study of LEVO and ESO was carried out by estimating different concentrations of LEVO (10, 25, 40 µg/ml) and ESO (4, 12, 24 µg/ml), 3 times on the same day and on 3 different days (second, third, fourth) and the results are reported in terms of C.V.

Detection limit and Quantitation limit

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the $3.3\sigma/S$ and $10\sigma/S$ criteria, respectively; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

Reproducibility

The absorbance readings were measured at different laboratory for sample solution using another spectrophotometer by analyst and the values obtained were evaluated using t- test to verify their reproducibility.

Determination of LEVO and ESO in their Combined Dosage

Sample preparation

A powder quantity equivalent to 75 mg LEVO and 40 mg ESO was accurately weighed and transferred to volumetric flask of 100 ml capacity. 60 ml of methanol was transferred to this volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through whatman filter paper (0.45µ). From this solution 1 ml was transferred to volumetric flask of 10 ml capacity. From this solution 2 ml was transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark to give a solution containing 15µg/ml of LEVO and 8 µg/ml of ESO. The resulting solution was analyzed by proposed method.

DERIVATIVE SPECTROSCOPY METHOD FOR ESTIMATION OF LEVO AND ESO IN THEIR COMBINED DOSAGE FORM

Owing to the high solubility of LEVO and ESO in the methanol and also there was no shift in the absorbance maxima of LEVO and ESO in above solvent. Hence methanol was selected as solvent.

For estimation of LEVO and ESO using derivative spectroscopy, zero crossing method was decided to be used. In this method two wavelengths are required. One wavelength is selected at which LEVO shows zero absorbance while other drug ESO shows considerable absorbance. The second wavelength is selected such that ESO shows zero absorbance while LEVO shows considerable absorbance.

The overlain derivative spectrum (second order) of LEVO and ESO at different concentrations revealed that at 260.75 nm different concentration of ESO possesses zero D2 absorbance whereas LEVO possesses significant D2 absorbance. In a similar manner, at 302.86 nm different concentrations of LEVO possess zero D2 absorbance whereas LEVO possesses significant D2 absorbance. Considering above facts, wavelength 302.86nm is selected for estimation of ESO and 260.75 nm is selected for the estimation of LEVO.

Calibration curves for LEVO and ESO were plotted between D2 absorbance and concentration. The following equations for straight line were obtained for LEVO and ESO.

Linear equation for LEVO: $y = 0.00007x - 0.00002$

Linear equation for ESO: $y = -0.0001x + 0.0001$

The developed second order derivative spectroscopy method was validated. The linear range, correlation coefficient, detection limit and standard deviation for LEVO and ESO by second order derivative spectrophotometry method are shown in Table 1. The LOD for LEVO and ESO was found to be 0.384 $\mu\text{g/ml}$ and 1.704 $\mu\text{g/ml}$ respectively. The LOQ for LEVO and ESO was found to be 1.166 $\mu\text{g/ml}$ and 5.16 $\mu\text{g/ml}$ respectively Accuracy was determined by calculating the recovery. The method was found to be accurate with % recovery 100.3 -101.7 % for LEVO and 100 -101.2% for ESO. Precision was calculated as repeatability and intra and inter day variation for both the drugs. The method was found to be precise with C.V. 0.21 - 0.35 for intraday (n=3) and C.V. 0.21 - 0.91 for inter day (n=3) for LEVO and C.V. 0.48 -1.72 for intraday (n=3) and C.V. 0.21- 1.28 for inter day (n=3) for ESO. The method was found to be reproducible when reproduced by different analyst at different times. The method was also found to be specific, as no interference observed when the drugs were estimated in presence of excipients.

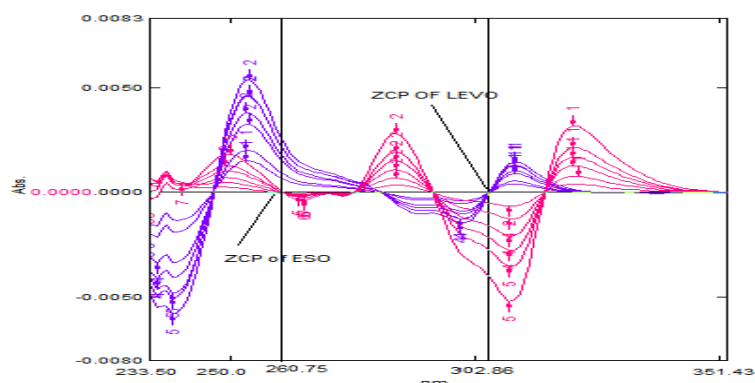


Figure: 2 Overlain second order derivative spectrum of LEVO and ESO in methanol

Table 1: Summary of Validation Parameters of derivative Spectrophotometric method

Parameters	LEVO	ESO
Recovery %	100.3 – 101.7 %	100 – 101.6 %
Repeatability(C.V.) (n=6)	0.19 – 0.81	0.83 -1.77
Precision		
Intra-day(n=3)	0.213 - 0.911	0.243 -1.017
Inter-day (n=3)	0.212- 0.914	0.249 - 1.960
Limit of Detection ($\mu\text{g/ml}$)	0.384 $\mu\text{g/ml}$	1.704 $\mu\text{g/ml}$
Limit of Quantitation ($\mu\text{g/ml}$)	1.16 $\mu\text{g/ml}$	5.16 $\mu\text{g/ml}$

Table 2: Statistical data for LEVO and ESO by Derivative Spectrophotometry method

Parameter	LEVO	ESO
Analytical wavelength	260.75	302.86
Range	10-40 $\mu\text{g/ml}$	4-28 $\mu\text{g/ml}$
Mean of Slope	0.00007	0.0001
Std deviation Intercept	0.00008	0.00005
Regression coefficient	0.998	0.998

Table 3: Precision data for LEVO absorbance at 260.75 nm

Conc. (µg/ml)	Intraday (Abs. ± S.D)	C.V.	Inter day (Abs. ± S.D)	C.V.
10	0.00063 ± 0.00005	0.911	0.00064 ± 0.00005	0.914
25	0.00164 ± 0.00005	0.350	0.00165 ± 0.00001	0.698
40	0.00270 ± 0.00005	0.213	0.00271 ± 0.00005	0.212

Table 4: Precision data for ESO absorbance at 302.86 nm

Conc. (µg/ml)	Intraday (Abs. ± S.D)	C.V.	Inter day (Abs. ± S.D)	C.V.
4	-0.0025 ± 0.00002	1.017	-0.0026 ± 0.00005	1.960
12	-0.00119 ± 0.00005	0.483	-0.00119 ± 0.00001	1.280
24	-0.00237 ± 0.00005	0.243	-0.00238 ± 0.00005	0.249

Table 5: Accuracy data for LEVO & ESO by Derivative spectrophotometric Method

% Level of Recovery	Amount of drug sample (µg/ml)	Amount of standard added (µg/ml)	Total amount of drug (µg/ml)	Amount of drug recovered ± SD (µg/ml)	% Recovery ± SD
LEVO (µg/ml)					
Unspiked	15	0			
50 %	15	7.5	22.5	7.62 ± 0.079	101.7 ± 0.435%
100 %	15	15	30	15.05 ± 0.045	100.3 ± 0.152%
150 %	15	22.5	37.5	22.6 ± 0.346	100.5 ± 0.115%
ESO (µg/ml)					
Unspiked	8	0			
50 %	8	4	12	4.01 ± 0.011	100.2 ± 0.577%

100 %	8	8	16	8.1 ± 0.064	101.2 ± 0.060%
150 %	8	12	20	12.2 ± 0.057	101.6 ± 0.264%

Table 6: Assay Results of Marketed Formulation

Formulation	Drug	Labelled claim (mg)	Amount taken (µg/ml) (n=3)	Amount found (µg/ml) (n=3)	% Label Claim ± S.D
Nexpro- L	LEVO	75	15	14.8 ± 0.115	99.11 ± 0.769
	ESO	40	8	7.9 ± 0.115	99.58 ± 1.443
Sompraz -L	LEVO	75	15	15.01 ± 0.115	100.06 ± 0.240
	ESO	40	8	8.03 ± 0.115	100.15 ± 0.202

CONCLUSION

The proposed second order derivative method provide simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous determination of LEVO and ESO in combined pharmaceutical formulation. The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The proposed methods can be used for routine analysis and quality control assay of LEVO and ESO in combined pharmaceutical formulation.

ACKNOWLEDGEMENT

Authors are thankful to Intas Pharmaceutical Pvt. Ltd. (Ahmedabad, Gujarat, India) and Torrent Pharmaceutical Pvt. Ltd. for providing gratis sample with the great pleasure. The authors also thankful to Indubhai Patel College of Pharmacy and Research Centre (Dharmaj, India) for providing the necessary facilities for research work and to all the staff members and friends for their guidance and help throughout the research work.

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