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DEVELOPMENT AND VALIDATION OF DUAL WAVELENGTH METHOD FOR SIMULTANEOUS ESTIMATION OF ESOMEPRAZOLE AND LEVOSULPIRIDE IN COMBINED CAPSULE DOSAGE FORM

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Abstract

Simple, sensitive, rapid, accurate and precise dual wavelength spectrophotometric method described for the simultaneous estimation of Esomeprazole (ESO) and Levosulpiride (LSP) in combined dosage form. The utility of dual wavelength data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. The principle for dual wavelength method is "The absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". The method was based on determination of ESO at the absorbance difference between 289.20 nm and 257.60 nm and LSP at the absorbance difference between 302 nm and 226.40 nm. The linearity was obtained in the concentration range of 4-24 µg/ml and 5-50 µg/ml for ESO and LSP respectively. The method was successfully applied to pharmaceutical dosage form because no interference from the excipients was found. The suitability of this method for the quantitative determination of ESO and LSP was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of ESO and LSP in capsule dosage form. The results of analysis have been validated statistically and by recovery studies.

INTRODUCTION

Esomeprazole is substituted benzimidazole 5-methoxy-2-[(R)-[(4-methoxy-3, 5-dimethylpyridin-2-yl) methane] sulfinyl]-1H-1,3-benzodiazole that function as proton pump inhibitors. It is anti-secretory drug effective for rapid healing peptic ulcer and corrosive esophagitis. Evosulpiride is benzamide class of drug N-{[(2S)-1-Ethylpyrrolidin-2-yl]methyl}-2-methoxy-5-sulfamoylbenzamide 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.[1-5] Combination of Esomeprazole and Levosulpiride is approved in 12th May - 2010 by CDSCO India and manufactured & marketed by Torrent Pharmaceutical LTD. as Nexpro-L Capsule with dosage regimen of 40 mg Esomeprazole and 75 mg Levosulpiiride. Combination is used in treatment of prevent conditions such as heartburn, ulcers, GERD and gastritis. Esomeprazole is official in US Pharmacopoeia[6] while Levosulpiride is not official in any pharmacopoeia. Deep literature survey reveals that numbers of analytical methods are reported for the estimation of Esomeprazole and Levosulpiride in single dosage forms. Reported methods for estimation of Esomeprazole are Spectrophotometry[7-8], derivative spectrophotometer, assay using HPTLC[9] and similarly for estimation of Levosulpiride are UV-spectroscopy[10], using RP-HPLC[11]. We could not trace a single analytical method for the estimation of these two drugs in combined dosage forms. So in present study simple, sensitive, specific, accurate and precise dual wavelength method is described for the estimation of these two drugs in combined capsule dosage form.

MATERIALS AND METHOD

Double beam UV-visible spectrophotometer (Shimadzu, model 1800) attached to a computer software UV-Probe 2.42 having two matched quartz cells with 1 cm light path, Electronic analytical balance, Shimadzu AUX-220, Volumetric flasks & Pipettes of Borosilicate Glass & Whatmann Filter Paper No. 41 were used in the study. Methanol was used as solvent of GR grade (Merck Ltd., Mumbai). All (zero cross for Diacerein). Apparatus and instruments were calibrated and validated as per calibration

and validation protocol specified before starting the experiment.

Preparation of Standard Solutions

Preparation of standard stock solution of Esomeprazole

Accurately weighed quantity of ESO10 mg was transferred to 100-ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 100 µg/ml.

Preparation of standard stock solution of Levosulpiride

Accurately weighed quantity of LSP 10 mg was transferred to 100-ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 100 µg/ml.

Procedure for determination of wavelength for measurement

In this method for estimation of both analyte, two wavelengths have been selected at which one analyte shows same absorbance and at this two wavelengths difference in absorbance is used for estimation of second analyte. The difference in absorbance between 289.20 nm and 257.60 nm (difference is zero for LSP) were plotted against the concentration of ESO. Similarly difference in absorbance between 302 nm and 226.40 nm (difference is zero for ESO) were plotted against the concentration of LSP. (Figure 1)

Preparation of Calibration Curves

Series A

Solutions of ESO ranging from 4-24 µg/ml were prepared by pipetting out 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 ml of the standard stock solution of ESO(100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol.

Series B

Solutions of LSP ranging from 5-50µg/ml Were prepared by pipetting out 0.5, 1, 2, 3, 4, and 5 ml of the standard stock solution of LSP(100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol.

Validation Parameters of Developed Method[12]

Validation of developed method was carried out as per ICH Q2 R1 guideline. Parameters such as Linearity, Accuracy,

Precision, LOD and LOQ were taken up as tests for analytical method validation.

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solutions of ESO and LSP. The Beer Lambert's concentration range was found to be 4-24 µg/ml for ESO and 5-50 µg/ml for LSP.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (50%, 100% & 150%). Average percent recovery for ESO and LSP were found to be 98.97%, 99.43, and 11.28% for ESO and 99.93, 100.33, and 98.03 for LSP respectively (table 2 & 3).

Precision

The repeatability, intraday and interday variations for determination of ESO and LSP were carried out three times in same day and for three consecutive days and % RSD were calculated. The method was found to be precise due to low values of %RSD.

LOD & LOQ

The LOD and LOQ of developed method were calculated by using equations:

Limit of Detection (LOD): 3.3 × σ/S

Limit of Quantification (LOQ): 10 × σ /S

Where, σ = The Standard deviation of the response,

S = Slope of calibration curve.

The results of all validation parameters obtained are shown in table no.3,4,5.

Simultaneous Estimation of ESO and LSP in Combined Capsule Dosage Form

Twenty capsules were weighed and average weight of content was determined & the content of capsules was powdered. The powder equivalent to 10 mg of ESO or 18.75 mg of LSP was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with methanol. The solution was filtered through Whatmann filter paper no. 41 and first few ml of filtrate were discarded. 1 ml of this solution was diluted to 10 ml with methanol six times.

The difference in absorbance of the resulting solution was measured at 289.20 nm and 257.60 nm for determination of ESO & at 302 nm and 226.40 nm for

determination of LSP. The concentration of each drug was calculated using calibration curve equation. The results obtained are shown in table no. 6.

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of ESO and LSP. In proposed Dual Wavelength Method, two wavelengths have been selected at which one analyte shows same absorbance and at this two wavelengths difference in absorbance is used for estimation of second analyte. The difference in absorbance between 289.20nm and 257.60 nm (difference is zero for LSP) were plotted against the concentration of ESO. Similarly difference in absorbance between 302 nm and 226.40 nm (difference is zero for ESO) were plotted against the concentration of LSP. The linearity of measurement was evaluated by analyzing different concentration of the standard solutions of ESO and LSP. The Beer Lambert's concentration range was found to be 4-24 µg/ml for ESO and 5-50 µg/ml for LSP. Percent label claim for ESO and LSP in capsule analysis was found to be 98% and 98.66%. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Percent recovery for found to be different levels (50%, 100% & 150%). Average percent recovery for ESO and LSP were found to be 98.97%, 99.43, and 11.28% for ESO and 99.93, 100.33, and 98.03 for LSP values of standard deviation and coefficient of variation were satisfactorily low indicating the accuracy of both the methods. Based on the results obtained, it is found that the proposed method is accurate, precise, reproducible & economical and can be employed for routine quality control of ESO and LSP in combined capsule dosage form.

CONCLUSION

A validated Dual Wavelength Method has been developed for the estimation of ESO and LSP in Capsule Dosage Form. Proposed method is simple, accurate and precise. The method is suitable for routine analysis of ESO and LSP in Capsule. The simplicity of this method allows for application in laboratories that lack sophisticated analytical instruments such as HPLC, LC–MS. Detection and quantification limit achieved,

describe the method is very sensitive. High recoveries and acceptable % RSD values confirm established method is accurate and precise. The analytical results demonstrate that their ability of the developed method to assay ESO and LSP. Hence, the method is recommended for routine quality control analysis of ESO and LSP.

Table 1 Linearity of Esomeprazole

Con.	Difference of Abs.
4	0.047
8	0.094
12	0.131
16	0.181
20	0.22
24	0.266

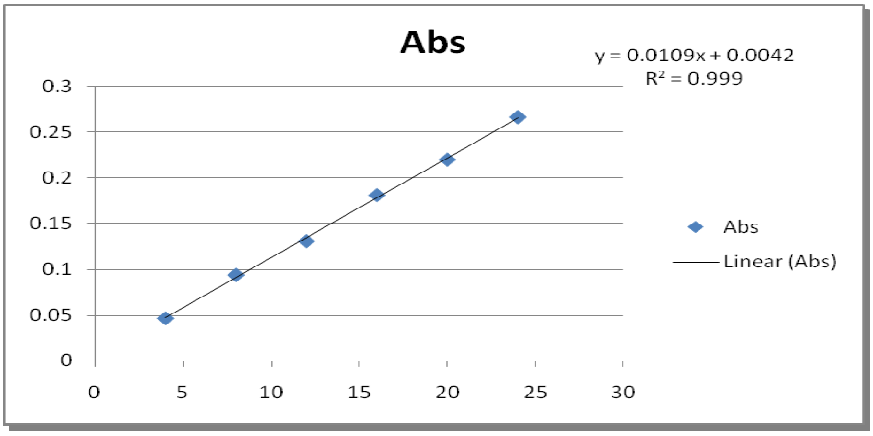

Table 2 Linearity of Levosulpiride

Con.	Difference of Abs.
5	0.225
10	0.36
20	0.698
30	1.104
40	1.401
50	1.797

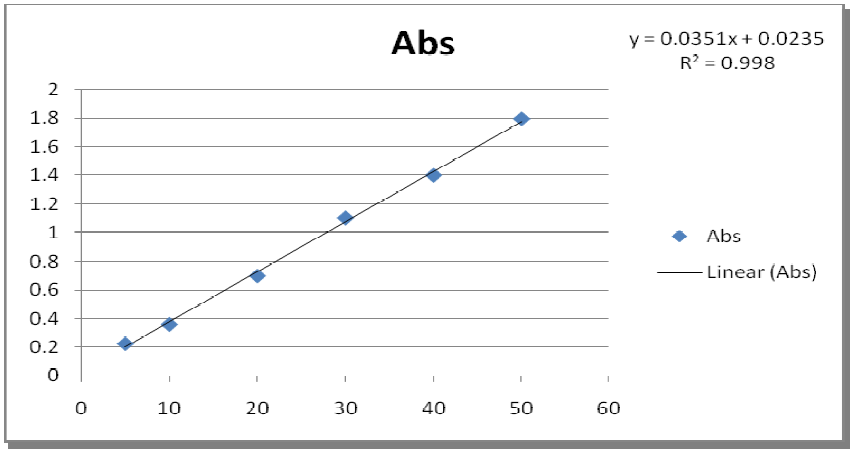

Table 3: Dual accuracy for Esomeprazole

Level of % Recovery	Amount of Standard Drug Added (µg/ml)	Abs at 289.2	Abs at 257.6	Difference of Abs.	% Recovery	%Avg Recovery	SD	%Rsd
	8+4	0.484	0.352	0.132	97.7			
50	8+4	0.485	0.351	0.134	99.23	98.97	1.170741	1.182925
	8+4	0.486	0.351	0.135	100			
	8+8	0.624	0.446	0.178	99.65			
100	8+8	0.625	0.448	0.177	99	99.43	0.375278	0.377429
	8+8	0.623	0.445	0.178	99.65			
	8+12	0.806	0.58	0.226	101.74			
150	8+12	0.807	0.583	0.224	100.82	101.28	0.46	0.454186
	8+12	0.805	0.58	0.225	101.28			

Table 4: Dual accuracy for Levosulpiride

Level of % Recovery	Amount of Standard Drug Added (µg/ml)	Abs. at 302	Abs at 226.4	Difference of Abs.	% Recovery	%Avg. Recovery	SD	%Rsd
	15+7.5	0.645	1.459	0.814	100			
50	15+7.5	0.644	1.457	0.813	99.96	99.93	0.083267	0.083325
	15+7.5	0.646	1.458	0.812	99.84			
	15+15	0.638	1.7	1.06	98.43			
10	15+15	0.637	1.71	1.07	99.38	100.33	1.299474	1.2952
	15+15	0.64	1.75	1.11	101			
	15+22.5	0.693	1.99	1.297	98			
150	15+22.5	0.695	2	1.305	97.35	98.03	1.381123	1.408878
	15+22.5	0.699	2.05	1.351	100			

Table 5: Regression analysis data and summary of validation parameters for proposed method

S.No.	Validation Parameters	Dual Wavelength Method	
		ESO	LSP
1.	Linearity Range	4-24 µg/ml	5-50 µg/ml
2.	Linearity Equation[p]	Y=0.0109x+0.0042	Y=0.0351x+0.0235
	Slope[b]	0.0109	0.0351
	Intercept[a]	0.0042	0.0235
3.	Correlation Coefficient (R^2)	0.999	0.9982
4.	Precision (% RSD)		
	Intraday (n=3)	1.031913	1.431994
	Interday (n=3)	0.966675	.604852
5.	Accuracy (n=3)	99.89	99.43
6.	LODs (µg/ml)	0.084	0.59
7.	LOQs (µg/ml)	0.256	1.766

[p]means Y = a + bc, where c is concentration in Og/mL, a – intercept, b – slope and Y – absorbance units.

Table 6: Analysis of market formulation

Capsule	Label Claim (mg)		Concentration. Found (mg)		% Assay	
	ESO	LSP	ESO*±SD	LSP*±SD	ESO*	LSP*
Nexpro-L	10	18.75	10.12±0.00028	18.50±0.0062	98	98.66

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