

SPECTROPHOTOMETRIC DETERMINATION OF NAPROXEN AND ESOMEPRAZOLE IN A LABORATORY MIXTURE BY SIMULTANEOUS EQUATION, ABSORPTION CORRECTION, ABSORPTION RATIO AND AREA UNDER CURVE METHODS

Neha A.Jain*, R.T.Lohiya and M.J.Umekar

S.K.B. College of Pharmacy, New kamptee, Nagpur, Maharashtra, India.

*Corres. author: niki_j19@yahoo.com

ABSTRACT: An accurate, specific and precise UV spectrophotometric method was developed for the simultaneous determination of naproxen (NP) and esomeprazole (EOZ) in a laboratory mixture of these two components. The method involves area under curve (AUC) method in the range 227-237nm and 296.5-306.5nm respectively, formation of simultaneous equation at 232nm and 301.5nm respectively, absorption correction method at 232nm λ_{max} of naproxen, 239.2nm isoabsorptive point of NP & EOZ & 301.5nm for absorption ratio method by using methanol as a solvent. The linearity for both naproxen and esomeprazole was in the range of 1-5 $\mu\text{g/ml}$ and 4-12 $\mu\text{g/ml}$ respectively. The % recovery was found to be 98.23% and 98.87% for naproxen and esomeprazole respectively indicating proposed method is accurate and precise for simultaneous estimation of naproxen and esomeprazole in bulk formulations.

KEY-WORDS: Naproxen, Esomeprazole, UV spectrophotometry, Simultaneous equation method, Absorption ratio, absorption correction method and Area under curve.

INTRODUCTION

Naproxen (NP) is chemically (S)-6-methoxy- α -methyl-2-naphthaleneacetic acid, is a non-steroidal anti-inflammatory drug (NSAID) which is used for the treatment of severe pain and inflammation. It acts by reducing the levels of prostaglandins, chemicals that are responsible for pain, fever and inflammation. Naproxen blocks the enzyme that makes prostaglandins (cyclooxygenase), resulting in lower concentrations of prostaglandins.^{1,2}

Esomeprazole (EOZ) is chemically bis(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1-H-enzimidazole-1-yl), a compound that inhibits gastric acid secretion. Esomeprazole is cost effective in the treatment of gastric oesophageal reflux diseases. It is S-isomer of omeprazole and is the first single optical isomer proton pump inhibitor. It provides better acid control than current racemic proton pump inhibitors and has a favorable pharmacokinetic profile relative to omeprazole.^{3,4,5}

Several analytical methods have been published for the determination of NP in pharmaceutical preparations and biological fluids. These methods included first derivative non-linear variable-angle synchronous fluorescence spectroscopy⁶, CE with electro spray mass spectrometry⁷, HPLC^{8,9} and capillary isotachopheresis^{10,11}, flow-injection analysis (FIA)¹² and FIA by using complex formation of NAPS^{13,14}.

A detailed survey of literature revealed the estimation of omeprazole by gas chromatographic method¹⁵, UV spectrophotometric method^{16,17}, TLC¹⁸ and several HPLC^{19,20} methods. However, there is no evidence in literature for simultaneous determination of NP and EOZ. Hence, in the present investigation Simultaneous

equation, Absorption correction, Absorption ratio and Area under curve methods was developed for the determination of NP and EOZ in combination from their laboratory mixture.

MATERIALS AND METHODS

Materials

Naproxen and esomeprazole were supplied by Dr. Reddy's lab as gift samples. A Jasco UV630 & Shimadzu1700 UV spectrophotometer with 1 cm matched quartz cells was used for estimation. All reagents were used of GR grade purchased from Loba chemie, Mumbai.

Methods

Standard Preparation:

Accurately weighed quantities (20 mg each) of NP and EOZ were dissolved separately in sufficient quantity of methanol in a 50 ml volumetric flask. The volume was adjusted up to the mark with methanol to obtain a stock solution of 100 µg/ml; each of NP and EOZ.

Preparation of laboratory mixture:

A bulk mixture of both drugs (NP and EOZ) was prepared using 20 mg of NP and 20 mg of EOZ. Common excipients which are used in tablet formulation were added in this laboratory mixture, triturated well and weighed. A powder equivalent to 20 mg of NP and 80 mg of EOZ was weighed accurately and transferred to 100 ml of volumetric flask, dissolved in sufficient quantity of methanol and volume was adjusted up to the mark with methanol. The sample solution thus prepared was filtered through Whatman filter paper no. 44, diluted with methanol to get the solution containing about 1µg/ml of NP and 4µg/ml of EOZ.

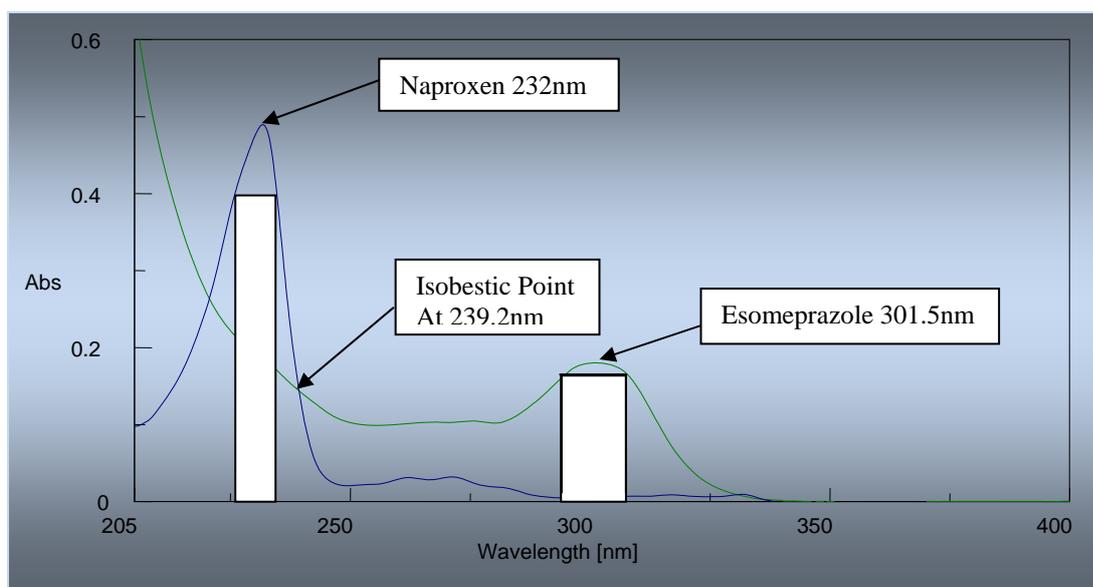
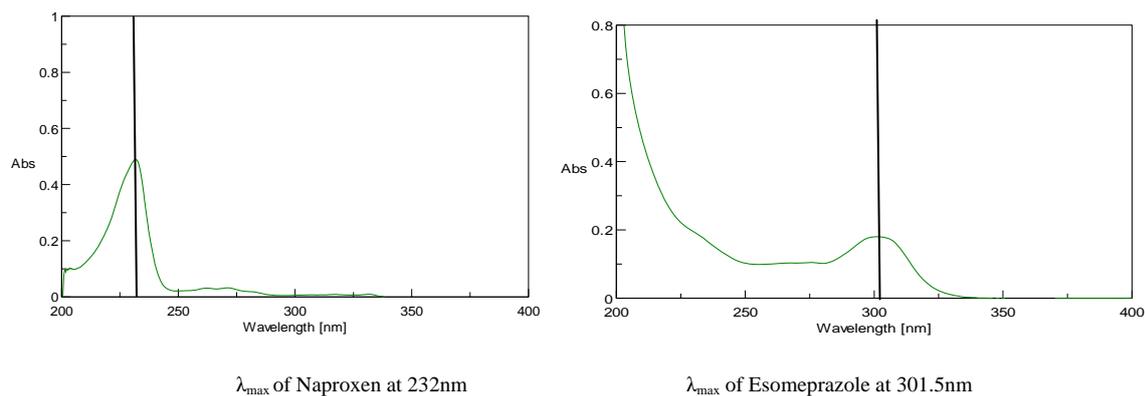


Fig..Overlain Spectra of Naproxen & Esomeprazole showing Abs. & AUC

Method development:

For the selection of analytical wavelength for the simultaneous estimation, the stock solutions of NP and EOZ were separately diluted with methanol, to obtain the concentrations of 10µg/ml each, and scanned in the wavelength range of 200-400 nm. The λ_{max} of NP and EOZ were found to be 232nm (λ₁) and 301.5 nm (λ₂) respectively. For the construction of calibration curve, standard solutions of NP and EOZ were diluted in the range of 1-5µg/ml and 4-12 µg/ml respectively.

In Simultaneous equation method, the absorbance of the solution was measured at 232nm and 301.5nm and concentration of the two drug was calculated using

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad (\text{Eqn.1}) \text{ and}$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad (\text{Eqn.2})$$

Where, C_x and C_y are concentration in g/100 ml of NP and EOZ respectively.

a_{x1} is the absorptivity of NP at 232nm, a_{x2} is the absorptivity of EOZ at 301.5nm,

a_{y1} is the absorptivity of NP at 232nm, a_{y2} is the absorptivity of EOZ at 301.5nm.

In Absorption correction method, isoabsorptive point was employed in which the absorbance was measured at two wavelengths, one being the isoabsorptive point of the two components and other being the wavelength of maximum absorption of one of the two components. From the overlain spectra of two drugs absorbances were measured at selected wavelength i.e. 239.2nm isoabsorptive point and 301.5nm, λ_{max} of EOZ [Figure1]. The absorbance and absorptivity values at the particular wavelengths were calculated and substituted in the following equation; to obtain the concentration

$$C_x = \frac{(Q_m - Q_y)}{(Q_x - Q_y)} \frac{A}{A^*} a_x \quad (3) \text{ and}$$

$$C_y = \frac{(Q_m - Q_x)}{(Q_y - Q_x)} \frac{A}{A^*} a_y \quad (4)$$

where, A=Absorbance of mixture at isoabsorptive point.

Q_m=Ratio of absorbance of laboratory mixture at 232nm and 301.5nm,

Q_x= Ratio of absorptivity of NP at 232nm and 301.5nm ,

Q_y= Ratio of absorptivity of PAR at 232nm and 301.5nm.

In method III, *Absorbance ratio method* uses the absorbances at two selected wavelengths, one at λ_{max} of one drug where other drug also shows considerable absorbance and other being the wavelength at which the first drug has practically nil absorbance 301.5nm is the corrected wavelength.

In Area under curve method, AUC involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelength 232nm and 301.5nm (Fig). Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. Suitable dilutions of standard stock solution (2µg/ml) of the drug were prepared and scanned in the spectrum mode from the wavelength range 400-200 nm and the calibration curve was plotted. The sampling wavelength ranges selected for estimation of NP and EOZ are 227-237nm (λ₁-λ₂) and 296.5-306.5nm (λ₃-λ₄) respectively. Mixed standard were prepared and their Area under the Curve were measured at the selected wavelength ranges.

Recovery studies were carried out at 80%, 100% and 120% level by adding a known quantity of pure drug to the preanalyzed laboratory mixture and the proposed method was followed. From the amount of drug found, percentage recovery was calculated.

RESULTS AND DISCUSSION

The proposed method of simultaneous determination of NP and EOZ showed absorptivity of 399.29 whereas EOZ showed absorptivity of 45.97. Linear regression of absorbance on concentration gave equation $y = 0.051x - 0.036$ with a correlation coefficient of 0.999 for NP and equation $y = 0.319x + 0.176$ with a correlation

coefficient of 0.999 for EOZ respectively. The low % RSD values of 0.414 for NP and 0.412 for EOZ were observed for analysis of 5 replicate samples, indicating precision and reproducibility. NP exhibits its maximum absorption at 232nm and obeyed Beer's law in the range of 1-5µg/ml and EOZ exhibits its maximum absorption at 301.5 nm and obeyed Beer's law in the range of 4-12µg/ml. The results of analysis and recovery studies are presented in the Table. The percentage recovery value 98.23% and 98.87% for NP and EOZ respectively indicates that there is no interference from the excipients present in laboratory mixture. The developed method was found to be sensitive, accurate, precise and reproducible and can be applicable for the analysis of NP and EOZ in laboratory mixtures.

TABLE: Analysis of dosages forms and Recovery studies.

Product	Drug	Labelclaim	% Estimated *	% RSD	% Recovery†	% RSD of recovery
Lab Mixture	NP	20 mg	100.97	0.41	98.23	1.23
	EOZ	80 mg	102.01	1.11	98.87	1.34

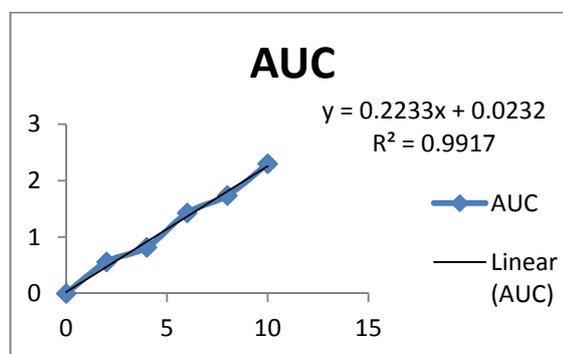
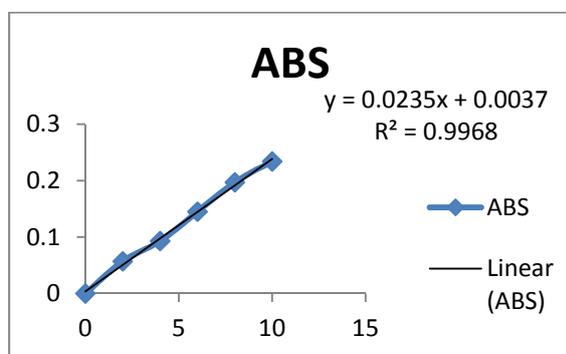
NP: Naproxen; EOZ: Eesomeprazole; RSD: Relative standard deviation;

* Indicates mean of three determinations (n=3);

†Indicates mean of three recovery studies at 80%, 100% and 120% level.

Table: Beer's Lambert study of lab mixture

Concentration	Absorbance	Area Under Curve
2	0.057	0.5591
4	0.093	0.8979
6	0.145	1.4266
8	0.197	1.7963
10	0.234	2.298



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