

was procured from the local drug market. Label claim of ESO and NAP in tablet is 20 and 500 mg, respectively.

Preliminary solubility studies

Solubility of ESO and NAP was determined at $25 \pm 1^\circ\text{C}$. Accurately weighed 10 mg ESO and NAP was added in different 10 mL volumetric flasks containing different solvents. It was found that both the drugs are having good solubility in methanol 50%.

Preparation of standard solution

Standard stock solutions were prepared by dissolving separately 100 mg of ESO and 500 mg of NAP in 50 mL of methanol 50% solution and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark (100 mL) with the same solvent to get a concentration of 1000 $\mu\text{g/mL}$ and 5000 $\mu\text{g/mL}$ for both the drugs respectively. Solutions were diluted with methanol 50% to obtain concentrations in the range 5 – 25 $\mu\text{g/mL}$ for ESO and 50 – 250 $\mu\text{g/mL}$ for NAP, respectively, for direct analysis.

Preparation of overlay spectra

Working standard solution from the standard stock solution prepared as stated above of 20 $\mu\text{g/mL}$ of ESO and 200 $\mu\text{g/mL}$ of NAP were scanned in the spectrum mode over the range of 200–400 nm against 50% methanol as blank and the overlaid spectra of the two were recorded. ESO showed an absorbance peak at 301.60 nm whereas NAP shows an absorbance peak at 330.20 nm. The overlaid spectra also showed isoabsorptive points at 311.50 nm (Fig. 2). Due to difference in absorbance maxima and having no interference with each other, both

drugs can be simultaneously estimated by simultaneous equation method (Method-1) and absorbance ratio method (Method-II).

Simultaneous equation method (Method I)

From the stock solution of 100 $\mu\text{g/mL}$, working standard solutions of drugs were prepared by appropriate dilution and were scanned in entire UV range to determine the λ_{max} values. Esomeprazole has λ_{max} of 301.60 nm while naproxen has λ_{max} at 330.20 nm, respectively (Fig. 2). Standard solutions were prepared having concentration 5 – 25 $\mu\text{g/mL}$ for esomeprazole and 50 – 250 $\mu\text{g/mL}$ for naproxen. The absorbances of these standard solutions were measured at 301.60 nm and 330.20 nm and calibration curves were plotted at these wavelengths. Two simultaneous equations (in two variables C_1 and C_2) were formed using these absorptivity coefficient values.

$$A_1 = (0.0487) C_1 + (0.0079) C_2 \quad (1)$$

$$A_2 = (0.00308) C_1 + (0.0065) C_2 \quad (2)$$

where, C_1 and C_2 were the concentrations of ESO and NAP measured in $\mu\text{g/mL}$, in sample solutions. A_1 and A_2 were the absorbances of mixture at selected wavelengths 301.60 nm and 330.20 nm, respectively.

By applying the Cramer's rule to equation 1 and 2, the concentration C_{ESO} and C_{NAP} , can be obtained as follows:

$$C_{\text{ESO}} = \frac{A_2 (0.0079) - A_1 (0.0065)}{A_2 (0.0079) - A_1 (0.0065)} \quad (3)$$

$$C_{\text{NAP}} = \frac{A_1 (0.00308) - A_2 (0.0065)}{0.000293} \quad (4)$$

Absorbance ratio method (Method II)

Q-absorbance method uses the ratio of absorbances at two selected wavelengths, one at isoabsorptive point and other being the λ_{max} of one of the two compounds. From the stock solutions, working standard solutions of esomeprazole (20 $\mu\text{g/mL}$) and naproxen (200 $\mu\text{g/mL}$) were prepared by appropriate dilution and were scanned in the entire UV range to determine the maximum absorbance (λ_{max}) and isoabsorptive point. Esomeprazole and naproxen have λ_{max} at 301.60 nm and at 330.20 nm, respectively. Both the drugs were found to have the same absorbance at 311.50 nm (isoabsorptive point). The wavelengths selected for analysis were 311.50 nm and 330.20 nm, respectively (Fig. 2). A series of standard solutions ranging from 5 to 25 $\mu\text{g/mL}$ for esomeprazole and 50–250 $\mu\text{g/mL}$ for naproxen were prepared and the absorbance of the solution were measured at 311.50 and 330.20 nm to plot a calibration curve of concentration *versus* absorbance. The

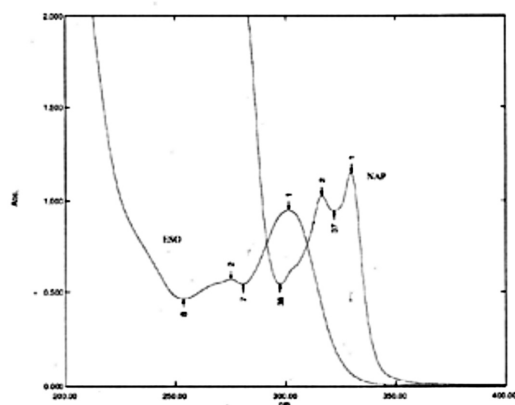


Figure 2. Overlaid spectra of ESO and NAP

Table 1. Results of linearity of esomeprazole (ESO) and naproxen (NAP).

PARAMETER	METHOD I		METHOD II	
	ESO	NAP	ESO	NAP
Working λ	301.6 nm	330.2 nm	311.5 nm	330.2 nm
Beer's law limit ($\mu\text{g/mL}$)	5–25	50–250	5–25	50–250
Correlation coefficient (r^2)*	0.9994	0.9999	0.9999	0.9999
Slope (m)*	0.0440	0.0068	0.0350	0.0068
Intercept (c)*	0.0015	0.0021	-0.0011	0.0021

* Average of five determinations, ESO = esomeprazole, NAP = naproxen

Table 2. Results of recovery studies on marketed formulations.

Recovery level %	% Recovery (mean \pm SD)*			
	METHOD I		METHOD II	
	ESO	NAP	ESO	NAP
80	98.50 \pm 0.064	98.90 \pm 0.234	98.50 \pm 0.064	97.59 \pm 0.358
100	96.70 \pm 0.204	99.10 \pm 0.176	95.98 \pm 0.061	98.03 \pm 0.274
120	99.70 \pm 0.055	98.40 \pm 0.214	98.24 \pm 0.098	98.48 \pm 0.507

*Average of 5 determinations. ESO = esomeprazole, NAP = naproxen.

Table 3. Results of validation (mean \pm SD).

PARAMETER		METHOD I		METHOD II	
		ESO	NAP	ESO	NAP
Precision (mean \pm SD)*	Repeatability	97.88 \pm 0.05	98.22 \pm 0.64	98.64 \pm 0.09	99.22 \pm 0.49
	Day to day	98.97 \pm 0.06	98.97 \pm 0.54	99.42 \pm 0.08	98.77 \pm 0.40
	Analyst to analyst	96.82 \pm 0.12	96.82 \pm 0.44	96.22 \pm 0.09	97.77 \pm 0.38
	Reproductibility	99.28 \pm 0.04	99.10 \pm 0.51	98.47 \pm 0.20	99.06 \pm 0.61

*Average of 5 determinations, ESO = esomeprazole, NAP = naproxen.

Table 4. Results of validation (%RSD).

PARAMETER		METHOD I		METHOD II	
		ESO	NAP	ESO	NAP
Precision (%RSD)*	Repeatability	0.662	0.643	0.096	0.493
	Day to day	0.066	0.540	0.080	0.403
	Analyst to analyst	0.122	0.441	0.092	0.384
	Reproductibility	0.049	0.517	0.206	0.617
	Robustness*	0.099	0.434	0.091	0.822

*Average of 5 determinations, ESO = esomeprazole, NAP = naproxen.

Table 5. Results of statistical parameters for tablet analysis.

	Drug	Label claim	Amount found	Mean*	S.D.*	%COV*	Std. error*
Method I	ESO	20	19.85	99.40	0.187	1.33	0.079
	NAP	500	498.64	98.09	0.780	0.641	0.124
Method II	ESO	20	19.92	99.39	0.070	0.748	0.013
	NAP	500	497.64	98.91	0.392	0.388	0.011

*Average of 5 determinations, ESO = esomeprazole, NAP = naproxen.

calibration curves were found to be linear in the concentration range under study.

The concentration of two drugs in mixture was calculated by using following equations:

$$C_{\text{ESO}} = \frac{Q_m - Q_x A_1}{Q_x - Q_x a_{x1}} \quad (5)$$

$$C_{\text{ESO}} = \frac{Q_m - Q_x A_2}{Q_y - Q_x a_{y1}} \quad (6)$$

where, A_1 and A_2 are the absorbances of mixture at 311.50 nm and 330.20 nm and a_{x1} (0.0349), a_{x2} (0.0087) and a_{y1} (0.00349), a_{y2} (0.0065) are absorptivities E (1%, 1 cm) of esomeprazole and naproxen at 311.50 nm and 330.20 nm and $Q_m = A_2/A_1$, $Q_y = a_{y2}/a_{y1}$ and $Q_x = a_{x2}/a_{x1}$.

Recovery studies

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e., 80%, 100% and 120%. The recovery studies were carried out by adding known amount of standard solution of ESO and NAP to preanalyzed tablet solutions. The resulting solutions were then re-analyzed by proposed methods. The whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 3 concentrations levels.

Precision studies

To evaluate precision at different parameter like repeatability, intermediate precision, and five dilutions in three replicates were analyzed in same day, in two different days by two analysts for day to day and analyst to analyst variation.

Robustness

As per ICH norms, small but deliberate variations by altering the pH and/or concentration of the solvent were made to check the methods capacity to remain unaccepted. The change was made in the combination of solvent system, containing 50%

methanol. Instead the 50 : 50 ratios of methanol and water, 60 : 40 methanol and water were used as solvent.

Analysis of tablet formulation

ESO: An amount of 10 mg was weighed from powdered mass of 20 tablets of VIMOVO (AstraZeneca) with the accuracy up to 0.1 mg. This powder was then transferred to 10 mL volumetric flask. The NAP present in this amount of tablet powder was 250 mg. The weighed amount of powder was poured with 8 mL of methanol 50% and sonicated for 15 min to solubilize the drug and then volume was made up to the mark with the same solvent. After sonication, filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with methanol 50% to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from both the methods. The procedure was repeated for three times.

RESULTS

For both the two methods linearity was observed in the concentration range of 5–25 µg/mL for esomeprazole and 50–250 µg/mL for naproxen (Table 1). The proposed methods were validated as per ICH guidelines. The accuracy of the method was determined by calculating the mean percentage recovery at 80, 100 and 120% level. The % recovery ranges from 95.98 to 99.70 for esomeprazole and naproxen for both two methods (Table 2). Precision was calculated as repeatability (SD and % RSD is less than 2) and inter and intraday variations (SD and % RSD is less than 2) for both the drugs. The repeatability data and reproducibility data are presented in Tables 3 and 4, respectively. Marketed brand of tablet was analyzed and amount of esomeprazole and naproxen determined by proposed

methods ranges from 98.09 to 99.40 as shown in Table 5.

DISCUSSION AND CONCLUSION

The proposed methods were found to be simple, accurate and rapid for the routine determination of esomeprazole and naproxen in tablet formulation. To study the validity and reproducibility of proposed methods, recovery studies were carried out. The methods were validated in terms of linearity, accuracy, precision, specificity and reproducibility. Both methods can be successfully used for simultaneous estimation of esomeprazole and naproxen in combined dosage form.

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Received: 07. 07. 2011