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Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Naproxen and Esomeprazole Magnesiumtrihydrate in Combined Pharmaceutical Formulation

Chandrakant Sojitra*, Abhishek Pathak, Sadhana Rajput

Quality Assurance Laboratory, Centre of Relevance and Excellence in Novel Drug Delivery System, Pharmacy Department, G. H. Patel Building, Donor's Plaza, The Maharaja Sayajirao University of Baroda, Fatehgunj, Vadodara, Gujarat, India – 390 002

Corres.author: sojitrapharma1610@gmail.com

Abstract: Four simple, rapid, precise and accurate spectrophotometric methods have been developed for simultaneous analysis of Naproxen (NAP) and Esomeprazole Magnesiumtrihydrate (ESO) in their combined dosage form. Method A, zero crossing first derivative spectrophotometry involves measurement of amplitudes at 276.4 nm (for NAP) and 322.2 nm (for ESO) in first derivative spectra. Method B, Dual wavelength method, involves measurement of difference in absorbance at 312 nm & 298 nm for estimation of NAP and 314 nm & 334.2 nm for estimation of ESO. Method C, ratio derivative spectrophotometry, involves division of spectra of NAP by one selected standard spectrum of ESO and then measuring amplitudes at 283.2 nm in ratio derivative spectra for estimation of NAP. Similarly, spectra of ESO are divided by one selected standard spectrum of NAP and then amplitudes at 295 nm in ratio derivative spectra are measured for estimation of ESO. Method D,simultaneous equation method applies measurement of absorptivities at two wavelengths, 335.2 nm and 319.6 nm, for ESO and NAP. The concentrations can be calculated from the derived equations. Developed methods were validated according to ICH guidelines. The calibration graph follows Beer's law in the range of 15 to 75 µg/ml for NAP and 2.8 to 6.0 µg/ml for ESO with R square value greater than 0.999. Accuracy of all methods was determined by recovery studies and showed % recovery between 98 to 102%. Intraday and interday precision was checked for all methods and mean %RSD was found to be less than 2 for all the methods. The methods were successfully applied for estimation of AML and IND in marketed formulation.

Keywords: Naproxen and Esomeprazole Magnesiumtrihydrate, Combined Pharmaceutical Formulation, Spectrophotometric Methods ,Simultaneous Estimation.

INTRODUCTION

Esomeprazole magnesium trihydrate (ES), bis(5-me thoxy-2- [(S) - [(4-me thoxy-3,5-dime thyl -2-pyridinyl)methyl]sulfinyl-1H-benzimidazole-1-yl) magnesium trihydrate (Figure a) is the S isomer of racemicomeprazole approved in February 2001 for use as a new pharmacological entity designed to improve the clinicaloutcome of available proton pump inhibitors in the management of acid-related disorders.

Naproxen is chemically 2-Naphthaleneacetic acid, 6-methoxy-α-methyl-,(s)-(+)-(s)-6-Methoxy-α-methyl-2-naphthaleneacetic acid(Figure b).

Naproxen is a nonsteroidal anti-inflammatory drug (NSAID) commonly used for the reduction of moderate to severe pain, fever, inflammation and stiffness. It works by inhibiting both the COX-1 and COX-2 enzymes. Like other NSAIDs, Naproxen is capable of producing disturbances in the gastrointestinal tract.

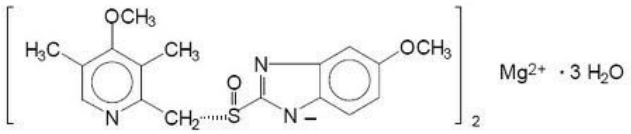

Fig a. Esomeprazole Magnesiumtrihydrate

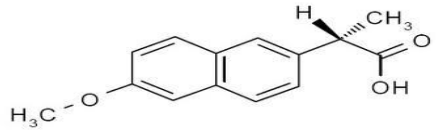

Fig b. Naproxen

EXPERIMENTAL

Instrumentation: Shimadzu UV-1700 double beam spectrophotometer connected to a computer loaded with Shimadzu UVProbe 2.10 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1 cm quartz cells over the range of 200-400 nm.The samples were weighed on electronic analytical balance (A×120, shimadzu).

Table 1: Instruments

Name	Model	Manufacture
UV/Visible spectrophotometer	UV-1700	Shimadzu
Electronic analytical balance	A×120	Shimadzu

Reagents and Chemicals

NaOH pellets (LOBA Chemiepvt ltd, Mumbai, India), Distilled water

0.1M NaOH was used as solvent and vehicle for UV Spectroscopy.

Preparation of solutions

Accurately weighed ESO and NAP (in quantities of 10 mg and 10 mg respectively) were transferred to two separate 10 ml volumetric flasks, dissolved with the use of 0.1M NaOH and volume was made up to the mark with 0.1M NaOH. From this, standard stocks solutions of NAP(1000 µg/ml) and ESO (1000 µg/ml) transferred 5 ml aliquots to 50 ml volumetric flasks and making up the volume with 0.1M NaOH& that will become 100 µg/ml. From this, 1.5, 3.0, 4.5, 6.0, and 7.5 ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with 0.1M NaOH. This gives 15 to 75 µg/ml of NAP. and for ESO from 10 µg/ml solution 0.8, 1.6, 2.4, 3.2 and 4 ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with 0.1M NaOH. To each of ESO solutions, 2 µg/ml standard addition was done to made 2.8 to 6.0 µg/ml of ESO.

Method A: Zero crossing first derivative spectrophotometry

The solutions of standard ESO and NAP were prepared in the range of 2.8 to 6.0 µg/ml and 15 to 75 µg/ml respectively. The absorption spectra of the solutions of ESO and NAP were recorded in the range of 200 nm to 400 nm and were stored in the memory of the instrument and transformed to first derivative with $\Delta\lambda$ = 4nm and scaling factor 10 (Fig 1.1.). At 276.4 nm, ESO is having zero crossing point and NAP can be determined. At 322.2 nm, NAP is having zero crossing point and ESO can be determined. The amplitudes at 276.4 nm were plotted against respective concentrations of NAP and the amplitudes at 322.2 nm were plotted against the respective concentrations of ESO for the preparation of calibration graph. Calibration graph for NAP and ESO are shown below (Fig 1.2.).

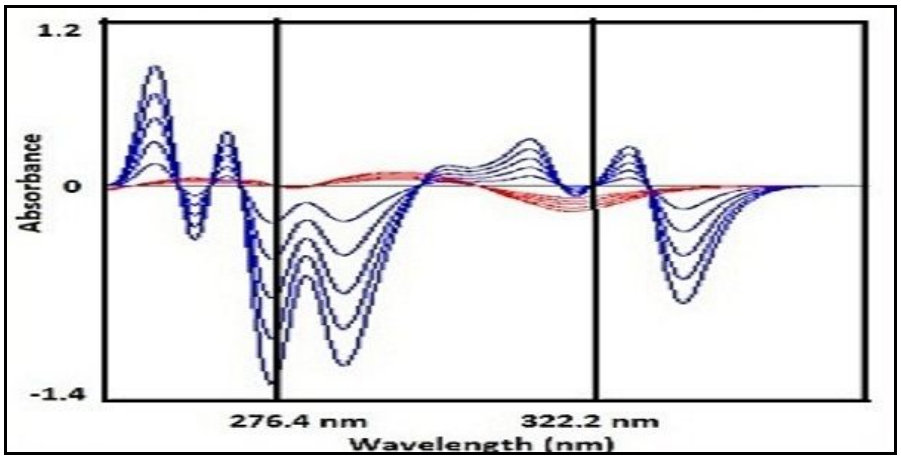

Fig 1.1, First derivative overlain spectra of NAP (15, 30, 45, 60, 75 µg/ml, blue) and ESO (2.8, 3.6, 4.4, 5.2, 6 µg/ml, red)

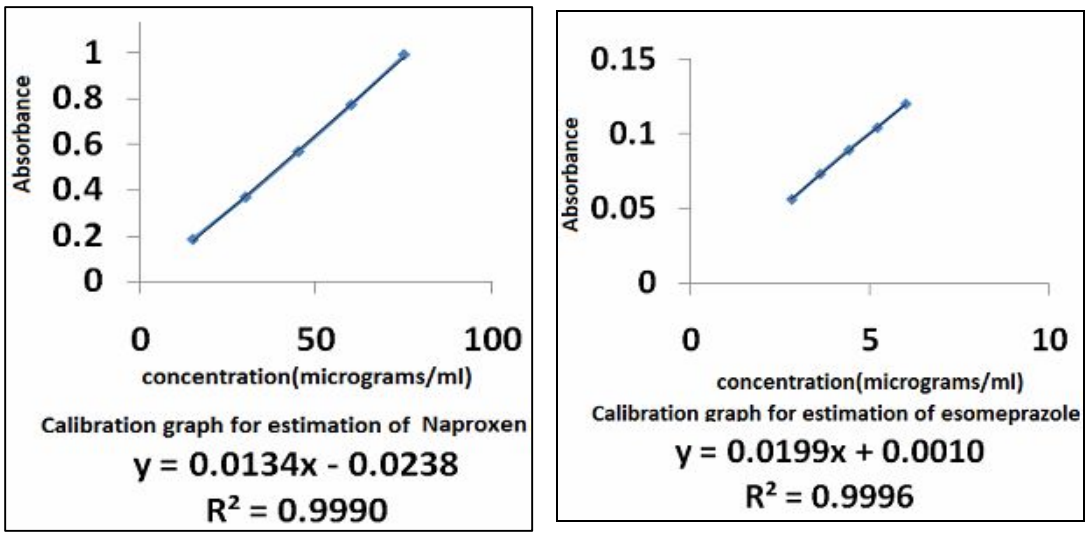

Fig 1.2, Calibration graphs of NAP and ESO by zero crossing first derivative method

Method B : Dual wavelength method

The solutions of standard ESO and NAP were prepared in the range of 2.8 to 6.0 µg/ml and 15 to 75 µg/ml respectively. The absorption spectra of the solutions of ESO and NAP were recorded in the range of 200 nm to 400 nm (fig 2.1). For estimation of each drug, difference in the absorbance at two wavelengths was measured in zero order spectra as the difference for other drugat this two wavelength is zero.For ESO, the difference in absorbance of 314 nm and 334.2 nm as the difference is zero for NAP were plotted against the concentration of ESO.Similarly, for the estimation of NAP, the difference in absorbance of 312 nm and 298 nm (difference is zero for ESO) were plotted against the concentration of NAP. Calibration graph for NAP and ESO are shown below (Fig 2.2).

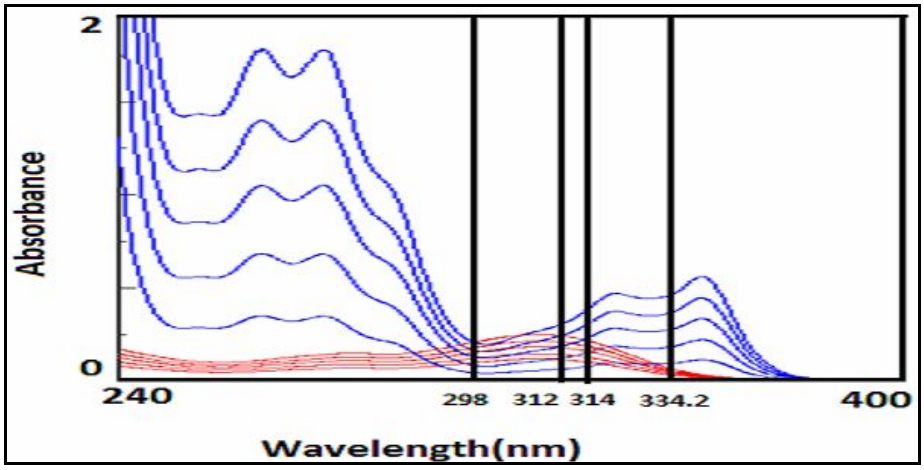

Fig 2.1, zero order overlain spectra of NAP (15, 30, 45, 60, 75 µg/ml, blue) and ESO (2.8, 3.6, 4.4, 5.2, 6 µg/ml, red)

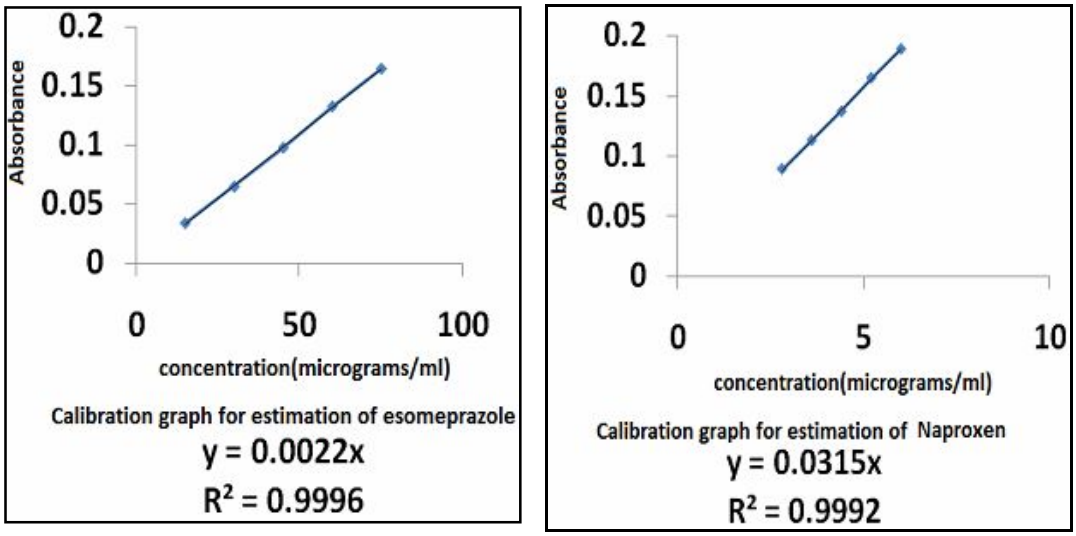

Fig 2.2, Calibration graphs of ESO and NAP by dual wavelength method

Method C : Ratio Derivative Spectrophotometry

In this method, the spectra of NAP and ESO were divided by one standard spectrum of ESO and NAP respectively. For selecting the standard solution as divisor, appropriate concentrations of NAP and ESO were tested and based on better signal to noise ratio values, 45 µg/ml of NAP and 4.4 µg/ml of ESO were selected as divisor concentration. The spectra of NAP ranging from 15 to 75 µg/ml were recorded in the region of 200 to 400 nm and were divided by standard spectrum of 4.4 µg/ml ESO to obtain ratio spectra. These ratio spectra were derivatised with$\Delta\lambda$ = 16 nm and scaling factor 1. Ratio derivative spectra are shown below (figure 3.1). Analytical wavelength of 283.2 nm was selected because of higher correlation co efficient for estimation of NAP. Calibration graph at this wavelength is plotted and shown in figure 8. Similarly, the spectra of ESO ranging from 2.8 to 6.0 µg/ml were recorded and divided by standard spectrum of 45 µg/ml NAP. These ratio spectra were derivatised with $\Delta\lambda$ = 16 nm and scaling factor 1. For estimation of ESO, analytical wavelength of 295 nm was selected. Ratio derivative spectra (Fig 3.1) and calibration graph (Fig 3.2) are shown below.

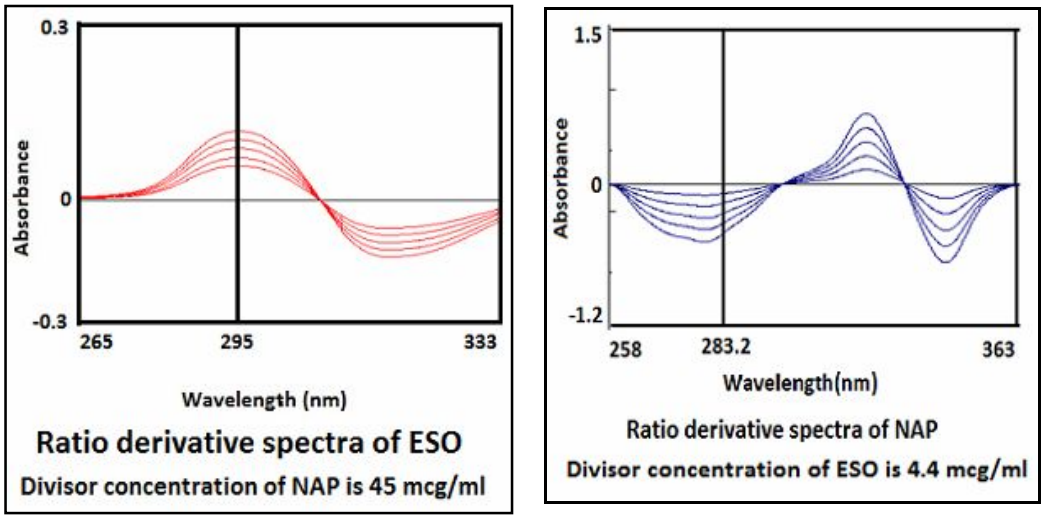

Fig 3.1, Ratio derivative spectra of ESO and NAP

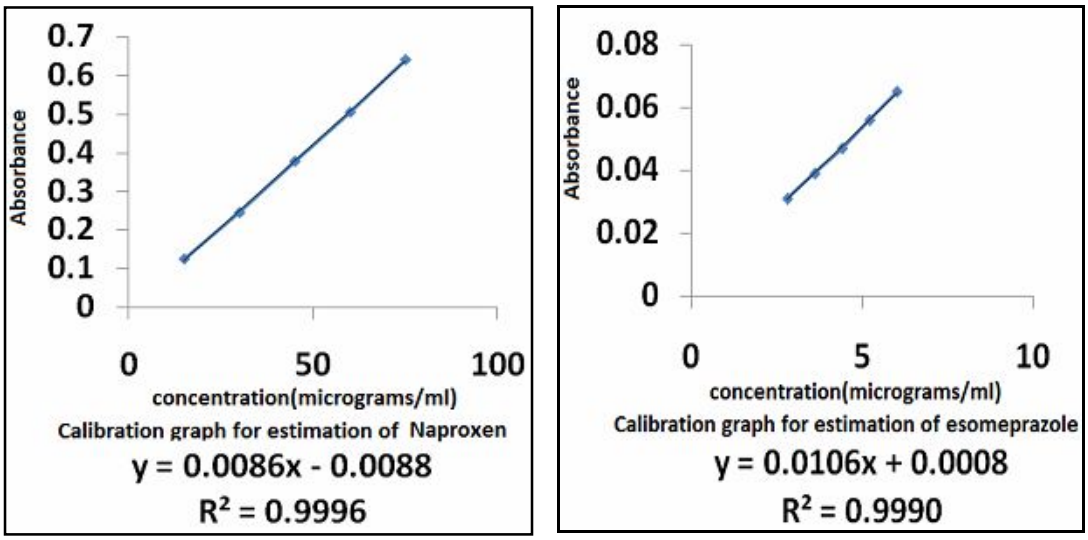

Fig 3.2, Calibration graphs of NAP and ESO by ratio derivative spectrophotometry

Method D: simultaneous equation method

From the stock solutions, working standard solutions of ESO (100 µg/ml) and NAP (100 µg/ml) were prepared. By appropriate dilutions, the solutions with concentrations 2.8-6 µg/ml (for ESO) and 15-75 µg/ml (for NAP) were prepared and scanned between 200 to 400 nm and transformed to first derivative with $\Delta\lambda = 4$ nm and scaling factor 50.For ESO and NAP, analytical wavelengths of 319.6 nm and 335.2 nm were selected respectively. Absorptivity of ESO and NAP were calculated at both the wavelengths.The concentrations of ESO and NAP can be calculated from following equations :

$$C_{x\,(ESO)} = (A_2\, a_{y1} - A_1\, a_{y2}) / (a_{x2}\, a_{y1} - a_{x1}\, a_{y2})$$
$$C_{y\,(NAP)} = (A_1\, a_{x2} - A_2\, a_{x1}) / (a_{x2}\, a_{y1} - a_{x1}\, a_{y2})$$

Assay of Commercial Formulation by Method A, B, C and D

10 tablets were powdered and an amount equivalent to 10 mg NAP and ESO were weighed and dissolved in 10 ml of 0.1M NaOH. Solutions were filtered using Whatmann filter paper grade 1. Appropriate dilutions were prepared in 0.1M NaOH taking suitable aliquots of the clear filtrates and subjected to analysis using all the four methods described above. The result of analysis is reported in Table 2.

Table 2: Results of Simultaneous Estimation of Marketed Formulation for Method A, B, C and D

Formulation :- VIMOVO		
Labelled claim :- NAP : ESO (375 mg : 20 mg)		
Method	**NAP***	**ESO***
A	99.88 ± 0.82 %	99.71 ± 1.34 %
B	98.77 ± 1.65 %	98.12 ± 0.97 %
C	99.95 ± 0.56 %	99.68 ± 0.77 %
D	98.95 ± 0.81 %	99.72 ± 0.65 %

* Mean value of five determinations

RESULTS AND DISCUSSION

Four Developed spectrophotometric methods for the simultaneous were validated according to ICH guidelines. The calibration graph follows Beer's law in the range of 15 to 75 µg/ml for NAP and 2.8 to 6.0 µg/ml for ESO with R^2 value greater than 0.999. Spectra of ESO were completely overlapped by NAP and derivatisation was used as a powerful tool for simultaneous determination. Accuracy of all methods was determined by recovery studies and showed % recovery between 98 to 102%. Intraday and interday precision was checked for all methods and mean %RSD was found to be less than 2 for all the methods. The results of validation parameters for all the four methods are reported in Table 3.

CONCLULSION

Four Spectrophotometric methods were developed for simultaneous estimation of NAP and ESO in their combined formulation without prior separation. Methods were found to be precise and accurate as can be reflected from validation data. Developed methods were successfully applied for estimation of NAP and ESO in marketed formulation.

Table 3: Summary of Validation Parameters by Developed Methods

Parameters		Method A		Method B		Method C		Method D	
		ESO	NAP	ESO	NAP	ESO	NAP	ESO	NAP
Analytical wavelength (nm)		322.2	276.4	295	283.2	314 & 334.2	312 & 298	335.2 & 319.6	
Beer's range (µg/ml)		2.8 to 6	15 to 75	2.8 to 6	15 to 75	2.8 to 6	15 to 75	2.8 to 6	15 to 75
Slope		0.0199	0.0134	0.0106	0.0086	0.0315	0.0022	0.1094	0.0408
Intercept		0.001	0.0238	0.0008	0.0088	0	0	0.0076	0.0147
Correlation coefficient		0.9996	0.999	0.999	0.9996	0.9996	0.9992	0.9994	0.9999
Intraday precision (%RSD)		1.025	1.472	1.08	0.897	0.91	0.857	0.85	1.2
Interday precision (%RSD)		1.52	1.96	1.31	0.994	0.95	1.03	0.98	1.3
LOD (µg/ml)		0.057	0.068	0.081	0.098	0.064	0.076	0.057	0.08
LOQ (µg/ml)		0.171	0.204	0.243	0.294	0.192	0.228	0.171	0.24
%RECOVERY	80% standard addition †	98.43	99.16	98.43	100.41	98.43	98.75	99.6	98.92
	100% standard addition †	101.25	102.33	101.25	101.33	101.25	102.5	98.96	100.8
	120% standard addition †	98.95	99.84	98.95	98.88	98.95	99.44	100.4	99.76

† Mean value of three determinations

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