Bulletin of Pharmaceutical Research 2016;6(1):28-35
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

RESEARCH PAPER

DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR THE SIMULTANEOUS ESTIMATION OF NAPROXEN AND ESOMEPRAZOLE IN BULK AND PHARMACEUTICAL FORMULATION

Deepa Moye Shrestha*, T. Srinivasa Rao, C. Sreedhar, H.G. Akkamma, G. Bharadwaja Reddy and Purushotam Kumar Yadav

Dept. of Pharmaceutical Analysis, Karnataka College of Pharamacy, Bangalore-560064, Karnataka, India.

**E-mail*: moyedipa123@gmail.com
Tel.: +91 8553573851.

Received: Apr 15, 2016 / Revised: Apr 29, 2016 / Accepted: Apr 30, 2016

A new, simple, rapid, sensitive and inexpensive RP-HPLC method has been developed for the simultaneous estimation of naproxen and esomeprazole in bulk drug and pharmaceutical formulations. The method was validated for linearity, precision, accuracy, LOD and LOQ according to ICH guidelines. Chromatographic separation was achieved on Waters XBridge C18 column (250 × 4.6 mm, 5*μ*) using isocratic mobile phase 20 mM ammonium acetate (pH 3.8), acetonitrile and methanol (45:44:11 *v/v*) at 228 nm. The flow rate was 1ml/min. The retention time was observed at 3.69 min for esomeprazole and 6.6 min for naproxen. The standard curve was linear over the range of 2-12 *μ*g/ml with the correlation coefficient of 0.9988 for esomeprazole and 0.995 for naproxen. The mean recoveries obtained for esomeprazole and naproxen were 100.06% and 99.8 to 100.01% respectively and RSD was less than 2%. Developed method was highly precise and convenient for routine analysis of naproxen and esomeprazole in bulk and tablet dosage forms.

Key words: Naproxen, Esomeprazole, RP-HPLC, Simultaneous estimation, ICH guidelines.

INTRODUCTION

Naproxen [2-(6-methoxynaphthalen-2-yl) propanoic acid] (**Figure 1a**), is a widely used non-steroidal anti-inflammatory drug (NSAIDs) that belongs to aryl acetic acid group which is used in the treatment of rheumatoid arthritis, headache, muscle aches, dental pain, arthritis, bursitis, gout attacks and menstrual cramps. It has been used for a long time because of its efficacy and safety profile (Ahmed *et al* 2010; Manrique-Moreno *et al* 2010).

It works by inhibiting both the COX-1 and COX-2 enzymes (Vetrichelvan and Suresh, 2012). The constitutive cyclooxygenase, COX-1, synthesizes prostaglandins necessary for normal gastrointestinal (stomach mucus protection with regulation of gastric acid) and renal function (Dubois *et al* 1998). The inducible cyclo-oxygenase, COX-2, generates prostaglandins involved in signaling pain and inflammation (Turini and DuBois, 2002). So, inhibition of COX-1 is thought to be associated with gastrointestinal and renal toxicity while inhibition of COX-2 provides anti-inflammatory activity (Masferrer *et al* 1996). This drug is recognized to be highly effective and clinically safe, but some side-effects such as gastrointestinal toxicity, nephrotoxicity, jaundice and hepatotoxicity have been reported (Yokoyama *et al* 2006).

Esomeprazole (**Figure 1b**), the S-isomer of omeprazole (a racemic mixture), is the first proton pump inhibitor to be developed as a single optical isomer (Lind *et al* 2000).

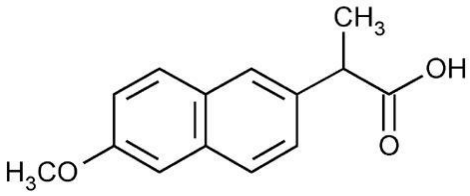

Fig. 1a. Structure of naproxen [2-(6-methoxy naphthalen-2-yl)propanoic acid]

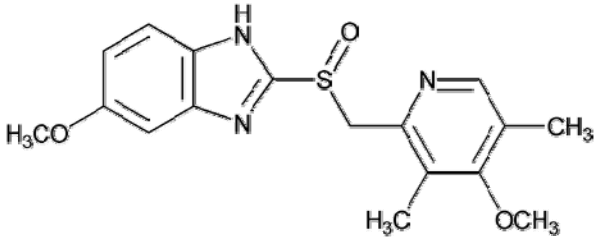

Fig. 1b. Structure of esomeprazole: [5-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl sulfinyl]-3*H*-benzoimidazole]

Proton pump inhibitors inhibit the gastric H^+/K^+-ATPase enzyme (the proton pump) and are the most potent suppressors of gastric acid secretion, diminishing daily production of gastric secretion and increasing intragastric pH (Miner *et al* 2003; Scott *et al* 2002). So, the combination of naproxen and esomeprazole proves beneficial to improve care and prevent NSAIDS associated gastric problems.

RP-HPLC and UV methods have been widely used for estimation of drug in bulk and dosage forms (Prasanthi *et al* 2011; Shukla *et al* 2011; Basaveswara Rao *et al* 2012; Singh *et al* 2012; Banerjee and Vasava, 2013; Singh and Dahiya, 2014).

Literature review also revealed that simultaneous analysis of naproxen and esomeprazole has been carried out by UV-Spectrophotometer (Mahaparale *et al* 2013; Patel *et al* 2012; Sojitra *et al* 2012), RP-HPLC (Jain *et al* 2011; Sojitra and Rajput 2012; Deshpande *et al* 2014; Ampati *et al* 2014; Saravanan *et al* 2014; Razzaq *et al* 2012), stability indicating simultaneous estimation of naproxen and esomeprazole by RP-HPLC (Reddy *et al* 2011), LC-MS/MS (Gopinath *et al* 2013) and UPLC method (Rao *et al* 2013; Bhavyasri *et al* 2013). The present work describes simple, specific, rapid, accurate and precise chromatographic method based on RP-HPLC mechanism for estimation of drugs in tablet dosage form.

EXPERIMENTAL

Instruments

The Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC-10AT vp pump) (4 MPa or 400 barr), rheodyne injector, UV variable wavelength detector, Standard cell and agilent syringe was used. The separations were achieved on a waters X- Bridge C18 column 5 μm 4.6 × 250 mm with UV detection at 228 nm. Analytical weighing balance (Shimadzu AUX 220) was used for weighing, sonicator (EQUITRON-230VAC, 50 Hz), vaccum pump (SUPER FIT), filtration kit (TARSONS) and Nylon membrane filter (Merck Millipore) for solvents and sample filtration were used throughout the experiment. Double beam UV Visible spectrophotometer (SHIMADZU-UV 1700) was used for wavelength detection. The EZ Chrome Elite software-single channel was used for acquisition, evaluation and storage of chromatographic data.

Reagents and chemicals

Naproxen and esomeprazole pure drugs were received as a gift sample from Karnataka Antibiotics and Pharmaceuticals Limited (KAPL), Bangalore, Karnataka, India.

The purity of the pure drugs was analyzed by using the IR spectrophotometer. Pharmaceutical formulation Vimovo tablet (Astrazeneca) (label claim containing 500 mg of naproxen and 20 mg of esomeprazole) was used in HPLC analysis. HPLC grade acetonitrile (Merck), methanol (Merck), analytical grade ammonium acetate was used as the solvents throughout the experiment. HPLC grade water was obtained in-house by using Direct-Q water purification system (Millipore, Milford, USA) was used in HPLC study.

Determination of working wavelength (λ_{max})

Ten milligram of naproxen standard drug was taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark. From this solution 0.1 ml is pipetted into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 μg/ml. Similarly, 10 mg of esomeprazole standard drug was taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark. From this solution 0.1 ml is pipetted into 10ml volumetric flask and made up to the mark with the methanol to give a concentration of 10 μg/ml.

Preparation of standard solution

Accurately weighed 25 mg of naproxen and 25 mg of esomeprazole is transferred in to 25 ml of volumetric flask and is dissolved in methanol and the volume were made up to the mark with the same solvent. This gave the concentration of 1000 μg/ml of naproxen and esomeprazole solution. From the above, six dilutions in between 2-12 μg/ml of working concentration made up by using mobile phase as a solvent.

Preparation of sample solution

Twenty tablets of Vimovo containing 500 mg of naproxen and 20 mg of esomeprazole were weighed and powdered for further study. The powder equivalent to 25 mg of naproxen and 25 mg of esomeprazole was accurately weighed and transferred to 25 ml volumetric flask.

After that drug mixture is dissolved in methanol. The volume is maintained with methanol and is sonicated for 10 min. The above solution was carefully filtered through Whatmann filter paper. From this solution, required dilutions for HPLC method were prepared by using methanol as a solvent. Triplicate of 20 μl injections were made for each concentration and chromatographed under specified condition at 24°C.

Optimized chromatographic condition

Waters X-Bridge C18 column (250 mm × 4.6 mm *i.d.*, 5 μm) column maintained at ambient temperature (25±1°C) was used as stationary phase. Mobile phase consisting ammonium acetate buffer pH 3.8, acetonitrile and methanol in ratio 45:44:11 *v/v*, at a flow rate of 1ml/min was used. The mobile phase was filtered using 0.45 μm filter paper and degassed for 10 min by sonication. Samples of 20 μl were injected into the HPLC system with the runtime of 10 min. Retention time of the drugs obtained under these conditions were 6.6 min for naproxen and 3.69 min for esomeprazole. For the quantitative analytical purposes the wavelength was set at 228 nm. Optimized chromatographic conditions for simultaneous estimation of naproxen and esomeprazole are summarized in **Table 1**.

Table 1. Optimized chromatographic conditions for simultaneous estimation of naproxen and esomeprazole

S. No.	Parameters	Combination of naproxen and esomeprazole
1	Mobile phase optimized	Ammonium acetate (buffer) : Acetonitrile : Methanol (45:44:11 *v/v*, pH 3.8 maintained by ortho-Phosphoric acid)
2	Stationary phase	C_{18} column 5 μm 250 × 4.6 mm (Waters X-Bridge)
3	Flow rate (ml/min)	1
4	Run time (min)	10
5	Column Temperature (°C)	25±1°C
6	Volume of Injection (μl)	20
7	Detection Wavelength (nm)	228 nm
8	Retention time (R_t)	6.6 min for naproxen and 3.69 min for esomeprazole

Method validation

Linearity

By using the working standard, aliquots of 2, 4, 6, 8, 10, 12 μg/ml were prepared with methanol. Six dilutions of each of the above mentioned concentrations were prepared separately and from these six dilutions, 20 μl of each concentration were injected into the HPLC system and their chromatograms were recorded.

Precision

The precision of the assay was determined in terms of intra and inter-day variation in the peak area for a set of drug solution 6 μg/ml, assayed six times on the same day and on different 2 days. The intra and inter day variation in the peak ratio of the drug solution was calculated in terms of co-efficient of variation (CV) and obtained by multiplying the ration of the standard deviation to the mean with 100 (CV = SD/MEAN × 100).

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted as either a conventional true value or an accepted reference value, and the value found. Accuracy of the method was performed at three levels, 80%, 100%, and 120% of the label claim of the tablet (20 mg of ESO and 500 mg of NAP). The amount spiked, amount recovered, percent recovery and

its mean were calculated.

$$\% \text{ Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

$$\text{Amount found} = \frac{\text{Mean test area}}{\text{Mean standard area}} \times \text{Standard conc.}$$

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes was compared with the signals of the blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

The above prepared solutions were scanned in UV between 200-400 nm using methanol as blank. The λ_{max} of naproxen and esomeprazole were found to be 228 nm and 302 nm respectively. Then, 228 nm was selected as common wavelength for the simultaneous estimation of both the drugs, as these are eluting in the same mobile phase with good absorbance along with good peak shape, height and intensity.

RESULTS AND DISCUSSION

Absorption maxima of naproxen and esomeprazole was achieved at 302.20 and 228.20 nm respectively (**Figure 2a,b**). Peak areas were recorded for all the peaks as shown in **Table 2** and a standard calibration curve of peak area against concentration was plotted. A linearity was obtained over six concentrations for both the drugs (**Figure 3a,b**). Recovery studies were used to determine the accuracy of the method (**Figure 4-6**).

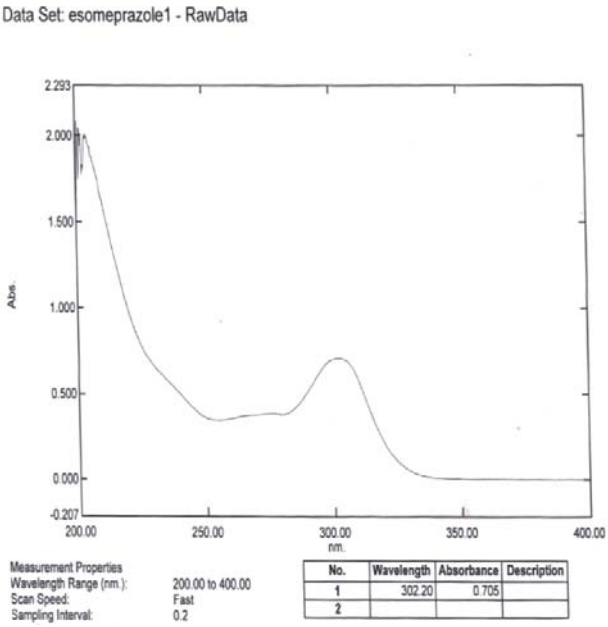

Fig. 2a. Absorption maxima of naproxen

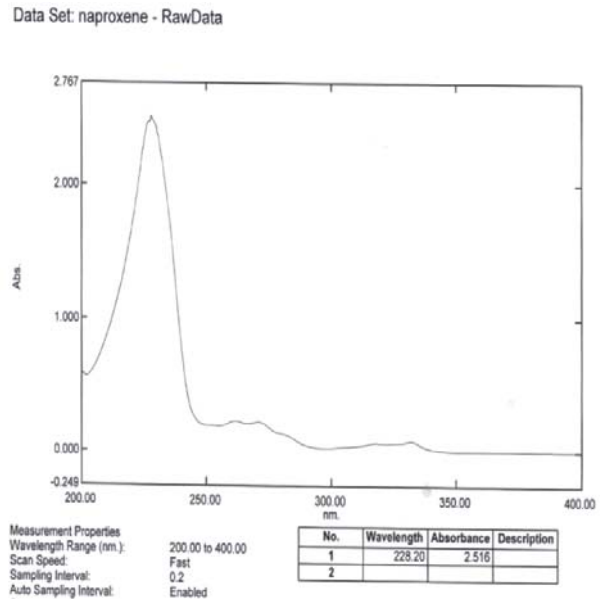

Fig. 2b. Absorption maxima of esomeprazole

Table 2. Calibration curve for simultaneous estimation of esomeprazole and naproxen

S. No.	Concentration	Peak area at 228 nm	
		Esomeprazole	Naproxen
1.	2 μg/ml	1542199	8893064
2.	4 µg/ml	2995860	18628489
3.	6 μg/ml	4511222	25339200
4.	8 μg/ml	6408594	38684275
5.	10 μg/ml	7881699	46682105
6.	12 μg/ml	9546070	56959467

The recovery studies were carried out three times over the specified concentration range. Accuracy was evaluated at three different concentrations equivalent to 80, 100 and 120%

of the active ingredient by calculating the recovery with %RSD. The low RSD values of table indicated the ruggedness of the method (**Table 3-5**). LOD and LOQ values for naproxen and esomeprazole are given in **Table 6** and System suitability parameter for simultaneous estimation of naproxen and esomeprazole are summarized in **Table 7**.

Table 3. Intraday precision of simultaneous estimation of naproxen and esomeprazole

Morning				
Injection (6 µg/ml)	**Areas**	**Average**	**SD**	**% RSD**
1	4511222	4511277	120.0422	0.002661
2	4511220			
3	4511240			
4	4511237			
5	4511200			
6	4511544			
Afternoon				
Injection (6 µg/ml)	**Areas**	**Average**	**SD**	**%RSD**
1	4511243	4511316	142.5351	0.001717
2	4511342			
3	4511229			
4	4511621			
5	4511220			
6	4511238			

Table 4. Interday precision of simultaneous estimation of naproxen and esomeprazole

Day-1				
Injection (6 µg/ml)	**Areas**	**Average**	**SD**	**% RSD**
1	4511247	4511197	77.45698	0.001717
2	4511027			
3	4511200			
4	4511243			
5	4511240			
6	4511222			
Day-2				
Injection (6 µg/ml)	**Areas**	**Average**	**SD**	**% RSD**
1	4511547	4511360	110.0426	0.002439
2	4511424			
3	4511345			
4	4511264			
5	4511256			
6	4511325			

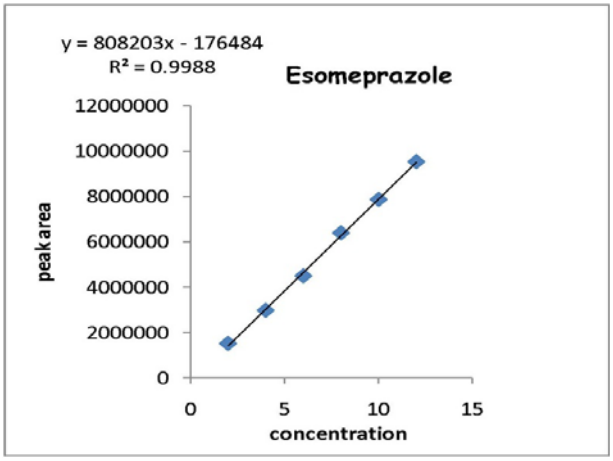

Fig. 3a. Linearity chart of Esomeprazole

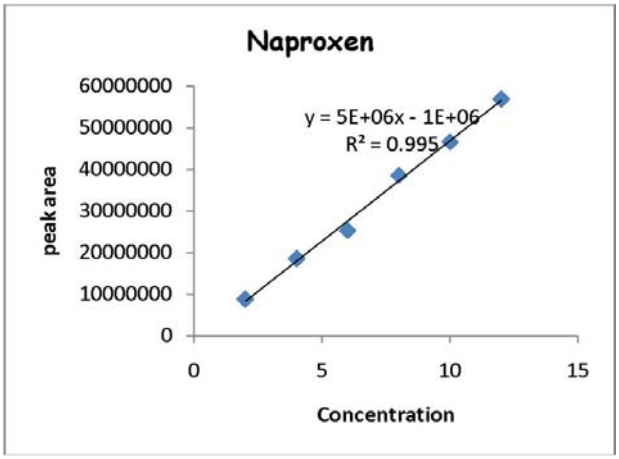

Fig. 3b. Linearity chart of naproxen

Table 5. Accuracy table for esomeprazole and naproxen

Level of percentage recovery	Amount standard drug added	Area response	Mean	Standard Deviation	Relative standard deviation	Total amount recovered Mg	% recovery
Esomeprazole							
80%	16	4511240 4511578 4511265	4511361	188.342	0.004175	20.032	100.016
100%	20	4511435 4511254 4511290	4511326	95.814	0.002124	20.02	100.06
120%	24	4511984 4511249 4511476	4511570	376.346	0.008342	20.01	100.06
Naproxen							
80%	400	25339278 25349980 25338898	25342719	6291.369	0.024825	499.15	99.83
100%	500	25337856 25356754 25339858	25344823	10381.21	0.04096	500.05	100.01
120%	600	25339209 25338976 25329998	25336061	5252.004	0.020729	499.9	99.8

Table 6. LOD and LOQ values for naproxen and esomeprazole

S. No.	Name of the drug	LOD (µg/ml)	LOQ (µg/ml)
1.	Naproxen	0.0189	0.063
2.	Esomeprazole	0.327	1.239

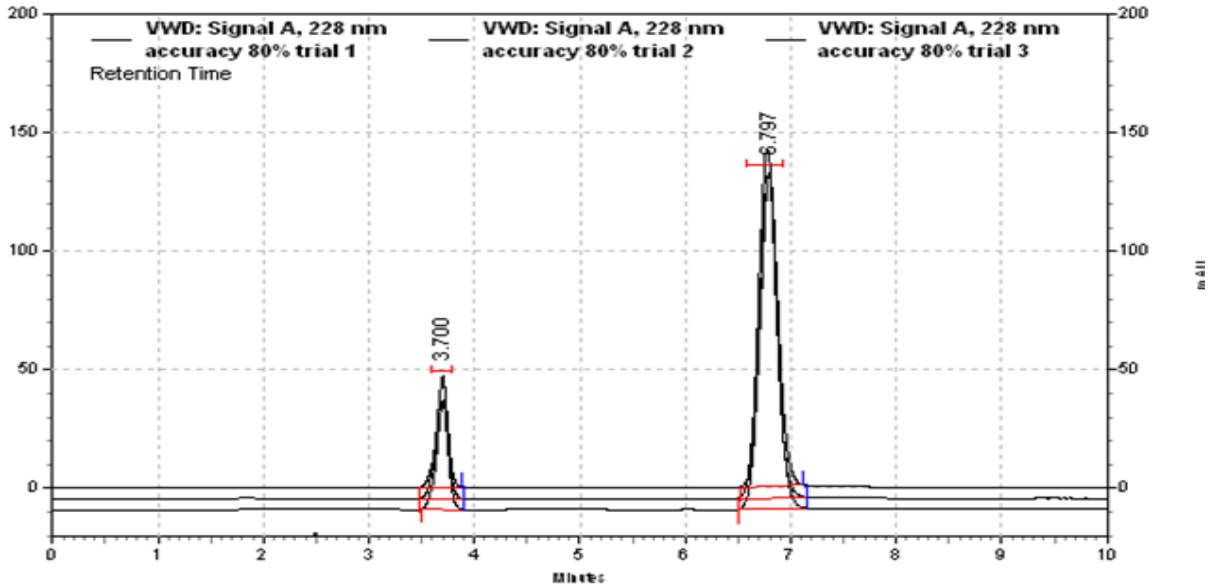

Fig. 4. Chromatogram for 80% accuracy

Table 7. System suitability parameter for simultaneous estimation of naproxen and esomeprazole

S. No.	Parameter	Method
1.	No. of theoretical plates per meter	5518 - Esomeprazole 7379 - Naproxen
2.	Tailing factor	0.88741 - Esomeprazole 1.04943 - Naproxen
3.	Capacity factor	0.833694
4.	Resolution	12.00900

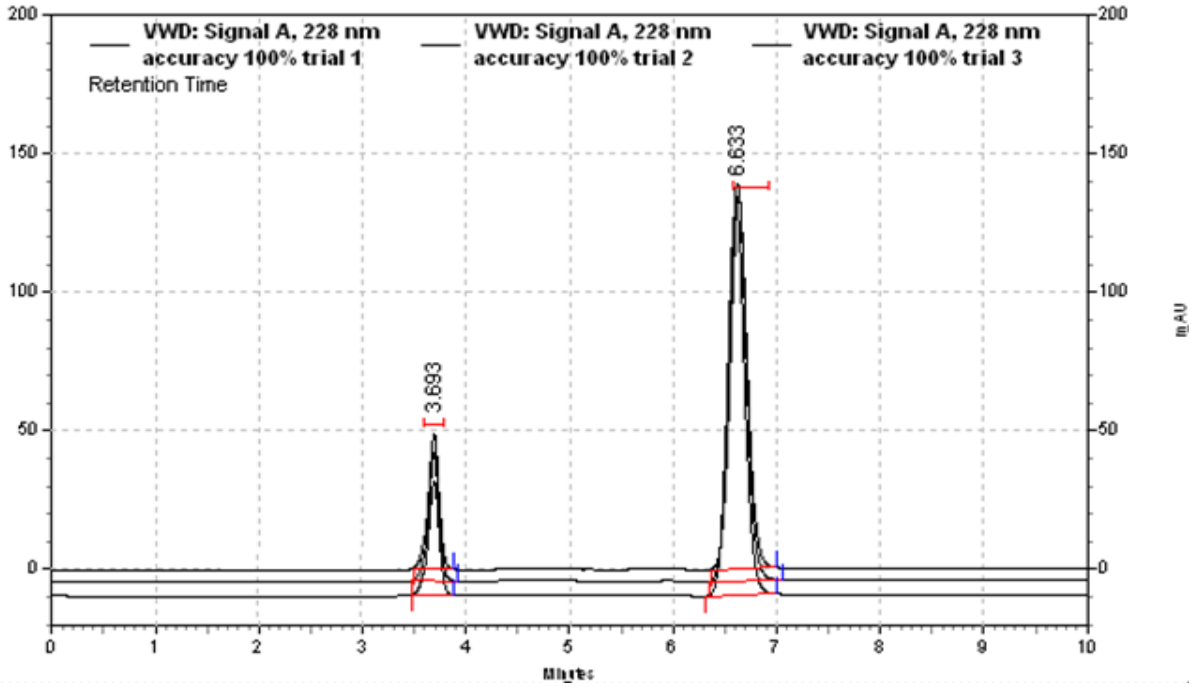

Fig. 5. Chromatogram for 100% accuracy

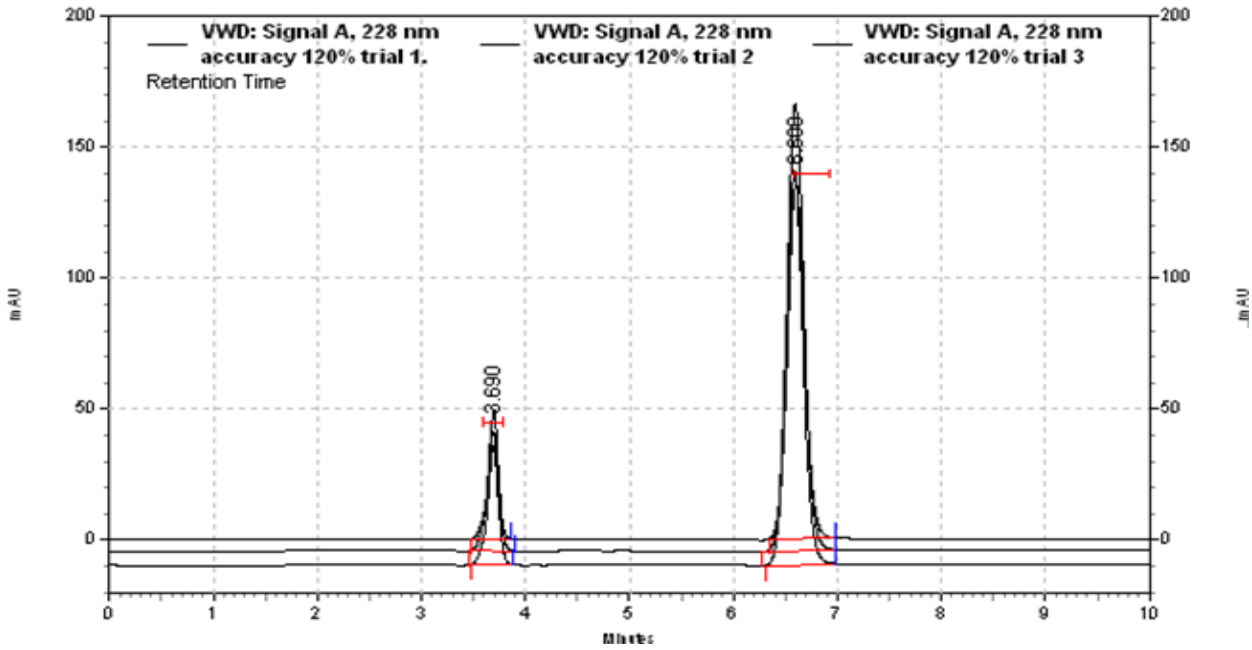

Fig. 6. Chromatogram for 120% accuracy

CONCLUSION

The studies revealed that developed RP-HPLC method was highly precise and convenient for routine analysis of naproxen and esomeprazole in bulk and tablet dosage forms and validated as per ICH guidelines.

REFERENCES

Ahmed I, Islam MR, Chowdhury JA, Islam MK and Rahman MH. In vitro release kinetics study of naproxen from swellable hydrophilic matrix tablets. *Bangladesh Pharm. J.* 2010;13(1):18-22.

Ampati S, Lagishetti S, Bairi AG. Development and validation of RP-HPLC method for simultaneous estimation of naproxen sodium and esomeprazole in pharmaceutical tablet dosage form. *Int. J. Pharm.* 2014;4(3):95-104.

Banerjee SK, Vasava NM. Simultaneous estimation of amlodipine and rosuvastatin in combined bulk forms by RP-HPLC using ultraviolet detection. *Bull. Pharm. Res.* 2013;3(1): 29-33.

Basaveswara Rao MV, Nagendrakumar AVD, Sivanadh M, Venkata Rao G. Validated RP-HPLC method for the estimation of telmisartan in tablet formulation. *Bull. Pharm. Res.* 2012;2(2):50-5.

Bhavyasri K, Murali Balaram V, Nageswarao R, Rambabu D, Ajitha M. Rapid simultaneous determination of naproxen and esomeprazole magnesium in combined tablets by validated ultra performance liquid chromatographic method. *J. Chem. Pharm. Res*. 2013;5(12):1230-6.

Deshpande SV, Mahaparale SP, Shinde SS, Funne SM. Development and validation of Esomeprazole and Naproxen in bulk and tablet dosage form by RP-HPLC method. *Int. J. Pharm. Anal*. 2014;2(5):449-53.

Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, Lipsky PE. Cyclooxygenase in biology and disease. *FASEB J.* 1998;12(12):1063-73.

Jain DK, Jain N, Charde R, Jain N. The RP-HPLC method for simultaneous estimation of esomeprazole and naproxen in binary combination. *Pharm. Methods* 2011;2(3):167-72. [DOI: 10.4103/2229-4708.90356]

Lind T, Rydberg L, Kyleback A, Jonsson A, Andersson T, Hasselgren G, Holmberg J, Rohss K. Esomeprazole provides improved acid control vs. omeprazole in patients with symptoms of gastro-oesophageal reflux disease. *Aliment Pharmacol. Ther.* 2000;14(7):861-7. [DOI: 10.1046/j.1365-2036.2000.00813.x]

Mahaparale SP, Mahaparale PR, Gonjari ID. Simultaneous spectrophotometric estimation of esomeprazole and naproxen in bulk and tablet dosage form. *Int. J. Res. Dev. Pharm. Life Sci.* 2013;2(2):377-82.

Manrique-Moreno M, Suwalsky M, Villena F and Garidel P. Effects of the nonsteroidal anti-inflammatory drug naproxen on human erythrocytes and on cell membrane molecular models. *Biophys. Chem*. 2010;147(1-2):53-8. [DOI: 10.1016/j.bpc.2009.12.010]

Masferrer JL, Isakson PC, Seibert K. Cyclooxygenase-2 inhibitors: a new class of anti-inflammatory agents that spare the gastrointestinal tract. *Gastroenterol. Clin. North Am*. 1996;25(2):363-72.

Miner P Jr, Katz PO, Chen Y, Sostek M. Gastric acid control with esomeprazole, lansoprazole, omeprazole, pantoprazole, and rabeprazole: a five-way crossover study. *Am. J. Gastroenterol*. 2003;98(12):2616-20. [DOI: 10.1111/j.1572-0241.2003.08783.x]

Patel M, Vohra J, Kakadiya J, Shah KH. Development and validation of simultaneous equation spectrophotometry method for simultaneous estimation of naproxen and esomeprazole magnesium trihydrate in tablet dosage form. *Int. J. Pharm. Res. Bio-Sci.* 2012;1(2):274-86.

Prasanthi V, Mary K, Narasimha Raju CH, Basaveswara Rao MV. Development and validation of new RP-HPLC method for determination of acetyl sulfisoxazole in bulk and pharmaceutical dosage forms. *Bull. Pharm. Res.* 2011;1(1): 47-53.

Rao TM, Prabhakar T, Girija Sankar G, Naidu PVL. Stability indicating assay of esomeprazole and naproxen in tablets by RP-UPLC PDA-method. *Int. J. Pharma Sci*. 2013;3(2): 205-10.

Razzaq SN, Ashfaq M, Khan IU, Mariam I. Development and validation of liquid chromatographic method for naproxen and esomeprazole in binary combination. *J. Chil. Chem. Soc.* 2012;57(4):1456-9. [DOI: 10.4067/S0717-97072012000400024]

Reddy PS, Sait S, Vasudevmurthy G, Vishwanath B, Prasad V, Reddy SJ. Stability indicating simultaneous estimation of assay method for naproxen and esomeprazole in pharmaceutical formulations by RP-HPLC. *Der Pharm. Chem.* 2011;3(6):553-64.

Saravanan S, Sudha T, Surendra B. Development and validation of reverse phase-high performance liquid chromatographic method for simultaneous estimation of naproxen sodium and esomeprazole magnesium trihydrate. *Drug Dev. Ther.* 2014;5(2):109-14.

Scott LJ, Dunn CJ, Mallarkey G, Sharpe M. Esomeprazole: a review of its use in the management of acid-related disorders. *Drugs* 2002;62(10):1503-38.

Shukla R, Shivkumar R, Shivan KN. Development of a UV-spectrophotometric method for the simultaneous determination of tramadol hydrochloride and paracetamol in bulk and marketed product. *Bull. Pharm. Res.* 2011;1(1):62-6.

Singh S, Dahiya R. Stability-indicating RP-HPLC method for estimation of atorvastatin calcium in solid dosage form. *Bull. Pharm. Res.* 2014;4(1):9-13.

Singh S, Yadav AK, Gautam H. First order derivative spectrophotometric determination of telmisartan in pharmaceutical formulation. *Bull. Pharm. Res.* 2012;2(2): 83-6.

Sojitra C, Pathak A, Rajput S. Development and validation of spectrophotometric methods for simultaneous estimation of naproxen and esomeprazole magnesium trihydrate in combined Pharmaceutical formulation. *Int. J. ChemTech Res*. 2012;4(2):602-8.

Sojitra C, Rajput S. Development and validation of RP-HPLC methods for simultaneous estimation of naproxen and esomeprazole magnesium trihydrate in combined pharmaceutical formulation. *Int. J. Pharm. Pharm. Sci*. 2012;4(3):533-7.

Turini ME, DuBois RN. Cyclooxygenase-2: a therapeutic target. *Annu. Rev. Med*. 2002;53:35-57. [DOI: 10.1146/annurev.med.53.082901.103952]

Vetrichelvan T, Suresh J. Simultaneous spectrophotometric estimation of naproxen and esomeprazole magnesium by area under curve method. *Int. J. Pharm. Res.* 2012;1(1): 1-3.

Yokoyama H, Horie T, Awazu S. Naproxen-induced oxidative stress in the isolated perfused rat liver. *Chem. Biol. Interact*. 2006;160(2):150-8. [DOI: 10.1016/j.cbi.2006.01.003]

*****