



Rapid simultaneous determination of aspirin and esomeprozole magnesium in combined tablets by validated ultra performance liquid chromatographic method

Yarram Rama Koti Reddy¹, Sudershan Reddy G¹, M. R. P. Reddy² and K. Mukkanti^{1*}

¹Centre for Chemical Science and Technology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad, India

²Centre for Materials for Electronics Technology IDA, HCL Post, Cherlapally, Hyderabad, India

ABSTRACT

A stability- indicating ultra Performance liquid chromatography (UPLC) method has been developed and validated for the simultaneous determination of Aspirin and Esomeprozole Magnesium in pharmaceutical preparations. An Agilent Zorbax XDB column (50X4.6mm i.d., 1.8 μ m particle size) was used. The mobile phase consisted of a mixture of 0.2% ortho Phosphoric Acid, methanol and acetonitrile in simple gradient elution Ultraviolet (UV) detection was performed at 210 nm. Total run time was 6 min; these two drugs were eluted at the retention times of 2.4 and 2.8 min for Esomeprozole Magnesium and Aspirin respectively. The method was validated in terms of linearity, range, specificity, accuracy, and precision, limit of detection (LOD) and limit of quantitation (LOQ). The linearity for both the drugs was found in the range of 32.5-97.5 μ g mL⁻¹ of Asp and 4-12 μ g mL⁻¹ for Eso. The % recoveries of Aspirin and Esomeprozole Magnesium were found to be 99.41 and 101.11, respectively. The utility of the procedure is verified by its application to marketed formulations that were subjected to accelerated degradation studies. The method distinctly separated the drug and degradation products even in actual samples. The products formed in marketed tablet dosage forms are similar to those formed during stress studies.

Key words: Method development, Validation, Simultaneous, Aspirin, Esomeprozole Magnesium and Stability-indicating.

INTRODUCTION

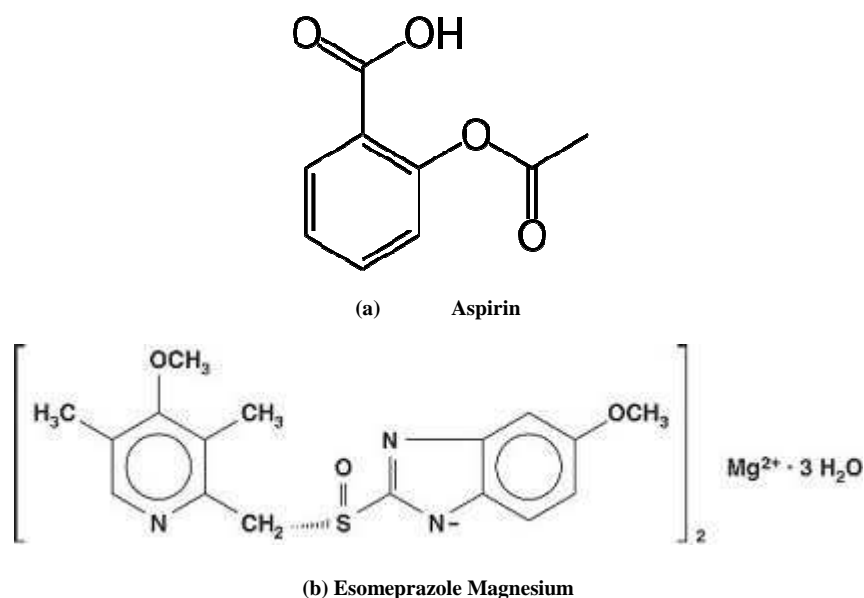
Aspirin, 2-acetoxy benzoic acid is cyclo oxygenase inhibitor. It is used as an analgesic, antipyretic, anti-inflammatory and anti thrombic agent [1] Aspirin is official in IP [2], BP [3] and USP [4]. Esomeprozole (ESO), S-isomer of omeprazole inhibits gastric acid secretion and is cost effective in the treatment of gastric oesophageal reflux diseases. It is the first single optical isomer proton pump inhibitor. It provides better acid control than current racemic proton pump inhibitors and has a favourable pharmacokinetic profile relative to omeprazole. It is chemically bis(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H- benzimidazole). The empirical formula is (C₁₇H₁₈N₃O₃S) 2 Mg x 3 H₂O, representing a molecular weight of 767.2 as a trihydrate and 713.1 on an anhydrous basis.

A literature survey revealed that high performance liquid chromatographic methods for Aspirin [5-6]. Aspirin can be estimation in pharmaceutical dosage forms with different combinations by high performance liquid chromatographic methods [7, 8, and 9]. Spectrometric method for the determination of aspirin in blood samples [10]. Simultaneous estimation of aspirin with Atrovastatin calcium by different techniques spectoflorimetry and HPTLC [11-16]. Esomeprozole magnesium can be estimated by different combination by RP-HPLC [17-18].

But so far, no chromatographic method has been reported for simultaneous determination of Aspirin and Esomeprozole in combined dosage forms; hence it is essential to development of chromatographic method for simultaneous estimation of two drugs in tablet formulation. As UPLC methods were widely used for routine analysis

of drugs because of its sensitivity and accuracy, so in the present work a new, simple, and specific RP-UPLC method was developed for simultaneous estimation of Aspirin and Esomeprozole in tablet dosage form.

Fig. 1: Chemical structures of Aspirin and Esomeprozole Magnesium



EXPERIMENTAL SECTION

Chemicals and Reagents

The Aspirin and Esomeprozole working standards are received as a gift samples from the Dr.Reddy's laboratories Hyderabad. HPLC grade acetonitrile, analytical grade ortho phosphoric acids were purchased from Merck (Mumbai, India). Water was prepared by Millipore MilliQ Plus water purification system. Commercial pharmaceutical preparation of Axanum combined tablets were purchased from the market. The declared content of tablets was Aspirin 81 mg and 20 mg Esomeprozole per tablet.

Instrumentation and Chromatographic Conditions

Analysis was performed with Waters Acquity UPLC system used consists of a binary solvent manager with universal loop injector with injection capacity 20 μ L. Detector consisted of photodiode array detector Waters 2996. Separation was carried out An Agilent Zorbax XDB C₁₈ column, 50 mm x 4.6 mm i.d with 1.8 μ m particles under reverse phase partition chromatographic conditions. The equipment was controlled by a PC with Empower-2 software. The work was carried out in an air conditional room maintained at a temperature of 25 \pm 2 $^{\circ}$ C. The mobile phase A was 0.2% ortho Phosphoric acid and acetonitrile and methanol in the ratio (50:50 v/v) was as solvent B used for mobile phase. The mobile phase prepared and degassed. The simple Gradient elution mode Mobile phase pumped at 0.7 ml min⁻¹. The eluants were monitored at 210 nm. The injection volume for samples and standards were 2 μ L. Acetonitrile and 0.1N Sodium hydroxide in the ratio, 50:50; v/v, respectively was used as diluent.

Sample Preparation

A commercial 10 tablet of Axanum (Asp 81 mg and 20 mg Eso) was weighed, placed in a 100 ml volumetric flask and dissolved in a mixture of acetonitrile and 0.1N sodium hydroxide solution (1:1). This solution was sonicated for 15 min at 25 $^{\circ}$ C and then topped off to volume. After shaking 5ml of the flask content were transferred in to 20 ml volumetric flask and diluted with acetonitrile and 0.1N sodium hydroxide solution (1:1). An aliquot was filtered through a nylon membrane filter.

Standard preparation

The standard stock solution of Aspirin and Esomeprozole were prepared by dissolving 50mg of each drug in 100ml of diluent from this solution, 10ml of solution were taken and diluted to 50 ml with the same to get a solution containing 100 μ g/ml of each drug. From the stock solutions, further dilutions were prepared by diluting required volume of solution with diluent and their area was noted by injecting 20 μ L into system. After that, a calibration curve was drawn between concentration against their respective area for Aspirin and Esomeprozole separately. From the calibration curve, it was found that Aspirin and Esomeprozole had linearity range between 5 and 50 μ g/ml.

Assay of tablet Formulation

For analysis of the tablets dosage form, 20 tablets were weighed individually and their average weight was determined. Then they were crushed to fine powders, and a powder equivalent to weight of 50mg of Aspirin was transferred into 100ml volumetric flask and dissolved in diluent. The solution was vigorously for 15min and filtered through whatman no.41 filter paper, and the residue was washed with diluent. Then volume was made up to the mark with diluent. The volume was made up to the mark with diluent. From this solution 10 ml of solution was taken and diluted to 50 ml with same to get 100 μ g/ml of Aspirin and corresponding concentration of Esomeprozole.

The solution contained aspirin and Esomeprozole in the proportion of 4:1.

From this solution 3.0ml of solution was transferred in 10ml volumetric flask and diluted with same to obtain a final concentration of 30 μ g/ml of aspirin and 7.530 μ g/ml of Esomeprozole. The amounts of Aspirin and Esomeprozole per tablet were calculating by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with tablet formulation. The result of analysis of tablet formulation is reported in Table 1.

RESULTS AND DISCUSSION

UPLC method development and Optimization

Column chemistry, solvent type, solvent strength (volume fraction of organic solvents in the mobile phase and pH of buffer solution), detection wave length, and flow rate were varied to determine the Chromatographic conditions giving the best separation. The mobile phase conditions were optimized so that the solvent and excipients did not interfere with the components. Other criteria such as time required for analysis, appropriate range for eluted peaks, assay sensitivity, solvent noise, and use of the same solvent system for extraction of drug from formulation matrices during drug analysis were also considered.

After trying different columns, the final choice of the stationary phase that gave a satisfactory resolution and run time was the reverse phase column An Agilent Zorbax XDB column (50X4.6mm i.d., 1.8 μ m particle size). A series of aqueous mobile phases containing buffer solution and different organic solvents are checked. The best results were obtained by use of a mixture of methanol and acetonitrile. The flow rate was determined by testing the effect of different flow on the peak area and resolution; a flow rate of 1.0ml/min found to be optimum. All experiments were carried out at ambient temperature.

To determine the appropriate wave length for simultaneous determination of aspirin and Esomeprozole solutions of these compounds in the diluent were scanned by a UV-Vis spectrophotometer (Shimazu 1700) in the range 200-400nm. From the overlaid UV spectra, suitable wave length considered for monitoring the drugs was 210nm. Solutions of each substance in the mobile phase were also injected directly for UPLC analysis, and the responses (peak area) were recorded at 210nm. It was observed there was no interference from the mobile phase or base line disturbance, and these two drugs absorbed well at 210 nm. It was, therefore concluded that 210nm is the most appropriate wavelength for analysis of both the drugs with suitable sensitivity.

Under optimum chromatographic conditions, the retention times obtained for Aspirin and Esomeprozole were 2.85 and 2.45 min respectively (Fig.1). The resolutions between two curves were 6.3 the result of capacity factor, tailing factor, and theoretical plate number are reported in Table 2.

The value obtained for these properties shows these chromatographic conditions are appropriate for separation and quantification of both compounds. The number of plates (N) is a measure of column efficiency, which shows the good separation efficiency of the column used.

Table 2. Result of assay of tablet formulation

Drug	Lable claim (mg/tab)	Amount found(mg/tab)	Lable claim (%)	SD	COV (%)	SE
Aspirin	81	79.93	99.57	0.2606	0.2609	0.1064
Esomeprozole	20	19.76	99.34	0.4210	0.4165	0.1697

Validation of the developed method

The method was validated for linearity, accuracy, precision, repeatability, selectivity, and specificity study. All the validation study was carried out by replicate injection of the sample and standard solutions.

Linearity

The linearity was determined for two drugs, Aspirin and Esomeprozole, separately by plotting a calibration graph of peak area against their respective concentration. From the calibration curve, it was clear that Aspirin and

Esomeprozole had linearity between 5 and 50 μ g/ml, where as Aspirin had a range between 5 and 30. The linear regression equation for two drugs was

Aspirin : $y =$

Esomeprozole : $y =$

Where y is peak area and x is concentration.

Accuracy

Accuracy of the developed method was confirmed by doing a recovery study as per ICH norms at three different concentration levels (80%, 100% and 120%) by replicate analysis (n=3). Standard drug solutions were added to a preanalyzed sample solution, and then percentage of drug content was calculated. The result of the accuracy study are reported in Table.3

Table 3. Results of recovery study

Drug	Amount taken(μ g/ml)	Amount added (μ g/ml)			Mean recovery		
		80%	100%	120%	80%	100%	120%
Aspirin	8	6.4	8	9.6	99.26	101.1	99.5
Esomeprozole	4	3.2	4	4.8	100.3	100.1	99.9

From the recovery study, it was clear that the method is very accurate for quantitative estimation of Aspirin and Esomeprozole in tablet dosage form because all the statistical results were within the acceptance range.

Precision and LOD and LOQ

Precision was determined by studying the repeatability and intermediate precision. Repeatability results indicate the precision under the same operating conditions over a short interval time and interassay precision. The standard deviation, coefficient of variance, and standard error were calculated for two drugs. The results are mentioned in table 5. Intermediate precision was carried out by doing intra and inter day precision studies. In the intraday study, the concentrations of the two drugs were calculated on the same day at an interval of 1 h. In the interday study, the concentrations of drug contents were calculated on three different days, and the study expressed within the laboratory variation in different days. In both intra and interday precision studies for the methods. COV% values were not more than 2.0%, which indicates good intermediate precision.

LOD and LOQ studies were carried out to evaluate the detection and quantization limits of the method to determine the presence of any impurity by using following equation.

$$\text{LOD} = 3.3\sigma/S$$

$$\text{LOQ} = 10\sigma/S$$

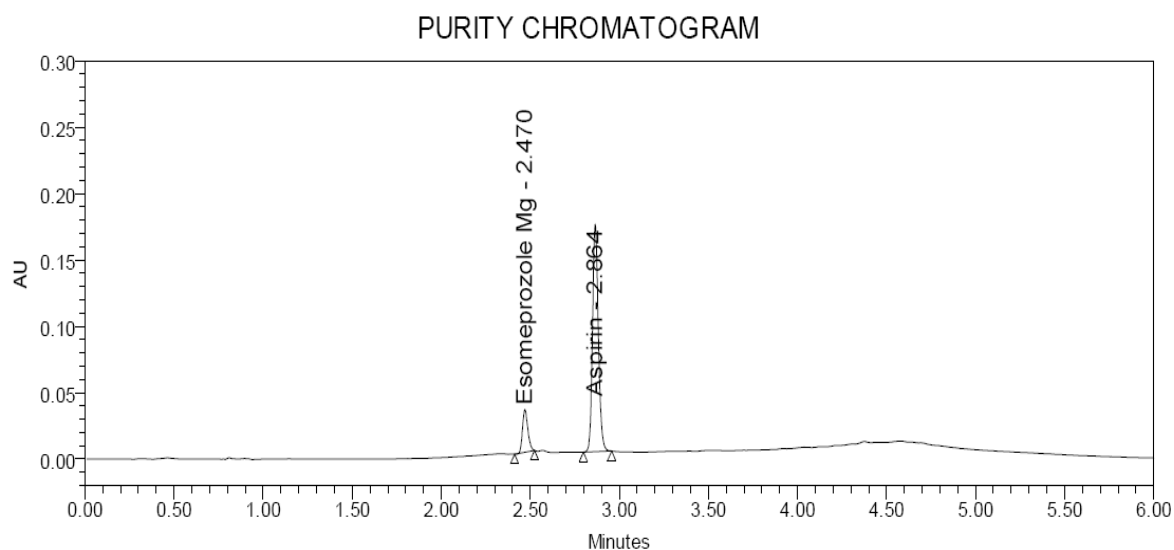
Where σ is the standard deviation and S is the slope of the curve.

The result is reported in Table 4. The developed method was precise for quantitative study because the precision study was found statistically significant.

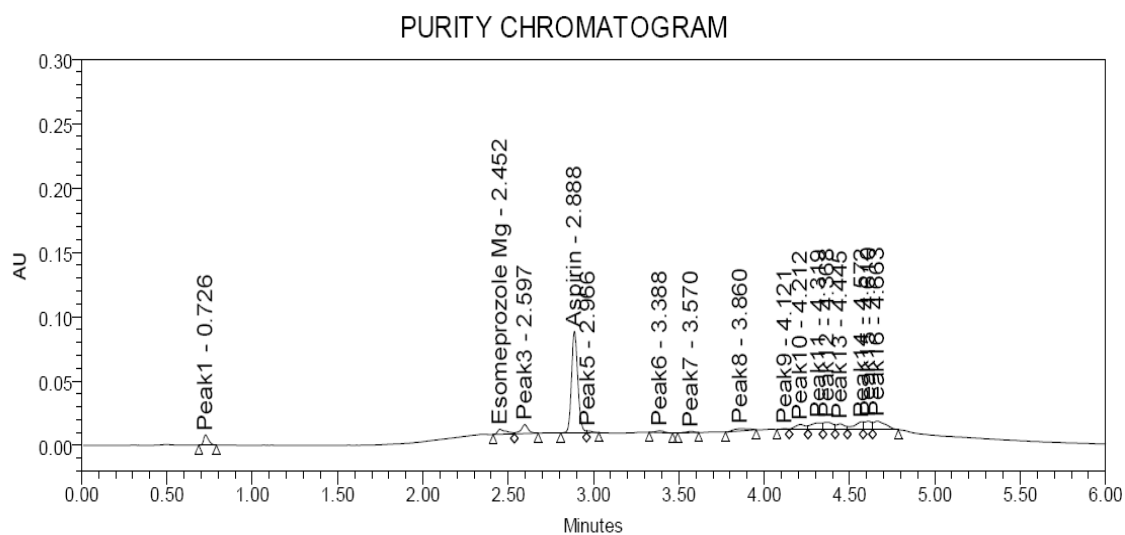
Selectivity and Specificity

To check the selectivity of the developed method, solutions of the three drugs were injected into the system, and three sharp peaks for Aspirin and Esomeprozole were obtained retention times of 2.85 and 2.45 min respectively in reference to placebo solution. Specificity of the method was assessed by comparing the chromatograms obtained from standard drugs with the chromatogram obtained from tablet solution. Because the retention time of two standard drugs and the retention time of the two drugs in sample solutions were the same, the method was specific. The developed method was specific and selective as no interference of excipients was found.

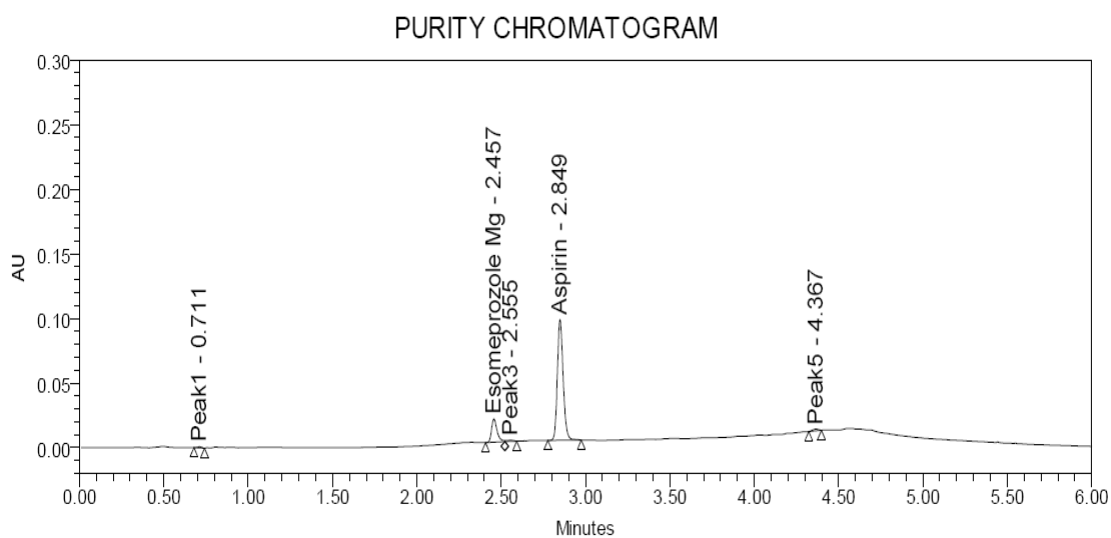
Fig. 2: A typical chromatograms obtained from Aspirin and Esomeprozole Magnesium tablets and from stressed samples



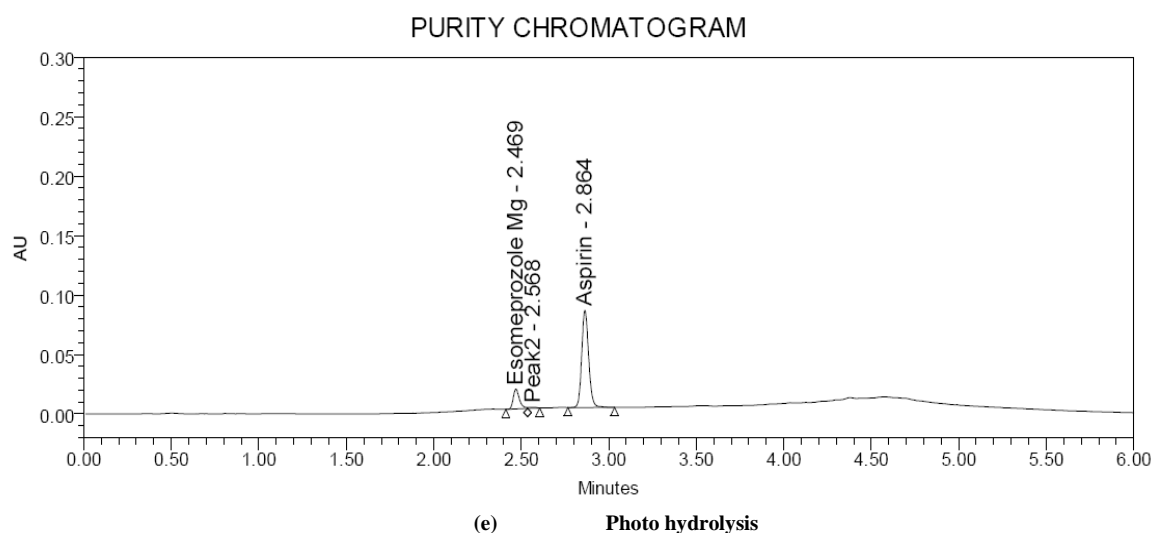
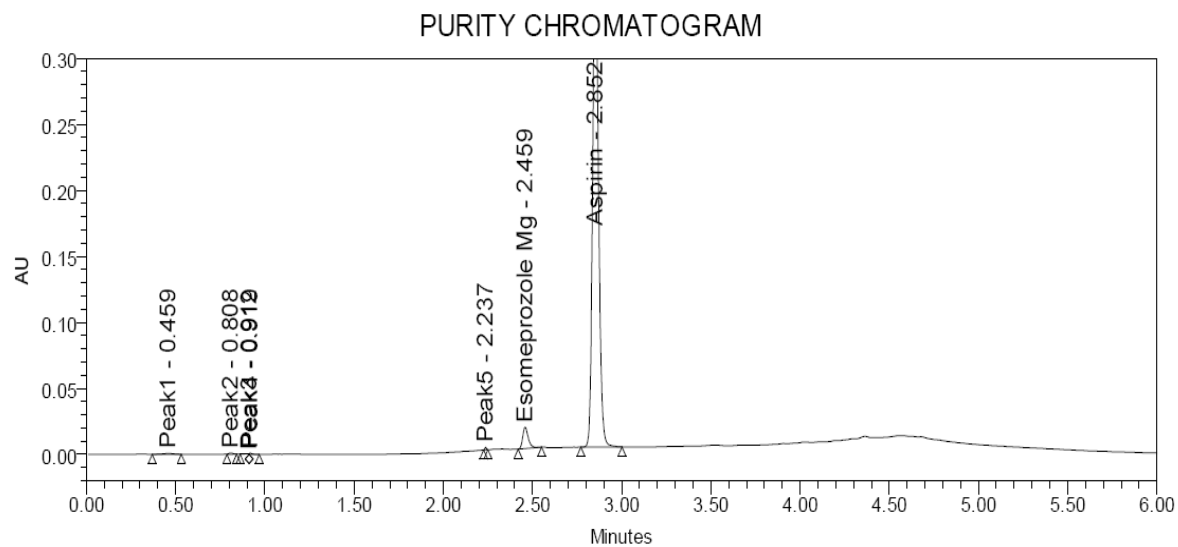
(a) **Chromatogram of Aspirin and Esomeprozole Magnesium in combined formulation**



(b) **Acid hydrolysis**



(c) **Base hydrolysis**



Forced Degradation Studies

Forced degradation studies were performed on Aspirin and Esomeprozole combined tablets to prove the stability indicating property of the method. The stress conditions employed for degradation study of Aspirin and Esomeprozole include acid hydrolysis (1 N HCl), base hydrolysis (1 N NaOH), water hydrolysis and oxidation (3% H₂O₂). For light studies, the monitoring period was 10 days whereas for heat, acid, base and water hydrolysis it was 48 h. Oxidation was carried out for 24 h. Peak purity of the principal peak in the chromatogram of stressed samples of Aspirin and Esomeprozole tablets was checked using photo diode array detector.

CONCLUSION

A new, reverse phase UPLC method has been developed for simultaneous quantization of Aspirin and Esomeprozole in tablet formulation. It has been shown that the developed method achieved accuracy, reproducibility, repeatability, linearity, precision, and selectivity, which prove the reliability of the method. The run time is relatively short, 6min, which enables rapid quantization of many samples in routine and quality control analysis of tablet formulation. The same solvent used throughout the analysis and no interference of any excipients matrices was found. The result shows that the method could find practical application as a quality control tool for the simultaneous estimation of two drugs from their combined dosage form in a quality control laboratory.

REFERENCES

- [1] Tripathi KD. Essentials of Medical Pharmacology. 5th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2004,560.

- [2] The Indian Pharmacopoeia, 4th ed. Vol. I, New Delhi: The controller of publications; 1996, 69.
- [3] British Pharmacopoeia, International edition, Vol. II. London: HMSO; 1993, 780.
- [4] United States Pharmacopoeia, National Formulary USP23, Asian Edition, Rockville, MD: United States Pharmacopoeial Convention, Inc; 1995, 133, 135, 1776, 1982.
- [5] Zenon Kokot and Kinga Burda, Simultaneous determination of salicylic acid and acetylsalicylic acid in aspirin delayed –release tablets formulation by second –derivative UV spectrophotometry. *J Pharma Bio Anal* 1998 Dec 1; 18(4-5):871-5.
- [6] Frieder K, Doris J, Horst G. Simultaneous determination of acetylsalicylic acid and salicylic acid in human plasma by HPLC. *J.Chrom.B* 1996 Feb23;677(1):172-7.
- [7] Gandhimathi M, Ravi TK, Abraham A, Thomas R, Simultaneous determination of Aspirin and isosorbide5-mononitrate in formulation by reversed phase high performance liquid chromatography *Pharma Bio Anal* 2003;32:1145-8.
- [8] Manoj K, Shanmuga pandiyan P, Anbazhagan S. RP-HPLC method for simultaneous estimation of atorvastatin and aspirin from capsule formulations. *Indian Drugs* 2004;41 suppl.5:284-289.
- [9] Manoj K, Shanmuga pandiyan P, Anbazhagan S. RP-HPLC method for simultaneous estimation of atorvastatin and aspirin from capsule formulations. *Indian Drugs* 2004;41 suppl.5:284-289.
- [10] Ahmed M, Biswas HU, Sadik G. Development of a spectrometric method for the determination of aspirin in blood samples. *J Med Sci.* 2001; 1 suppl.2:61-62.
- [11] Damle M C, Sinha P K, Bothra K G, A validated stability indicating HPTLC method for the determination of Aspirin and Clopidogrel bisulphate in combined dosage form, *Eurasian journal of Analytical Chemistry*, 2009, Vol.4:152-160.
- [12] Suvillan C, Sherma J, Determination of Salicylamide in pharmaceutical tablets by HPTLC with UV absorption densitometry, *Acta Chromatographica*, No.16:153-164.
- [13] Shah DA, Bhatt KK, Mehta RS, Shankar MB, Baldania SL, Development and Validation of an RP-HPLC method for the determination of Atorvastatin Calcium and Aspirin in capsule dosage form, *Indian Journal of Pharm. Sci.* 2007, 69(4):546-549.
- [14] Panchal HJ, Suhagia BN, Patel NJ, Simultaneous HPTLC analysis of Atorvastatin Calcium, Ramipril and Aspirin in capsule dosage form, *JPC-Journal of Planar Chromatography*, Vol.22:265-271.
- [15] Hassan HH, Fawzy AEY, Mohamad EM, Ghassan MS, Nada MS (2008). Stability-Indicating Spectrofluorimetric and RP-HPLC Methods for the Determination of Aspirin and Dipyridamole in their Combination. *Open Spectrosc J.*, 2: 19-28.
- [16] Mishra P, Archana D (2006). Simultaneous determination of clopidogrel and Aspirin in pharmaceutical dosage forms, *Indian J. Pharm Sci.*, 68(3): 365-368.
- [17] Kumar S.T., Kumar B.K, Kumar A. S, Mohan M., NandaS.S., VenkateshwarRao P. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Esomeprazole and Domperidone in Pharmaceutical Dosage Form *Journal of Pharmacy Research* 2011,4(11),4097-4099.
- [18] Zarpakar, SS. and Kanyawar, NS. 2002. Simultaneous estimation of domperidone and omeprazole in pharmaceutical dosage by reverse phase high performance liquid chromatography. *Indian Drugs*, 39: 217-21.