ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF OMEPRAZOLE AND ASPIRIN USING REVERSE PHASE HPLC METHOD IN BULK AND DOSAGE FORM

Abstract:

A new simple, accurate, precise and reproducible RP-HPLC method has been developed for the simultaneous estimation of Aspirin and Omeprazole in bulk and pharmaceutical dosage form using C18 column (Agilent, 250 x 4.6 mm, 5 μm) in isocratic mode. The mobile phase consisted of Methanol & 0.1 M Dipotassium Phosphate buffer (pH 3) in the ratio of 60:40 v/v. The detection was carried out at 256 nm . The method was linear over the concentration range for Omeprazole 50-250 μg/ml and for Aspirin 10-50 μg/ml. The recoveries of Omeprazole and Aspirin were found to be 100.07 and 100.06% respectively. The validation of method was carried out utilizing ICH-guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form.

Keywords: Omeprazole, Aspirin, reverse phase HPLC, validation.

Introduction:

Aspirin (ASP) is chemically 2-(acetyloxy)-benzoic acid (Figure 1). It is nonselective cyclooxygenase inhibitor used as an antipyretic, analgesic, anti-inflammatory, and antithrombotic agent. Esomeprazole magnesium (ESO) is S-isomer of omeprazole and proton pump inhibitor. It is magnesium, bis [5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazolato] (Figure 2). It is used in treatment of peptic ulcer disease, NSAIDS-associated ulceration and Zollinger-Ellison syndrome, used as antiulcerative. ASP and ESO in combined dosage form are used in cardiovascular disorder and cerebrovascular disorders [1–3]
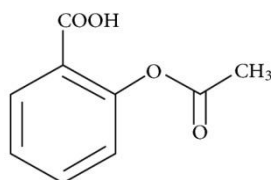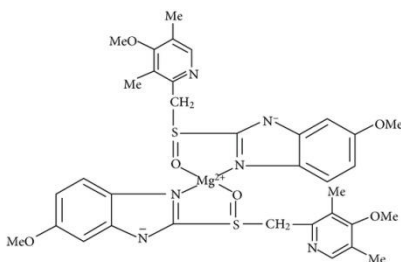

Figure 1: Structure of aspirin.

Figure 2: Structure of esomeprazole .

The review of literature revealed that various analytical methods involving spectrophotometry [5-7], HPLC [8-11] and HPTLC [12] have been reported for ASP in single form and in combination with other drugs. Several analytical methods have been reported for ESO in single form and in combination with other drugs including spectrophotometry [13, 14], HPLC [15, 16], and HPTLC [17].

The present work describes the development of a simple, precise, accurate, and reproducible HPLC method for the simultaneous estimation of ASP and ESO in combined dosage form. The

developed method was validated in accordance with ICH Guidelines [1&18] and successfully employed for the assay of ASP and ESO combine dosage form.

Materials

ASP and OMP were received gratis from Hetero drugs, Hyderabad and were used as received. HPLC grade Methanol was purchased from SD Fine Chem Pvt. Ltd. (Mumbai, Maharashtra). Ultra-pure water was obtained from ELGA (Bucks, UK) water purification unit. Waters total recovery vials (Waters, Milford, MA, USA) were of glass type 1, class A with 950 μL maximal injectable volumes. All other chemicals were of analytical reagent grade.

EXPERIMENTAL WORK:

Chromatographic conditions

The HPLC system (LC Waters, Milford, MA, USA) consisted of quaternary gradient system (600 Controller), in-line degasser (Waters, model AF), photodiode array detector (Water, 2998 model) and auto sampler (Waters, model 717 plus). Data was processed using Empower Pro software (Waters, Milford, MA, USA).

Isocratic elution of the mobile phase 0.1 M Dipotassium Phosphate buffer (pH 3) and Methanol in the ratio of 40:60 v/v with the flowrate of 1 ml/min. Separation was performed on a Waters C_{18} (250 x 4.6 mm i.d, 5 μ particle size) analytical column and a pre-column to protect the analytical column from strongly bonded material. Integration of the detector output was performed using the Waters Empower software to determine the peak area. The contents of the mobile phase were filtered through a 0.45 μm membrane filter and degassed by sonication before use. Mobile phase was used as diluents.

The flow rate of the mobile phase was optimized to 1 ml/min which yields a column back pressure of 110–112 kg/cm. The run time was set at 6 min and a column temperature was maintained at 35°C. The volume of injection was 10 μl, prior to injection of the analyte, the column was equilibrated for 30–40 min with the mobile phase. The eluents were detected at 256 nm. The developed method was validated in terms of specificiy, linearity, accuracy, limit of detection (LOD), limit of quantification(LOQ), intra-day and inter-day precision and robustness for the assay of ASP and OMP as per ICH guidelines.

Preparation of standard solutions

ASP and OMP were weighed (10 mg each) and transferred to two separate 10 ml volumetric flasks and dissolved in 5 ml of water and make up the volume up to the mark with mobile phase. Working standards of the drugs were prepared from this solution.

Preparation of sample solution:

Twenty tablets (Yosprala, Make:Aralez Pharmaceuticals) were weighed. Anaccurately weighed amount of the finelypowdered tablets equivalent to 10mg was made up to 10mL with mobile phase. The solution was filtered followed by serial dilution to the required concentrations for each experiment.

Results and Discussion:

Method Development:

Number of mobile phase and their different proportions were tried and finally was selected as 0.1 M Dipotassium Phosphate buffer (pH 3) and Methanol in the ratio of 40:60 v/v appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The results of system suitability parameters were shown in table 2. The chromatogram of working standard solution is shown in Fig 3. The summary of Chromatographic conditions was given in table 1.

Table 1: Summary of Chromatographic conditions

S. No	Parameter	Description/Value
1.	Stationary Phase	Water's C18 (250X4.6X5)
2	Mobile Phase	0.1 M Dipotassium Phosphate buffer (pH 3) and Methanol in the ratio of 40:60 v/v
3	Flow rate	1 ml/min
4	Detection Wavelength	256 nm
5	Detector	Photo diode array
6	Injection	auto sampler -Waters, model 717 plus
7	Rt's	Omeprazole – 2.323Min Aspirin – 4..342 Min
8	Injection volume	10 μl
9	Column Temperature	35 °C
10	Run time	6 mins
11	Diluent	Mobile Phase
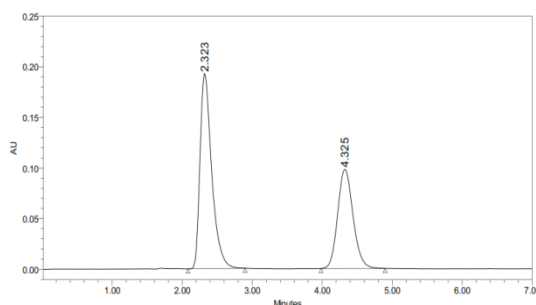
Fig.3 Typical Chromatogram of Omeprazole & Aspirin

Table 2: System suitability parameters

S. No	Parameter	Result	
		Omeprazole	**Aspirin**
1	Retention Time	2.323 min	4.325 min
2	Tailing	1.079	1.189
3	Theoretical Plates (n)	5076	7837
4	Resolution factor (R)	3.08	
5	Similarity Factor	1.0124 (Limit: 0.98 – 1.2)	

Method Validation:

Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantitiesof pure standards at three different levels in 50%, 100%and 150% to the pre analysed sample formulation. From the amount of drug found, amount of drug recoveredand percentage recovery were calculated which sense to conformation that the proposed method was accurate. The results were tabulated in Table 3.

Table 3: Results of Accuracy

S. No	% Concentration (at specific level)	Omeprazole			Aspirin		
		Amount added (µg)	Amount found (µg)	Mean % Recovery	Amount added (µg)	Amount found (µg)	Mean % Recovery
1	50	75	75	100*	15	15	100*
2	100	150	149.25	99.13**	30	30	100**
3	150	225	224.89	99.69*	45	44.55	99*

*Mean % Recovery of 6 replicates; **Mean % Recovery of 3 replicates

Precision

The intraday and interday precision of the proposed methodwas determined by analyzing mixed standard solution of OMP and ASP at concentration 150 µg/mL and 30 µg/mL, 3 times on the same day and on 3 different days. The results shown in table 4 were reported in terms of relative standard deviation.

Table 4: Results of Precision (%Assay)

Sample No.	Omeprazole		Aspirin	
	Sample Area - 1	% Assay - 1	Sample Area - 2	% Assay - 2
1	2194758	100.06	1456296	100
2	2195700	99.49	1457422	100
3	2196191	99.14	1456513	98
4	2195326	100.27	1454579	99
5	2200951	100.27	1451483	99
6	2196585	100.39	1455259	99
Average Assay:		100	Average Assay:	99
STD		0.51	STD	0.82
% RSD		0.51	% RSD	0.83

Linearity

Calibration graphs were constructed by plotting peak area vs concentration of ASP and OMP and the regression equations were calculated. The calibration graphs were plotted over 5different linear concentrations in the range of 10-50µg/ml for ASP and 50-250 µg/ml for OMP. Aliquots (10 µl) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n =6)]. The linearity graphs were shown in fig 4 & 5.
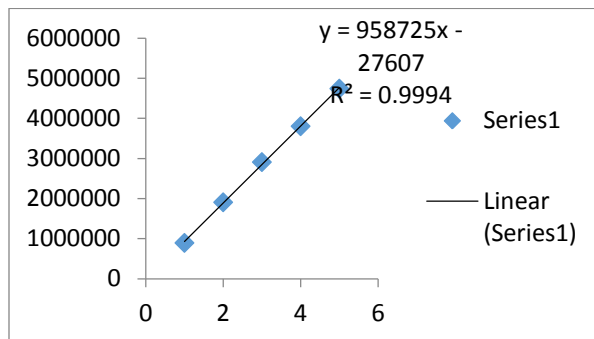

y = 958725x - 27607
R^2 = 0.9994

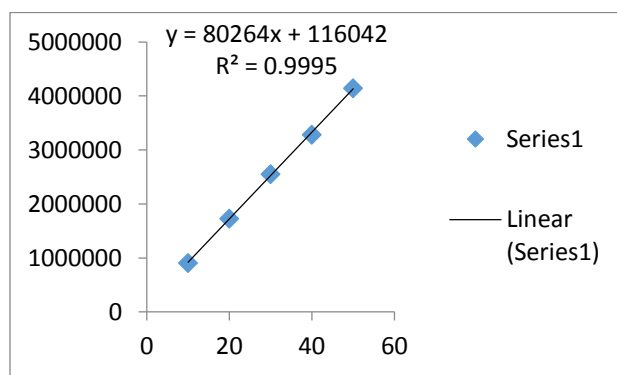Fig 4: Linearity of Omeprazole

$$y = 80264x + 116042$$
$$R^2 = 0.9995$$

Fig 4: Linearity of Aspirin

Limit of detection (LOD) and limit of quantitation(LOQ):

The limit of detection (LOD) and limit of quantitation (LOQ) of ASP and OMP were determined by calculating the signal-to-noise(S/N) ratio of 3:1 and 10:1, respectively according to International Conference on Harmonization guidelines.LOD values for ASP and OMP were found to be 3.08and 3.041 µg/mL respectively. LOQ values for C were found to be 10.37 and 9.79µg/mL respectively.

Assay of the tablet dosage form

The proposed validated method was successfully applied to determine ASP and OMP in tablet dosage form. The result obtained for ASP and OMP were comparable with corresponding labeled amounts. The results were tabulated in table 4.

Conclusions

The proposed method has advantage of simplicity and convenience for the separation and quantitation of ASP and OMP in the combination which can be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographicprocedure. The method is accurate, precise,rapid and selective for simultaneous estimation of Aspirin and Omeprazole in tablet dosage form. Hence itcan be conveniently adopted for routine analysis.

Acknowledgments

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