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Research Article

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF ASPIRIN AND ESOMEPRAZOLE MAGNESIUM IN BINARY MIXTURE

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ABSTRACT

Objective: A simple, accurate, rapid and precise RP-HPLC method for the simultaneous estimation of Aspirin and Esomeprazole magnesium in binary mixture has been developed and validated.

Method: Separation of drugs was achieved from HPLC Column (hypersil 250 x 4.6 mm C18 column) with a mobile phase consisting of HPLC grade Methanol: Acetonitrile (90:10 v/v) at a flow rate of 1 ml/min with UV detection at 240nm. The method was validated with respect to linearity, sensitivity, accuracy, precision and robustness as per the International Conference on Harmonisation (ICH) guidelines. The method was specific and it was observed that no interference with diluents.

Result: The retention times of Aspirin and Esomeprazole magnesium were 1.92 ± 0.3min and 3.40 ± 0.05min respectively. The linearity was established over the concentration range of 10-140µg/ml and 5-40µg/ml with correlation coefficients (r^2) 0.9977 and 0.9985 for Aspirin and Esomeprazole magnesium respectively. The mean recoveries were found to be in the range of 99.05%-100.75% and 99.21% -100.76% for Aspirin and Esomeprazole magnesium respectively. Conclusion: The %R.S.D. values for intraday precision study and inter-day study were <1.0%, confirming that the method was sufficiently precise. The method can be successfully employed for the simultaneous determination of Aspirin and Esomeprazole magnesium in binary mixture.

Keywords: Aspirin, Esomeprazole magnesium, HPLC, Simultaneous determination, Validation.

INTRODUCTION

Aspirin (ASP) is chemically 2-(acetyloxy)-benzoic acid (Figure 1). It is non-selective cyclo-oxygenase inhibitor used as an antipyretic, analgesic, anti-inflammatory and antithrombotic agent. It reduces non-fatal myocardial infraction.[1-8] It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), United states pharmacopoeia (USP) and European Pharmacopoeia (EP). It is estimated by acid-base titration method as per IP, BP, USP & EP [9-11] Literature review reveals that HPLC[13,14], UV spectrophotometric[15] methods has been reported for estimation of ASP in pharmaceutical dosage forms. Esomeprazole Magnesium[1-8] (ESO) is S-isomer of omeprazole and Proton pump inhibitor. It is chemically Di-(S)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2 pyridinyl)methyl]-sulfinyl]-1Hbenzimidazolemagnesium trihydrate (Figure 1 b).It is used in treatment of peptic ulcer disease, NSAIDS- associated ulceration and Zollinger- Ellison syndrome used as Anti-ulcerative. ESO and its tablet dosage form is official in IP, USP & EP and estimated by Liquid Chromatographic method[8]. Literature review also reveals that UV spectrophotometric[16,17] HPLC[18-21] methods has been reported for the estimation of Esomeprazole in pharmaceutical dosage forms. Literature survey does not reveal any HPLC method for simultaneous determination of ASP and ESO in Pharmaceutical dosage form. The present developed method is simple, rapid, precise and accurate for simultaneous determination of both drugs in binary mixture as per International Conference on Harmonization (ICH) guidelines[12].

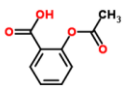

Fig. 1a: Structure of Aspirin (ASP)

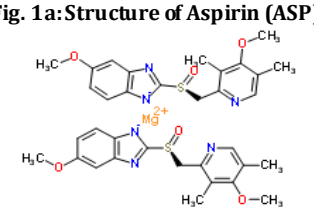

Fig. 1b: Structure of Esomeprazole magnesium (ESO)

MATERIALS AND METHODS

Chemicals and reagents

Pure drug samples of Aspirin and Esomeprazole magnesium and Methanol, Acetonitrile of AR Grade and all other chemicals were provided by West coast pharma, Ahmedabad, Gujarat, India.

Chromatographic conditions

The isocratic mobile phase consisted of methanol: acetonitrile in the ratio of 90:10 (v/v), flowing through the column at a constant flow rate of 1 ml/min. A hypersil C_{18} column (250 x 4.6mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for two drugs, 240nm was selected as the detection wavelength for UV-PDA detector. The HPLC system (Shimadzu) was operated at room temperature 25 ^{0}C.

Preparation of standard stock solutions

Accurately weighed Aspirin (10 mg) was transferred to 10 mL volumetric flask, dissolved in and diluted with methanol up to the mark (1000µg/mL). This solution was further diluted with methanol to obtain final concentration of ASP 100µg/ml. For preparation of ESO stock solution, accurately weighed Esomeprazole magnesium (10 mg) was transferred to 10 mL volumetric flask, dissolved in and diluted with methanol up to the mark (1000µg/mL). For preparation of working standard solution, 1 ml of stock solution of ESO (100µg/ml) and 4 ml of stock solution of ASP(100µg/ml) were transferred to 10 ml volumetric flask and diluted with methanol upto the mark to obtain final concentration containing 10µg/ml of ESO and 40µg/ml of ASP.

Preparation of sample solution

Powder mixture equivalent to 80 mg of aspirin and 20 mg of Esomeprazole was transferred in 100ml volumetric flask containing 50mL methanol, sonicated for 5 min and diluted to mark with same solvent to obtain 0.8mg/ml of ASP and 0.2mg/ml of ESO. The resulting solution was filtered using watmann filter paper. From the above solution 1mL was transferred into 10mL volumetric flask and diluted to mark with same solvent. So, Resultant solution was found to contain 20µg/ml of Esomeprazole magnesium and 80µg/ml of aspirin.

Method Validation

Linearity

The calibration curve was linear over the concentration range of 10-140µg/ml for ASP and 5-40µg/ml for ESO.

Precision

Precision of the method was determined in the terms of intra-day and inter-day variation (%RSD). Intra-day precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days.

Accuracy

To the pre-analyzed sample a known amount of standard solution of pure drug (ASP and ESO) was spiked at three different levels (50%, 100% and 150%). These solutions were subjected to re-analysis by the proposed method.

Sensitivity

The sensitivity of measurement of ASP and ESO by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by equation. Based on the standard deviation of the response and the slope, LOD and LOQ were estimated using the formulae:

LOD= 3.3 σ/S

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

LOQ = 10 σ/S

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

LOD and LOQ were determined from the standard deviations of the responses for six replicate determinations.

Specificity

Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate, lactose,) were spiked in to a pre weighed quantity of drugs .The chromatogram was taken by appropriate dilution and the quantities of drug were determined. The specificity of the HPLC method is illustrated in Fig. 3. Where complete separation of ASP and ESO in presence of tablet excipients.

Repeatability

Repeatability of sample application was assessed by injecting 20µg/ml and 80µg/ml of drug solution of Esomeprazole magnesium and Aspirin respectively six times.

System suitability

The suitability of the chromatographic system was tested before each stage of validation. Five replicate injections of standard preparation were injected and resolution, asymmetry, number of theoretical plates and relative standard deviation of peak area were determined as shown in table 9.

RESULTS AND DISCUSSION:

Method development

The HPLC procedure was optimized for simultaneous determination of ASP and ESO. The mobile phase Methanol: Acetonitrile (90:10 v/v) resulted in good resolution and sharp and symmetrical peaks. Using a reversed-phase C18 column, the retention times for ASP and ESO were observed to be 1.92 ± 0.3 for ASP and 3.4 ± 0.05 for ESO respectively. Total time of analysis was less than 5 min. The maximum absorption of ASP and ESO together as detected at 240 nm and this wavelength was chosen for the analysis (Fig. 2)

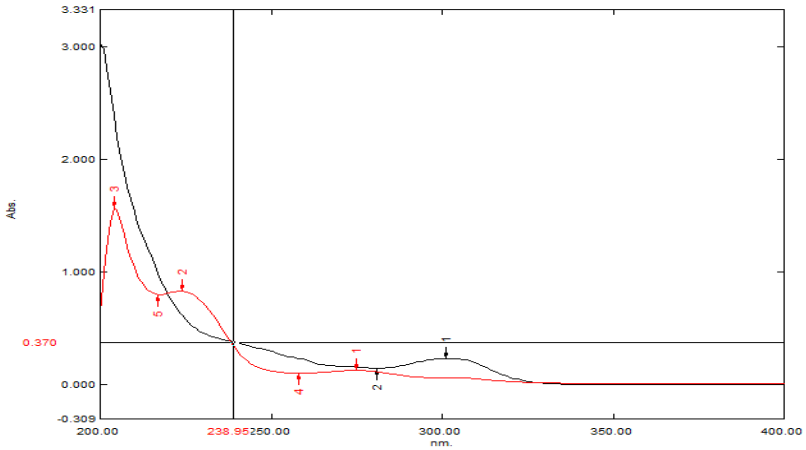

Fig. 2: Overlain spectrum of 20 µg/ml ASP and 5 µg/ml ESO

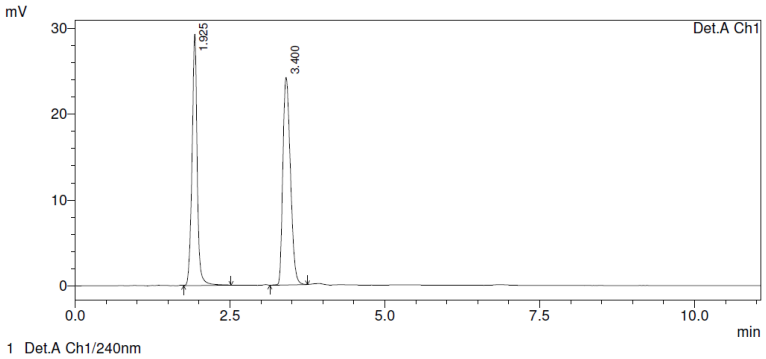

Fig. 3: Chromatogram of mixed standard solution containing 10 µg/ml ASP and 5 µg/ml ESO

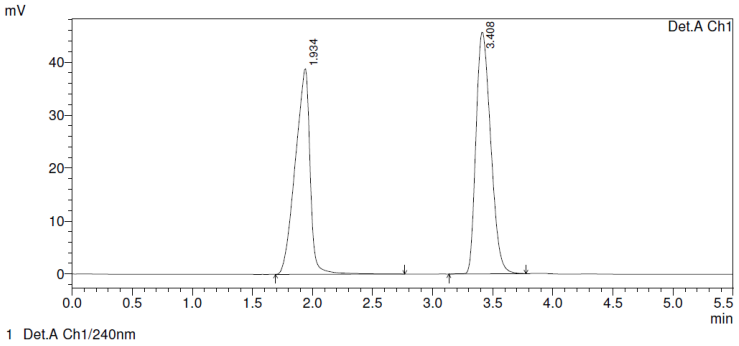

Fig. 4: Chromatogram of sample solution of 20 µg/ml ASP and 5 µg/ml ESO

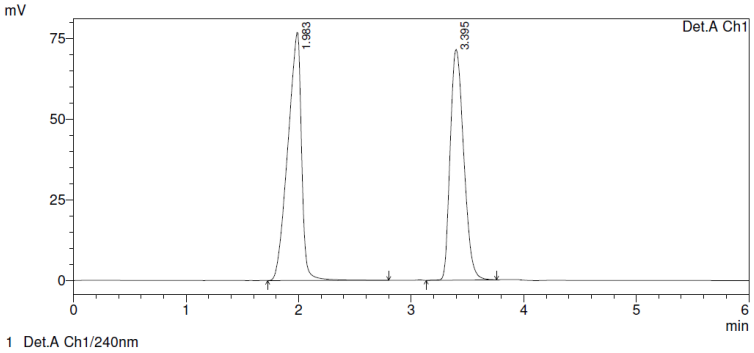

Fig. 5: Chromatogram of 30 µg/ml ASP and 15 µg/ml ESO in the presence of excipients.

Validation

Linearity

Linear regression data for the calibration plots revealed good linear relationships between area and concentration over the ranges 10-140 µg/ml for ASP and and 5-40 µg/ml for ESO. The linear equations for the calibration plots were y = 15463x + 20876 and y = 41817x - 20910 with Regression (r^2) being 0.9977 and 0.9985 for ASP and ESO, respectively (Fig. 6,7) (Table 1, 2 and 3)

Precision

The precision of the method was expressed as relative standard deviation (RSD %). The %R.S.D. values for intra-day precision study and inter-day study listed in (Table 4 and 5) were <1.0%, confirming that the method was sufficiently precise.

Accuracy

When the method was used for accuracy and subsequent analysis of both the drugs, and spiked with 50, 100, and 150% of additional pure drug, the recovery was found to be 99.05- 100.75% for ASP and 99.21- 100.76% for ESO (Table 6 and 7).

Specificity & selectivity

Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate, lactose,) were spiked in to a pre weighed quantity of drugs .The chromatogram was taken by appropriate dilution and the quantities of drug were determined. The specificity of the HPLC method is illustrated in Fig. 5. Where complete separation of ASP and ESO in presence of excipients.

Table 1: Result of Calibration readings for ASP

Concentration (µg/ml)	Area Mean (n=6) ± SD	%RSD
10	168988 ± 1096.93	0.649118
20	330888 ± 4147.23	1.253362
40	647824 ± 7521.68	1.161069
60	100892 ± 9627.41	0.954227
80	122903 ± 11769.78	0.957643
100	151902 ± 15034.93	0.989774
120	191341 ± 10956.46	0.572612
140	219040 ± 4082.90	0.186399

Table 2: Result of Calibration readings for ESO

Concentration (µg/ml)	Area Mean (n=6) ± SD	%RSD
5	205505 ± 85.36978	0.041541
10	389155.5 ± 401.348	0.103133
15	598229.2 ± 1602.95	0.267951
20	812038.7 ± 5461.27	0.672539
25	1038123 ± 7503.20	0.722767
30	1212088 ± 11159.5	0.920684
35	1467957 ± 8175.04	0.5569
40	1644230 ± 5234.91	0.318381

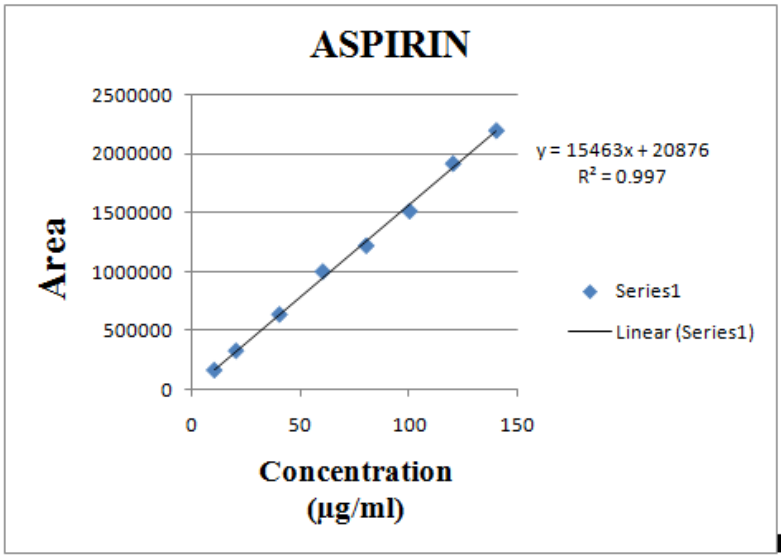

Fig. 6: Calibration curve of ASP in Methanol at 240 nm

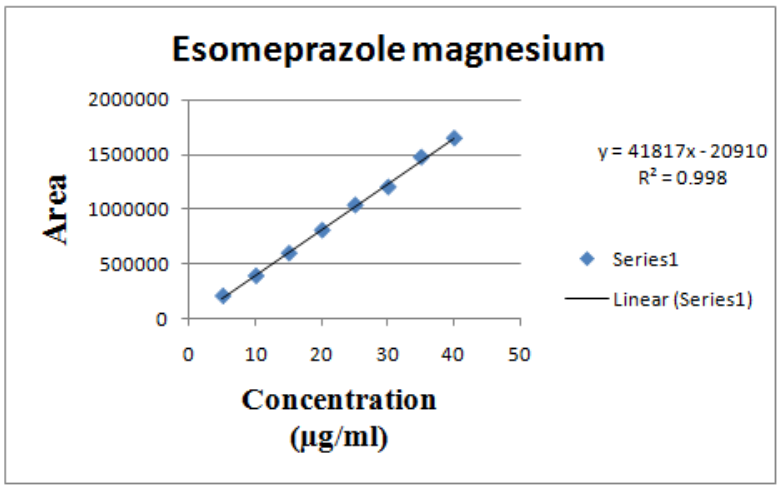

Fig. 7: Calibration curve of ESO in Methanol at 240 nm

Table 3: Statistical Data of ASP and ESO

Parameters	Results	
	ASP	ESO
Linear Range(µg/ml)	10-140	5-40
Slope	15437.17	41716.83
Intercept	27342	17504.83
Std. Deviation of Slope	43.51283	109.9426
Std. Deviation of Intercept	5477.771	3013.965
Limit of Detection(µg/ml)	0.999905	0.315539
Limit of Quantifications(µg/ml)	3.030017	0.956179
Regression Equation	y = 15463x + 20876	y = 41817x - 20910
Co-Efficient of Determination (r^2)	0.9977	0.9985

Table 4: Intra-Day and Inter-Day study of ASP

Concentration (µg/ml)	Intra-Day Area Mean (n=3) ± SD	%RSD	Inter-Day Area Mean (n=3) ± SD	%RSD
20	332806 ± 901.5337	0.270889	328027.7 ± 4059.65	1.237595
60	1005512 ± 3304.57	0.328646	1010472 ± 5828.35	0.576795
100	1512529 ± 269.495	0.017818	1509198 ± 5947.35	0.394074

Table 5: Intra-Day and Inter-Day study of ESO

Concentration (µg/ml)	Intra-Day Area Mean (n=3) ± SD	%RSD	Inter-Day Area Mean (n=3) ± SD	%RSD
5	206555.3 ± 905.858	0.438555	205682.7 ± 1160.01	0.563983
15	598772.7 ± 2251.11	0.375954	602902.7 ± 8060.85	1.337008
25	1043429 ± 5589.05	0.535643	1045071 ± 12975.77	1.241616

Table 6: Determination of Accuracy for ASP

Concentration of Sample Taken (µg/ml)	Concentration of Pure API spiked (µg/ml)	Total Concentration (µg/ml)	Mean Total Concentration Found (n=3) (µg/ml)	%Recovery Mean (n=3)	%RSD
40	20	60	60.45	100.75	0.6721
40	40	80	79.55	99.43	0.5721
40	60	100	99.05	99.05	1.05
Average				99.743	

Table 7: Determination of Accuracy for ESO

Concentration of Sample Taken (µg/ml)	Concentration of Pure API spiked (µg/ml)	Total Concentration (µg/ml)	Mean Total Concentration Found (n=3) (µg/ml)	%Recovery Mean (n=3)	%RSD
10	5	15	14.88	99.21	0.384
10	10	20	19.91	99.59	0.245
10	15	25	100.76	100.76	0.634
Average				99.853	

Table 8: Repeatability study of ASP and ESO

Concentration	ASP 80 (µg/ml)	ESO 20 (µg/ml)
Area	122049	807119
	1220330	807209
	1230229	807195
	1220699	807905
	1221429	807795
	1220122	807565
Mean	1222206	807464.7
± SD	3956.349	337.8317
%RSD	0.323705	0.3041839

Table 9: System suitability parameters

Parameter	Aspirin	Esomeprazole
Retention time*	1.92 ± 0.3 min	3.40 ± 0.05 min
Resolution	7.497 ± 0.9	
No. of theoretical plate*	2303.242 ± 26.72	3403.723 ± 17.58
Tailing factor*	1.039 ± 0.04	1.357 ±0.02
HETP*	65.126	44.069

Table 10: Assay Result of Synthetic mixture

Parameters	ASP	ESO
Actual Concentration (µg/ml)	80	20
Concentration Obtained (µg/ml)	79.55	19.91
%Purity	99.43	99.59
%RSD	0.5721	0.245

Table 11: Validation Parameters

	Summary of Validation Parameters	
	ASP	ESO
Recovery (%)	99.743	99.853
Repeatability (%RSD)	0.3237	0.3041
Precision (CV)		
Intra-day (n=3)	0.003286	0.00376
Inter-day (n=3)	0.005768	0.00564
Specificity	Specific	Specific
Selectivity	Selective	Selective

Analysis of ASP and ESO in synthetic mixture

Content of ASP AND ESO found in the synthetic mixture from the proposed method are shown in table 10. The % purity was 99.43% for ASP and 99.59% for ESO.

Sensitivity

The LOD and LOQ were calculated by equation. The LOD and LOQ values were 0.999 and 3.030µg/ml for ASP and 0.3155 and 0.9561µg/ml for ESO.

Repeatability

The % RSD for peak area values of ASP and ESO were found to be 0.3237 and 0.04183 respectively, as given in Table 8.

CONCLUSION

The developed HPLC method is simple, precise, accurate and reproducible and can be used for simultaneous determination of ASP and ESO in pharmaceutical dosage forms. The method was validated as per International Conference on Harmonization (ICH) guidelines.

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