



RESEARCH ARTICLE

**Development and Validation of HPTLC Method for Simultaneous Estimation of
Esomeprazole Magnesium and Aspirin in Bulk and Synthetic Mixture
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ABSTRACT

A simple, accurate, and precise HPTLC method has been developed and validated for the simultaneous estimation of Esomeprazole magnesium (ESO) and Aspirin (ASP) from bulk drug and Synthetic mixture. The method employed TLC aluminum plates precoated with silica gel 60 GF₂₅₄ as the stationary phase. The solvent system comprised Acetate: Toluene: Glacial acetic acid (4.5:0.5:0.05 v/v/v). This system was found to give good result for both the drugs (R_f value: of ESO 0.22cm and ASP 0.78cm). Spectrodensitometric scanning-integration was performed at a wavelength of 239nm. The calibration curve was found to be linear within the concentration range of 200ng/spot to 800ng/spot for both the drugs. The regression data for calibration curve shows good linear relationship with r² = 0.9981 and 0.9990 for ASP and ESO respectively. The method was validated in accordance with the requirements of ICH guidelines. The method was successfully applied for determination of drug in bulk and synthetic mixture. Thus, the proposed method can be used successfully for routine analysis of ESO and ASP from bulk and Synthetic mixture.

KEYWORDS

Validation, HPTLC, Esomeprazole Magnesium, Aspirin

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 Spectrophotometric¹⁵ methods has been reported for estimation of ASP in pharmaceutical dosage forms.

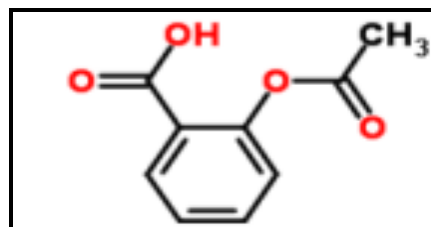


Figure 1: Structure of Aspirin

Esomeprazole Magnesium¹⁻⁸ (ESO) is S-isomer of omeprazole and Proton pump inhibitor. It is chemically Di-(S)-5-methoxy-2-[[[4-methoxy-

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3,5-dimethyl-2-pyridinyl)methyl]-sulfinyl]-1H-benzimidazole magnesium trihydrate (Figure 2). 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 method⁸.

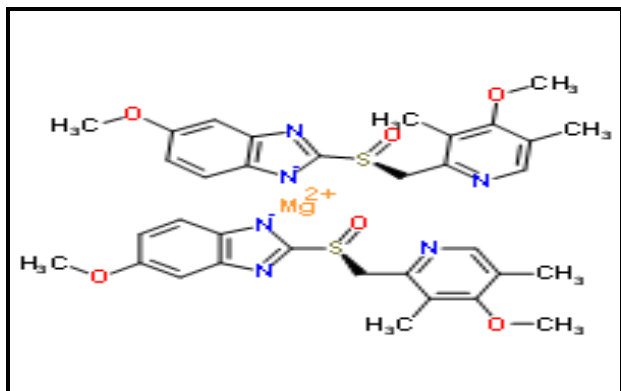


Figure 2: Structure of Esomeprazole magnesium
Literature review also reveals that UV Spectrophotometric^{16,17} HPLC¹⁸⁻¹⁹ methods has been reported for the estimation of Esomeprazole in pharmaceutical dosage forms. Literature survey does not reveal any HPTLC method for simultaneous determination of ASP and ESO in Pharmaceutical dosage form. The present developed method is simple, rapid, precise and accurate for simultaneous determination of both drugs in synthetic mixture as per International Conference on Harmonization (ICH) guidelines.

MATERIALS AND METHOD

Chemicals and Reagents

Pure drug samples of Aspirin and Esomeprazole magnesium and Methanol, Acetonitrile of AR Grade and all other chemicals were provided by Department of Pharmaceutical Sciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India.

Chromatographic Conditions

Stationary phase was Precoated Silica gel G60 F₂₅₄ aluminum Sheets 10×10 cm²; layer thickness 0.2 mm activated the TLC plates by prewashing with Toluene and activated in Oven at 50^oC for 5minute. The Optimized Mobile

phase was Ethyl Acetate: Toluene: Glacial acetic acid (4.5:0.5:0.05 v/v/v). Chamber saturation time: 30 minute at ambient temperature and migration distance was 75mm. The detection was done at 239nm.

Preparation of Standard Stock Solutions

Accurately weighed 10mg of ASP and ESO were transferred into 10ml volumetric flask individually, dissolved and diluted up to the mark with Toluene to get stock solution having 1000µg/ml concentration of ASP and ESO. 1 ml of each from standard stock solutions of ASP and ESO was transferred to 10 ml volumetric flask and diluted to 10 ml with Toluene to get ASP and ESO working standard solution having 100µg/ml concentration. To obtain calibration curve, working standard solutions ranging from 2.0 – 8.0 µl was applied by Hamilton syringe with the help of Linomat V applicator on TLC plate that gave concentration in the range of 200-800 ng/spot for both the drugs.

Preparation of Sample Solution

Powder mixture equivalent to 80mg of aspirin and 20mg of Esomeprazole magnesium was transferred in 100ml volumetric flask containing 50ml Toluene 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 whatman filter paper. From the above solution 1ml was transferred into 10ml volumetric flask and diluted to mark with the same solvent. So, Resultant solution was found to contain 20µg/ml of Esomeprazole magnesium and 80µg/ml Aspirin. 10µl of this solution applied on TLC plate followed by development and scanning at 239 nm.

Method Validation

Linearity

The calibration curve was linear over the concentration range of 200-800ng/spot for both ASP and 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:

$LOD = 3.3 \sigma/S$ Where, σ = the standard deviation of the response

S = the slope of the calibration curve

$LOQ = 10 \sigma/S$ 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 HPTLC 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 200ng/spot and 800ng/spot

of Eesomeprazole magnesium and Aspirin respectively six times and statistical data was calculated.

RESULT AND DISCUSSION

Method Development

The solvent system was developed and optimized using trial and error method. Various proportions of different solvents as mobile phase were tried to get resolution of both the compounds. The optimized mobile phase was Ethyl acetate: Toluene: Glacial acetic acid:: 4.5:0.5:0.05 (v/v/v). The optimized mobile phase could resolve both the compounds apart from each other and the bands obtained were compact too. The maximum absorption of ASP and ESO together as detected at 239 nm and this wavelength was chosen for the analysis (Fig. 3).

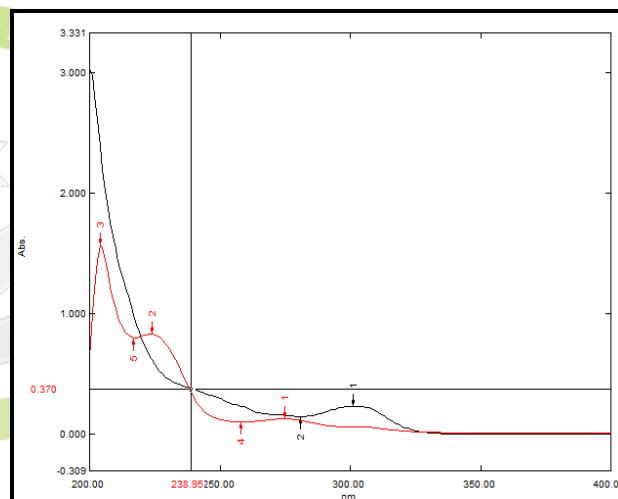


Figure 3: Overlain spectrum of 20µg/ml ASP and 5µg/ml ESO

The optimized solvent system yielded a symmetrical peak for the both drugs with R_f 0.22 and 0.78 for ESO and ASP respectively. The HPTLC chromatogram of ESO and ASP is shown in Figure 4.

The developed method was then validated and successfully applied for quantification of ESO and ASP from the formulation. Precision, expressed in terms of %RSD was determined in terms of intra-day and inter-day precisions, analyzing the drugs at three different concentrations, determining each concentration thrice summarized in Table 1 and 2.

Table 1: Intra- day Precision Data for ESO and ASP

Conc. (ng/spot) ASP	Area Mean (n=3) \pm SD ASP	%RSD ASP	Conc. (ng/spot) ESO	Area Mean (n=3) \pm SD ESO	%RSD ESO
600	6265.5.68	0.516	200	1689.81	0.929
700	7089.84	0.599	300	2163.22	0.785
800	8018.95	0.367	400	2613.06	0.727

Table 2: Inter- day Precision Data for ESO and ASP

Conc. (ng/spot) ASP	ASP Mean Area (n=3) \pm SD	%RSD ASP	Conc. (ng/spot) ESO	ESO Mean Area (n=3) \pm SD	%RSD ESO
600	6279.28	0.902	200	1698.01	1.3
700	7093.69	0.77	300	2165.15	1.09
800	8022.64	0.672	400	2618.49	0.82

Table 3: Recovery data for determination of ESO and ASP

Conc. of Sample Taken (ng/spot)	Conc. of Pure API spiked (ng/spot)	Total Conc. (ng/spot)	Mean Total Conc. Found (n=3) (ng/spot)	%Recovery Mean (n=3)
200	100	300	300.91	100.3
200	200	400	398.67	99.56
200	300	500	1989.36	100.46
Average				99.80

Table 4: Results of sensitivity data for ASP and ESO

Parameter	Results	
	ASP	ESO
LOD (ng/spot)	3.77	17.46
LOQ (ng/spot)	11.44	52.90

To ensure accuracy of the method, recovery studies were performed by standard addition method at three different levels I, II and III (50%, 100%, and 150%), to the pre-analyzed samples and the subsequent solutions were re-analyzed. At each level, three determinations were performed and the results obtained are shown in Table 3.

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 values obtained are shown in Table 4.

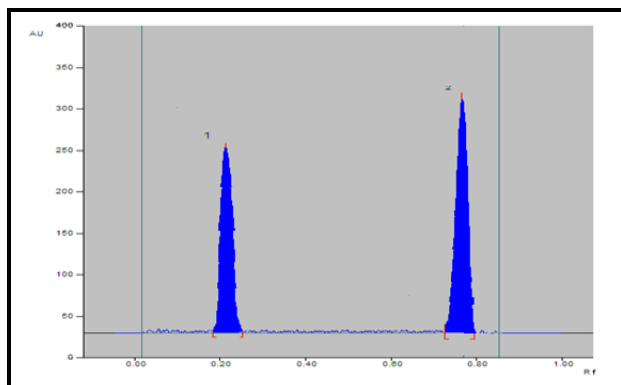


Figure 4: A Typical Chromatogram of ESO and ASP

The repeatability study was carried out and the result from that regression analysis obtained are summarized in Table 5.

The method was applied to the synthetic mixture and the Chromatogram of the synthetic mixture is shown in Figure 5.

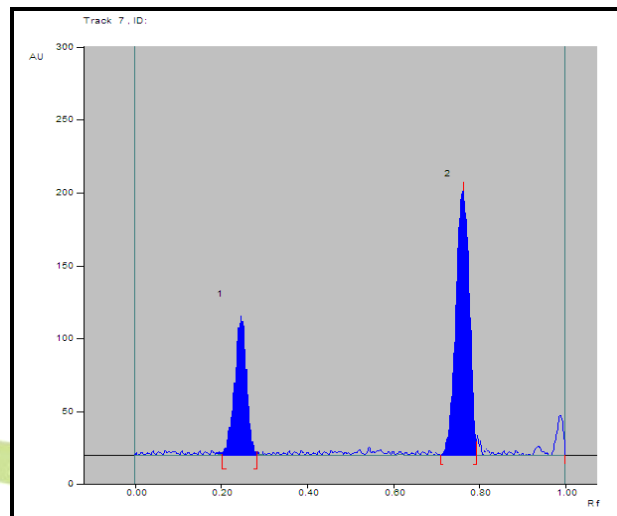


Figure 5: Typical HPTLC Chromatogram of ESO and ASP synthetic mixture

The peak purity of ASP and ESO were assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., r (S, M) and r (M, E) Figure 6.

Table 5: Regression Analysis Data of ESO and ASP

Parameters	Results	
	ASP	ESO
Linear Range(ng/spot)	200-800	200-800
Slope	9.40	4.70
Intercept	562.13	754.76
Std. Deviation of Slope	0.0244	0.0247
Std. Deviation of Intercept	16.169	12.052
Limit of Detection (ng/spot)	3.77	17.46
Limit of Quantitation (ng/spot)	11.44	52.90
Regression Equation	$y = 9.4019x + 562.13$	$y = 4.7088x + 754.75$
Co-Efficient of Correlation (r^2)	0.9981	0.9990

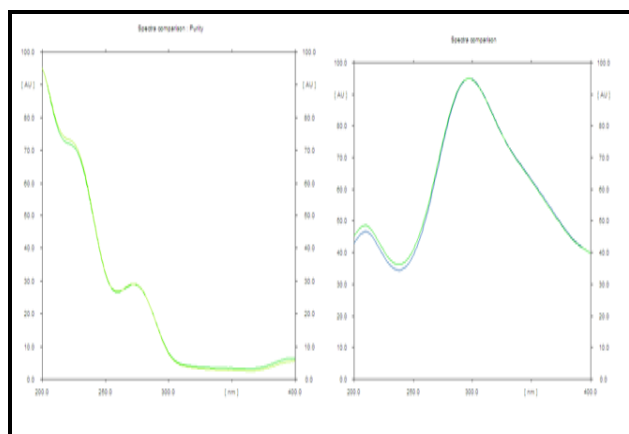


Figure 6: Purity Comparison Spectra of a. ASP and b. ESO in standard and synthetic mixture

CONCLUSION

In this proposed method the linearity was observed in the concentration range of 200-800ng/spot for both ASP and ESO with coefficient of correlation, $r^2 = 0.998$ and $r^2 = 0.999$ for ASP and ESO, respectively at 239nm. The result of the analysis of combined mixture by the proposed method was found to be highly reproducible and reliable. The additives present in mixture of the assayed samples did not interfere with determination of ASP and ESO. So, the developed HPTLC method is simple, precise and accurate and can be used for simultaneous determination of ASP and ESO in pharmaceutical dosage forms.

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