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Research Article

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Development and Validation of HPTLC Method for Simultaneous Quantification of Aspirin and Omeprazole in Bulk and Marketed Formulation

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ABSTRACT

A simple High Performance Thin Layer Chromatography (HPTLC) method has been developed and validated for determination of Aspirin (ASP) and Omeprazole (OME) simultaneously in tablet dosage form (Yosprala). The samples were applied on an aluminum TLC plate pre-coated with silica gel. For separation, Toluene: Ethyl Acetate: Methanol: Glacial acetic acid (2.0:6.0:0.5:0.1, v/v/v/v) was used as a mobile phase. The drugs were separated satisfactorily with Rf values of 0.38 ± 0.02 and 0.69 ± 0.01 for OME and ASP, respectively. Detection was performed densitometrically in absorbance mode at 280 nm. The regression analysis for the calibration plots showed linear relationship with R2 of 0.999 and 0.998 for ASP and OME, respectively in the concentration range of 200-1400 ng/spot for ASP and 100-700 ng/spot for OME. The limit of detection and quantitation were 17.33 and 52.53 ng/spot, respectively for ASP and 7.48 and 22.66 ng/spot, respectively for OME. The proposed developed HPTLC method can be applied for identification and quantitative determination of OME.

Keywords: Aspirin, Omeprazole, HPTLC, Validation

INTRODUCTION

Myocardial infarction indicated as a heart attack occurs when blood flow stops to a part of the heart causing damage to the heart muscle. Yosprala, a fixed-dose combination is available containing an anti-platelet agent Aspirin and the proton pump inhibitor Omeprazole (Figure 1) [1-5].

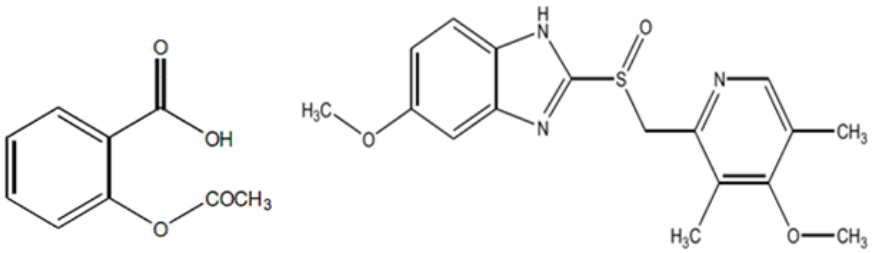

Figure 1. Chemical structures of aspirin and omeprazole

Literature survey reveals only one analytical methods reported for quantitative estimation of the aspirin and omeprazole are UV [6] and only one method was reported for reverse phase HPLC [7] in combination. To the best of our knowledge some methods were reported for aspirin and omeprazole for combination with other drugs [8-11] but no any HPTLC method has been reported for the quantitative estimation of the aspirin and omeprazole. So, the aim of the present method was to develop and validate HPTLC method for determination of aspirin and omeprazole simultaneously.

MATERIALS AND METHODS

Chemicals and Reagents

Standard sample of Aspirin was given as a gift sample from Sidmak, Valsad, India and Omeprazole was given as a gift sample from Mangalam drugs, Vapi. Methanol, Ethyl acetate, Toluene and Glacial acetic acid of AR-grade were purchased from Merck, Mumbai, India.

Instrumentation and Chromatographic Conditions

For the proposed method linomat-V of Camag was used as a sample applicator was used with TLC scanner-4. Twin trough developing chamber of diameter containing 10 × 10 cm was used. Hamilton syringe of 100 µl was used to apply a sample, Pre-coated silica gel 60 F254 aluminium plates, 10 × 10 cm having a thickness of 100 µm was used as a stationary phase. The HPTLC plate was prewashed by methanol and activated at 60°C for 5 min prior to chromatography; samples were spotted in the form of bands having 6 mm of width. Toluene: Ethyl Acetate: Methanol: Glacial acetic acid (2.0: 6.0: 0.5: 0.1, v/v/v/v) was selected as a mobile phase for proposed method on trial and error bases. 10 µl volume was applied for each chromatographic run. Slit dimension was kept at 4 mm × 0.30 mm with 20 mm/s scanning speed. Distance travel from solvent front was kept at 90 mm. Densitometric detection was done using a UV detector at 280 nm.

EXPERIMENTAL WORK

Preparation of Solutions for Analysis

Aspirin stock solution: Aspirin standard stock solution containing 200 µg/ml was prepare in 100 ml volumetric flask by dissolving 20 mg of aspirin and then dilute into 100 ml with methanol.

Omeprazole stock solution: Omeprazole standard stock solution containing 100 µg/ml was prepare in 100 ml volumetric flask by dissolving 10 mg of aspirin and then dilution to 100 ml with methanol.

Preparation of sample solution: Accurately weigh a weight of tablet powder equivalent to 20 mg of aspirin was taken into the 100 ml volumetric flask, about 50 ml of methanol was added into the flask and sonicated for 15 min. The solution was further diluted up to mark with methanol, mixed well and filtered to obtain solution containing 200 µg/ml for ASP, 100 µg/ml for OME.

Selection of Wavelength

Standard stock solutions of 200 µg/ml of aspirin and 100 µg/ml of omeprazole were taken. After chromatographic development, bands were scanned over the wavelength range of 200-400 nm at scan speed 100 mm/s and the overlain spectra were recorded. Single wavelength showing maximum absorbance was selected as a detection wavelength for HPTLC method. Overlain UV spectra are shown in Figure 2.

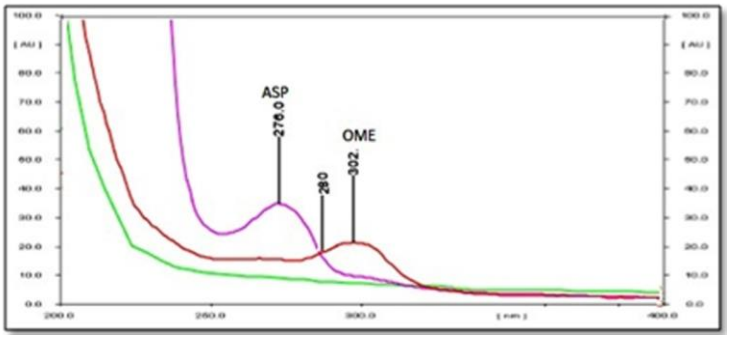

Figure 2. Overlain UV spectra of aspirin and omeprazole

Validation

Validation of the developed HPTLC method was carried out with respect to ICH guideline which is described as follows: [12].

Specificity: For evaluation specificity of the optimized method, injections of diluent, mobile phase, injections of standard solution containing aspirin (800 ng/spot) and omeprazole (400 ng/ml) and injection of test solution of the same concentration were injected. Any interference from blank and excipients of the tablets was checked by comparing of Rf value of standard and test solutions (Figure 3).

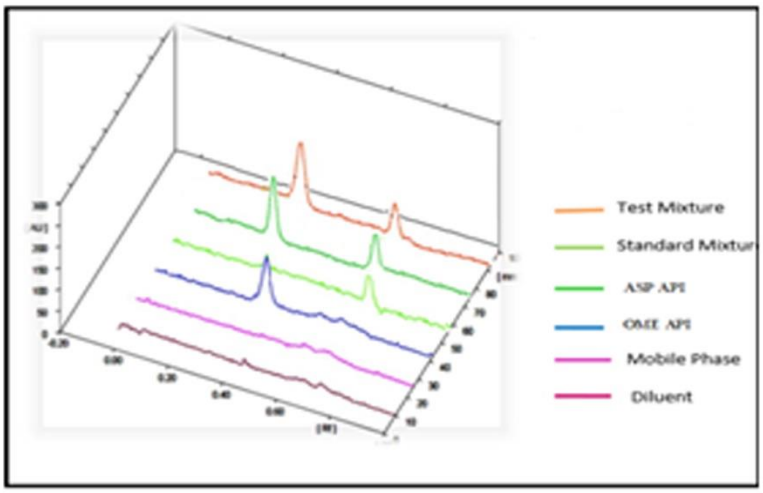

Figure 3. 3D Chromatogram of specificity study

Linearity and range: Calibration curves were plotted over the concentration range of 100-700 ng/spot and 200-1400 ng/spot for OME and ASP respectively by preparing 10, 20, 30, 40, 50, 60 and 70 µg/ml solutions for omeprazole from stock solution by withdrawing 1, 2, 3, 4,5, 6 and 7 ml and 20, 40, 60, 80, 100, 120 and 140 µg/ml solutions for aspirin by withdrawing 1, 2, 3, 4, 5, 6 and 7 ml from stock solution. The calibration curves were constructed by plotting peak areas (Y- axis) against the concentrations (X - axis). Figure 4 describes chromatogram of standard solutions for linearity study. The correlation coefficient and equation is shown in Figures 5 and 6 and Tables 1 and 2 for aspirin and omeprazole respectively.

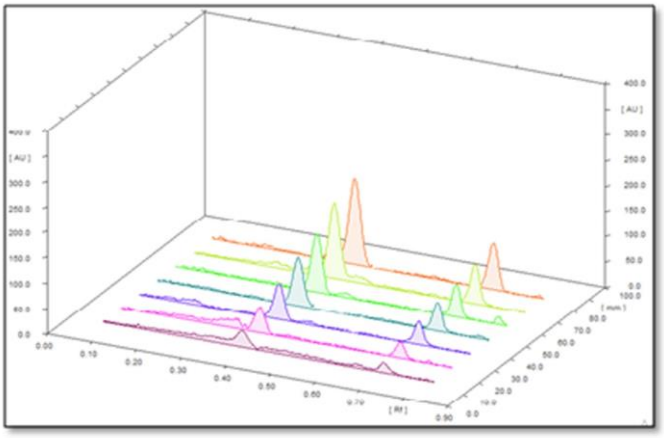

Figure 4. 3D Chromatogram of linearity study

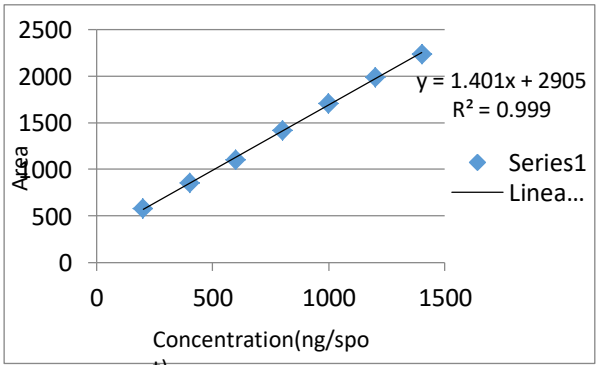

Figure 5. Calibration curve of aspirin

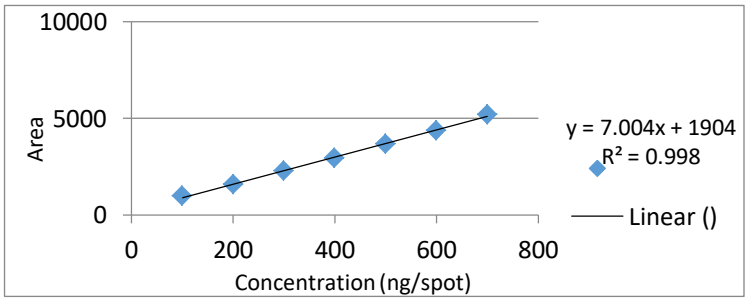

Figure 6. Calibration curve of omeprazole

Table 1. Results of quantitative determination for aspirin

Parameters	Results
Linearity and Range	200-1400 ng/spot
Regression coefficient (R^2)	0.999
Y – intercept ± SD	2904 ± 25.35
Slope ± SD	1.403 ± 0.0020
LOD	17.33
LOQ	52.53

Table 2: Results of quantitative determination for Omeprazole

Parameters	Results
Linearity and Range	100-700 ng/spot
Regression coefficient (R^2)	0.998
Y-intercept ± SD	1901 ± 15.39
Slope ± SD	6.879 ± 0.0057
LOD	7.48
LOQ	22.66

Limit of detection (LOD) and limit of quantitation (LOQ): Both parameters were calculated by following equations and reported in Tables 1 and 2 for aspirin and omeprazole respectively.

$LOD=3.3 \times \sigma/S$ and $LOQ=10 \times \sigma/S$

Accuracy (%Recovery study): The accuracy of the proposed method was determined by standard addition method by calculating the percentage recoveries of all three drugs. The accuracy was evaluated in triplicates, at three different concentrations levels of 50, 100 and 150 % of the active ingredients, by adding different concentration of Aspirin and Omeprazole standard to the known amount of sample and calculating the % recovery for both the drugs. For aspirin recovery studies were carried out by spiking three different amount of ASP standard (400 ng/spot, 800 ng/spot, 1200 ng/spot) to the dosage form (100 ng/spot), and for Omeprazole spiking OME standard (200 ng/spot, 400 μg/spot and 600 ng/spot) to the dosage form (200 ng/spot) by standard addition method. Results for % recovery study were reported in Table 3.

Table 3. Result of % recovery of accuracy study for proposed method

Drug	Level (%)	Amount of sample (ng/spot)	Amount of std. spiked (ng/spot)	Total amount (ng/spot)	Amt. found (avg.)	% Recovery (avg.)
Aspirin	50	200	400	600	600	100
	100		800	1000	1002.8	100.28

	150		1200	1400	1402.1	100.15
Omeprazole	50	100	200	300	302.88	100.32
	100		400	500	498.65	99.73
	150		600	700	700.49	100.07

Precision: Intraday and interday precision was performed by freshly prepared solutions for both the drugs. For study of this parameter standard solution of ASP and OME of 3 different concentrations (200, 400 and 600 ng/spot for ASP; 400, 800 and 1200 ng/spot for OMP) were prepared and analyze 2 times on the same day for repeatability. For interday study, same concentration of solutions were prepared but measured on 2 different days. For determination of result %RSD was calculated. Results for intraday as well as inter day precision study were reported in Table 4.

Table 4. Result of precision study for aspirin and omeprazole

Drug	**Concentration (ng/spot)**	**Intraday (% RSD)**	**Intraday (% RSD)**	**Interday (Day 2)**	**Interday (day 3)**
Aspirin	400	0.2323	0.1400	0.2883	0.4510
	800	0.0949	0.0948	0.1217	0.0934
	1200	0.0851	0.1269	0.1413	0.1098
Omeprazole	200	1.1811	0.5481	1.0420	0.7293
	400	0.3714	0.3118	0.2630	0.4012
	600	0.2963	0.1971	0.1778	0.2596

Robustness: The robustness of the method was evaluated by varying method parameters such as saturation time (13 min and 17 min); Distance from solvent front (8 cm and 10 cm) and by changing wavelength (278 nm and 282 nm) It was assessed by using the three replicates of one standard concentration (800 ng/band of ASP, and 400 ng/band of OME) and calculating the values of mean area and % RSD. The effects of changes observed were reported in Table 5.

Table 5. Result of robustness study for aspirin and omeprazole

Parameters	**Average (%RSD)**					
	Area of ASP	**Area of OMP**	**Rf of ASP**	**Rf of OMP**	**% content of ASP**	**% content of OMP**
Distance from solvent front	1.5962	0.9949	0.8356	0.5356	0.2981	0.3649
Saturation time	1.5912	1.2461	0.9790	0.5356	0.3452	0.3210
Wavelength	1.6213	1.009	0.8356	0.8306	0.4542	0.5123

Analysis of marketed formulation: From sample solution of aspirin (200 ppm) and omeprazole (100 ppm) withdraw 4 ml of solution and dilute it with 10 ml of methanol to obtain 80 ppm (800 ng/spot) of aspirin and 40 ppm (400 ng/spot) of omeprazole. The results were shown in Table 6.

Table 6: Results of Analysis of marketed formulation

Drug	Concentration (ng/spot)	Area	Mean of area	Amount obtained	% Assay
Aspirin	800 ng/spot	2838.60 2831.30 2834.20	2834.70	794 ng/spot	99.25
Omeprazole	400 ng/spot	1364.54 1357.31 1352.30	1358.05	403 ng/spot	100.75

RESULTS AND DISCUSSION

The proposed method was validated as per ICH guidelines. The calibration curve was obtained for a series of concentration in the range of 200-1400 ng/spot and 100-700 ng/spot for aspirin and omeprazole respectively. And it was found to be linear. The linear regression equation was y=1.401x-2905 with correlation coefficient value 0.999 for aspirin and y=7.004 x+1904 with correlation coefficient value 0.998 for omeprazole which were within the acceptance range. Specificity was studied for the examination of excipients present in the tablet dosage form. The results indicated that in the assay no any interference was found and safe Rf values were obtained as a standard of 0.38 and 0.69 for omeprazole and aspirin respectively. Accuracy was found by recovery study from prepared samples with standard solution. Recovery was carried out by standard addition method at three different levels which are 50%, 100% and150%. The % recovery was calculated and was found to be 100, 100.28 and 100.15 for aspirin and 100.32, 99.73 and 100.07 for omeprazole respectively. Accuracy was found to be within the acceptance range of 98-102%. This showed that the recovery of aspirin and omeprazole by proposed method was found to be satisfactory. The precision was measured in terms of repeatability, which was determined by three concentrations of sample within the day and next two days for inter day precision. For both the types %RSD was calculated and was found within the acceptance limit ± 2.0% for intraday and interday precision. Robustness was performed by using three parameters of the actual procedure. The % RSD was calculated which was within the acceptable range Not More Then 2.0%. The validated method was applied for the assay of commercial tablets of aspirin and omeprazole, Yosprala (81 mg aspirin, 40 mg omeprazole). The % assay was calculated from standard calibration curve. The result found to be 99.25% for aspirin and 100.75% for omeprazole were observed for good agreement within the labeled content. Hence, the method developed in the present research is found to be accurate, rapid, simple, sensitive, rugged, and precise. Hence the developed method can be successfully applied for the estimation of aspirin and omeprazole in bulk and tablet dosage form.

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