Vol 5, Issue 4, 2018 ISSN 2349-7041

Research Article

APPLICATION OF TLC DENSITOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF ASPIRIN AND OMEPRAZOLE IN PHARMACEUTICAL PREPARATION

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ABSTRACT

Objective: Thin layer denistometric method has been developed for simultaneous determination of aspirin and omeprazole in their new pharmaceutical dosage form. Methods: The densitometric separation process was performed on precoated TLC aluminum silica gel 60 GF254 plates using toluene – ethyl acetate- methanol (3:5:2, by volume) as mobile phase and UV detection was carried out at 240 nm. Results: All the bands of aspirin and omeprazole have been well resolved from each other with significantly different Rf values. Conclusion: The proposed method has been successfully applied for the simultaneous determination of aspirin and omeprazole in the pharmaceutical formulation. Also, the method has been validated with respect to linearity, limits of detection and quantification, accuracy, precision and specificity.

Keywords: Aspirin; Omeprazole; Simultaneous analysis.

INTRODUCTION

Aspirin (ASP), **Figure 1** is a non-steroidal, anti-inflammatory and an anti-platelet drug which suppresses the normal functioning of platelets through irreversibly blocking the formation of thromboxane A_2 in platelets, producing an inhibitory effect on platelet aggregation during the lifetime of the affected platelet (8–9 days). ASP is useful for reducing the incidence of heart attacks, unstable angina, ischemic stroke or transient ischemic attack [1, 2]. Various methods have been reported for the determination of ASP including HPLC [3, 4], gas chromatography [5], Raman spectroscopy [6], UV spectrophotometry, spectrofluorimetry [7, 8] and electrochemically [9, 10].

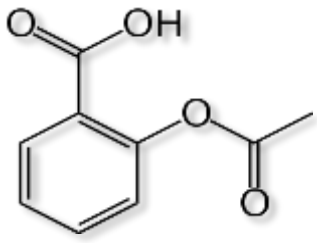

Fig. 1: Structure formula of Aspirin

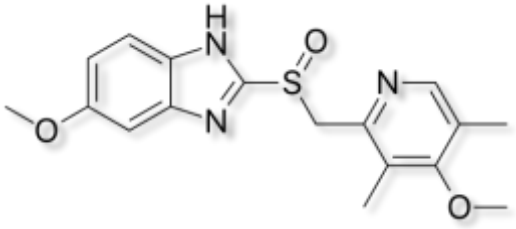

Fig. 2: Structure formula of OPZ

Omeprazole (OPZ), **Figure (2)** is a proton pump inhibitor used in the treatment of gastro esophageal reflux and peptic ulcer diseases [11]. Several methods have been reported for determination of OPZ either individually or in combination with other drugs including spectrophotometry [12, 13], spectrofluorimetry [14], electrochemically [15, 16] and HPLC [17, 18].

Yosprala tablet, a new combination of ASP and OPZ, was recently approved to protect high risk patient of developing aspirin-associated gastric ulcers [19].

The literature review revealed that there is no TLC densitometric method was developed for simultaneous determination of ASP and OPZ in their new pharmaceutical preparation.

The aim of this work was to develop sensitive and valid TLC densitometric method for simultaneous determination of ASP and OPZ in pure form and tablet.

EXPERIMENTAL

Materials and reagents

Pure ASP (99.25%), OPZ (99.75%) and Yosprala ® tablets nominally containing 81mg of ASP / 40mg of OPZ per tablet were kindly supplied by National Organization for Drug Control and Research, Giza, Egypt. Ethyl acetate, methanol, and toluene, HPLC grade (Sigma-Aldrich, Germany).

Apparatus

Camag TLC scanner 3, with wincats computer software (Switzerland). Precoated TLC plates, silica gel 60 GF_{254} (20 x 20 cm), (Fluka chemie, Switzerland). Hamilton 50-µL micro syringe (Germany). UV lamp with short wavelength (240 nm.) (Desega-Germany). Chromatographic tank (25 x 25 x 9 cm).

Standard solutions

A standard solution of 100µg/mL of ASP and OPZ was prepared by dissolving 10 mg of the drug powder in 50 mL of methanol using a two separated 100-mL volumetric flask and completing to volume with methanol.

Procedures

TLC densitometric conditions

TLC densitometric analysis was performed on precoated 20 x 20 cm TLC aluminum silica gel 60 GF_{254} plates. ASP and OPZ samples were applied to the plates using spotted 1 cm apart from each other and 1 cm apart from the bottom edge using Hamilton micro syringe (50µL). The chromatographic tank was pre-saturated with the mobile phase for 20 min. and then developed by ascending chromatography using toluene – ethyl acetate- methanol (3:5:2, by volume) as a mobile phase. The plates were air dried and detected under UV lamp 240 nm.

Construction of calibration curves

In a series of 10-mL volumetric flasks, aliquots of ASP (100 µg/mL) and OMP solution (100 µg/mL) equivalent to (10- 100 µg) of ASP and (4- 80 µg) of OPZ were transferred and diluted to volume with methanol. 10 µL of each drug solution were applied to a TLC plate following the optimum conditions and scanned at 240 nm. .The band areas were plotted versus the corresponding drug concentrations (ng/ml) to get the calibration graphs. Then, the corresponding regression equations were derived.

Procedure for pharmaceutical preparation

Five Yosprala ® tablets (81mg of aspirin/40mg of omeprazole per tablet) were weighed , finely powdered then Accurate weight of powder equivalent to one tablet was accurately weighed, transferred to 100 mL volumetric flask and the volume was made up to 50 mL with methanol. The solution was shaken vigorously and sonicated for 30 min and filtered. The volume was completed to 100 mL with methanol to produce a stock solution labeled to contain 0.81 and 0.4mg/mL of aspirin and omeprazole respectively. Necessary dilutions of the stock solution were made with the developing system to get different concentrations of ASP and OPZ covering the concentration range. 10 µL aliquots were injected and eluted with the mobile phase under the optimum densitometric conditions. Tablet contents of both drugs were calculated using the corresponding regression equation.

RESULTS AND DISCUSSION

TLC densitometric method has been developed and validated for the simultaneous determination of ASP and OPZ in bulk powder and in a new approved pharmaceutical dosage form.

METHOD DEVELOPMENT AND OPTIMIZATION

TLC densitometric technique is a simple and accurate separating technique for the analysis of drugs in their mixtures. The proposed method is based on the difference in the retardation factor (Rf) between ASP and OPZ. In order to achieve the best separation and sharp symmetric peaks, the method has been optimized. Different developing systems with different ratios were tried. Initially, solvent systems with different ratios such as chloroform- methanol-acetonitrile, methanol- acetonitrile, ethyl acetate -methanol-toluene and toluene- acetonitrile were tried. The mobile phase of toluene – ethyl acetate (3:5 v/v) gave closely spots of aspirin and omeprazole which with poor resolution and less symmetric pattern. In order to enhance the TLC densitometric resolution, 2 mL of methanol was added to the mobile phase. The best separation with well-defined spots was obtained after the mobile phase became toluene – ethyl acetate- methanol (3:5:2, by volume). Different band dimension were tested to obtain sharp and symmetrical separated peaks. The optimum band width chosen was 5 mm and the inter-space between bands was 15 mm. Different scanning wavelengths were tried such as 240, 340 and 372 nm**.** A wavelength of 240 nm has been selected since it provided a good sensitivity with sharp detection for both aspirin and omeprazole. After optimization of the TLC densitometric conditions, the plates were visualized under UV lamp at 240 nm, where spots were appeared at Rf of 0.30 for ASP and 0. 71 for OPZ as shown in **Figure (3).**

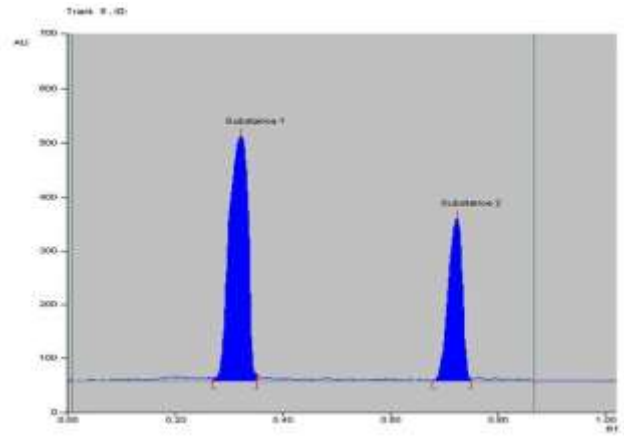

Figure (3): TLC chromatogram of ASP (20 µg/mL) and OPZ (20 µg/mL).

METHOD VALIDATION

The proposed method was validating according to ICH guidelines. The validation parameters were checked regarding linearity, limit of quantification, limit of detection accuracy, precision, specificity and system suitability [20].

Linearity

The calibration graphs for the method were constructed by plotting the spot area values of each dug versus drug concentrations in ng/spot. The regression plots were found to be linear over the range of 10-100 ng/spot for ASP and 4-80 ng/spot for OPZ as in Table (1).

Limit of detection and limit of quantification

Limit of detection (LOD) was lowest concentration of analyte in the sample that could be detected under the stated experimental condition and Limit of quantification (LOQ) the lowest concentration of the active ingredients in a sample that could be determined with accepted precision and accuracy. According to ICH recommendation, the approach based on the standard deviation (SD) of the response and slope (S) was used for determining the detection and quantification limits. LOD can be calculated according to formula LOD = 3.3 (SD/S) and LOQ = 10(SD/S). The signal to noise ratio was determined. The results were given in Table (1).

Accuracy and precision

The accuracy of the method was determined by applying the general procedure for determination over three concentrations levels covering the linearity range of each drug (20, 40, 60 ng/spot). The results in **Table (1)** indicated the accuracy of the proposed method.

Precision of the method, calculated as the percent of relative standard deviation (%RSD), was assessed by triplicate determination of three concentration levels covering the linearity range of each drug (20, 40, 60 ng/spot) within one day for repeatability and on three successive days for Inter mediate precision. The small values of %RSD indicated high precision of the method as shown in **Table (1)**.

Specificity

The specificity of the method was confirmed by perfect simultaneous determination of ASP and OPZ. TLC chromatograms revealed that ASP and OPZ were well clearly separated from each other confirming the selectivity and specificity of the method. Moreover the standard addition technique was applied to check the specificity of the described method. It was done by adding known quantities of ASP or OPZ in their pure forms to already analyzed pharmaceutical preparations and the percent recovery (%R) of the pure added concentrations was calculated. The data listed in Table (2) proved that the proposed method could selectively analyze the drugs without any interference from any excipients.

System suitability

To confirm that, the TLC densitometric system was working correctly during the analysis process, various parameters such as resolution factor (Rs), retention factor (k) and tailing factor (T) were evaluated. The results obtained, as shown in **Table (3)**, revealed that the described TLC densitometric conditions allowed complete base line separation between ASP and OPZ bands with minimum tailing.

Table 1: Regression and validation data for determination of ASP and OPZ by the proposed TLC densitometric method

Parameters		Proposed TLC densitometric method ASP	OPZ
Wavelength (nm)		240	240
Linearity range (ng/spot)		10 – 100	4 – 80
- Slope (b)		813.6444	299.7369
- Intercept (a)		-230.2932	145.5514
Coefficient of determination (r^2)		0.9998	0.9999
LOD (ng/spot)		1.686	0.961
LOQ (ng/spot)		5.110	2.912
Accuracy (%R)[a]		100.38	98.75
Precision (%RSD)[a]	Repeatability	0.781	0.543
	Intermediate precision	1.352	0.982

[a] **Values for 3 determinations of 3 different concentrations**

Table 2: Recovery study of ASP and OPZ by adopting standard addition technique via the proposed TLC densitometric method

Drug	Pharmaceutical taken(ng/spot)	Pharmaceutical found(ng/spot)	Pure added (ng/spot)	Pure found (ng/spot)	%Recovery
ASP	16.2	16.25[a]	20	20.33	101.65
			40	40.42	101.05
			60	59.82	99.70
Mean± %RSD					100.80± 0.999
OPZ	8	7.96[a]	20	19.67	98.35
			40	39.95	99.88
			60	58.98	98.30
Mean± %RSD					98.84±0.898

Table 3: System suitability testing parameters for the determination of ASP and OPZ by the proposed TLC densitometric method

Parameters	Obtained value ASP	OPZ
Retardation factor (Rf)	0.30	0.71
Retention factor (K)	9.00	1.70
Tailing factor (T)	0.834	0.810
Resolution (Rs)	5.6	

Application to pharmaceutical formulation

The simultaneous determination of ASP and OPZ in Yosprala® tablets was determined by the proposed method. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives as shown in Table (4).

Table 4: Determination of ASP and OPZ in YOSPRALA® tablets by the proposed TLC densitometric

Parameters	Proposed method ASP	OPZ
n[a]	5	5
Average (%Recovery)	100.43	99.87
SD	1.024	1.121
%RSD	1.221	1.342

[a] **Average of five determinations**

CONCLUSION

In this work, TLC- densitometry technique provides a simple, straightforward method for separating aspirin and omeprazole. The suggested method found to be accurate, selective and sensitive It could be applied for routine analysis of studied drugs in the pure form or in the pharmaceutical formulation..

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