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SIMULTANEOUS ESTIMATION OF CILOSTAZOL AND ASPIRIN IN SYNTHETIC MIXTURE USING HPTLC METHOD

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ABSTRACT

A high performance thin layer chromatography (HPTLC) method was developed and validated for quantitative determination of cilostazol and aspirin in synthetic mixture. The analysis was performed using a mobile phase composed of methanol : ethyl acetate : toluene : solvent ether (1:2:2:0.5). The calibration graphs were linear correlation coefficient (r) > 0.995 in the studied concentration range of 100 to 800 ng/mL for cilostazole and 75-600 ng/mL for aspirin for HPTLC. Relative standard deviation of all the parameters for intraday and interday precision is less than 2% (0.75-1.25) and the accuracy was greater than 98%. So the proposed method is precise and accurate and can be applied directly and easily to the cilostazol and aspirin in synthetic mixture.

Key words: Cilostazol, Aspirin, Simultaneous, HPTLC

INTRODUCTION

Cilostazol is the drug of the class phosphodiesterase-3 inhibitor. It is US FDA approved drug. Cilostazol is not official in any pharmacopoeia and no official method is available for cilostazol estimation. Literature survey revealed HPLC and LC/MS/MS methods for the estimation of cilostazol and its metabolites in biological fluids and pharmaceutical formulations. Aspirin is analgesic, antipyretic and anti-inflammatory. In IP'2003, USP-24 and BP'2005 aspirin is estimated by titrimetric and HPLC method.

Combination of cilostazol with aspirin is not available in market that's why estimation methods for this combination are not found. Cilostazol and aspirin are co-

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administered in patients with co-existent intermittent claudication and coronary artery disease. Therefore, in future, combination of these two drugs may come in market.

There are no simple Spectrophotometric, derivative spectrophotometric and HPTLC methods and estimation of cilostazol and aspirin in synthetic mixture is important and therefore, it is thought of interest to develop a simple, sensitive, accurate and precise method for their analysis is a mixture.

EXPERIMENTAL

Chemicals and materials

Analytical grade methanol was obtained from Finar Laboratories. Ethyl acetate, toluene and solvent ether obtained from Merck India Limited, Mumbai. TLC aluminum plate percoated with silica gel 60 F_{254} (10 cm ×10 cm) was obtained from E. Merck Limited, Mumbai.

Instrument

A Camag HPTLC system equipped with Linomat IV sample applicator was used. Camag TLC scanner 3 was used for scanning and integration software and CATS 4.02 (Switzerland) was used for interpretation of data.

Standard preparation:

Stock solution of 1000 μg/mL cilostazol and aspirin were prepared in 100 mL of methanol.

Chromatographic condition

The experiments were performed on a precoated silica gel G60 F_{254} HPTLC plates (10 cm×10 cm) using mobile phase comprising of ethyl acetate, toluene, methanol and ether (2 : 2 : 1 : 0.5). The plates were prewashed by methanol and activated at 110^0 C for 20 min prior to chromatography. Samples were applied as bands 6 mm long at 6 mm intervals, under a stream of nitrogen. Ascending development to a distance of 8 cm was performed in saturated 20 cm × 10 cm twin trough TLC development chamber (Camag) at room temperature. The plate was scanned and quantified at 257 nm using slit dimension of 4.0 × 0.45 mm.

Linearity of detector response

Varying concentrations of the drug (100 ng/mL to 900 ng/mL) were prepared form

the stock solution. The above concentrations were applied on the chromatographic plate. The plate was developed with mobile phase comprising of ethyl acetate, toluene, methanol and ether (2 : 2 : 1 : 0.5) in twin trough chamber, to a distance of 8 cm. After removal from chamber, the plate was scanned. From the result obtained, the peak area was calculated. A linear relationship was observed between the concentrations of peak in the range of 100 ng/mL to 900 ng/mL.

Sample preparation

Twenty tablets were weighed and average weight of each tablet was calculated. An accurately weighed amount of sample equivalent to 100 mg of drug was transferred in to a 100 mL volumetric flask and extract was filtered through Whatman filter paper and residue was washed with 10 mL of methanol. The extract and washing were pooled and transferred to 100 mL volumetric flask and volume was made up to 100 mL with methanol.

Recovery studies

To study the accuracy and precision of the method, recovery experiment was performed by the method of standard addition to determine, if there are positive or negative interferences from excipients present in the formulation. The recovery of the added standard was studied at three different levels, each being analyzed in a manner similar to as described for assay. Each set of additions was repeated five times and the recovery of added standard was calculated.

RESULTS AND DISCUSSION

Chromatogram

HPTLC method has been not reported so far for simultaneous determination of cilostazol and aspirin. Several mobile phases were tried to accomplish good separation of cilostazol and aspirin. Using the mobile phase methanol : ethyl acetate : toluene : ether (1 : 2 : 2 : 0.5 v/v) and 10 × 10 cm HPTLC silica gel G60 F_{254} aluminum plate, better separation was attained, where R_f values were found to be 0.24 for aspirin and 0.72 for cilostazol.

Validation of the proposed method

Linearity and range

Linear correlation was obtained between peak areas and concentrations of cilostazol and aspirin in concentration range of 200- 700 ng/spot and 75- 525 ng/ spot,

respectively. Characteristic parameters for regression equation and correlation were obtained. The linearity of the calibration curves was validated by the high value of correlation coefficients of the regression.

Table 1. Data of recovery study for cilostazol and aspirin by HPTLC method

Drug	Amount taken (ng/spot)	Amount added (ng/spot)	Amount found (ng/spot)	% Recovery ± S. D. (n = 3)
Cilostazol	100	25	125.34	100.27 ± 1.63
	100	50	149.69	99.79 ± 0.89
	100	100	198.61	99.30 ± 1.24
Aspirin	75	25	98.4	98.4 ± 1.87
	75	50	123.8	99.04 ± 0.51
	75	75	149.7	99.8 ± 1.12

Table 2. Method precision data for analysis of cilostazol and aspirin by HPTLC method

Cilostazol (200 ng/spot)	R_f value		Peak area	
Aspirin (150 ng/spot)	Aspirin	Cilostazol	Aspirin	Cilostazol
1	0.24	0.71	589	1628
2	0.24	0.71	579	1611
3	0.23	0.72	596	1597
4	0.24	0.71	573	1671
5	0.24	0.72	593	1649
Mean	0.248	0.726	586	1631.2
SD	0.0044	0.0055	9.695	29.533
RSD (%CV)	1.25	0.73	1.654	1.810

Slope: 5.8947, Intercept: 466.87, Correlation Co-efficient: 0.9996

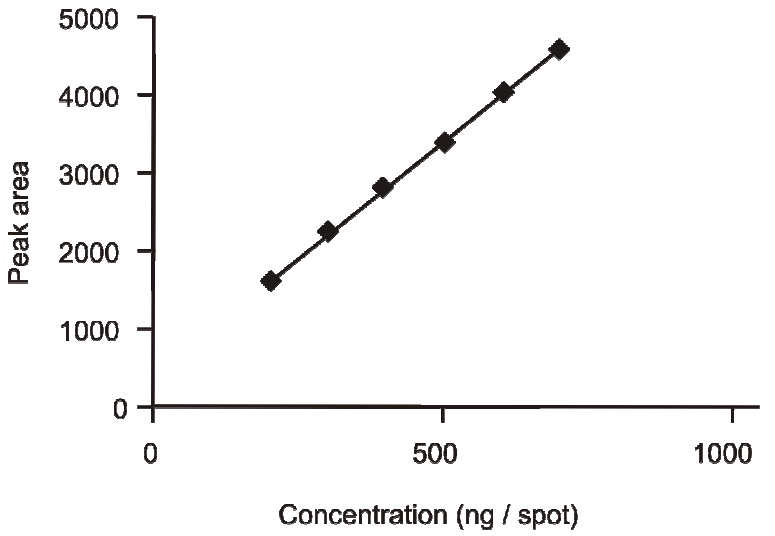

Fig. 1: Calibration curve of cilostazol

Slope: 2.1538, Intercept: 153.1, Correlation Co-efficient: 0.99561

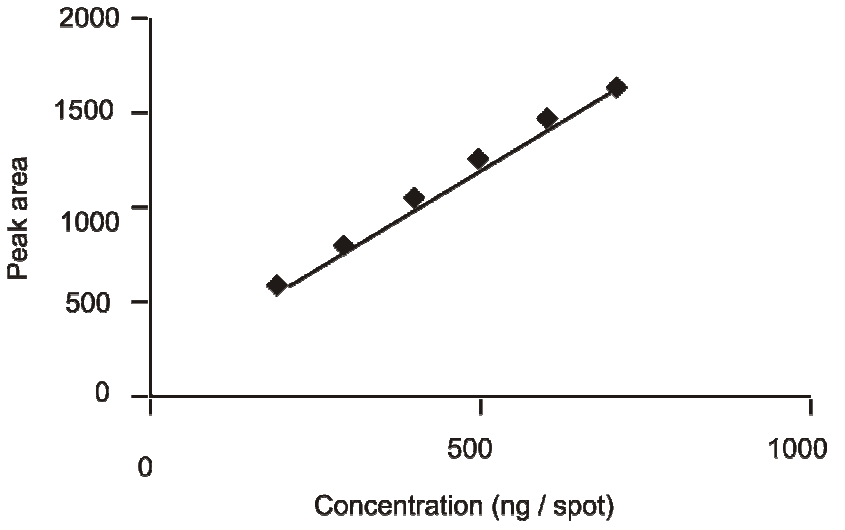

Fig. 2: Calibration curve of aspirin

Table 3. Intra-day data for analysis of cilostazol and aspirin by HPTLC method

Concentration		Intra-day precision			
		Cilostazol		Aspirin	
Cilostazol (ng/spot)	Aspirin (ng/spot)	Mean ± S.D (n = 3)	% C. V.	Mean ± S. D. (n = 3)	% C. V.
200	150	1630 ± 23.05	1.41	589 ± 9.68	1.64
300	225	2239 ± 24.99	1.12	762 ± 10.06	1.32
400	300	2850 ± 42.46	1.48	1034 ± 17.39	1.68
500	375	3456 ± 18.87	0.54	1257 ± 10.81	0.85
600	450	3989 ± 25.03	0.62	1470 ± 11.51	0.78
700	525	4580 ± 79.56	1.73	1633 ± 18.37	1.12

Table 4. Inter-day data for analysis of cilostazol and aspirin by HPTLC method

Concentration		Inter-day precision			
		Cilostazol		Aspirin	
Cilostazol (ng/spot)	Aspirin (ng/spot)	Mean ± S.D. (n = 3)	% C. V.	Mean ± S.D (n = 3)	% C. V.
200	150	1726 ± 36.71	1.41	553 ± 9.39	1.69
300	225	2267 ± 37.85	1.66	803 ± 12.61	1.57
400	300	2812 ± 16.90	0.60	1062 ± 10.35	0.97
500	375	3408 ± 17.88	0.52	1276 ± 15.86	1.24
600	450	4048 ± 24.13	0.59	1498 ± 11.06	0.73
700	525	4627 ± 42.56	0.92	1625 ± 24.91	1.53

Table 5. Application of proposed HPTLC method to the determination of synthetic mixture

Formulation	Drug	Amount taken (mg)	Amount found (mg)	% Amount found ± S. D. (n =3)
Synthetic mixture	Cilostazol	100	100.81	100.81 ± 1.12
	Aspirin	75	74.79	99.72 ± 0.91

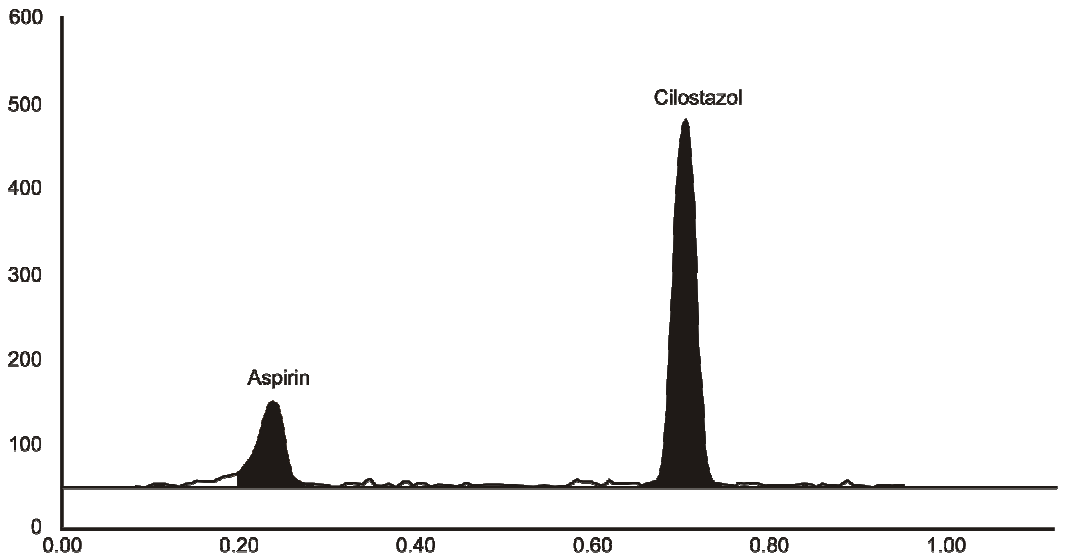

Fig. 3: Chromatogram of cilostazol and aspirin with corresponding R_f values at 240 nm

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