Int. J. Chem. Sci.: 6(1), 2008, 73-79

SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF CILOSTAZOL AND ASPIRIN IN SYNTHETIC MIXTURE

J. V. PATEL*, C. N. PATEL, I. S. ANAND, P. U. PATEL[a] and P. H. PRAJAPATI

Shri Sarvajanik Pharmacy College, MEHSANA (Guj.) INDIA.
[a]Shree S. K. Patel College of Pharma Education and Research, Ganpat Vidyanagar, Kherva, MEHSANA-382711 (Guj.) INDIA

ABSTRACT

Two simple, rapid, accurate and economical methods have been developed for the simultaneous estimation of aspirin and cilostazol in the synthetic mixture. Aspirin has absorbance maxima at 226 nm and cilostazol has absorbance maxima at 257 nm in methanol. The linearity was observed in the concentration range of 2-24 μg/mL for the both aspirin and cilostazol. First method is based on the simultaneous equations; Absorbances of both the drugs were determined at 226 nm (λ_{max} of aspirin) and at 257 nm (λ_{max} of cilostazol). The method was validated in terms of accuracy (99.30 ± 0.97, 101.58 ± 0.89). Second method is based on Q-absorbance ratio; absorbances of both the drugs were determined at 226 nm (λ_{max} of aspirin) and at isoabsorptive point (239.5 nm). Q-absorption ratio method was validated in terms of accuracy (98.05 ± 1.20, 99.12 ± 1.00). The proposed methods were found accurate, reproducible and economical for the routine analysis of both the drugs in the synthetic mixture.

Key words: Spectrophotometric, Cilostazol, Aspirin

INTRODUCTION

Cilostazol is chemically 6-[4-l(-cyclohexyl-lH-tetrazol-5-yl-butoxyl] 3-4-dihydro-2(1H) - quinolinone[1]. Cilostazol is the member of the phosphodiesterase inhibitor-3, approved by the US Food and Drug Administration (FDA) in 1999. It has been proved effective in significantly improving walking distances among patients with claudication[2-4]. Aspirin is chemically 2-(acetyloxy) benzoic acid; Acetyl salicylic acid; salicylic acid acetate[5]. Aspirin is official in IP[6], BP[7], USP[8] and in the extra pharmacopoeia[9].

A survey of literature revealed HPLC[10, 11] and LC/MS/MS[12] method for the determination of cilostazol in biological fluids and tablets. Cilostazol and aspirin are

* Author for correspondence; E-mail: jayesh_pharma@yahoo.com

co-administred in patients with co-existent 1C and coronary artery disease[13, 14] and therefore, in future combination of these two drugs comes in market.

In the present investigation an, attempt has been made to develop two simple, economical, accurate and reproducible spectrophotometric methods for the simultaneous estimation of cilostazol and aspirin in the synthetic mixture. First method is based on simultaneous equation method and second method is based on Q-absorbance ratio. The proposed methods were successfully applied for simultaneous estimation of cilostazol and aspirin in the synthetic mixture.

EXPERIMENTAL

Materials and methods

Shimadzu model 1601 double beam UV-visible spectrophotometer with a pair of 10 mm matched quartz cells was used to measure absorbance of the resulting solutions. Sartorius CP224S analytical balance, an ultrasonicater (Frontline FS 4). cilostazol (Cadila Pharma Ltd., Ahmedabad), aspirin and methanol, AR grade (Merck India limited, Mumbai) were used in the study.

Preparation of standard solutions and synthetic mixture

Standard cilostazol stock solution (100 µg/mL): Accurately weighed cilostazol (10 mg) was transferred in 100 mL volumetric flask, dissolved in methanol and diluted to the mark with methanol.

Standard aspirin stock solution (100 µg/mL): Accurately weighed Aspirin (10 mg) was transferred in 100 mL volumetric flask, dissolved in absolute alcohol and diluted to the mark with absolute alcohol.

Preparation of synthetic mixture of cilostazol and aspirin

The synthetic mixture of cilostazol and aspirin was prepared in the ratio of 1 : 0.7. Accurately weighed cilostazol (10 mg) and aspirin (7.5 mg) were transferred in 100 mL volumetric flask and dissolved in methanol (70 mL). Common excipients, which are used in the tablet formulation, were added in this mixture and sonicated for 20 minutes. This solution was filtered through the Whatman filter paper No. 41 and the residue was washed thoroughly with methanol. The filtrate and washings were combined and diluted to the mark with methanol to get solution having cilostazol (100 µg/mL) and aspirin (75 µg/mL).

Selection of wavelength for estimation of cilostazol and aspirin

The standard stock solutions of cilostazol and aspirin were scanned in the range of 200 nm to 400 nm against methanol as a blank. Maximum absorbance was obtained at 257 nm and 226 nm for cilostazol and aspirin, respectively. Isoabsorptive point was found at 239.5 nm (Fig. 1).

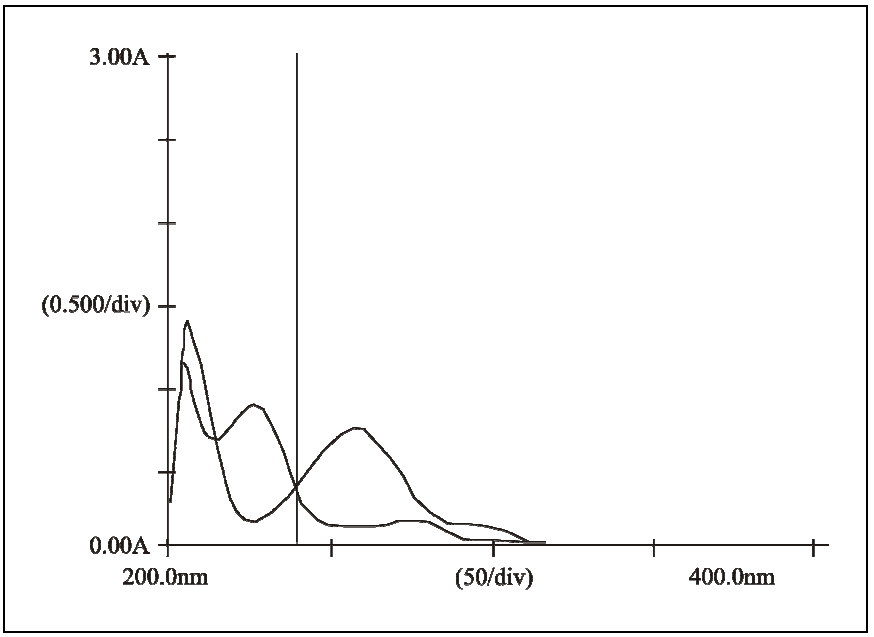

Fig. 1: Overlain spectra of cilostazol and aspirin showing isoabsorptive point at 239.5 nm

Calibration curve for cilostazol and aspirin

A calibration curve was plotted over a concentration range 4-24 μg/mL for both cilostazol and aspirin. Accurately measured standard stock solution of cilostazol (0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 and 2.4 mL) and standard stock solution of aspirin (0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 and 2.4 mL) were transferred into a series of 10 mL of volumetric flasks and diluted to the mark with methanol. Absorbance of each solution was measured at both the wavelength 257 nm and 226 nm. Calibration curves were constructed for cilostazol and aspirin by plotting absorbance versus concentrations at both wavelengths. Each reading was average of three determinations. In Q- absorbance ratio method, absorbance of each solution was measured at the three wavelengths 226 nm, 237 nm and 239.5 nm. Calibration curves were constructed for cilostazol and aspirin by

plotting absorbance versus concentrations at three wavelengths. Each reading was average of three determinations.

Accuracy

Accuracy was determined in term of percent recovery. The proposed method was applied to determine cilostazol and aspirin in the synthetic mixture. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the synthetic mixture with three different concentrations of standards.

Analysis of the synthetic mixture

The absorbance of final sample solution was measured against methanol as a blank at 226, 239.5 and 257 nm. The amount of cilostazol and aspirin were calculated using simultaneous equations as well as Q-absorbance ratio method.

RESULTS AND DISCUSSION

Table 1. Summary of validation parameters for simultaneous equation and Q-absorption ratio methods

Parameters	257 nm		226 nm		239.5 nm
	Aspirin	Cilostazol	Aspirin	Cilostazol	Cilostazol and aspirin
Beer's law limit (µg/mL)	4-24	4-24	4-24	4-24	4-24
Molar absorptivity (lit/mole/cm)	0.050×10^4	1.768×10^4	0.837×10^4	0.321×10^4	0.512×10^4 (Asprin) 1.049×10^4 (Cilostazol)
Sandel's sensitivity (µg/mL/cm^2/0.001)	0.3571	0.0208	0.0214	0.1149	0.03521
LOD (µg/mL)	0.378	0.486	1.206	1.359	0.899
LOQ (µg/mL)	1.126	2.181	3.928	2.915	2.548
Regression equation (y = mx + c)	y = 634.99 x = -0.0064	17557 +0.003	9206.2 –0.0278	3303.1 +0.0003	0.035 –0.0594
Correlation coefficient (r^2)	0.9954	0.9990	0.9994	0.9949	0.9925

Calibration curves for cilostazol and aspirin over concentration range of 4-24 μg/mL were plotted and molar absorptivity for both the compounds were calculated at three wavelengths 226 nm (λ_{max} of aspirin), 239.5 nm (isoabsorptive point) and 257 nm (λ_{max} of cilostazol). The linearity of the calibration graphs was validated by the high value of correlation coefficients of the regression (Table 1). LOD for cilostazol and aspirin were found to be 0.58 μg/mL and 0.378 μg/mL respectively while LOQ for cilostazol and aspirin were found to be 2.181 μg/mL and 2.08 μg/mL, respectively by both the methods. These data show that both the methods are sensitive in the determination of cilostazol and aspirin.

Accuracy

The percent recoveries obtained were 99.30 to 101.58 and 100.08 to 101.14 for cilostazol and aspirin, respectively by simultaneous equation method; 98.05 to 99.05 and 98.12 to 98.59 for cilostazol and aspirin, respectively by Q-absorbance ratio method (Table 2). The low value of SD indicates that both the methods are accurate.

Analysis of the synthetic mixture

Table 2. Data of recovery studies by simultaneous equation and Q-absorption ratio methods

Content	Amount taken (μg/mL)	Amount added (μg/mL)	% Recovery ± SD n = 5 by simultaneous equation method	% Recovery ± SD n = 5 by Q - absorption ratio method
Cilostazol	5	5	101.00 ± 1.14	98.05 ± 1.20
	5	10	99.30 ±0.97	98.57 ± 1.05
	5	15	101.58 ± 0.89	99.05 ± 0.90
Asprin	5	5	100.12 ± 0.99	98.59 ± 1.18
	5	10	100.08 ± 1.18	99.12 ± 1.00
	5	15	101.4 ± 1.21	98.14 ± 1.02

In the simultaneous equation method-concentration of cilostazol and aspirin in the synthetic mixture were found out by solving following equations.

$$C_c = \frac{A_2 a_{a1} - A_1 a_{a2}}{A_{c2} a_{a1} - a_{m1} a_{r2}} \quad \text{and} \quad C_c = \frac{A_1 a_{c2} - A_2 a_{c1}}{a_{c2} a_{a1} - a_{c1} a_{a2}}$$

Where; C_c, C_a = concentration of cilostazol and aspirin in the sample solution

A_1, A_2 = Absorbances of the sample solution at 226 nm and 257 nm, respectively

A_{c1} and a_{c2} = Molar absorptivities of cilostazol at 226 nm and 257 nm, respectively

A_{a1} and a_{a2} = Molar absorptivities of aspirin at 226 nm and 257 nm, respectively

In the Q- absorbance ratio method, concentration of cilostazol and aspirin in the sample solutions were calculated using equations $C_{c2}= (Q_o-Q_a/Q_c-Q_a) \times A_3/a_{c3}$ and $C_{p2} = A_3/a_{a3}-C_{c2}$, where A_1 and A_3 are absorbances of sample solution at 257 nm and 239.5 nm; and a_{c3} and a_{a3} are molar absorptivity of cilostazol and aspirin at 239.5 nm; aci and aai are molar absorptivity of cilostazol and aspirin at 257 nm. $Q_o=A_1/A_3$, $Q_c=a_{cl}/a_{c3}$ and $Q_a=a_{a1}/a_{a3}$.

The proposed validated methods were successfully applied to determine cilostazol and aspirin in the synthetic mixture. The % recoveries for cilostazol and aspirin obtained were 101.58 ± 0.89, 101.14 ± 1.21 by simultaneous equations method and 98.57 ± 1.05, 98.59 ± 1.18 by Q-absorption ratio method, respectively (Table 3). No interference of the cxcipients with the absorbance appeared; hence, the proposed methods were applicable for the quantitative determination of cilostazol and aspirin in synthetic mixture. The proposed methods are based on simultaneous equation and absorption ratio and only require measurement of absorbance at selected wavelengths. The proposed methods were found to be simple, rapid, economical, accurate and precise. They are particularly useful for routine in-process quality control and simultaneous quantification of cilostazol and aspirin in combined synthetic mixture.

Table 3. Analysis of the synthetic mixture by simultaneous equation and Q-absorption ratio methods

Content	% Amount found ± SD n = 5 by Simultaneous equation method	% Amount found ± SD n = 5 by Q-absorption ratio method
Cilostazol	100.48± 1.20	99.01 ± 1.58
Aspirin	99.39± 1.53	101.15 ± 1.63

REFERENCES

1. S. Budavari; Edn, in.; The Merck Index, 13th Ed., Merck & Co., Inc., Whitehouse

Station, NJ, (2001) p. 395.

2. H. G. Beebe, D. L. Dawson, B. S. Cutler, J. A. Herd, D. E. Strandness, E. B. Bortey, W. P. Forbes, A New Pharmacological Treatment for Intermittent Claudication Results of a Randomized, Multiceter Trial, Arch. Inter. Med., **159**, 2041 (1999).
3. S. R. Money, J. A. Herd, J. Isaacsoh, M. Devidson, B. Culter, J. Heckman and W. P. Forbes, Effect of Cilostazol on Walking Distance in Patients with Intermittent Claudication Caused by Peripheral Vascular Disease, J. Vase. Surg., **27**, 267 (1998).
4. D. L. Dawson, B. S. Culter, M. H. Meissner, Jr DE. Strandness Cilostazol has Beneficial Effects in Treatment of Intermittent Claudication Results from a Multicenter, Randomized Prospective, Double Blind Trial. Circulation, **98**, 678 (1998).
5. S. Budavari; Ed., in.; The Merck Index, 13th Ed., Merck & Co., Inc., Whitehouse Station, NJ, (2001) p. 856.
6. Indian Pharmacopoeia. The Controller of Publications on Behalf of Govt. of India, Ministry of Health and Family Welfare, New Delhi, 3rd Edition, Vol. 1, 69 (1996).
7. British Pharmacopoeia, HMSO Publications Center, Landon, International Edition, Vol. 1, 176 (2005).
8. The United States Pharmacopoeia, United States Pharmacopeia Convection, INC. Asian Edition, 161 (2000).
9. Martindale, The Extra Pharmacopoeia, The Royal Pharmaceutical Society, 31st Edition, 17
10. J. Pharma Biomed. Anal., **24**, 381 (2001)
11. J. Liq. Chromato. & Related Technol., Taylor & Francis, **27**, 2603 (2004)
12. S. L. Bramer, P. N. V. Tata., S. S. Vengurlekar and J. H. Brisson, J. Pharma. Biomed. Anal., **26**, 637 (2001).
13. S. Mallikaarjun, W. P. Foreb and S. L. Bramer, Interaction Potential and Tolerability of the Coadministration of Cilostazol and Aspirin, Clin. Pharmacokinetic, **37** (suppl-2), 87 (1999).
14. E. Susman. Platelet Function Response to Single and Multiple Platelet Inhibitors in PAD Patients: A Prospective Study, 14th Annual Meeting of the Society for Vascular Medicine and Biology (2003), Chicago.

Accepted : 08.08.2007