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Simultaneous estimation of Aspirin and Ticlopidine hydrochloride in combination by derivative spectroscopy

Syed Fazlur Rahman[1], Ritu Vivek Kimbahune[1]*, G.Mubeen[1], Shaik Mohammed Maaz[1], Prachi Kabra[2]

[1]Department of Quality Assurance, Al-Ameen College of Pharmacy, Hosur Road, Bangalore, 560027, Karnataka, (INDIA)

[2]Department of Quality Assurance, Nargund College of Pharmacy, Dattatreya Nagar, Bangalore, 560085, Karnataka, (INDIA)

E-mail : rituvivekk@gmail.com

ABSTRACT

Two methods for simultaneous estimation of Aspirin and Ticlopidine hydrochloride in combination have been developed using 0.1N hydrochloric acid as a solvent. The sample solution was scanned in the range 190-400nm at fast speed. The resulted spectrum was converted into first and second order derivative (using $\Delta\lambda$=16, scaling factor 100). In second order derivative spectroscopy Aspirin was measured at 303nm while Ticlopidine hydrochloride was determined at 288nm. In second method, amplitudes of first order derivative were measured at 279.2 and 288.2nm and the concentration of Ticlopidine and Aspirin were estimated by using simultaneous equation method. The linearity ranges for Aspirin and Ticlopidine hydrochloride were found to be 20-200µg/ml and 100-700µg/ml respectively at 279.2nm, 288.2nm for first order and 288nm, 303nm for second order derivative method. The accuracy of the methods was assessed by recovery studies was found to be 95.95% and 101.49% for second order derivative method and112.22% and 99.14% for simultaneous equation method for Aspirin and Ticlopidine hydrochloride respectively. These methods have been found to be simple, accurate and rapid; does not require preliminary separation and can therefore be used for routine analysis of both drugs in quality control laboratories.

KEYWORDS

Aspirin;
Ticlopidine hydrochloride;
Second order derivative;
Simultaneous equation.

INTRODUCTION

Aspirin (ASP) chemically is 2-(Acetoxy) benzoic acid, an antipyretic, analgesic, anti-inflammatory, anti-coagulant, antiplatelet and fibrinolytic agent, used for the relief of fever, aches and pains associated with many conditions[1]. This is also prescribed to prevent heart attack or a stroke as well as toprevent blood clots and for blood thinning. It is official in Indian Pharmacopoeia (IP)[2], British Pharmacopoeia (BP)[3], and United States Pharmacopoeia (USP)[4].

Ticlopidine hydrochloride (TCP) chemically is 5-(2-Chlorobenzyl)-4, 5, 6, 7-tetrahydrothieno[3, 2-c]pyridine hydrochloride, used for blood thinning and to reduce risk of stroke. It is official in British Pharmacopoeia[3] and Japanese Pharmacopoeia[5].

Ticlopidine hydrochloride in combination with Aspirin is prescribed to prevent blood clots and also in

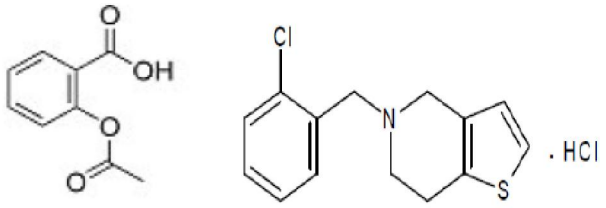

Figure 1 : Chemical structure of Aspirin and Ticlopidine hydrochloride

other conditions such as blood circulation problems in leg or heart disease[1].

Literature survey revealed that few spectrophotometric methods[6, 7] and many RPHPLC, SIHPLC[8, 9] HPLC- MS[10, 11], methods have been reported for Aspirin and Ticlopidine hydrochloride as individual or in combination with other drugs like Esomeprazole[12], Prasugrel[13], Amlodipine besylateandAtenolol[14], Metoprolol[15], Methocarbanol[16], Rosuvastatin[17], Dipyridamole[18], Clopidrogel Bisulphate and Atorvastatin calcium[19], Salicylsalicyclic acid, Salicylic acid in human plasma and urine[20], paracetamol in pharmaceutical dosage forms as well as in biological fluids. Spectrophotometric methods like first order derivative, area under curve, ratio derivative spectroscopyhave been reported for simultaneous estimation of Aspirin and Ticlopidinehydrochloride using methanol as solvent[21]. The present manuscript describes simple, accurate, precise, rapid and economic spectrophotometric methods for simultaneous estimation of ASP and TCP in mixture using 0.1N HCl as a solvent.

MATERIALS AND METHODS

Chemicals and reagents

ASP and TCP bulk powder were kindly gifted by Alta Laboratories Limited, Raigad and Aarti Drugs Limited, Sion, Mumbairespectively. Ticlid tablets (Taj Pharmaceuticals) were procured from local pharmacy. Calibrated glass wares and 0.1 N HClwere used throughout the work.

Apparatus

A shimadzu model 1700 (Japan) double beam UV/ Visible spectrophotometer with 1 cm matched quartz cells connected to a computer running UV-Probe 2.32 software for absorbance measurement and treatment of data was used along with Acculab digital balance for weighing.

Preparation of standard stock solutions

An accurately weighed quantity of ASP (100mg) and TCP (100mg) were transferred to a separate 100ml volumetric flasks and dissolved and diluted to the mark with 0.1N HCl to obtain standard solution having concentration of ASP (1000µg/ml) and TCP (1000µg/ml). The ASP stock solution was further diluted to get concentration 200µg/ml.

Method I: Second order derivative method

The standard solutions of ASP and TCP were scanned separately in the UV region of 190-400 nm to determine λmax of both drugs. Thewavelengths of absorption of ASP and TCP were found be 227nm (λmax), 276nm and 232nm (λmax), 268nm, 275nm, respectively. The serial dilutions of stock solutions having concentration 20 to200µg/ml of ASP and 100 to 800µg/ml of TCP were prepared in 0.1N HCl. All these solutions were scanned in the UV region of 190-400 nm at fast speed and the obtained spectra were transformed to second order derivative (D^2) using 16nm as wavelength difference ($\Delta\lambda$=16) and 100 as scaling factor. The amplitudes of TCP (D^2) were measured at 288nm (zero crossing of ASP) while the amplitudes (D^2) at 303nm were used for measuring ASP (zero crossing of TCP). The amplitudes (D^2) of the respective spectra were plotted against concentrations. The concentrations of ASP and TCP in sample solution were determined by solving the respective equations generated by calibration curves at 208 nm and 303 nm.

C_{TCP}= 0.0009/ Amplitude (D^2) at 288 nm

C_{ASP}=0.0011/ Amplitude (D^2) at 303 nm

Method II: Simultaneous equation method

The zero order spectra obtained from serial dilutions of ASP and TCP were transformed to first order derivative (D^1) using 16nm as wavelength difference ($\Delta\lambda$=16) and 100 as scaling factor. The amplitudes (D^1) of TCP and ASP were measured at 279.2 nm and 288.2 nm and plotted against concentrations. The calibration curves were obtained for both ASP and TCP at both wavelengths. The concentrations of ASP and TCP in sample solution were determined by solving following simultaneous equations

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$$C_x = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2}$$

$$C_y = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2}$$

Where, C_X - Concentration of TCP; C_y -concentration of ASP; A_1, A_2 - Amplitudes (D^1) of the mixture 279.2 nm and 288.2nm respectively; ax_1, ax_2– Slopes (10^4) of calibration curves of Xat 279.2nm and 288.2nm respectively; ay_1, ay_2 – Slopes (10^4) of calibration curves of Y at 279.2nm and 288.2nm respectively

Validation of the proposed methods

The proposed methods were validated according to the International Conference on Harmonisation (ICH) guidelines Q2(R1)[22].

Precision

The intraday precision of the proposed methods was determined by analysing the three sets of samples three times on the same day. Reproducibility of the methods were checked by performing the assay three times by two different analysts.

Accuracy (Recovery Study)

The accuracy of the method was determined by calculating recovery of ASP and TCP by the standard addition method. Known amounts of standard solutions of ASP and TCP were added at 50, 100 and 150% level to pre-quantified sample solutions of ASP and TCP. The amounts of ASP and TCP were estimated by both methods and recovery of standard drugs was calculated by subtracting the assay value of sample from spiked sample value. The experiment was repeated for three times for both methods.

Linearity and Range

The linearity for the developed methods were checked by determining correlation coefficient (R^2) of calibration curves of ASP and TCP over a concentration range of 20-200µg/ml and 100-800µg/ml respectively for first and second order derivative amplitudes.

The range was determined by considering absorbance at selected wavelengths, Beer's law limitation and instrument conditions.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by using the following equations

LOD =3.3× σ/S, LOQ = 10× σ/S;where, σ = the standard deviation of the intercept of calibration curve and S =slope of the calibration curve.

Analysis of ASP and TCP in combined form (Powder)

Accurately weighed 100mg of ASP and 250mg of TCP were mixed proportionately (in 1: 2.5 ratio) and triturated to fine powder. Accurately weighed 35mg of the mixture was transferred in 100 ml volumetric flask, dissolved in few ml of 0.1N HCl by sonicating for 15min and volume was made to the mark. This solution was filtered using whatman filter paper No.41 and the first 15ml of filtrate was discarded. The filtrated was mixed thoroughly and 5ml of the filtrate was further diluted to 50ml with 0.1N HCl. This solution was scanned in region of 190-400nm at fast speed using 0.1N HCl as blank. The obtained spectrum was transformed to first order and second order using 16 nm as wavelength difference ($\Delta\lambda$=16) and 100 as scaling factor. The amplitudes (D^1 and D^2) were measured at respective wavelengths and the concentrations of the two drugs were estimated using equations mentioned in method I and II.

The analysis procedure was repeated for six times and results are depicted in table.

RESULTS AND DISCUSSION

The stock solution of ASP and TCP were prepared in water and further it was diluted with different solvents like water, methanol, 0.1 N HCl and 0.1 N NaOH. The satisfactory results were obtained in 0.1 N HCl; hence it was selected as solvent for development of simultaneous estimation of ASP and TCP by UV-Visible spectrophotometry.

The solutions of ASP and TCP in 0.1 N HCl was stored for three days to check the stability of the solutions. The TCP solution was found to be stable for three days while absorbance of ASP was started decreasing and it was found that ASP solution was stable for about 1hr.

The overlay spectra of ASP and TCP were studied carefully (Figure 2). The wavelengths of absorption of

ASP and TCP were found be 227nm (λmax), 276nm and 232nm (λmax), 268nm, 275nm, respectively.

The zero order spectra of ASP and TCP were transformed to first and second order derivative using various wavelengths differences like 2nm, 4nm, 8nm and 16nm to get accurate and reproducible zero crossing for both drugs. The wavelength difference of 16nm and 100 scaling factor were showed satisfactory results.

The second order derivative spectra of TCP showed zero amplitude at 303 nm and ASP was found to be of zero amplitude at 288nm (Figure 3). Hence calibration curves for TCP and ASP were constructed by measuring amplitudes (D^2) of second order derivative at 288nm and 303nm respectively as shown in Figure 4 and 5. The linear regression equations obtained from these curves were used for calculation of ASP and TCP in the sample solution.

The overlay spectra of first order derivative of ASP and TCP were found to satisfy the criteria for simultaneous equation method (Figure 6). Hence the calibra-

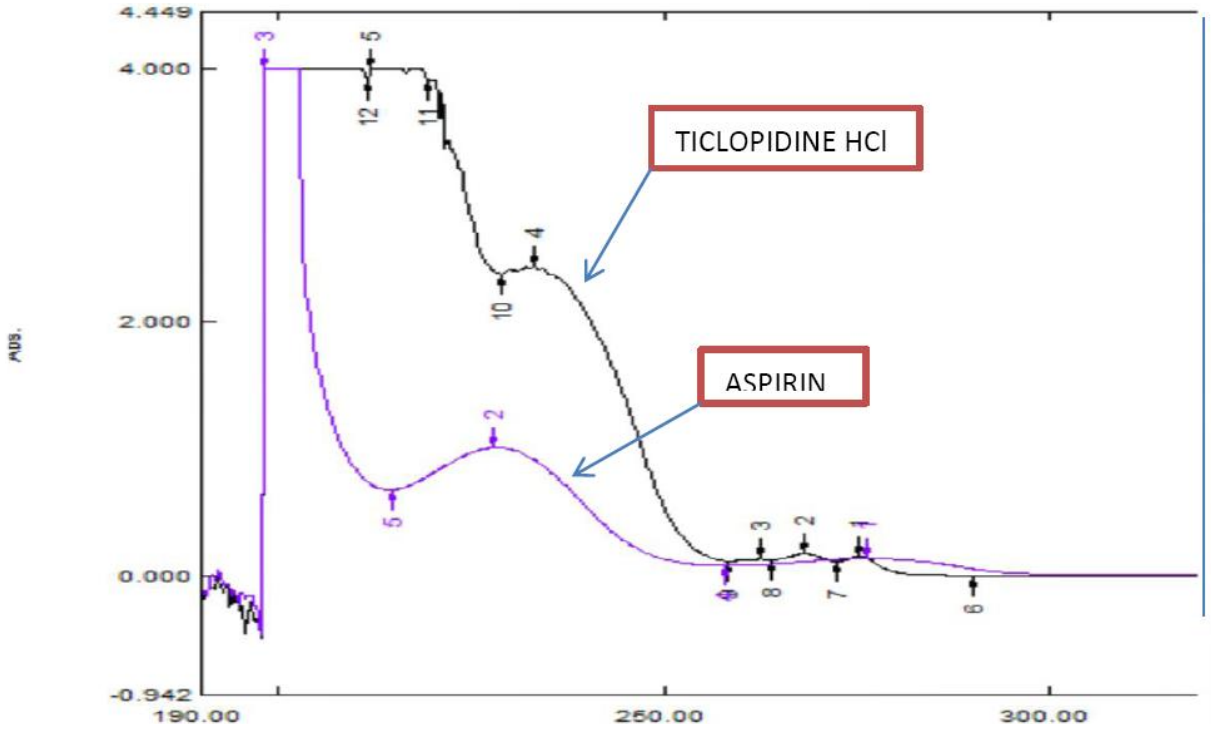

Figure 2 : Overlain spectra of Aspirin and Ticlopidine HCl

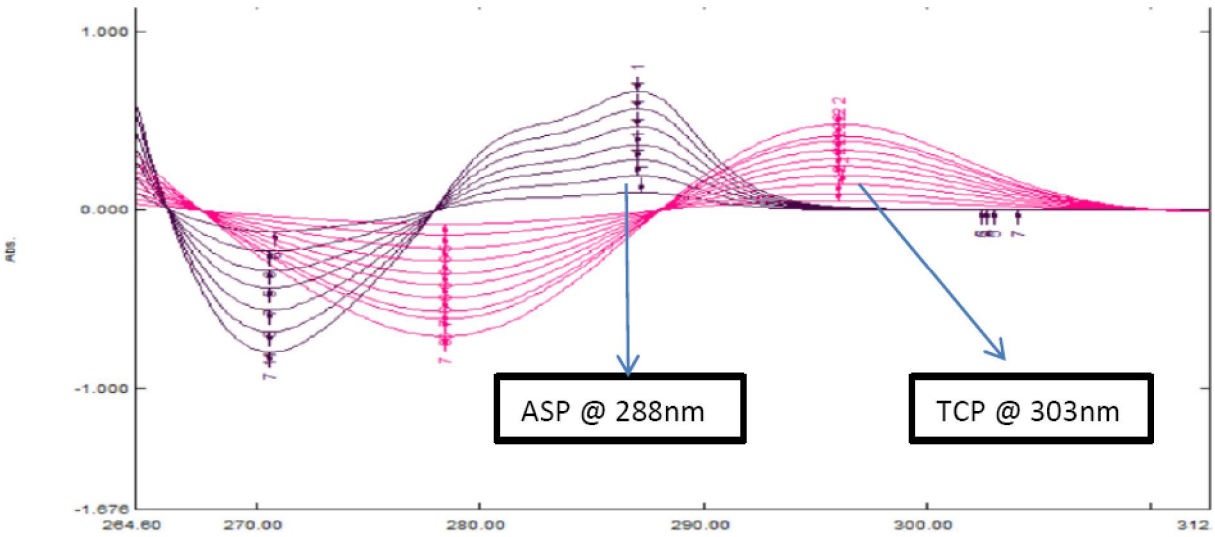

Figure 3 : Second order overlain spectra of Aspirin and Ticlopidine HCl

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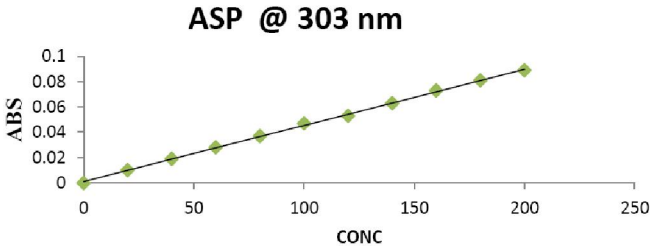

Figure 4 : Aspirin calibration curve

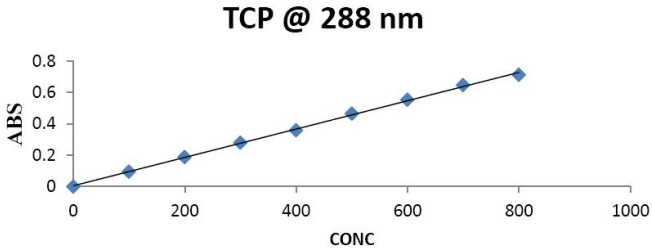

Figure 5 : Ticlopidine HCl calibration curve

tion curves of ASP and TCP were prepared by using amplitude of first order derivative at 279.2 nm and 288.2 nm. The concentration of ASP and TCP was determined using simultaneous equations mentioned in method II. The values for ax_1, ax_2, ay_1 and ay_2used were slopes of regression equation obtained from first order derivative of ASP and TCP. The calibration curves for ASP and TCP are as shown in Figure 8 and 9.

To simplify the calculation, slopes were calculated for per cent solution by multiplying with 10^4. The obtained values, 76 and 36 were used as $ax_1$and ax_2 while 95 and 241were used as $ay_1$and ay_2 values respectively. The results of assay of tablets are given in TABLE 1.

The validation of developed method had been carried out for accuracy, precision, linearity, and range. The accuracy was determined by recovery method and it was found 112.2 % and 95.95 % for ASP and 99.14 % and 101.49 % for TCP for method I and II respectively as given in TABLE 2

TABLE 1 : Results of analysis of tablets

Drugs	Second order derivative method (I) (n=6)	Simultaneous equation method (II) (n=6)
ASP	95.77%	90.69%
TCP	98.25%	95.55%

Precision was determined by intra-day and reproducibility studies. The RSD of these studies was found to be less than 3% for TCP and found to be about 6 % for ASP (TABLE 3). As discussed earlier, ASP solution was stable for about hour, the % RSD for ASP

TABLE 2 : Results of the recovery studies

Level of recovery (%)	Second order derivative method (I)		Simultaneous equation method (II)	
	ASP	**TCP**	**ASP**	**TCP**
50	90.90	100.71	108.99	99.03
100	99.99	103.69	116.85	100.45
150	96.96	99.75	111.80	97.95
Mean	95.95	101.49	112.22	99.14
%RSD	3700	1.517	3.337	2.699

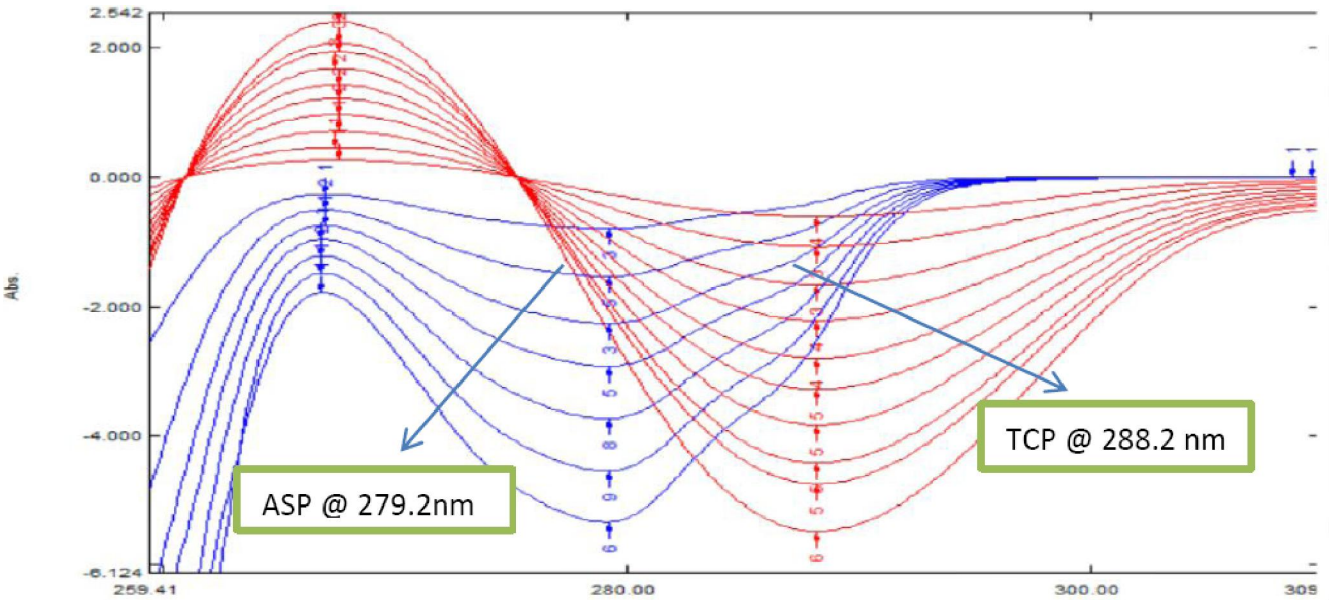

Figure 6 : First order overlain spectra of Aspirin and Ticlopidine HCl

TABLE 3 : Results for precision studies

Study	ASP			TCP		
	Variability	Mean (n=3)	RSD	Variability	Mean (n=3)	RSD
Interday	0 Hr	0.325	6.093	0 Hr	0.383	4.884
	3 Hr	0.300		3 Hr	0.365	
	6 Hr	0.285		6 Hr	0.345	
Robustness	Analyst 1	0.323	1.215	Analyst 1	0.381	2.465
	Analyst 2	0.324		Analyst 2	0.386	

was found to be high. Hence inter-day studies were not performed. The reproducibility was analysed by performing the assay of same sample by different analysts and evaluated on the basis of F and t- test. F-test values were calculated as 1.591 and 1.267 for ASP and TCP respectively at the degree of freedom 2, 2 and it was found that there is no significant difference between the precision of the results produced by two analysts at 10% probability (Standard value is 9.00 at 10 % probability). The t-test values for ASP and TCP were found as 4.97 and 0.195. There is no significant difference among results of TCP but significant difference was found among the results of ASP at degree of freedom 4 and 10% probability (1.533). This may be due to the degradation of ASP while waiting for analysis more than one hour.

Both the methods were found to be linear in the range 20-200µg/ml and100-800µg/ml for ASP and TCP respectively. The LOD and LOQ were found to 0.373µg/mland 1.134µg/ml for ASP and 0.760µg/ml and 2.303µg/ml for TCP. (TABLE 4)

The developed method was found to fast and simple for simultaneous estimation of ASP and TCP in combination.

TABLE 4 : Regression analysis data and summary of validation parameter of the calibration curves

Parameters	First Order Derivative		Second Order Derivative	
	ASP	TCP	ASP	TCP
Wavelength (nm)	279.2	288.2	303	288
Beers Range (µg/ml)	20-200	100-800	20-200	100-800
Regression equation (y= mx)	0.0072	0.036	0.0011	0.0009
Correlation coefficient (R^2)	0.9993	0.9987	0.9981	0.9991
LOD (µg/ml)	0.3743	0.7606	0.0471	0.3667
LOQ (µg/ml)	1.1343	2.3030	1.4285	1.111

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