

SPECTROPHOTOMETRIC SIMULTANEOUS DETERMINATION OF ASPIRIN AND TICLOPIDINE IN COMBINED TABLET DOSAGE FORM BY FIRST ORDER DERIVATIVE SPECTROSCOPY, AREA UNDER CURVE (AUC) AND RATIO DERIVATIVE SPECTROPHOTOMETRIC METHODS.

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ABSTRACT

A simple, rapid, economical, precise and accurate method for simultaneous determination of Aspirin (ASP) and Ticlopidine (TIC) in combined dosage form has been developed. The first method was First Order Derivative Spectroscopy (Method A) in which derivative amplitudes were measured at selected wavelengths. Second method was Area Under Curve (Method B) and third method was Ratio Derivative spectroscopy (Method C). Methanol was used as solvent for all the three methods. The amplitudes at 232.98 nm and 239.50 nm in the first order derivative spectra were selected to determine ASP and TIC, respectively. The wavelength ranges 234.15-238.88 nm and 215.30-219.50 nm were selected to determine ASP and TIC by AUC method. Amplitude at 224.61nm and 234.50 nm were selected in the ratio derivative spectra to determine ASP and TIC, respectively. Beer's law was obeyed in the concentration ranges of 2-10 μ g mL⁻¹ and 5-25 μ g mL⁻¹ ASP and TIC, respectively in all three methods. The % assay for commercial formulation was found to be in the range 99.12% – 101.21 % for ASP and 98.92 – 100.20 % for TIC by the proposed methods. Recovery was found in the range of 98.74 – 101.24 % for ASP and TIC in the Formulations. The results of analysis have been validated statistically and recovery studies confirmed the accuracy and reproducibility of the proposed methods which were carried out by following ICH guidelines.

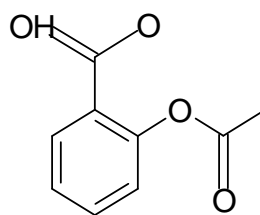
Keywords: Aspirin, Ticlopidine, First order derivative spectroscopy, Area under curve, Ratio derivative spectroscopy.

INTRODUCTION

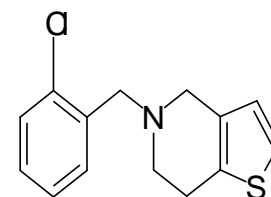
Aspirin is also known as acetylsalicylic acid is a salicylate drug, often used as an analgesic, antipyretic, anti-inflammatory and also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecule together to create a patch over damage of the walls within blood vessels. Chemically it is 2-acetoxybenzoic acid and is a nonsteroidal anti-inflammatory drug (NSAIDs) and shows inhibition of the enzyme cyclooxygenase and it is official in Indian Pharmacopoeia, The United States Pharmacopoeia and British Pharmacopoeia¹⁻⁴. Ticlopidine is an antiplatelet drug of the thienopyridine class. Chemically it is 5-(2-chlorobenzyl) 4,5,6,7-tetrahydrothieno [3,2-c] pyridine and it is official in British pharmacopoeia^{3, 4}. It is an adenosine diphosphate (ADP) receptor inhibitor, inhibits platelet aggregation by altering the function of platelet membranes thus prolongs bleeding time. It decreases incidence of stroke in high risk patients.

Literature survey revealed that there are various methods have been reported for estimation of ASP such as UV spectrophotometry, HPTLC, GC, Fluorimetry individually and in combined dosage form with other drugs⁵⁻¹⁰. For TIC various analytical methods have been reported for its individual estimation includes UV spectrophotometry, HPLC, IR spectroscopy¹¹⁻¹⁴. Literature survey also reveals that there is no spectrophotometric method available for the determination of these analytes in combination; therefore the aim of the study was to develop simple, rapid, accurate, reproducible and economic derivative, AUC and

Ratio derivative spectrophotometric methods for simultaneous estimation of ASP and TIC from its formulation. The proposed methods were validated as per the International Conference on Harmonization (ICH) analytical method validation guidelines¹⁵.



ASPIRIN



TICLOPIDINE

MATERIALS AND METHODS

Instrumentation

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells was used. All weighing were done on electronic balance (Model Shimadzu AUW-220D), Ultrasonicator model 5.5L150H.

Reagents and chemicals

Spectroscopic grade Methanol was purchased from Merck Specialties Private Ltd. Mumbai. Tablet used for analysis were provided by JCPL Pharma, Jalgaon (B. No. T 01) containing ASP 100 mg and TIC 250 mg per tablet. ASP and TIC are available in the ratio of 2:5, respectively in

formulation and were used in same ratio for preparation of calibration curves. Pharmaceutical grade of ASP (% purity 99.96) and TIC (% purity 99.84) were kindly supplied by Vapi Care Pharma Pvt. Ltd., Vapi Gujarat and were used without further purification.

Preparation of Standard Stock Solutions and calibration Curve

Standard stock solution of pure drug containing 1000 $\mu\text{g mL}^{-1}$ of ASP and 1000 $\mu\text{g mL}^{-1}$ of TIC were prepared separately in the methanol. The working standard solutions of these drugs were obtained by dilution of the respective stock solution in the distilled water. Series of solutions with conc. 2-10 $\mu\text{g mL}^{-1}$ and 5 – 25 $\mu\text{g mL}^{-1}$ of ASP and TIC respectively were used to prepare calibration curve. Solutions were scanned and proposed methods were applied.

Preparation of Sample Stock Solution and Formulation analysis

A quantity of powder from twenty tablets equivalent to 100 mg of ASP (250 mg of TIC) was weighed and transferred to 25 ml flask containing 20 ml of methanol and ultrasonicated for 5 min and solution was filtered through

Whatman paper No. 41 into a 100 mL volumetric flask. Filter paper was washed with same solvent, adding washings to the volumetric flask and volume was made up to the mark with the solvent. The solution was suitably diluted with distilled water to have (6 $\mu\text{g mL}^{-1}$ and 15 $\mu\text{g mL}^{-1}$ of ASP and TIC).

Theoretical Aspects

Method A: First Order Derivative Spectroscopy

To determine derivative amplitude for ASP and TIC, solutions of decreasing and increasing concentrations of ASP and TIC were prepared in combination and scanned in the range 200 - 300 nm at 0.2 band widths and 300 nm/min scan speed. These spectrums were converted to first order derivative spectra by using instrument mode with filter size 9 and interval 1.2. After observing the derivative amplitudes of first order derivative spectra (Fig-1), it was observed that there is proportionate increase in amplitude at 239.50 nm with increase in concentration of TIC. On similar basis, it was observed that there is proportionate increase in amplitude at 232.98 nm with increase in concentration of ASP. Therefore λ_{max} 232.98nm and λ_{max} 239.50 nm were assigned to ASP and TIC, respectively.

Figure 1: First Order Derivative Spectra of Aspirin and Ticlopidine.

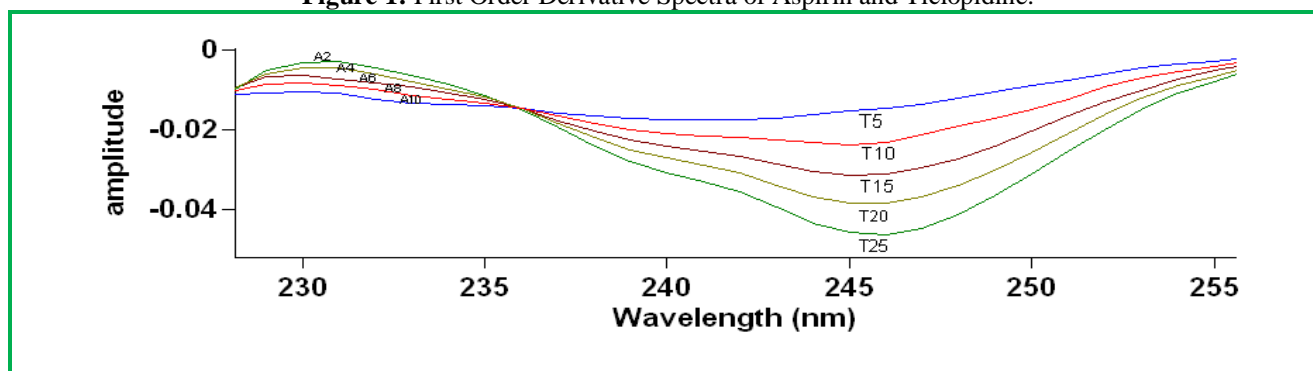


Table 1: Optical characteristics of the methods and results of precision and formulation analysis by the proposed methods

Parameter	ASPIRIN			TICLOPIDINE		
	Method A	Method B	Method C	Method A	Method B	Method C
λ (nm)	232.98	234.15-238.88	224.61	239.50	215.30-219.50	234.50
Beer's law limit ($\mu\text{g mL}^{-1}$)	2-10	2-10	2-10	5-25	5-25	5-25
Regression Equation ($y = mx + c$)	Slope (m)	0.0008	----	0.0004	0.0005	----
	Intercept (c)	0.0043	----	0.00002	0.0142	----
Correlation coefficient	0.996	----	0.999	0.999	----	0.999
Precision (%R.S.D.)	Repeatability (n=5)	0.62	0.88	1.06	1.22	0.92
	Intra-day (3×5 times)	0.78	0.54	0.73	1.09	0.55
	Inter-day (3×5 days)	0.97	0.69	1.20	1.06	0.96
	Analyst	1.05	0.92	0.45	1.01	0.37
Tablet Analysis % Assay, %RSD) n=6 Tablet	99.12, 0.52	99.83, 0.83	101.21, 1.08	100.20, 0.64	99.91, 1.02	98.92, 0.49

Figure 2: Overlay spectrum of ASP and TIC in Methanol. ASP (2-10µg mL⁻¹) and TIC (5-25 µg mL⁻¹) for AUC

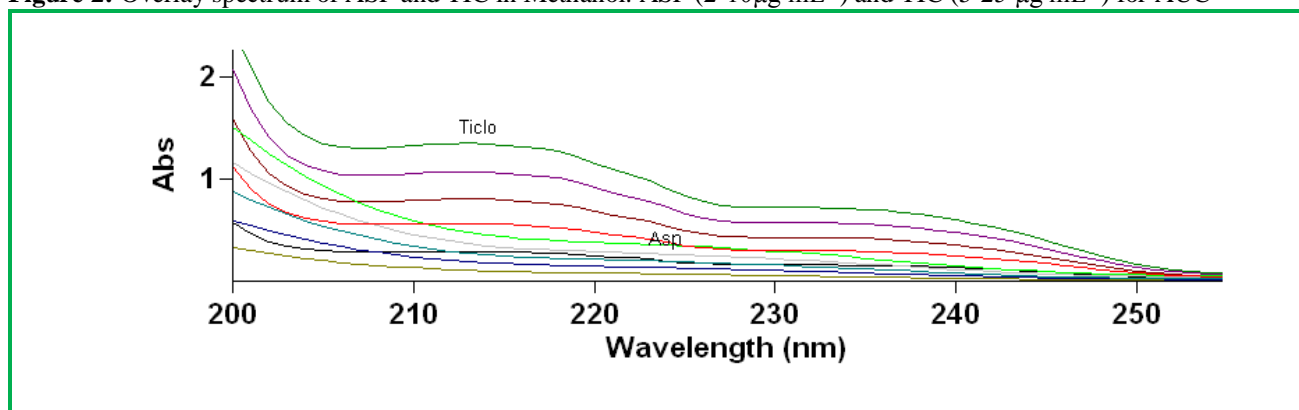


Table 2: Result of Recovery studies by the proposed methods

Formulation Used	Recovery Level	Recovery of	% Mean Recovery, % RSD by using		
			Method A	Method B	Method C
Tablet	50%	Aspirin	99.96, 0.82	100.89, 0.76	99.69,0.78
		Ticlopidine	100.12, 1.07	99.42, 0.58	98.74,0.56
	100%	Aspirin	100.84, 0.64	99.02, 1.22	100.92,1.08
		Ticlopidine	98.40, 0.65	100, 0.47	100.47,0.57
	150%	Aspirin	100.07, 0.36	99.67, 0.54	99.24,0.92
		Ticlopidine	100.96, 0.93	101.09, 1.26	101.24,0.34

Method B: Area under Curve

For the simultaneous determination using the area under curve (AUC) method, suitable dilutions of the standard stock solutions (1000 µg/mL) of ASP and TIC were prepared separately in methanol. The solutions of drugs were scanned in the range of 200-300 nm. For Area Under Curve method, calibration curve was plotted and the sampling wavelength ranges selected for estimation of ASP and TIC are 234.15-238.88 nm (λ1-λ2) and 215.30-219.50 nm (λ3-λ4) and area were integrated between these selected wavelength ranges for both drugs (Fig 2), which showed linear response with increasing concentration hence the same wavelength range were used for estimation of tablet formulations. By using integrated areas two simultaneous equations were constructed and solved to determine concentrations of analytes. Concentration of two drugs in mixed standard and the sample solution were calculated using equation (1) and (2).

$$C_{ASP} = A_2 \times a_{ay1} - A_1 \times a_{y2} / a_{x2} \times a_{y1} - a_{x1} \times a_{y2} \dots\dots (1)$$

$$C_{TICLO} = A_2 - a_{x2} \times C_{ASP} / a_{y2} \dots\dots\dots (2)$$

Where,

a_{x1} (885.30) and a_{x2} (1617.83) are absorptivities of ASP at (λ1-λ2) and (λ3-λ4) respectively.

a_{y1} (1737.26) and a_{y2} (2129.13) are absorptivities of TIC at (λ1-λ2) and (λ3-λ4) respectively.

A1 and A2 are Absorbances of mixed standard at (λ1-λ2) and (λ3-λ4) respectively.

C_{ASP} and C_{TIC} are the concentrations in g /100 mL¹⁶.

Method C: Ratio Derivative Spectroscopy

The method involves dividing the spectrum of mixture by the standardized spectra of each of the analyte to get ratio spectra and first derivative of ratio spectrum was obtained, which was independent of concentration of divisor. The concentration of active compounds are then determined from calibration graph obtained by measuring amplitude at points corresponding to minima or maxima. Using appropriate dilutions of standard stock solution, the two solutions were scanned separately. The ratio derivative spectra of different ASP standards at increasing concentrations were obtained by dividing ASP+TIC scans with the stored spectrum of the standard solution of TIC (15µg mL⁻¹) (Figure 3). Wavelength 224.61 nm was selected for the quantification of ASP in ASP+TIC mixture. The ratio and ratio derivative spectra of the solutions of TIC at different concentrations were obtained by dividing ASP+TIC scans with the stored standard spectrum of the ASP (6 µg mL⁻¹) (Figure 3b). Wavelength 234.50 nm was selected for the quantification of TIC in ASP+TIC mixture. Measured analytical signals at the selected wavelengths were proportional to the concentrations of the drugs. Calibration curves were prepared from the measured signals at the selected wavelength and concentration of the standard solutions. The amount of ASP (C_{ASP}) and TIC (C_{TIC}) in tablets was calculated by using following equations-

At 224.61nm:

$$C_{ASP} = (\text{Ratio derivative amplitude for ASP} - (-.00002)) / .00040 \dots\dots (3)$$

At 234.50nm:

$$C_{TICLO} = (\text{Ratio derivative amplitude for TIC} - (-0.0057)) / 0.2644 \dots\dots(4)$$

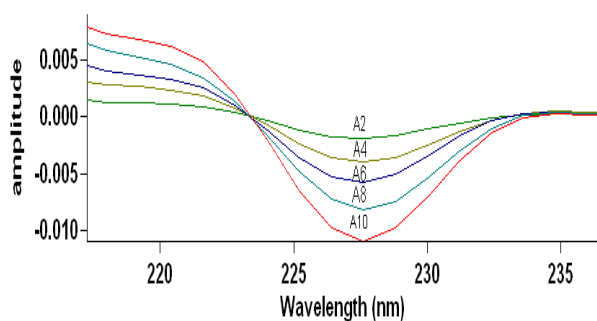


Figure 3a: First Order Ratio Derivative Spectra of Aspirin Using Ticlopidine ($15\mu\text{g mL}^{-1}$) as divisor

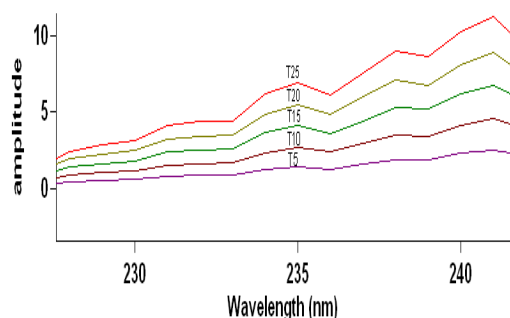


Figure 3b: First Order Ratio Derivative Spectra of Ticlopidine using aspirin ($6\mu\text{g mL}^{-1}$) as divisor

Recovery Studies

The accuracy of the proposed methods was checked by recovery study, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (50 %, 100 % and 150 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was $3\mu\text{g mL}^{-1}$ of ASP and $7.5\mu\text{g mL}^{-1}$ of TIC for all three methods.

Precision of the Method

Precision of the method was determined by repeating assay six times. To study intraday precision, method was repeated 5 times in a day and the average % RSD was calculated. Similarly the method was repeated on five different days and average % RSD was calculated

RESULTS AND DISCUSSION

The proposed methods for simultaneous estimation of ASP and TIC in combined dosage form were found to be accurate, simple and rapid. Since not a single method was reported for simultaneous analysis of the two drugs earlier, the developed methods can be used for routine analysis of two drugs in combined dosage forms. Area under curve method involves formation and solving of simultaneous equation. Once the equations are formed, then only measurement of the area of sample solution at two wavelength ranges and simple calculations are required. Practically no interference from tablet excipients was observed in these methods. The values of % RSD and

correlation of coefficient for First order Derivative spectra (Tablet) were found to be (% RSD 0.49- 1.08) and correlation coefficient was 0.999 for ASP and TIC. The result of recovery studies for tablet was found to be in the range of 98.40 -100.96% for method A, 99.02-100.89 for method B and 98.74- 101.24 for method C. It indicates that there is no interference due to excipients present in the formulation. It can be easily and conveniently adopted for routine quality control analysis. All three methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic and are validated as per ICH guidelines.

CONCLUSION

The proposed methods are simple, precise, accurate, economic and rapid for the determination of ASP and TIC in combined tablet dosage forms. Analysis of authentic samples containing ASP and TIC showed no interference from the common additives and excipients. Hence, recommended procedure is well suited for the assay and evaluation of drugs in commercial tablets. It can be easily and conveniently adopted for routine quality control analysis.

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