OPEN ACCESS

Review Article

Method Development and Validation for simultaneous Estimation of Aspirin and Ticlopidine HCL in Bulk and Tablet Formulation by using UV Spectrophotometer

Shubhangi Bhalerao*, Dr. Rishikesh. S. Bachhav, Netra Adhav

Pharmaceutical Quality Assurance, R. G. Sapkal college of Pharmacy, Nashik

ABSTRACT

A simple,economical,rapid,accurate,precise method for simultaneous estimation was developed and validated. UV Spectrophotometric estimation of Aspirin and Ticlopidine HCL of the bulk and tablet formulation is done in which distilled water was used as a solvent and methanol is used as a co-solvent. Method used is- Simultaneous Equation Method which was carried out at the wavelength of 275 nm and 214 nm for Aspirin and Ticlopidine HCL respectively. The linearity range was found to be 25-125 µg/ml and 5-25 µg/ml.The recovery studies were performed and percent recoveries were found to be 99.66±0.3932 % and 99.72± 0.2535 % for Aspirin and Ticlopidine HCL. So proposed method was found to be simple,accurate, linear and specifc.

INTRODUCTION

The number of drugs introduced into the Market increasing every year. Very often there is a time lag from the date introduction of into the market of the date of its inclusion in pharmalopacias. This happens because of the possible uncertainties in the reports continuous and wider usage of these drugs, of new toxicities. Under these Conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. Therefore, it becomes necessary, to develop newer analytical method for such drug. Method Validation is the process used to confirm that the analytical procedure employed for a specific test suitable for its intended use. Results from method validation Can be used to judge the quality, reliability and consistency of analytical results. . It is process of defining an analytical requirement and confirm that method under consideration has performance Capabilities consistent with what the application requires. Method validation evolves from method development and So the two activities are often closely tied with the validation study employing the techniques and steps in analysis's defined by method.

---

*Corresponding Author: Shubhangi Bhalerao

Address: *Pharmaceutical Quality Assurance, R. G. Sapkal college of Pharmacy, Nashik*

Email ✉ : bhaleraoshubhangi30@gmail.com

Analytical methods need to be validated or revalidated:

- before their introduction into routine use
- Whenever the Conditions change for which the method has been validated. (e.g. Samples with different matrix or instrument with different characteristics.)
- Whenever the method is changed and the change is outside the original scope of the method

Analytical chemistry

"Pharmaceutical analytical chemistry may be defined as the branch of practical chemistry which deals with the resolution, separation, identification, determination and purification of given sample of medicine or pharmaceutical. Analytical chemistry may be defined as the art of determining the components of material in terms of the element or compound contained. Its application extended to all parts of an industrial society. It can be splits into two main types as

1. **Qualitative**
2. **Quantitative**

UV Visible Spectrophotometry

Principle

UV spectroscope has received electromagnetic radiation. The wavelength and intensity of the electromagnetic radiation molecules absorb energy and this energy can bring about translational. Rotational or vibration motion or ionization of the molecules depending upon the frequency of the electromagnetic radiation they receive Excited molecules are unstable and quickly drop down to ground state again giving off the energy they absorbed or emitted can be recorded to get a spectrum. Spectral analysis yields qualitative and quantitative information about the matter under study.

An UV Visible spectrophotometer measures the intensity of light passing through sample (P) and compress it to the intensity of light before it passes through the sample (P_0) expressed in absorbance (A) or transmittance (T). It uses light over the ultraviolet range (185 to 400 nm) and visible range (400 to 800 nm) of electromagnetic spectrum.

$A= -\log T=\log P_0 /P =\varepsilon bc$

Beer – Lambert Law

Lambert's Law: It states that when a beam of monochromatic radiation passes through a homogeneous absorbing medium, the rate of decrease of intensity of radiation with thickness of absorbing medium is proportional to the intensity of the incident radiation.

Beer's Law: This law states that when a beam of monochromatic radiation is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the intensity of incident radiation as well as the concentration of the solution.

Types of Spectrophotometers:

Single Beam Instrument

It consists of a tungsten or deuterium lamp, a filter or a monochromator for wavelength selection, matched cells that can be placed alternately in the radiation beam, transducer, an amplifier, and a readout device. Normally, a single-beam Instrument requires a stabilized voltage supply to avoid errors resulting from changes in the beam intensity during the time required to make the 100% measurement and determine %T for the analyte.

Double-Beam Instrument

Many modern photometers and spectrophotometers are based on a double-beam design. Figure b) illustrates a double- beam-in-space instrument in which two beams are formed in space by a V-shape mirror called a beam splitter. One beam passes through the reference solution to a photodetector, and the second simultaneously traverses the sample to a second, matched detector. The two outputs are amplified, and their ratio is determined electronically or by a computer and displayed by the readout device.

Instrumentation:

A spectrophotometer consist of 2 devices, a spectrometer and a photometer. A spectrophotometer is a device that produces, typically disperses and measures light. A photometer indicates the photoelectric detector that measures the intensity of light.
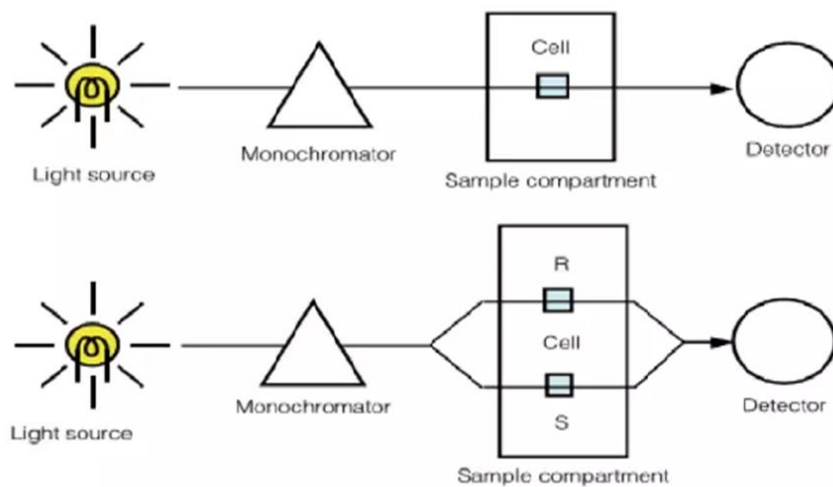
Figure no. 1: a) single beam instrument b) double beam instrument

Spectrophotometric Methods

Following Spectrophotometric methods are used for the analysis of a combination sample-
1. Simultaneous equation method
2. Absorbance ratio method (Q- Absorbance method)
3. Derivative Spectrophotometric method
4. Difference spectrophotometry
5. Solvent extraction method
6. Absorbance correction for interference
7. Ratio derivative spectra method
8. Orthogonal polynomial method
9. Multi-variate method
10. Area Under Curve

Simultaneous Equation Method

If a sample contains two absorbing drugs (x and y) each of which absorbs at the λmax of the other, then it may be possible to determine both drugs by the technique of simultaneous equation (Vierordt's method) provided that certain apply.

The information required is: The absorptivity of x at λ1and λ2, ax1 and ax2 respectively. The absorptivity of y at λ1, and λ2, ay1 and ay2 respectively. The absorbance of the diluted samples at λ1, and λ2, A1 and A2 respectively.

Let Cx and Cy be the concentrations of x and y respectively in the diluted samples. Two equations are constructed based upon the fact that at λ1, the absorbance of the mixture is the sum of the individual absorbance of x and y.

Cx = A2ay1 - A1ay2/ax2ay1-ax1ay2
Cy =A1ax2-A2ax1/ax2ay1-ax1ay2

Validation of Analytical Method:

✓ Method validation process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. This process establishes, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. Methods need to be validated or revalidated as follows:

Before their introduction into routine use

✓ Whenever the condition change for which the method has been validated, e.g. instrument with different characteristics.

✓ Whenever the method is changed and the change is outside the original scope of the method

✓ When quality control indicates the equivalence method is changing with time

The International Conference of Harmonization (ICH) has developed a consensus text on validation of analytical procedures: The document include definition for eight characteristics.

1. Specificity
2. Accuracy
3. Precision
   - Repeatability
   - Intermediate precision
   - Reproducibility
4. Linearity
5. Range
6. Robustness
7. Ruggedness
8. System Suitability Testing

Characteristics of Validation

1. Specificity

Specificity is the ability to assess unequivocally the analytes in the presence of components, which may be expected to be present. Typically, these might include impurities, degradants. Matrix etc.

2.Accuracy

The accuracy of an analytical procedure expresses the closeness of an agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed as trueness,

% Bias - (Measure value - True value / True value) x100

3.Precision

The precision of analytical procedure expresses closeness of agreement (degree of scatter)between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. It may be considered at three levels: it is expressed as standard deviation or coefficient of variation.

%RSD = (standard deviation / mean) x100

- Repeatability

Repeatability expresses the precision under the same operating conditions over a small interval of time. Repeatability is also termed intra-assay precision.

- Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analyst, different equipment etc.

- Reproducibility

Reproducibility expresses the precision between laboratories.

4. Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

4.1 Based on Signal to Noise Ratio

A signal to noise ratio between 3 or 2:1 is generally considered acceptable for estimating in the detection limit

4.2 Based on the Standard Deviation of the Response and the Slope

Detection Limit (DL) = 3.3 σ / S

Where σ= the standard deviation of the response

S= the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, for example :

4.2.1 Based on the Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analysing an appropriate number of blank samples and calculating the standard deviation of these responses.

4.2.2 Based on the Calibration Curve

A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

4.3 Based on Visual Evaluation

It may be used for non–instrumental methods but may also be used with instrumental methods.

5. Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products.

5.1. Based on Signal to Noise Approach

This approach can only be applied to analytical procedures that exhibit baseline noise. Determination of the signal to noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal to noise ratio is 10:1.

5.2. Based on the Standard Deviation of the Response and the Slope

Quantitation Limit (QL) = 10 σ/S

Where, σ= the standard deviation of the response

S= the slope of the calibration curve

Slope S may be estimated from the calibration curve of the analyte.

5.2.1. Based on Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analysing an appropriate number of blank samples and calculating the standard deviation of these responses.

5.2.2. Based on the Calibration Curve

A specific calibration curve should be studied using samples, containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

Linearity And Range

The linearity of an analytical procedure is the ability (within a given range) to obtain test results, which are directly proportional to the concentration of an analytes in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of analytes in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Robustness

The robustness of an analytical procedure is a measured of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Ruggedness

The Ruggedness of an analytical procedure is the reproducibility of test result obtained by the analysis of the same sample under variety of condition, such as different laboratories, different analyst, different assay temperature, different

System Suitability Testing:

System Suitability is an integral part of many analytical procedure. The tests are based on the concept that the equipment, electronics, analytical operation, and sample to be analysed constitute an integral system that can be evaluated as such.

ICH Guidelines

Table no 1: ICH Guidelines for Method Development and Validation

Guidelines	Title
Q1A (R2)	Stability testing of new drug substances and product
Q1B	Stability testing: Photo stability testing of new drug substances and products
Q1E	Evaluation of stability data
Q2	Analytical Validation
Q2 (R1)	Validation of analytical procedures: Text And Methodology
Q2(R2)	Analytical procedure development and revision of Q2 R1 Analytical Validation

Drug Profile:

Aspirin:

Structure
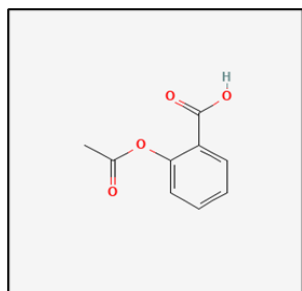
IUPAC Name: 2-(Acetyloxy)benzoic acid

Molecular formula - $C_9H_8O_4$

Molecular Weight - 180.16 g/mole

Solubility: 1 gm dissolves in 300 ml H_2O at 25^oC (Slightly soluble)

1 gm dissolves in 100 ml H_2O at 37^oC (Sparingly soluble)

1 gm dissolves in 5ml alcohol (Freely soluble)

1 gm dissolves in 17 ml chloroform (Soluble)

1 gm dissolves in 10-15 ml ether (Soluble)

Decomposition by boiling H_2O or when dissolved in solution of alkali hydroxides and carbonates, inorganic salts soluble in H_2O (especially Calcium salts) but decomposed quickly.

Mechanism of action – It acetylates the enzyme COX1 and TX synthase- inactivating them irreversibly. TXA_2 activates platelets to change shape and release mediator rich granules which promote aggregational it inhibits the release of ADP from platelets and their sticking to each other.

LD50 orally in mice, rats, 1.1,1.5/kg

pK(25°) - 3.4 g

UV max - (0.1 NH_2SO_4) 229 nm; ($CHCl_3$) 277 nm

Ticlopidine HCl

Structure
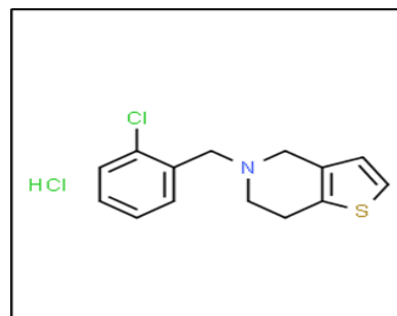
IUPAC Name : 5-[(2-Chlorophenyl 1)-methyl]-4,5,6,-tetrahydrothieno[3,2-C]Pyridine

Molecular Formula- $C_{14}H_{14}ClNS.HCl$

Molecular Weight- 300.25 g/mole

Solubility Almost Soluble in H_2O (Sparingly) Soluble in 95% alcohol,methanol,chloroform Insoluble in ether

Mechanism of action – First thienopyridine drug that acts by blocking the $P2Y_{12}$ type of purinergic receptors on the surface of platelets and inhibits ADP – induced aggregation.

LD50 in mice (mg/kg/24 hrs) 55 iv;>300 orally

pKa - 7.64

UV max- H_2O: 214,268,295 nm (A_{1cm} 303.8, 13.14,2 respectively)

MATERIALS AND EQUIPMENT:

In the present work a simple ,selective, rapid, Precise, UV Spectrophotometric method for estimation of Aspirin and Ticlopidine HCL has been developed and validated.

Materials
Active Pharmaceutical Ingredients

Table no 2: API and Supplier

Sr. No	API	Supplier
1	Aspirin	AVCOP Sangamner
2	Ticlopidine HCL	AVCOP Sangamner

Marketed Formulation: Lab Mixture

Reagents

Table no.3 List of Chemicals/Reagents

Sr. No.	Name of chemicals	Grade
1	Water	Distilled Water (Pharmaceutical Analysis lab)
2	Methanol	AR

Instruments

Table no 4: List of Instruments/Equipment's

Sr no.	Name of Instrument	Manufacturer	Model
1	Digital Analytical Balance	Shimadzu	AUX220
2	UV Spectrophotometer	Shimadzu	1800
3	Infrared Spectrophotometer	Bruker Optics	Alpha T

RESULTS AND DISCUSSION:

Physical characteristics:

Solubility of Drug:

Solubility profiling of Aspirin and Ticlopidine HCL was done by using different solvents such as Water and Methanol and examined using UV spectroscopy.

Table no 5: Solubility of Drug in different solvent

Sr no.	Solvent	Solubility of Aspirin	Solubility of Ticlopidine HCL
1	Water	Slightly Soluble	Sparingly Soluble
2	Methanol	Freely Soluble	Sparingly Soluble

FTIR Spectroscopy

The FTIR spectroscopy uses infrared radiation which is a type of molecular absorption spectroscopy. In this a spectrum of % Transmittance v/s Wavenumber is obtained. An IR spectrum of the Aspirin and Ticlopidine HCL was obtained using ATR technique.
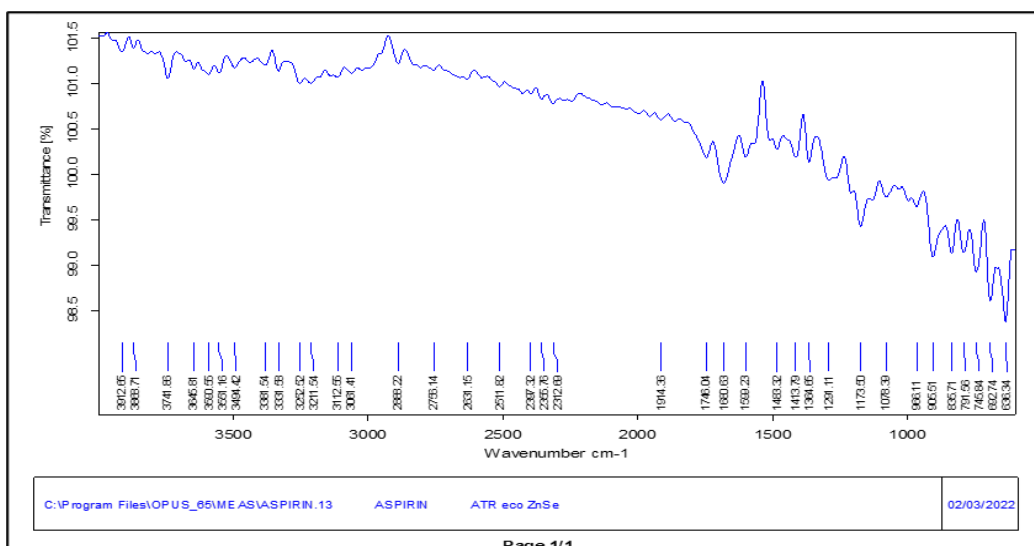
Fig no. 2 FTIR spectrum of Aspirin

Table no 6: IR Frequencies of Aspirin

Sr no.	Functional Group	Observed Ranges (cm^{-1})	Standard Ranges (cm^{-1})
1	C-H (Ar) bend	905.51	900-860
2	C=O (acid) str	1680.63	1700-1680
3	C=O (ester) str	1746.04	1775-1755
4	C-O (ester) str	1291.11	1310-1250
5	C=C skeletal vibration	1599.23	1600
6	CH$_3$-CO- deformation	1364.65	1364-1354
7	O-H (acid) str	3112.55	3300-2500
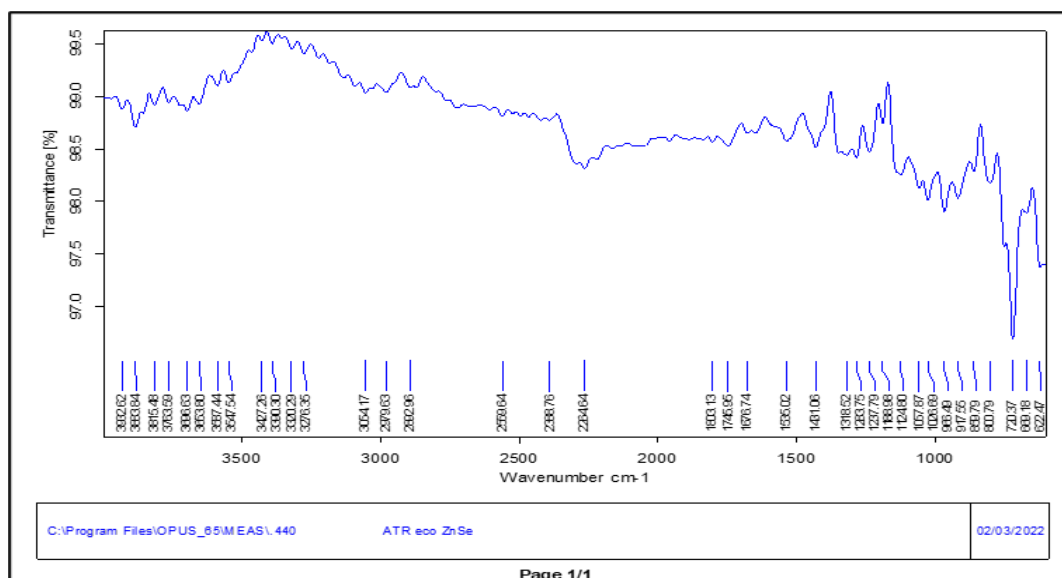
Fig no.3 FTIR Spectrum of Ticlopidine HCL

Table no 7: IR Frequencies of Ticlopidine HCL

Sr no.	Functional Group	Observed Ranges (cm^{-1})	Standard Ranges (cm^{-1})
1.	CH$_2$ bend	1431.06	1485-1445
2.	C-N	1188.98	1220-1020
3.	C-Cl str	720.37	760-540
4.	=C-H bend	1057.87	1225-950
5.	C-S str	720.37	710-570

UV spectrophotometric method

Selection of wavelength

Different concentration of solution of both the drugs was prepared. These solutions are scanned between 200-400 nm using distilled water as a reference solvent. In UV spectrophotometer214 nm and 275 nm were selected for the determination of simultaneous equation method of Ticlopidine HCL and Aspirin.
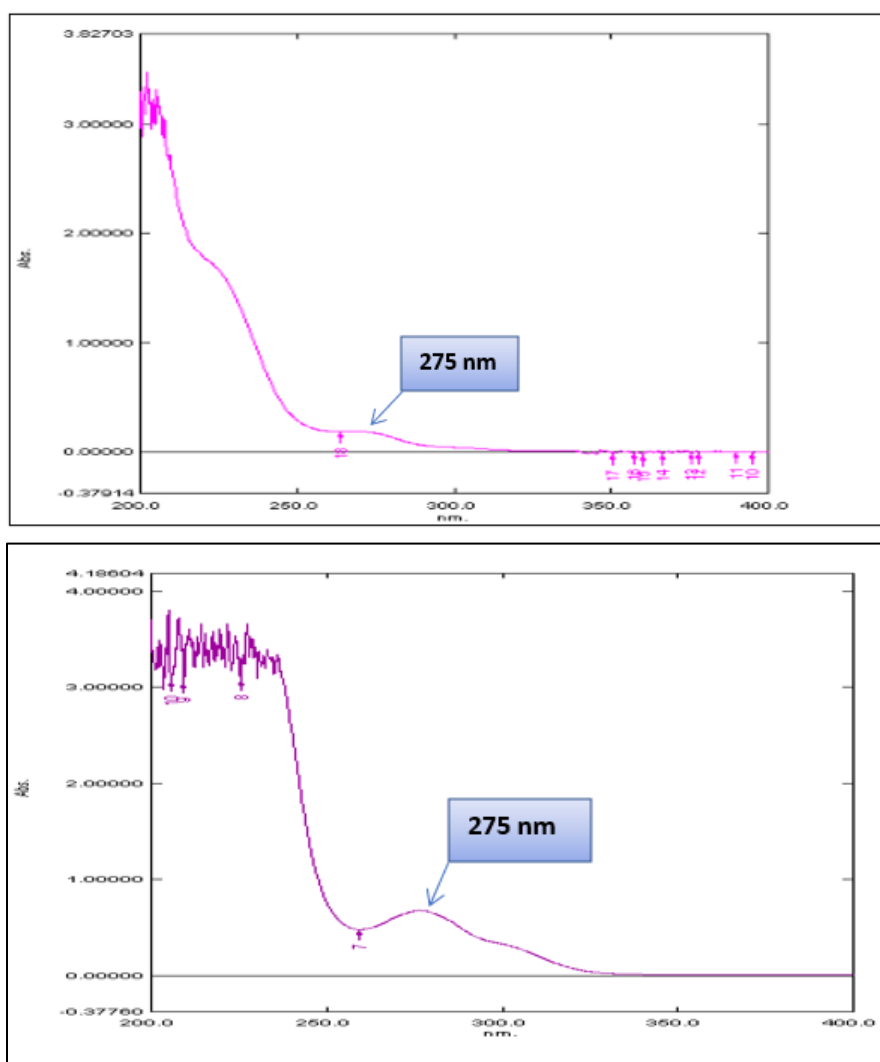
Fig no 4: UV spectrum of aspirin
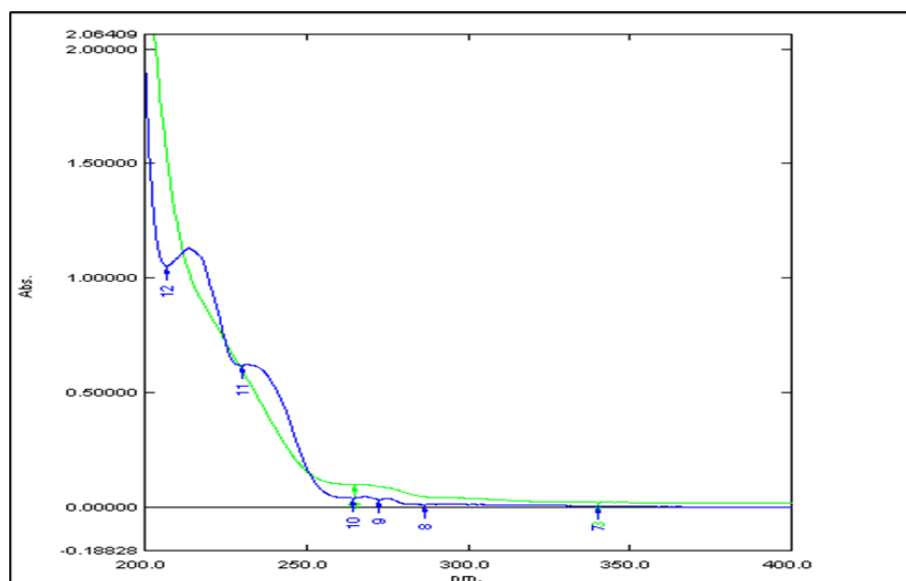
Fig. 5 Overlay Spectra of Aspirin and Ticlopidine HCL
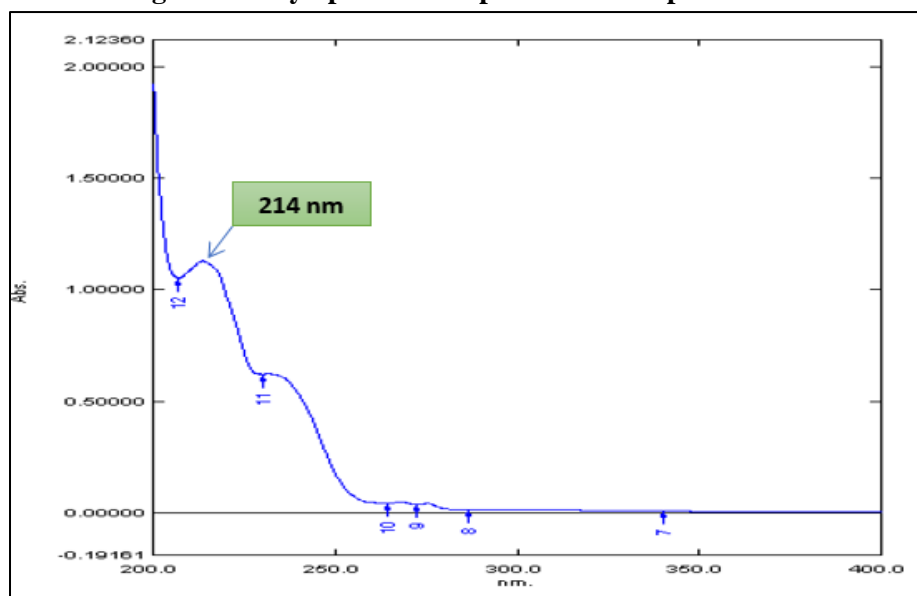
Fig no 6: UV spectrum of Ticlopidine HCL

Absorptivity coefficient for the two drugs were determine at both the selected wavelengths. The value obtained as a mean of three independent determinations were used for forming the simultaneous equation. The concentration of drugs x (Ticlopidine HCL) and y (Aspirin) in sample solution were determined using the following formula

$Cx = A2ay1 - A1ay2/ax2ay_1-ax1ay_2$

$Cy =A1ax2-A2ax1/ax2ay1-ax1ay2$

Where Cx and Cy are the concentration of Ticlopidine HCL and Aspirin. A1 and A2 are the absorbance of sample solution at 214 nm and 275 nm respectively, ax1 and ax2 are the absorptivity of Ticlopidine HCL at 214 nm and 275 nm, ay1 and ay2 are the absorptivity of Aspirin at 214 nm and 275 nm.

Table no 8: Result of Simultaneous Equation Method

Sr. no	Value	Simultaneous Equation Method
1	C_x	50.223
2	C_y	11.643
3	ax_1	0.04717
4	ax_2	0.00195
5	$ay1$	0.04336
6	$ay2$	0.006538
7	A1	2.4556
8	A2	0.08613

Table no 9 Linearity data of Aspirin

Sr. no.	Concentration (µg/ml)	Absorbance (275 nm)
1	25	0.235
2	50	0.4626
3	75	0.6777
4	100	0.8784
5	125	1.1230
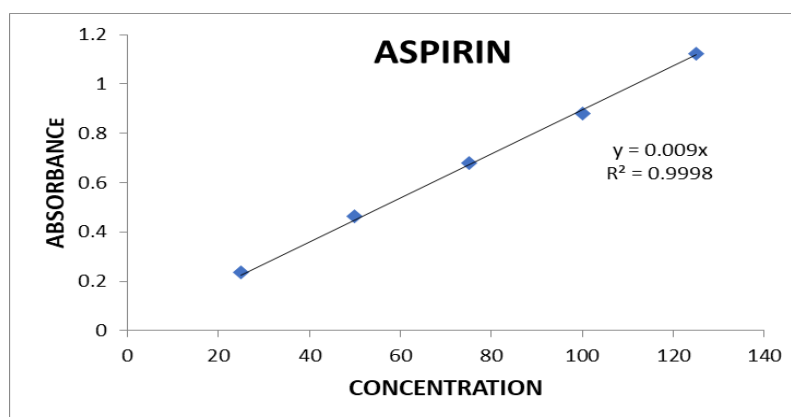
Fig no 7 Calibration curve of Aspirin

Table no 10 Linearity data of Ticlopidine HCL

Sr. no	Concentration µg/ml	Absorbance 214 nm
1	5	0.2475
2	10	0.4359
3	15	0.6019
4	20	0.7988
5	25	1.0027

Fig no 8 Calibration curve of Ticlopidine HCL

Method Validation

Validation of analytical method is a process to established that the performance characteristic of developed method meet the requirement of the analytical application.

Linearity: Linearity study was performed by preparing solutions of 25-125 µg/ml for Aspirin and 5-25 µg/ml For Ticlopidine HCL. The calibration curve was plotted for each drug with concentration versus absorbance separate.
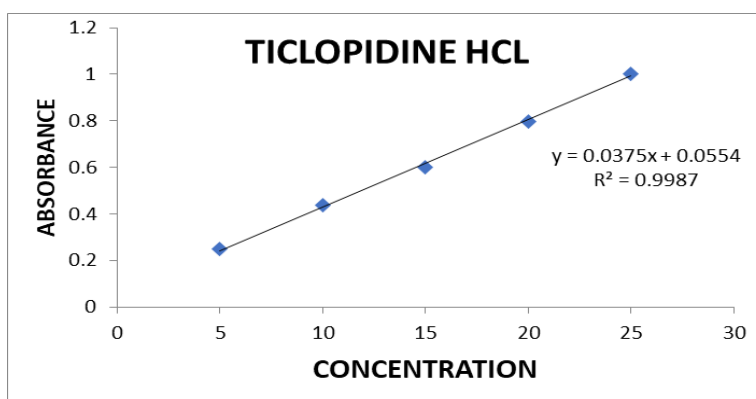
Table no 11 Optical characteristics and another parameter

Parameter	Aspirin	Ticlopidine HCL
λ_{max}wavelength (nm)	275	214
Linearity range (µg/ml)	25-125	5-25
Slope (m)	0.008767	0.03747
Intercept (c)	0	0.0554
Regression Coefficient R^2	0.9986	0.9987
Limit of detection (µg/ml)	0.8158	0.6830
Limit of quantitation(µg/ml)	2.4724	2.0698

Pre-analysis Study of the Marketed Formulation:

Table no 12 Pre-analysis Study

Aspirin				Ticlopidine HCL			
Conc µg/ml.	Std. (Abs.)	Marketed (Abs.)	Actual Conc. (µg/ml)	Conc µg/ml.	Std. (Abs.)	Marketed (Abs.)	Actual Conc. µg/ml
6	0.0802	0.0801	5.99	15	0.6012	0.6010	14.99
	0.0802	0.0799	5.97		0.6012	0.6008	14.99
	0.0802	0.0803	6.0074		0.6012	0.6013	15.00
		Mean	5.989			Mean	14.99
12	0.1245	0.1240	11.95	30	1.214	1.212	29.95
	0.1245	0.1248	12.02		1.214	1.210	29.90
	0.1245	0.1241	11.96		1.214	1.209	29.87
		Mean	11.97			Mean	29.90
18	0.1902	0.1910	18.07	45	1.912	1.910	44.95
	0.1902	0.1905	18.02		1.912	1.913	45.023
	0.1902	0.1898	17.96		1.912	1.911	44.97
		Mean	18.01			Mean	44.981

Precision

The Precision of the method was evaluated by intraday and interday variation studies. In intraday studies working solutions of standard and sample were analysed thrice in a day and the percentage of relative standard deviation (%RSD) was calculated. In case of variation the working solutions of standard and sample were analysed on three consecutive days and the percentage of relative standard deviation (%RSD) was calculated.

Table no 13 Intraday – Interday Precision data

	Dilutions	Aspirin				Ticlopidine HCL			
		Intraday N=3		Interday N=3		Intraday N=3		Interday N=3	
		Abs	Conc.	Abs	Conc.	Abs	Conc.	Abs	Conc.
	1	0.2368	25	0.2353	25	0.2320	5	0.239533	5
	2	0.2312	25	0.2352	25	0.2437	5	0.278333	5
	3	0.2357	25	0.2358	25	0.2290	5	0.244033	5
	SD	0.0029		0.0005		0.002747		0.00363	
	%RSD	1.237		0.2074		1.1878		1.453759	
	1	0.4641	50	0.4606	50	0.4735	10	0.481033	10
	2	0.4635	50	0.4637	50	0.4743	10	0.480733	10
	3	0.4625	50	0.4608	50	0.4800	10	0.455633	15
	SD	0.0029		0.0016		0.002389		0.003744	
	%RSD	0.6352		0.3357		1.0014		0.802161	
	1	0.675	75	0.6727	75	0.631633	15	0.644833	15
	2	0.674	75	0.6752	75	0.630133	15	0.665767	15
	3	0.6772	75	0.6722	75	0.6159	15	0.652933	15
	SD	0.0025		0.0034		0.009696		0.006909	
	%RSD	0.3764		0.4988		1.545844		1.058798	
Mean SD	0.0028			0.0018		0.004944		0.004761	
Mean%RSD	0.7497			0.3473		1.245048		1.104906	

Accuracy

To confirm the accuracy of proposed method, Recovery studies were carried at 3 different levels (50%,100%,150%) as per US FDA .The amount of Aspirin and Ticlopidine HCL were estimated by developed method.

Table no 14 Result of Recovery study

Level of Recovery	Amount Taken (µg/ml)				Actual Concentration (µg/ml)		Recovered Concentration (µg/ml)		% Recovery	
	ASP		TICLO		ASP	TICLO	ASP	TICLO	ASP	TICLO
	Std	Mark	Std	Mar						
50%	12	6	30	15	17.99	44.99	6.028	14.99	100.47	99.98
	12	6	30	15	17.97	44.99	5.961	14.97	99.36	99.80
	12	6	30	15	18.00	45	6.00	14.99	100.10	99.94
	Mean								99.97	99.90
100%	12	12	30	30	23.95	59.95	11.94	29.86	99.55	99.54
	12	12	30	30	24.02	59.90	11.96	29.91	99.68	98.71
	12	12	30	30	23.96	59.87	12.02	30.01	100.17	100.05
	Mean								99.8	99.43
150%	12	18	30	45	30.07	89.95	17.72	44.85	98.46	99.68

12	18	30	45	30.02	90.02	17.85	44.94	99.21	99.87
12	18	30	45	29.96	89.97	18	44.97	100	99.94
Mean								99.22	99.83
							Mean % recovery	99.66	99.92
							SD	0.3932	0.2535

LOD and LOQ:

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal to noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ based on the third approach and calculated with the following equations:

$LOD = 3.3\sigma/S$

$LOQ = 10\sigma/S$,

where σ= the standard deviation of response

S= the slope of the corresponding calibration curve.

Table no 15 LOD and LOQ

Parameter	Aspirin	Ticlopidine HCL
LOD	0.8158	0.6830
LOQ	2.472	2.0698

Ruggedness

Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from lab to lab and analyst to analyst.

Table no 16 Ruggedness data

Sr No.	Aspirin		Ticlopidine HCL	
	Concentration	Absorbance	Concentration	Absorbance
1	25	0.2357	5	0.2750
2	50	0.4607	10	0.4527
3	75	0.6707	15	0.6523
4	100	0.8877	20	0.8456
5	125	1.1219	25	1.061
SD	0.004021		0.009823	
% RSD	0.6017		1.643538	

Robustness:

Robustness tests examine the effect operational parameters have on the analysis results. If the influence of the parameter is within a previously specified tolerance the parameter is said to be within the methods robustness range.

Table no 17 Robustness data

	Aspirin				Ticlopidine HCL			
	at 274 nm		at 276 nm		at 213nm		at 215 nm	
	Conc.	Abs.	Conc.	Abs	Conc.	Abs	Conc.	Abs
1	25	0.2362	25	0.2344	5	0.2525	5	0.243033
2	50	0.4596	50	0.4611	10	0.4447	10	0.433
3	75	0.6773	75	0.6769	15	0.65	15	0.638333
4	100	0.8771	100	0.877	20	0.918333	20	0.895667
5	125	1.1182	125	1.1221	25	1.248667	25	1.239667
	SD	0.002504	SD	0.002384	SD	0.007848	SD	0.008802
	%RSD	0.5276	%RSD	0.3883	%RSD	1.310845	%RSD	1.381186

CONCLUSION:

The proposed methods are simple, precise, accurate, economic and rapid for the determination of ASP and TIC in combine tablet dosage form. Maximum recovery obtain by this developed method and the percent RSD for each component is not more than 2%. For the Precision, Robustness, Ruggedness and accuracy, the percent RSD is not more than 2%. Hence proposed UV method is cost effective precise and accurate for the simultaneous determination of Aspirin and Ticlopidine HCL in combine dosage form & is suitable for analysis of pharmaceutical preparations.

REFERENCE

1. Skoog, Holler, Crouch, "Principles Of Instrumental Analysis", Seventh Edition,2016,Cengage Learning, Page No. 746-755.
2. Gurdeep R. Chatwal, Sham K. Anand, "Instrumental Methods Of Chemical Analysis", Fifth Revised Edition(Reprint), 2007 Himalaya Publishing House, Page No2.167-2.170.
3. Y.R. Sharma, "Elementary Organic Spectroscopy-Principles And Chemical Applications'", Fifth Revised Edition 2013,S. Chand &Company Pvt. Ltd, Page No.76,144-147.
4. Indian Pharmacopoeia 2010-Volume I ,Government Of India-Ministry Of Health & Family Welfare, Indian Pharmacopoeia Commission, Ghaziabad, Page No.23,117.
5. International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use: Q2(R1)-Validation Of Analytical Procedures: Text And Methodology, Parent Guideline Dated 27 October 1994 ,Incorporated In November 2005, Page No. 4,5,6,8,10,11,12,13.
6. A.H. Beckett. J.B. Stenlake "Practical Pharmaceutical Chemistry", Fourth Edition-Part Two, British Library Cataloguing In Publication Data, Page No. 255,284-299.
7. Manohar A. Potdar, "Pharmaceutical Quality Assurance",Fifth Edition 2016, Page No.8.31.
8. Raman.N. Kachave, Sanjay R. Chaudhari , Mayura Kale, Rajendra D. Wagh, Inventi Rapid: Pharm Analysis And Quality Assurance,"Development And Validation Of Ticlopidine Hcl And Aspirin In Bulk Drug And Its Tablet Formulation By Uv Spectroscopy",Vol.2013., Issue 4.
9. Shraddha C. Guajarati, Aditi R. Shaha , Swati C. Jagdale, Prasanna A. Datar, Vishnu P. Chaudhari, Bhanudas S. Kuchekar ,International Journal Of Pharmaceutical Sciences Review And Research "Spectrophotometric Simultaneous Determination Of Aspirin And Ticlopidine In Combine Tablet Dosage Form By First Order Derivative Spectroscopy, Area Under Curve (Auc) And Ratio Derivative Spectrophotometric Methods".
10. Sayyed Fazlur Rehman, Ritu Vivek Kimbahune, G. Mubeen, Shaik Mohammed. Maaz ,Prachi Kabra Analytical Chemistry-An Indian Journal,"Simultaneous Estimation Of Aspirin And Ticlopidine Hydrochloride In Combination By Derivative Spectroscopy",Vol.13, Issue
11. R.B. Kakde ,A.G.Barsagade, N.K. Chaudhary And D.L.Kale, Asian Journal Research Chemistry 2(1):Jan-March. 2009 "Spectrophotometric Method For Estimation Of Ticlopidine Hcl In Bulk And Dosage Form.
12. M. Dacic A. Uzunovic, A. Kunic, S.Pilipovic And A. Sapcanin Springer Nature Switzerland AG. "Uv Visible Determination Of Acetylsalicylic Acid In Aspirin Tablet Using Different Solvents And Conditions".
13. The Merck Index-An Encyclopaedia Of Chemicals, Drugs, And Biologicals 13th

Edition, Published By Merck Reasearch Laboratories Division Of Merck & Co., Inc. Page No.145-146 , 9508.

14. Pavia, Lampman, Kriz, Vyvyan, 'Introduction To Spectroscopy',4th Edition, Published By Brooks/Cole Cengage Learning. Page No. 381.

15. Kd Tripathi ''Essentials Of Medical Pharmacology '',8th Edition,Published By Jaypee Brothers Medical Publishers (P) Ltd, Page No 677 -678.

16. M. Dolores, E. A. Morales-Hipólito, J. A.Garduño-Rosas,A. Villaseñor And R.López-Arellano,Indian Journal Of Pharmaceutical Sciences,''Development And Validation Of An Alternate Stability-Indicating UV Spectrophotometric Analytical Method For Aspirin In Tablets''.

17. Robert A. Nash, Alfred H.Wachter,''Pharmaceutical Process Validation'',3rd Edition,Published By Marcel Dekker, Inc.,Page No.507,522.

18. Ramole Rina, Mohini Baile, Ashish Jain, Systemic Review Pharmacy,''A Review : Analytical Method Development And Validation'',Vol 12,Issue 8 Apr.2021, Page No 450.

19. Sahar Samir Mohammed Journal of Al-Nahrain University, ''Comparative Analytical Study for Determination of Acetylsalicylic Acid in Bulk and in Pharmaceutical Formulations'',Vol 16(1),March,2013