RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF RECENT RESEARCH IN PHARMACY

IJRRP

DEVELOPMENT AND VALIDATION OF CURRENT STATISTICAL APPROACH FOR ESTIMATING ACETAZOLAMIDE IN PHARMACEUTICAL DOSAGE FORM

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Received- 06/July/2020 **Revised-** 27/July/2020 **Accepted-** 10/August/2020 **Published-** 30/September/2020

ABSTRACT

In this work 3 very sensitive and effective (Zero order, AUC, HPLC and spectroscopy) has been developed to assess the Acetazolamide in bulk & pharmaceutical formulation. For the measurement of Acetazolamide in pharmaceutical formulations, a simple, precise and accurate Zero-order relative Spectroscopy approach was established and tested. Going to weigh 100 mg of Regular Acetazolamide in 10 ml volumetric flask with DMSO, prepared the stock solution. The final solution for stock was made with distilled water to produce 10000 mcg/ml. As per the protocol more dilutions have been prepared. The solution for the drug showed maximum absorbance at 262 nm. The linearity was observed within the range of concentrations of 100-600 mcg/ml. The Coefficient of Correlation was 0.999. It was noticed that the regression model is Y=0.0003c + 0.00075. The results were validated for predictability, accuracy, detection limit, linear range and roughness. The detection limit and quantitation limit for Acetazolamide estimation were found to be 2.34 (mcg/ml) and 20.65 (mcg/ml), respectively. Acetazolamide restoration was reported to be in the 89.0 percent range – 100 percent.

KEYWORDS: Acetazolamide, Chromatography, validation, Spectroscopy

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INTRODUCTION

Analytical chemistry [1] is also defined as the field of chemistry, which is liable for describing matter composition, both quantitatively (how much is present) and qualitatively (what is present). Analytical chemistry is not a distinct scientific division but rather the application of scientific expertise.

Pharmaceutical analysis [2] is the chemical division engaged in the differentiation, detection and assessment of the relative quantities of the materials that make up a example of matter. It is primarily engaged in the qualitative analysis or discovery of mixes and measurable analyses of the ingredients used in bulk processing and pharmacological. The technique [3] working in quantitative investigation is founded upon the measurable efficiency of appropriate chemical reactions and either the quantity of reagent used to complete the feedback, or the quantity of the reaction product gained is measured.

Quality [4] is essential in every good or service, but as it includes life, it is critical in medicine. Unlike ordinary consumer products, medicines cannot have a second rating. Superiority governor is a philosophy which aims to deliver a perfect product through a series of measures designed to prevent and eradicate faults at various production stages. Physio-chemical methods [5, 6] are used to analyze the actual effect that results from chemical reactions. Among the most important physic-chemical methods are the optical (Polarimetry, Fluorescence methods of analysis etc.) and chromatographic (Paper, Column, GLC, TLC, HPLC) methods. Methods like nuclear magnetic resonance and paramagnetic resonance

are increasingly common. The most effective methods available are the synthesis of mass spectroscopy with gas chromatography and liquid chromatography. Chemical approaches include gravimetric and volumetric techniques founded on dynamic creation; acid-mediated reactions, redox and precipitation. It also used in clinical research in the non-aqueous medium and complexometry. The number of new medications is continuously on the rise. This needs new ways of monitoring their efficiency. Modern pharmaceutical research requires the criteria that accompany.

1. The examination should take a nominal time.
2. The precision of the examination should meet the demands of Pharmacopoeia.
3. The examination should be inexpensive.

The physic-chemical approaches of investigation these criteria, a benefit of which is their abstract existence and can be used to analyses biological mixtures with a complex construction. In such, observable spectrophotometry is usually favored by small-scale enterprises in particular because the expense of the device is smaller, and the maintenance issues are negligible [7].

Instrumentation of Chemical Analysis

Contributory approach is an interesting and informative aspect of chemical research, dealing with all fields of chemistry and with numerous other aspects of unadulterated and applied sciences. Analytical instrumentation pays a significant part in the development & review of new goods, as well as in consumer and environmental safety. This instrumentation has lower concentration levels that are necessary to ensure safe food, narcotics, and water. Analytical chemists are increasingly using instrumental techniques to conserve time, prevent isolation of chemicals and gain greater precision. Maximum instrumental techniques are suitable into one of the four principle areas. For suitability and better thoughtful introduction is divided into three parts,

A) Chromatography

B) Validation

C) Spectroscopy

Additional sections such as objectives, methodology, results, discussion, and conclusion have been shared into two parts,

A) UV Spectrophotometry

B) High Performance Liquid Chromatography [8].

METHODOLOGY

Part A: UV Spectroscopy

Method A: Zero Order Derivative Spectroscopy

Analytical Wavelength Selection

Reasonable dilutions from the normal stock solution were able for medication and the solutions were tested in the 200-400 nm wavelength range. The absorption spectra thus obtained where derivative from the system zero order and AUC.

Stock Solutions Preparation

Weighed and moved to a 10 ml volumetric flask, the regular acetazolamide 100 mg was dissolved in DMSO. The flask was shook and the amount was made up to the DMSO level to have a 10000 mcg/ml solution.

Analytical Concentration Ranges Selection

Relevant aliquots were pipetted in up to 10 ml volumetric flasks from the acetazolamide normal stock solution and dilutions were made with purified water to achieve working regular concentration solution of 100 – 600 mcg/ml. The analytical concentration range for the regular solution was found to be 100 – 600 mcg/ml, and those values were stated in **Table 1**.

Calibration curve for Acetazolamide (150 - 650 mcg/ml)

Sufficient volume of aliquots from regular acetazolamide stock solutions is moved into various 10 ml size volumetric flasks. The amount of purified water was calibrated to the level to achieve concentrations of 150, 250, 350, 450, 550 and 650 mcg/ml. The absorbance spectra of each solution were calculated at 262 nm against distilled water as blank and the graphs of absorbance against concentration were plotted and displayed in **Figure 1**. The equation for regression and the coefficient for correlation has been calculated and is described in **Table 2**.

Sample Preparation for Purpose of Acetazolamide from Dosage Form

Weighed 20 tablets and finely crushed. The acetazolamide powder equal to 25 mg was correctly measured and transferred to a 25 ml volumetric regular flask. In DMSO the contents have been dissolved and sonicated for five minutes. This solution was made up to the

DMSO level to get 1000 mcg/ml solution. To achieve the concentration 100 mcg / ml, an aliquot of 1 ml of test solution was diluted to 10 ml with water in 10 ml normal volumetric flask. Filtered carefully by 0.45-micron filter paper (no. 41), then used to approximate acetazolamide.

Spectrophotometric Method Validation

Range & Linearity

The linearity of the analytical method is its ability to produce test results which are directly proportional to the analyte concentration in the sample within a given range which was presented in **Figure 2**. The spectrum of analytical method is the distance between the upper & lower analyte levels, which has been shown to be calculated beyond an acceptable degree of precision, accuracy and linearity.

Precision

An analytical method's precision is the degree of agreement between individual test results when the procedure is consistently extended to multiple homogenous sampling samples. It gives an overview of the outcome of the random error and was expressed as percent RSD.

Accuracy

Accuracy is the closeness of the test outcomes that the process produces to the true value. 20 tablets were weighed and powdered to research the precision and examination of the same was done. Recovery experiments were performed by applying known quantity of regular drug solution (400 mcg/ml) to the sample. Recovery percentages were measured and listed in **Table 3**.

Intra and Inter-day Precision

A outcome difference within the same day (intra–day), result variance within days (intra-day), result variance within days (inter-day) was studied. Analyzing acetazolamide at 262 nm for six times in the same day determined intraday accuracy. Inter-day accuracy was determined by evaluating 262 nm once every day for six days and the percentage of RSD was estimated and shown in **Table 4**.

Ruggedness

With variations in analytical conditions such as different laboratory such as different laboratory environments and different analysts, the solutions were prepared and evaluated and stated in **Table 5**.

Method B: Area Under Curve Method

Standard Stock Solution Preparation

Standard stock solution preparation was same as described in method A.

Selection of analytical wavelength range for Area Under Curve

Appropriate dilutions were prepared for drug from stock solution and the solutions were then scanned in the wavelength ranges of 200 - 400 nm. The absorption spectra obtained was showing the absorption maxima [λ_{max}] at 262 nm and Area Under Curve [AUC] in absorption spectra were measured between the wavelength range 254 to 270 nm which illustrated in **Figure 3**.

Selection of Analytical Concentration Range

Selection of analytical concentration range was made same as described in method A by measuring AUC between 254 nm to 270 nm instead of absorbance at 262 nm. For the standard solutions analytical concentration range was found to be 100-600 µg/ml and those values were reported in **Table 6**.

Calibration Curve for the Acetazolamide (100 - 600 µg/ml)

Calibration curve for the Acetazolamide was prepared same way as described in method A by measuring AUC between 254 nm to 270 nm instead of absorbance at 262 nm and is shown in **Figure 4**. The regression equation and correlation coefficient were determined which are presented in **Table 7**.

Sample Preparation for Determination of Acetazolamide from Dosage Form

Sample preparation for determination of Acetazolamide from dosage form was same as described in method A.

Validation of Spectrophotometric Method

All the validation parameters are same as described in Method A.

Part B: High Performance Liquid Chromatography

An easy and sensitive RP-HPLC approach for quantitative evaluation of acetazolamide in bulk medication and pharmaceutical formulations has been developed in the present investigation.

Experimental

Instrumentation

An isocratic High-Performance Liquid Chromatography with auto sampler and DAD r PDA detector, using EZ chrome Elite tools, was used. We used Qualisil C18 column gold (4.6X 150mm, 5 nm).

Reagents

Ranbaxy Laboratories (Ahmadabad, India) had kindly gifted the reference standard acetazolamide. Without further purification the normal medicines were being used. Methanol was procured from qualigens and other HPLC type reagents, Mumbai was used in the experiment. The mobile process consists of a combination of 50:50 (V/V) of methanol and vapour.

Preparation of Working Stock Solution of Acetazolamide

Weighed and transfer correctly 10 mg of acetazolamide operating normal into a 10 ml volumetric flask apply around 5 ml of diluent and sonicate to fully dissolve a render volume with the same solvent (stock solution) up to the point. Additional pipette 0.4 ml of the above stock solution into a 10 ml volumetric flask and dilute with diluent up to the point. Mix well and filter by 0.45 microns size filter.

Chromatographic Condition

- Equipment: Agilent High performance liquid chromatography armed with Auto Sampler and DAD or PDA detector.
- Column: Qualisil gold C18 (4.6 x 150mm, 5 µm)
- Flow rate: 1 ml/ min
- Wavelength: 220 nm
- Injection volume: 20 ±l
- Column oven: Ambient
- Run time: 8.0 min

Assay Procedure

Functional regular solutions containing 10 – 60 mcg/ml of acetazolamide is prepared by diluting the stock solution with the mobile step necessary. Five times twenty ul aliquot of each solution was inserted into the tank, and the chromatograms were reported and shown in **Figure 5**. They considered the retention period to be 5.20 min calibration graph was created by plotting mean peak area as a function of Acetazolamide concentration.

Analysis of Formulation

Twenty tablets are measured precisely and finely pulverized. Tablet powder equal to 0.01 mg of acetazolamide was reliably measured and dissolved in 50 ml of methanol in a 100 ml volumetric flask and diluted to the water level for 1000 mcg/ml concentration.

From this, 0.4 ml of the above stock solution was pipetted into a 10 ml volumetric flask and diluted to the mobile step level for 40 mcg/ml concentration for RP-HPLC process. The resulting solution was filtered into filter paper in Whatman. The final solution was pumped in three times into the chromatographic method.

Validation of Analytical Method

Validation of an analytical method is the mechanism by which laboratory experiments determine that the output feature of the method satisfies the criteria for the analytical procedure expected. Quality characteristics are represented with empirical criteria in mind.

Accuracy

The accuracy of a method was inferred by establishing the precision and linearity of the standards and given in **Table 12**.

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution were made and the response factor of drug peak and % RSD were calculated and present in **Table 13**. The chromatogram was shown in **Figure 6**.

In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and response factor of drugs peak and % RSD were calculated shown in **Table 13**. From the data obtained, the developed method was found to be precise.

Linearity

The linearity of the method was demonstrated over the concentration range of 10-60 mcg/ml of the target concentration. Aliquots of 10, 20, 30, 40, 50 and 60 mcg/ml were prepared from above prepared stock solution. Different concentrations of the pure drug were injected into

the chromatographic system. Calibration curve of Acetazolamide was constructed by plotting peak area vs. applied concentration of Acetazolamide. The obtained results shown an excellent correlation between peak area and concentration of pure drug within the concentration range and it has shown in **Figure 7**. The correlation coefficient for the average area at each level versus concentration of analyte was calculated and is presented in **Table 14**. and their calibration parameters were shown in **Table 15**.

Limit of Detection (LOD)

Detection limit is calculated by evaluating samples of established analyte concentrations and by deciding the minimum amount at which the analyte may be identified reliably. From the regular stock solution 0.4 ml was piped out into 10 ml volumetric flask, and the amount was mobile process up to the level.

Pipetted 1 ml of 100 mcg / ml solution into a 10 ml volumetric flask from this solution and dilute with diluent up to the point. Total dilution was accomplished by pipetting 1.51 ml (10 mcg / ml) of the above solution into a 10 ml volumetric flask and diluted with diluent up to the target. The solution was pumped, and **Figure 8** revealed chromatogram. The concentration level for acetazolamide was found to be 1.51 mcg/ml. the LOD findings are seen in **Table 17**.

Limit of Quantitation (LOQ)

The LOQ values were determined by multiplication with three times, depending on the LOD intensity (1.51 mcg/ml). pipetted out from the regular stock solution 0.4 ml was inserted into 10 ml volumetric jar, and the amount was rendered up to the handheld step level. Pipetted 1 ml of 100 mcg/ml solution into a 10 ml volumetric flask and diluted with diluent when labeled. Additionally, pipetted 4.6 ml of the above diluted solution into a 10 ml volumetric jar and dilute with diluent to the target. The solution was pumped, and **Figure 9** revealed chromatogram. The quantitation value for acetazolamide was determined to be 4.6 mcg / ml. the LOQ findings are displayed in **Table 18**.

Ruggedness

An analytical methods roughness is the degree of reproducibility of test findings produced by examining the same samples under a range of standard test conditions, such as various labs, different researchers, different methods, different lots of reagents, different assay hours, different assay temperatures, different days etc. weighed and dissolved a sample of acetazolamide equal to 0.01 mg in a 100 ml volumetric flask comprising mobile phase 50 ml, sonicated for a few minutes and the final amount was rendered with mobile phase. The samples were inserted into the column; reported chromatogram as seen in **Figure 10**. ruggedness findings were seen in **Table 19**.

Robustness

An analytical procedure's robustness is a measure of its capacity to remain unaffected by minor, yet intentional changes in process parameters and offers an indicator of its efficiency during daily use.

For robustness determination, a variety of system parameters, such as pH, column temperature, volume of injection, flow rate, wavelength detection or composition of the mobile process are varied within a realistic range, and the quantitative influence of the variables is determined.

System Suitability

Machine-suitability checks are an important part of the creation of methods which are used to ensure the chromatographic devise works properly. Retention period (Rt), number of theoretical plates (N), and tailing factor (T) were measured at a concentration of 100 mcg/ ml for six duplicate injections of the compound. The finding shown in **Table 16**. Had been within acceptable limits.

RESULTS

Method A: Zero Derivative Order Spectroscopy

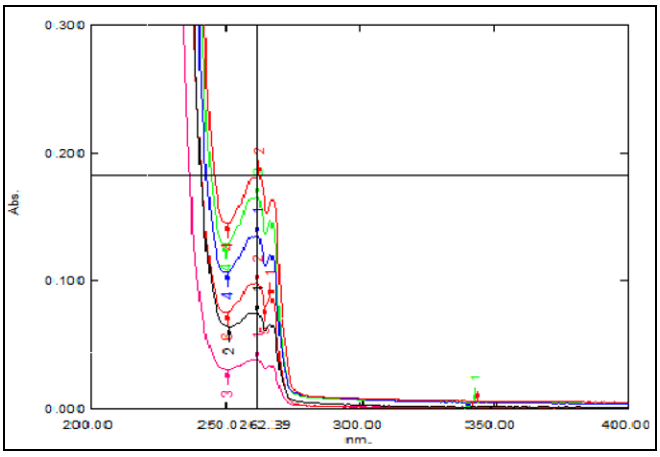


Figure 1: Zero Order Spectra of Acetazolamide showing Absorbance at 262 nm

Table 1: Calibration Curve Results at 262 nm for Acetazolamide through Spectroscopy

Concentration (mcg / ml)	OD at 262 nm
150	0.051
250	0.072
350	0.099
450	0.158
550	0.173
650	0.200

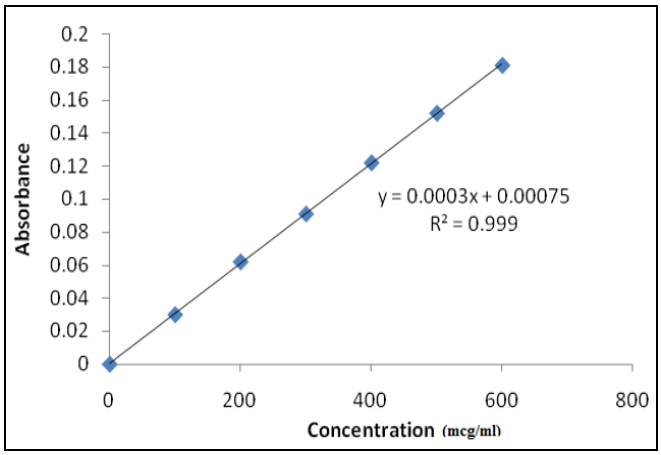


Figure 2: Linearity curve for Acetazolamide at 262 nm by Zero order Spectroscopy

Table 2: Maximum Circumstances, Optical Properties and Numerical Details of the Zero Order Regression Spectroscopy Equation

Parameter	UV
Beer's law limits (mcg/ml)	100-600
Correlation coefficient(r^2)	0.999
Intercept (a)	0.00075
Intraday Precision (% RSD**)	0.678
detection Limit (mcg/ml)	3.44
Quantitation limit (mcg/ml)	10.45
coefficient (L mol^{-1} cm^{-1})	0.0003×10^4
Equation of Regression (Y*)	Y = 0.002 C + 0.0062
Sensitivity of Sandell's (mcg/cm^2-0.001 absorbance units)	3.305
Slope (b)	0.0003
σ_{max} (nm)	262

*Y = bx + a where x is the concentration of Acetazolamide in mcg/ml and Y is the absorbance at the respective λmax.
**Average of Six determinations

Table 3: Determination of Quality results by Zero order Spectroscopy for Acetazolamide at 262 nm

Used brand	Sample (mcg/ml)	Drug added (mcg/ml)	Quantity Recovered	% Recovery ± SD**	% RSD
BANZEL	400	250	401.82	110.89 ±0.16	1.45
	400	450	646.34	109.09 ± 0.88	1.08
	400	650	881.82	140.32 ± 13	0.37

**Average of Six determinations

Table 4: Expression of Acetazolamide Accuracy by Zero-Order Derivative Spectroscopy at 262 nm

Quantity mcg / ml	Intra-day Mean of ab. ± SD**	% RSD	Inter-day Absorbance Mean ± SD**	% RSD
150	0.1205 ± 0.02055	1.05	1.029 ± 0.055	2.85
250	0.1605 ± 0.44075	1.00	0.061 ± 0.019	1.06
350	0.2011 ± 0.10075	0.03	7.09 ± 0.089	0.19
450	0.1003 ± 0.54782	0.14	4.12 ± 0.009	1.70
550	0.1000 ± 0.55126	0.25	8.152 ± 0.001	5.76

**Average of six determinations

Table 5: Ruggedness Results for Acetazolamide at 262 nm by Zero Order Derivative Spectroscopy

Tablet	Label claim (mg)	Analyst I		Analyst II	
		Amount found (mg)	Recovery ± SD** (%)	Amount found (mg)	Recovery ± SD** (%)
BANZEL	450	498.087	88.087±0.48	199.52	79.52 ± 0.34

**Average of Six determinations

Method B: Area Under Curve Spectroscopy

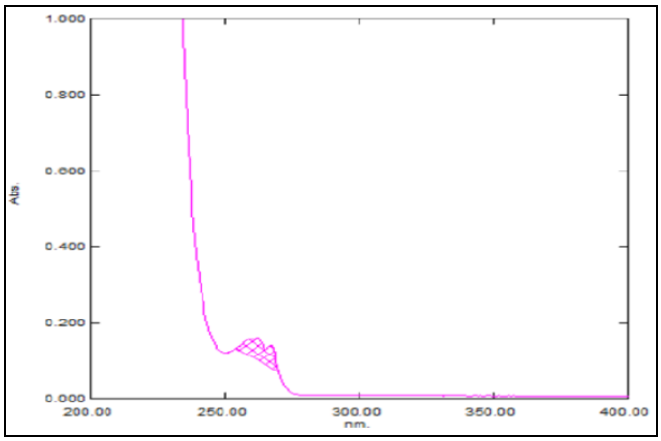


Figure 3: Typical Zero Order Spectra of Acetazolamide showing Area Under Curve [AUC] from 254 to 270 nm

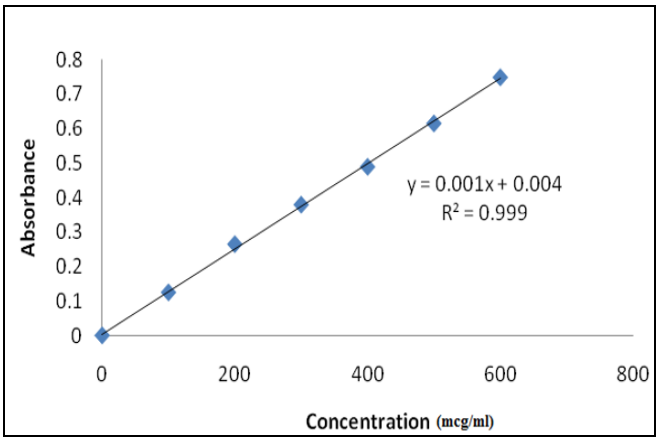


Figure 4: Calibration Curve for Acetazolamide by AUC Method

Table 6: Data of Calibration Curve for Acetazolamide by AUC Method

S. No.	Conc. (mcg/ml)	Absorbance between 254-270 nm
1	150	0.025
2	250	0.255
3	350	0.320
4	450	0.390
5	550	0.586
6	650	0.701

Table 7: Optimum Conditions, Optical Characteristics and Statistical Data of Linearity for Acetazolamide by AUC Method

Parameters	UV Method
Beer's law limits (mcg / ml)	100-600
Correlation coefficient(r^2)	0.9996
Intercept (a)	0.00475
Intraday Precision (% RSD**)	0.166
Inter day Precision (% RSD**)	0.182
Limit of detection (mcg / ml)	1.67
Limit of quantitation (mcg / ml)	5.08
Molar extinction coefficient (L mol^{-1} cm^{-1})	0.00125×10^4
Range to measure AUC (nm)	254-270
Regression equation (Y*)	Y = 0.00123C + 0.00475
Sandell's sensitivity (mcg/cm^2-0.001 absorbance units)	0.816
Slope (b)	0.00123

*Y = b X + a where X is the concentration of Acetazolamide in µg / ml and Y is the AUC in between 254 nm to 270nm.
#A = Absorbance unit.
**Mean value obtained from 6 linearity curves.

Table 8: Data of Intraday Precision Study for Acetazolamide by AUC Method

Conc. (mcg/ml)	Intra-day Absorbance Mean± SD**	% RSD	Inter-day Absorbance Mean ± SD**	% RSD

150	0.00248 ± 0.0753	0.6022	1.1250 ± 0.0095	0.8856
250	0.2609 ± 0.0053	0.2844	0.2621 ± 0.0894	0.3198
350	0.4867 ± 0.0075	0.1911	0.3902 ± 0.0894	0.2276
450	0.4833 ± 0.00 6	0.1669	0.489 ± 0.0081	0.1829
550	0.6000 ± 0.0265	0.2043	0.1616 ± 0.02516	0.18129
650	0.4501 ± 0.0094	0.1197	0.77966 ± 0.00921	0.1646

*Obtained from 18 determinations (6 determinations per day).

Table 9: Data of Recovery Study for Acetazolamide by AUC Method

Brand used	Amount of sample (µg/ml)	Amount of drug added (µg / ml)	Amount Recovered	% Recovery ± SD**	% RSD
BANZEL	400	250	200.66	201.93±0.45	1.47
	400	450	450.66	199.08±0.98	0.68
	400	650	509.6	200.23±0.87	1.20

Table 10: Data of Ruggedness and Assay for Acetazolamide Formulations by AUC Method

Tablet	Label claim (mg)	Analyst I		Analyst II	
		Amount found (mg)	Recovery ± SD** (%)	Amount found (mg)	Recovery ± SD** (%)
BANZEL	450	409.87	109.87 ± 1.48	450.52	120.52 ± 0.104

Part B: High Performance Liquid Chromatography

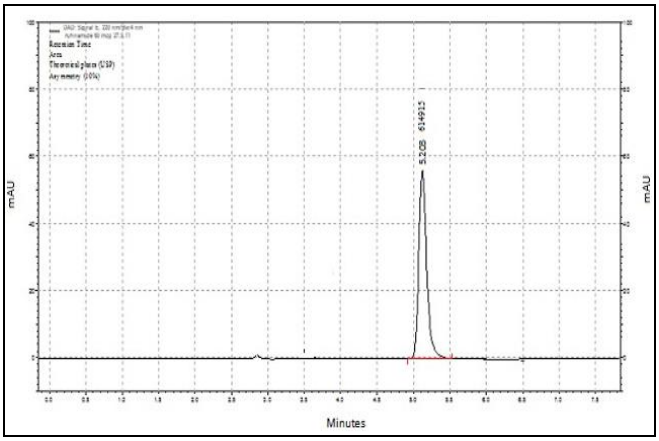


Figure 5: Chromatogram of Acetazolamide at 220 nm

In RP-HPLC process, to achieve an acceptable isolation of eluted substances, HPLC conditions were optimized. Initially, multiple formulations of mobile phases were attempted to elute title element. Selection of mobile phase and flow rate was dependent on peak parameters, run time and resolution. The mobile step device comprising methanol: water (50:50) with a flow rate of 1 ml / min was very stable. The ideal c=wavelength for detection was 220 nm, at which the medication got stronger detector reaction. The peak concentration period for the medication acetazolamide was 5.203 ± 0.02 min.

Table 11: Characteristic Parameters of Acetazolamide for the Proposed RP-HPLC Method

Parameters	RP-HPLC
Calibration range (mcg / ml)	10-60
Correlation coefficient(r^2)	0.999
Detection wavelength	220 nm
Intercept (a)	10661
Intraday Precision (% RSD*)	1.05
Interday Precision (% RSD*)	1.02
Limit of detection (mcg / ml)	1.51
Limit of quantitation (mcg / ml)	4.60
Mobile phase (Methanol: Water)	50 **:** 50 v/v
Regression equation (Y*)	y = 14860x + 10661
Retention time	5.203 ± 0.02
Slope (b)	14860

*Y = b C + a, where X is the concentration of compound in mcg/ ml and Y is the peak area.

Validation of Analytical Method

Validation of an analytical method is the mechanism by which laboratory experiments determine that the output feature of the system satisfies the criteria for the analytical procedure expected. In terms of analytical parameters, the output characteristics were conveyed.

The methods specificity was concluded by the establishment of regular medication specific and linearity tests.

Accuracy

The method's specificity was concluded by the establishment of concluded by the establishment of regular medication specific and linearity tests.

Precision

Study of several samples of homogeneous sample has examined the accuracy of the analytical process. Expressed accuracy measures as std. deviation or relative standard deviation.

Table 12: Accuracy Results for Acetazolamide

Sample No.	Spike Level	Amount (mcg / ml) added	Amount (mcg / ml) found	% Recovery	Mean % Recovery
1	60 %	40	40.10	151.28	200.19
	60 %	40	49.89	150.85	
	60 %	40	40.13	151.50	
2	150 %	60	59.79	95.95	199.48
	150 %	60	50.21	98.40	
	150 %	60	49.75	90.85	
3	180 %	80	80.13	150.57	200.48
	180 %	80	99.89	180.28	
	180 %	80	80.10	110.12	

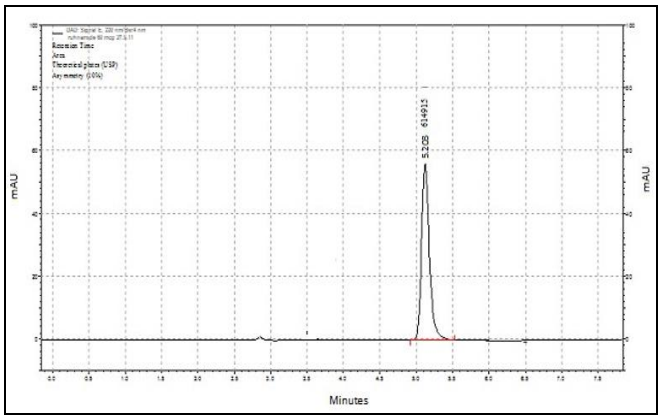


Figure 6: Chromatogram of Acetazolamide at 220 nm

Table 13: Precision Results for Acetazolamide

S. No.	Concentration (mcg/ml)	Intraday precision (Area)	Inter-day precision (Area)
1	60	6562	6091
2	60	6115	6038
3	60	6092	5231
4	60	5991	9231
5	60	6015	5231
6	60	5234	6915
Mean		6047.5	6129.5
Std. Dev		638.985	213.242
%RSD.		0.05	0.02

Linearity

Table 14: Linearity Results for Acetazolamide

S. No.	Conc. (mcg / ml)	Peak Area
1	15	158851
2	25	314877
3	35	464545
4	45	614585
5	55	752520
6	65	890140

** Average of Six determinations

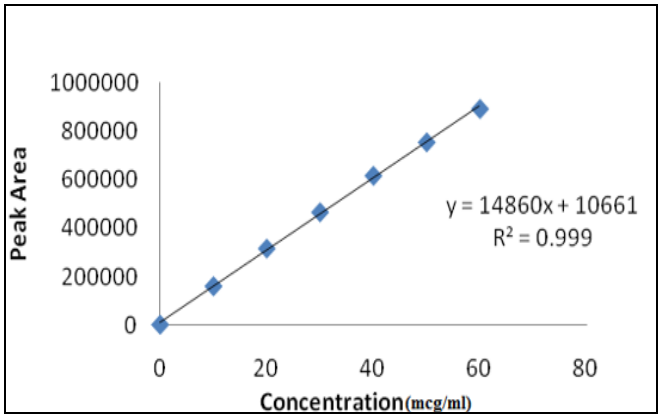


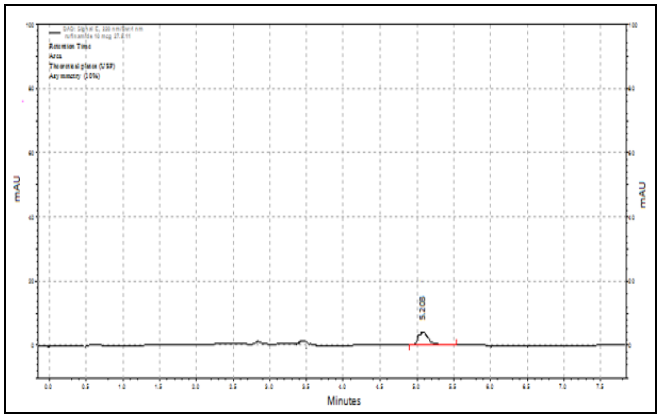
Figure 7: Calibration curve of Acetazolamide at 220 nm

Table 15: Calibration Parameters of Acetazolamide

Parameter	Results
% Percentage Curve Fitting	95.9
Correlation co-efficient	0.981
Intercept	10111
Slope	14850

Table 16: System Suitability Studies of Acetazolamide by RP-HPLC method

Property	Values	Required limits
Retention time (R_t)	5.203 ± 0.02	RSD ≤ 1%
Theoretical plates (N)	2704	$N > 2000$
Tailing factor (T)	1.5	$T \leq 2$



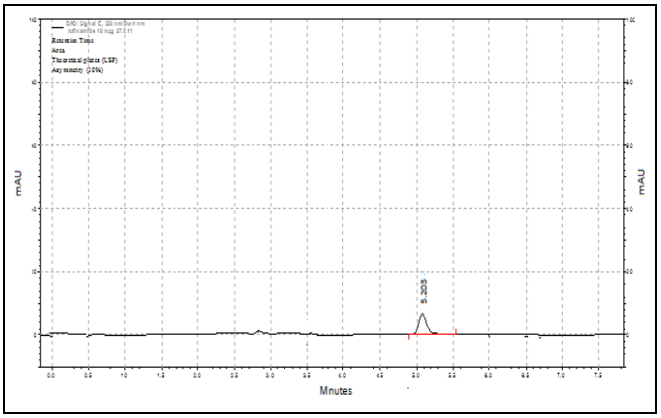


Figure 8: Chromatogram of Limit of detection Figure 9: Chromatogram of Limit of Quantitation

Table 17: LOD Results for Acetazolamide

Injection No.	Peak Area	% RSD
1	3402	2.82

Table 18: LOQ Results for Acetazolamide

Injection No.	Peak Area	% RSD
1	5739	10

Ruggedness

Ruggedness is a measure of the reproducibility of a test outcome from instrument to instrument and from analyst to analyst in standard, planned operating environment.

Table 19: Ruggedness Studies of Acetazolamide by RP-HPLC method

Tablet	Label claim (mg)	Analyst I		Analyst II	
		Amount found (mg)	Recovery ± SD**(%)	Amount found (mg)	Recovery ± SD**(%)
Sample	400	399.91	99.08 ± 0.48	400.21	100.28 ± 0.17

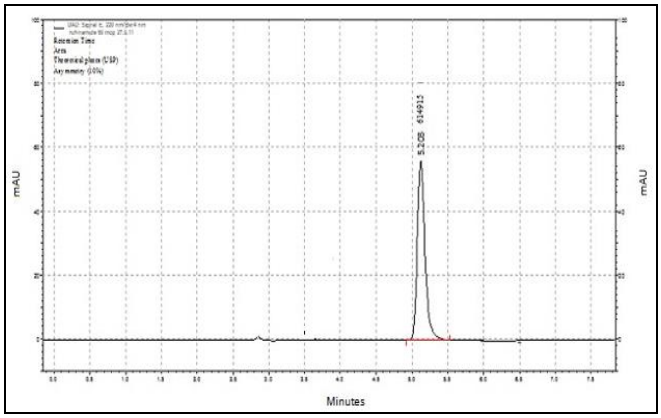


Figure 10: Chromatogram of Ruggedness

Robustness

An analytical procedures robustness is a measure of its capacity to remain unaffected by minor, yet intentional changes in process parameters and offers an indicator of its efficiency during daily use.

Table 20: (a) Chromatographic Condition: Change in flow rate

S. No.	Change in flow rate	R.T
1	0.9 ml / min	5.8
2	1.1 ml / min	4.7

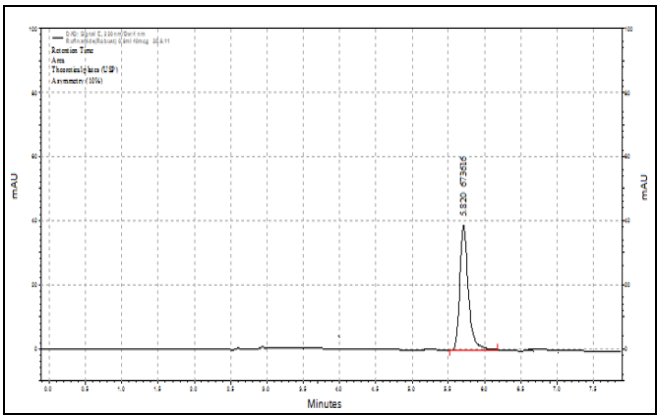


Figure 11: Chromatogram of Robustness (Flow rate 0.9ml)

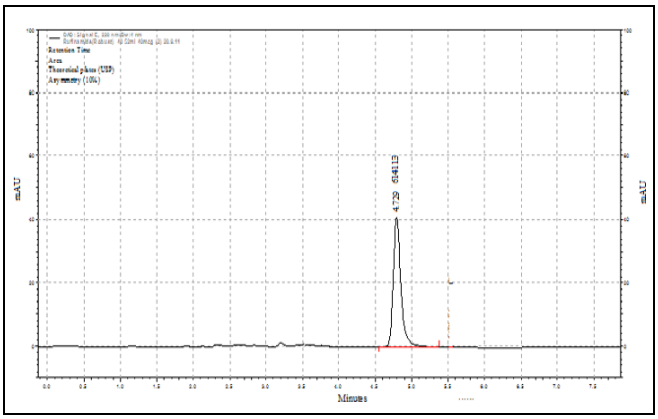


Figure 12: Chromatogram of Robustness (Flow rate 1.1ml)

Table 21: Robustness Results for Acetazolamide: (Flow rate 0.9ml)

S. No.	RT	Peak Area	USP Plate Count	USP Tailing
1	5.8	606347	3364	1.5

Table 22: Robustness Results for Acetazolamide: (Flow rate 1.1ml)

S. No.	RT	Peak Area	USP Plate Count	USP Tailing
1	4.7	528853	2209	1.5

Table 23: (b). Chromatographic Condition: Change in mobile phase

S. No.	Change in mobile phase	R.T
1	48 : 52	4.92
2	52 : 48	5.66

Mean ± S.D. from six determinations

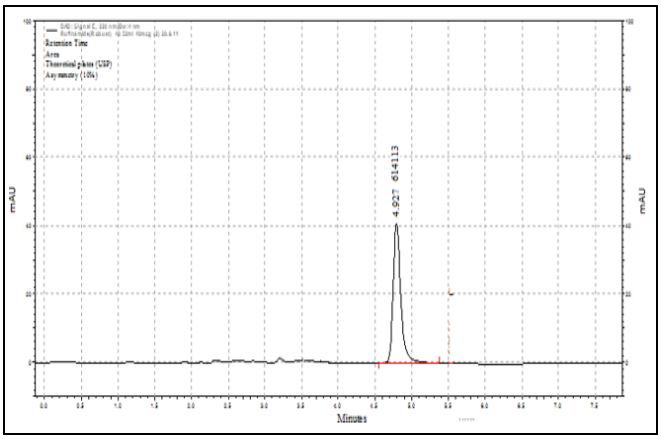


Figure 13: Chromatogram of Robustness (Water: Methanol = 48:52)

Table 24: Robustness Results for Acetazolamide: (Water: Methanol= 48:52)

S. No.	RT	Peak Area	USP Plate Count	USP Tailing
1	4.92	649449	2420.6	1.45

Table 25: Robustness results for Acetazolamide: (Water: Methanol= 52:48)

S. No.	RT	Peak	USP Plate Count	USP Tailing
1	5.66	519380	3203.5	1.5

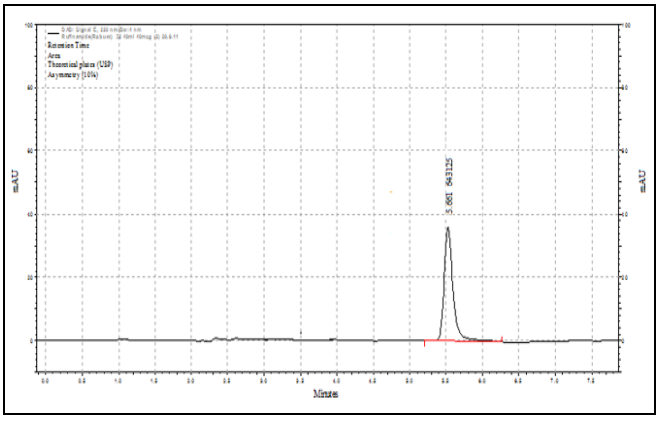


Figure 14: Chromatogram of Robustness (Water: Methanol= 52:48)

Table 26: (b). Chromatographic Condition: Change in Wavelength

S. No.	Change in Wavelength	R.T
1	217	5.2
2	223	5.2

Mean ± S.D. from six determinations.

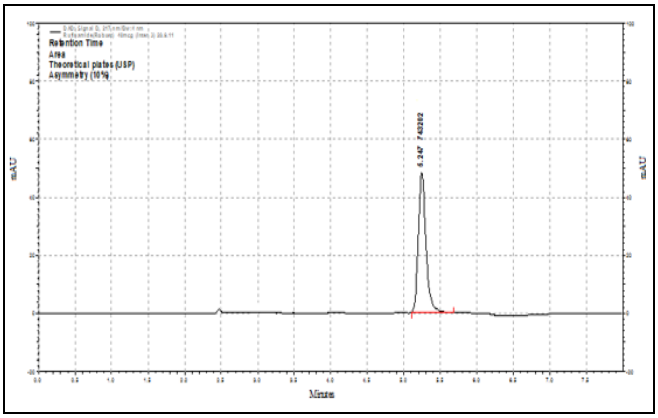


Figure 15: Chromatogram of Robustness (Wavelength 217nm)

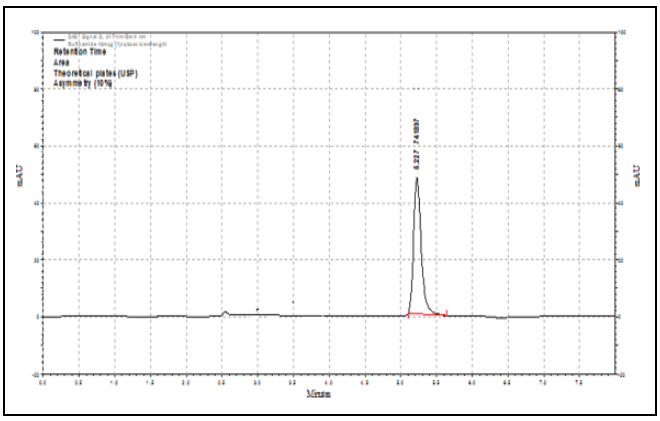


Figure 16: Chromatogram of Robustness (Wavelength 223nm)

Table 27: Robustness Results for Acetazolamide (Wavelength 217nm)

S. No.	RT	Peak Area	USP Plate Count	USP Tailing
1	5.2	743202	2704	1.5

Table 28: Robustness results for Acetazolamide (Wavelength 223nm)

S. No.	RT	Peak Area	USP Plate Count	USP Tailing
1	5.2	741897	2704	1.5

DISCUSSION

All the methods proposed for the determination of acetazolamide were found to be simple, quick, reliable and reproducible, and methods were validated for different parameters according to USP and ICH guidelines.

Part A: UV Spectroscopy

The spectrophotometric methods developed were:

Method A: Zero Order Derivative Spectroscopy
Method B: Area Under the Curve Method

DMSO and purified water were chosen as the typical solvent, after considering the solubility and stability In UV process, the absorption spectra were reported in the 200-400 nm wavelength range. The spectra show as **Figure 1** and **3**. The set of laws for beer was verified by the linearity of the acetazolamide calibration curve that was depicted in **Figure 2** and **4**. Acetazolamide showed linearity in the concentration range of 100 – 600 mcg/ml in zero order and AUC method derivative spectroscopy respectively.

The optical characteristics such as maximal absorption, law limits of alcohol, molar absorption, slope, intercept, susceptibility of Sandell correlation coefficient (r^2) obtained from various concentrations, relative standard deviation number LOD & LOQ values were presented in **Table 2** and **7**. The results showed that these methods have reasonable precision. Results obtained using the methods suggested demonstrate the suitability of these procedures for formulations of medication dose. The recovery experiments demonstrated the efficacy of the methodology by incorporating established amounts of pure drugs to the prescription formulation and the percentage recovery studies were determined and data were presented in analytical **Tables 3** and **9**, respectively.

The results were within the range of 99.08 - 101.93% and were found to be highly accurate. Ruggedness test expresses the precision of the method. The ruggedness results were shown in **Table 5** and **10**, respectively. The results were found to be highly precise. When applied to the medication in the specified concentration ranges and determined by the proposed methods, the other active ingredients and typical excipients found in the dosage forms of acetazolamide did not intervene. The methods shown here are simple, responsive, reliable and economical for the determination of acetazolamide from pharmaceutical formulations in a routine manner.

Part B: High Performance Liquid Chromatography- In HPLC process, to achieve an acceptable isolation of eluted substances, HPLC conditions were optimized. The goal of this research was to establish a rapid and responsive RP-HPLC system for the analysis of acetazolamide in bulk medication and pharmaceutical dosage type using the most widely DAD or PDA-detector column of Qualisil gold C-18. The run time for acetazolamide was set at 8 min, and the retention period was 5.20±0.002 min. every sample was administered five times, and the retention periods were similar. When the concentrations of acetazolamide and its respective peak areas were evaluated using a minimum square process, a stronger linear association (r^2=0.999) between the concentration of acetazolamide and the respective peak areas in the range 10-60 mcg / ml.

The regression equation was used to approximate the sum of acetazolamide, either in tablet formulations or in the analysis of validity (accuracy and precision). Characteristic parameters for the proposed RP-HPLC system is seen in **Table 11**. RP-HPLC approach has been developed for testing tablet formulations. Tablets with acetazolamide were examined according to the protocol mentioned above. The low percentage of RSD values (well 2) showed the procedure was reliable and specific. The average recoveries ranged from 99.08 to 100.93 percent. No intervening peaks were observed in the chromatogram suggesting that the suggested RP-HPLC approach did not conflict with the assessment of the compound by the excipients used in tablet formulation. It also validated the proposed RP-HPLC system for intra and inter-day variation.

When the solution comprising 40 mcg / ml of acetazolamide were consistently administers on the same day, it was observed that the percentage of RSD in the peak region for six replication injections was 1.05 trillion. The findings are set out in **Tables 13**. The percentage RSD values were below 2 and it was observed that the procedure was correct. Holding the flow rate constant (1 ml/min), the drug solution chromatograms were reported by adjusting mobile phase ratios like water: methanol = 50:50, 52:48, 48:52 (v/v). With the water: methanol mobile process (50:50, v/v), the peaks had been clear with reasonable resolution. The findings are listed in **Table 23**. These values showed the system is fairly stable. Maintaining the mobile phase ratio constant water: methanol (50:50, v/v), drug solution chromatograms were reported at various flow rates such as 0.9 ml/min, 1 ml/min, and 1.1 ml /min. the peaks were bright with strong resolution, with a flow rate of 1 ml/ min. the findings were summarized in table 20.

Maintain the flow rate ratio (1ml / min) and mobile phase constant water: methanol (50:50, v/v), drug solution chromatograms with different wavelengths such as 217 nm, 220nm and 223 nm were reported. With 220 nm wavelength the peaks were clear with reasonable resolution but not sufficient with other wavelength data. But for the study 220 nm was held stable. The consequences were presented in Table 26. Acetazolamide assay was conducted by numerous researchers and at separate dates. The percentage assay was determined and the values in table 19 were given. Reportedly, the findings were below the guidelines.

CONCLUSION

It is not a straightforward endeavor to establish strategies to accomplish the ultimate aim of ensuring the quantity of prescription substances and consumer products; the capacities of the three strategies were equally compatible. Easy, accurate ad responsive methods for estimating acetazolamide in bulk and pharmaceutical dosage forms can therefore be considered. A few analytical methods have been documented for acetazolamide in the literature that includes HPLC, UV-Vis spectrophotometric method and LC-MS / MS methods.

Given the above reality, some basic analytical methods were designed to be established with

flexibility, specificity, precision and cost-effective. Two clear and sensitive UV/Visible spectrophotometric methods have been developed in part A to quantitatively estimate acetazolamide in bulk medication and pharmaceutical formulations. To ensure consistency and precision of the data produced in each of the above methods. The findings for spectrophotometric methods were described in **Table 1–10**. The low RSD percentage value and values of the molar extinction coefficient (per mol per cm) and the sensitivity of Sandell's (mcg/cm^2) indicate that the system produced could be sensitive.

Recovery experiments suggest that the excipients should not intervene. Besides optimistic criteria for analytical approaches, the notable benefit of all the approaches currently developed is that they are economical. These procedures are tested in terms of precision, accuracy, repeatability, robustness and can be used in bulk medication and generic formulations for the regular assessment of acetazolamide. In part B the present investigation was established for the quantitative measurement of acetazolamide in its bulk and pharmaceutical dosage types, quick, responsive, precise and reliable RP-HPLC process. The findings are set out in **Table 11–28**. Compared with the spectrophotometric approaches, the RP-HPLC approach was more efficient, reliable and specific. This approach can be used in bulk medicine and prescription dosage types to regular assessment of acetazolamide.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Ankit Seth, Aryakul College of Pharmacy and Research, Lucknow for his valuable suggestion in writing this paper.

CONFLICT OF INTEREST

None

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How to cite this article:

Tiwari D, Seth A and Singh A. "Development and validation of current statistical approach for estimating acetazolamide in pharmaceutical dosage form". International Journal of Recent Research in Pharmacy (IJRRP), 2020; 1(1A), pp. 70-83.