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Spectrophotometric Determination of Acetazolamide in Bulk and Tablet Dosage Form by Area Under Curve and First Order Derivative Methods

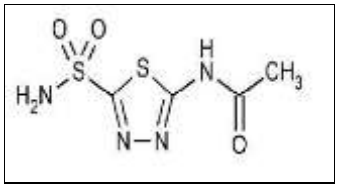
Dudhe P.B.*, Lahane A. V., Borhade K. D., Shelke P.S., Chavare P.D.

Department of Pharmaceutical Chemistry, Sinhgad College of Pharmacy, Vadgaon (Bk), Pune-411 041, India

Abstract : A simple, reproducible and economical two spectrophotometric methods have been developed for determination of acetazolamide in tablet dosage form. All solutions were prepared by using methanol as an solvent. Method A is area under curve (AUC), in which area was integrated in the range of 253.00 nm – 273.00 nm and in method B i.e. first order derivative spectroscopy, absorbance values were measured at λmin = 248.83 nm, λmax = 278.94 nm and λzero cross = 263.89 nm. For both methods linearity was established in the range of 5µg/ml - 30µg/ml (Method A: R^2 = 0.9991 and Method B: R^2 = 0.9991). validation studies were performed by ICH Q2(R1) guideline for method A and B. Accuracy, precision, assay, limit of detection (LOD) and limit of quantitation (LOQ) studies were done for both methods and results was found within acceptable limit. Proposed methods were applied successfully for determination of acetazolamide from pharmaceutical dosage form.

Introduction

Chemically acetazolamide (fig. 1) is N-(5-sulfamoyl-1,3,4 – thiadiazol – 2 – yl) acetamide. The molecular formula of drug is $C_4H_6N_4O_3S_2$ and molecular weight is 222.237 g/mol.[15]Acetazolamide used as an carbonic anhydrase inhibitor, diuretic and anticonvulsant. Acetazolamide potently inhibit carbonic unhydrase both membrane bound and in cytoplasmic form, resulting in nearly complete abolition of $NaHCO_3$reabsorption in the proximal tubule.[13] Acetazolamide is white to faintly yellowish in colour available in powder form having melting point in the range of258°C - 259°C.[2]



FigureNo.1-Chemical StructureofAcetazolamide

Acetazolamide is sparingly soluble in cold water. The drug is sensitive to light and stored between 15°C to 30°C in well closed container.[19] From literature survey of drug it is shown that only few RP – HPLC methods are available for its determination.[11,12,17,18] And there is no UV – spectrophotometric methods are

available for the quantification of acetazolamide so, the proposed methods are established for its determination from bulk and pharmaceutical dosage form.

Experimental:

Materials andMethods:

HPLC grade methanol was used to prepare solutions, Acetazolamide 250 mg tablets were purchased from pharmacy store in Pune. Shimadzu UV 1800 (Japan) with matched quartz cells, connected to computer loaded with UV Prob Software. Single pan electronic balance (Shimadzu, ATY 224) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonication (Spectralab UCB 40, India). Calibrated volumetric glasswares (Borosil) were used to perform study.

Method Development:

Preparation of Acetazolamide Standard Stock Solution:

Acetazolamide standard stock solution was prepared by weighing 10 mg of drug which was transferred into100 ml volumetric flask and volume was make up by methanol upto the mark. The resulting solution was of 100 µg/ml concentration, from this standard stock solution further solutions were prepared in the range of 5µg/ml to 30µg/ml.

Wavelength Selection:

Acetazolamide 15µg/ml working standard solution scanned between 400.00nm – 200.00 nm in UV spectrophotometer by using methanol as blank after baseline correction. 263.00 nm wavelength was selected for analysis.

Area Under Curve (AUC): Method A:

This method involves calculation of integrated value of absorbance with respect to wavelength in indicated range. Area calculation processing it emcalculates the area bounded by the curve and horizontal axis. Here horizontal axis represents baseline.

$$\text{Area calculation}(\alpha+\beta) = \frac{\lambda 1}{\lambda 2} Ad\lambda$$

Whereas, α is area of portion bounded by curved at a and a straight line connecting the start and end point, β is area of portion bounded by a straight line connecting the start and end point on curve data and horizontall axis, $\lambda 1$ and $\lambda 2$ are wavelengths representing start and end point of curve region. In this study area was integrated between wavelength ranges from 253.00nm - 273.00nm.

First Order Derivative Spectrophotometry: Method B:

Acetazolamide 5 to 30µg/ml solutions were prepared and scanned in spectrum mode from 400.00 nm – 200.00 nm. The first order derivative spectra were analysed and absorbance recorded at zero crossing wavelength i.e.263.89 nm. Calibration curve was constructed to obtain regression equation.

Preparation of Calibration Curve:

Suitable concentrations of acetazolamide in the range of 5, 10, 15, 20,25 and 30 µg/ml were prepared by using methanol as an solvent. For area under curve method above solutions scanned in the range of 253.00 nm – 273.00 nm and absorbance were recorded. Calibration curve plotted for absorbance against concentration to produce regression equation. For first order derivative method all solutions were analysed at zero crossing wavelength i.e.263.89 nm and absorbance were recorded and regression equation obtained.

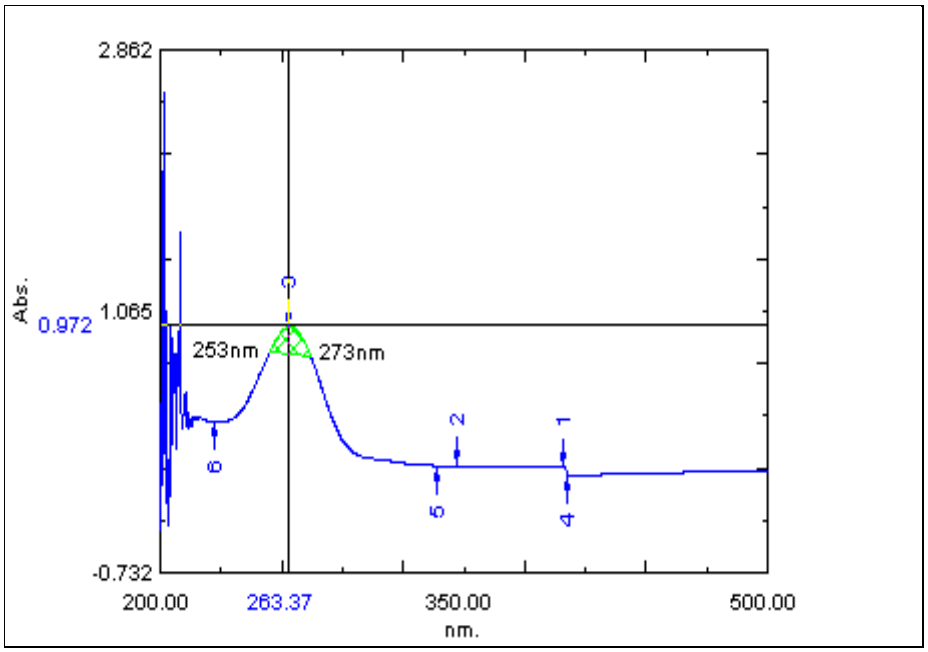


Figure No.2 : Area Under Curve Plot of 30 µg/ml Acetazolamide Solution.

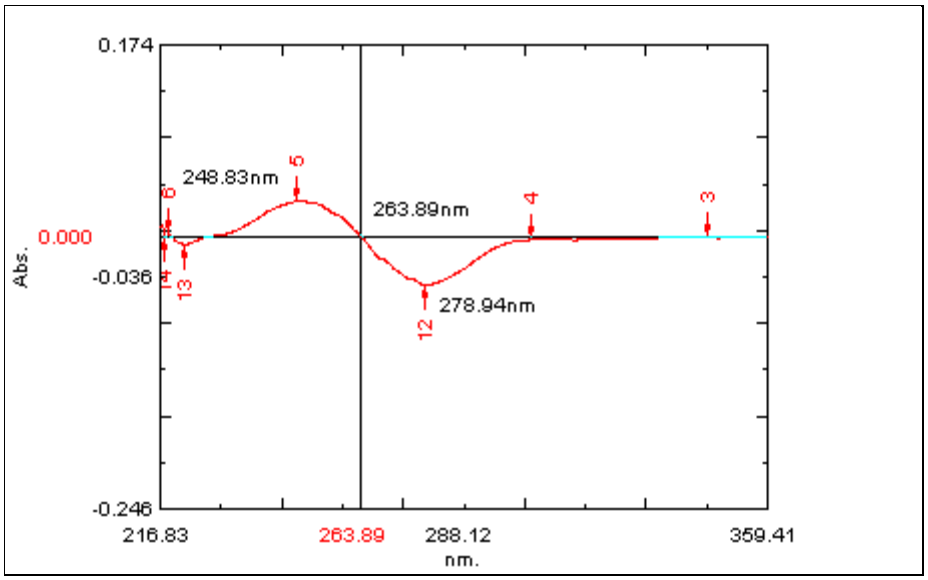


Figure No.3 : First Order Derivative Spectrum of Acetazolamide (30 µg/ml).

Assay of Acetazolamide (250 mg) Tablets:

Twenty tablets weighed and their average weight determined. Tablets were crushed into fine powder, from this 10 mg powder weighed and transferred into 100 ml volumetric flask. To this 70 ml of methanol was added and sonicated for 30 minutes to dissolve completely. After attaining room temperature volume was make up with same solvent, and shaked well to obtained homogeneous solution.

After discarding first 20 ml of solution tablet solution was filtered . Resulting solution was 100 µg/ml sample stock solution, which was further diluted with methanol to obtained working stock solutions. Working stock solutions were prepared in triplicate and scanned in 263.00nm.

Table No. 1: Assay of 250mg Acetazolamide Tablet:

Method	Label claim	Amount taken	Amount found (mg/tab)	Mean % Assay ±SD/%RSD (n=3)
A (AUC)	250mg	10 mg	10.00 mg	100.02% ±0.98
B (First Order Derivative)	250 mg	10 mg	9.944 mg	99.44%± 1.08

n =number of determinations, SD = standard deviation, % RSD = % Relative Standard Deviation.

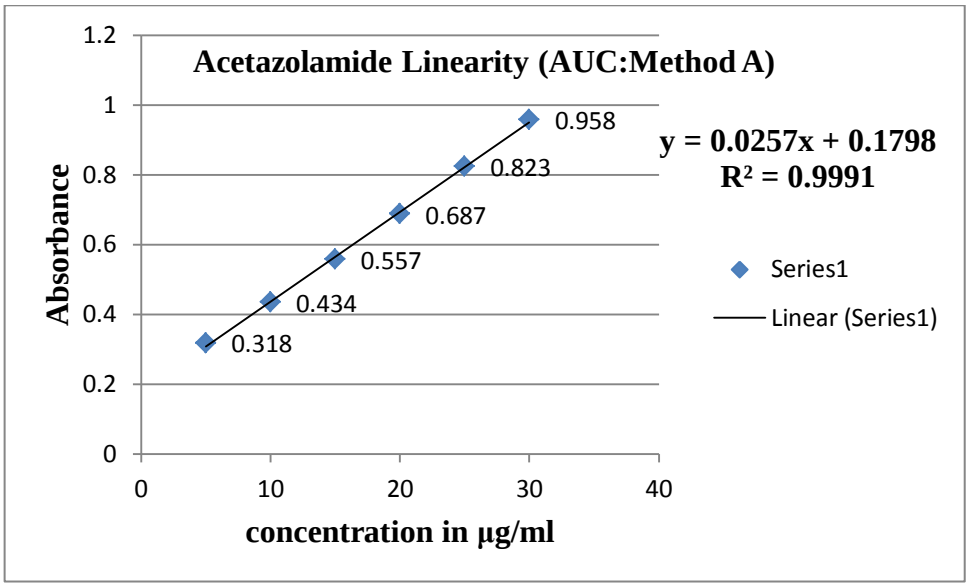


Figure No.4 : Calibration Curve of Acetazolamide: Method A

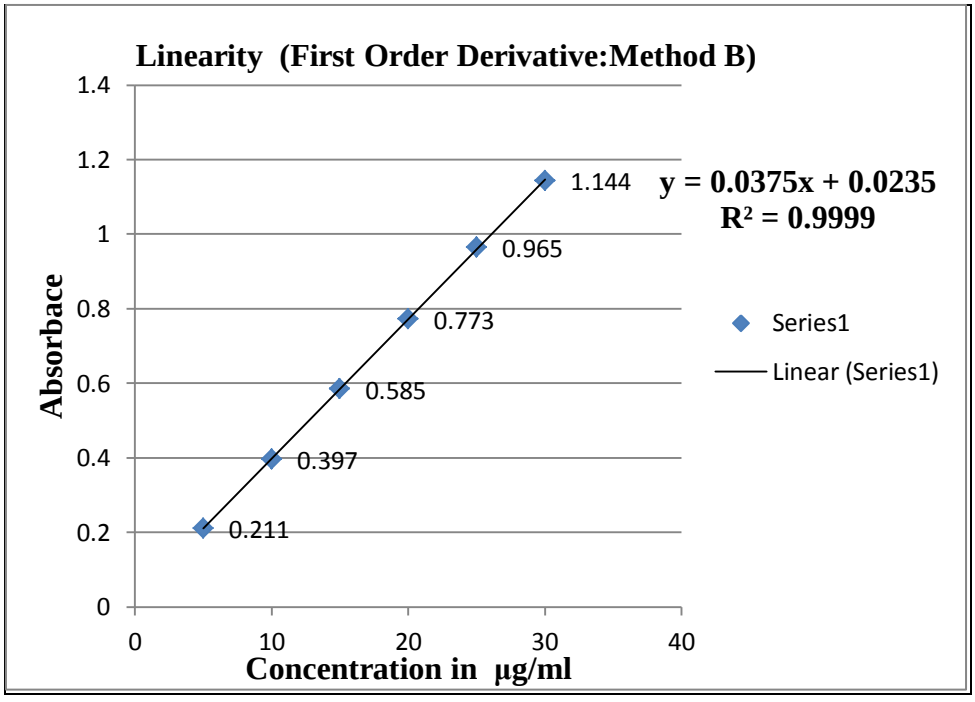


Figure No.5 : Calibration Curve of Acetazolamide: Method B

Analytical Method Validation:

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its Intended purpose. Present method was validated according to ICH Q2 (R1) guideline for range, linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ).

Linearity and Range:

Solutions of acetazolamide 5 – 30µg/ml were prepared and analysed at 253.00 nm – 273.00 nm for area under curve method and at 263.89 nm zero crossing wavelength for first order derivative method. The absorbance were plotted against concentration to establish regression equation for both methods.

Method Precision:

Repeatability:

Six solutions of 10 µg/ml acetazolamide were prepared and analysed by area under curve and first order derivative method at 253.00 nm–273.00 nm and 263.89 nm wavelength respectively. Percent relative standard deviation (%RSD) calculated and reported in table no. 3.

Table No. 2: Calibration Data of Acetazolamide:

Concentration (µg/ml)	Absorbance: MethodA (Area Under curve)	Absorbance: Method B (First Order Derivative)
5	0.318	0.309
10	0.434	0.418
15	0.557	0.557
20	0.687	0.669
25	0.823	0.803
30	0.958	0.915

Intermediate Precision (Reproducibility):

The intraday and interday precision was performed by analysing 15 µg/ml, 20 µg/ml and 25 µg/ml solution of acetazolamide in triplicate for each concentration on same day and different days respectively. Percentrelative standard deviation determined and reported in table no. 3.

Table No. 3: Results of Acetazolamide Precision:

Precision	Method A (% RSD)	Method B (%RSD)
Repeatability(n=3)	1.75	1.31
Intraday (n=3)	1.27	0.82
Interday (n=3)	0.74	0.68

n =number of determinations, % RSD = % Relative Standard Deviation.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The calibration curves were plotted for six sets of 5 - 30µg/ml solutions of acetamide. Standard deviation was calculated and put into LOD and LOQ formula to get results.

$$LOD = 3.3 \times \frac{SD}{S}$$

$$LOQ = 10 \times \frac{SD}{S}$$

Where, SD is standard deviation of y-intercept of the calibration curves, S is mean slope of six calibration curves.

Table No.5: Results of LOD and LOQ:

Method	Method A (Area Under Curve)	Method B (First Order Derivative)
LOD(µg/ml)	1.28	1.34
LOQ(µg/ml)	3.89	4.06

Accuracy:

Acetazolamide solutions were prepared in triplicate for each 80%, 100% and 120% level of accuracy. Absorbance were measured by both methods by using their specific wavelength range. Percent recovery and percent relative standard deviation determined and tabulated in table no. 4.

Table No.4: Acetazolamide Recovery Data:

Test sample (µg/ml)	Accuracy Level (%) (n = 3)	Amount of standard drug added (µg/mL)	Mean % Recovery ± SD/%RSD (Method A)	Amount of standard drug added (µg/mL)	Mean % Recovery ± SD/%RSD (Method B)
10	80	8	99.90 ± 0.99	8	99.71 ± 1.53
	100	10	99.25 ± 0.70	10	99.90 ± 0.77
	120	12	99.49 ± 0.71	12	99.75 ± 1.10

n =number of determinations, SD = standard deviation, % RSD = % Relative Standard Deviation.

TableNo.6: Results for Validation Parameters of Acetazolamide by UV – Spectroscopic Method:

Validation Parameter	Method A (Area Under Curve)	Method B (First Order Derivative)
Range	253.00 nm – 273.00 nm	263.89 nm
Linearity range	5 – 30 µg/ml	5 – 30 µg/ml
Regression equation ($y = mx + c$)	0.0257 x + 0.1798	0.0246 x + 0.1821
Slope (m)± SD	0.0257 ± 0.00024	0.0246 ± 0.001
Intercept (c)± SD	0.1798 ± 0.010	0.1821 ± 0.011
Correlation coefficient (R^2)± SD	0.9991 ± 0.00079	0.9991 ± 0.0024
Repeatability (n=6) (% RSD)	1.75	1.31
Intraday(n=3)(% RSD)	1.27	0.82
Interday(n=3)(% RSD)	0.74	0.68
Accuracy (Mean % Recovery)	99.54	99.78
LOD (µg/ml)	1.28	1.34
LOQ (µg/ml)	3.89	4.06

n =number of determinations, SD = standard deviation, % RSD = % Relative Standard Deviation.

Result and Discussion:

Two new methods i.e. area under curve (AUC) and first order derivative spectroscopy were attempted to develop for determination of acetazolamide from pharmaceutical dosage form. The regression equation for both methods are as follows:

Method A: AUC $= \int_{253}^{273} Ad'\lambda 0.0257x + 0.1798$ $R^2 = 0.9991$

Method B: First Order Derivative $= \frac{d'A}{d'\lambda}$ $0.0246x + 0.1821$ $R^2 = 0.9991$

Where, $\int_{253}^{273} Ad'\lambda$ is area under curve between 253.00nm – 273.00nm, $\frac{d'A}{d'\lambda}$ is amplitude difference, x is concentration and R^2 is correlation coefficient. The both methods are found to be linear as R^2 values was 0.9991 for method A and 0.9991 for method B. Percent relative standard deviation for both methods found within limits which indicates that the present methods are precise.

At each 80%, 100% and 120% level percent recoveries found to be 99.54% and 99.78% for method A and method B respectively, which shows that both methods are accurate. For tablet dosage form of acetazolamide assay was found to be 100.02% and 99.44% for method A and B respectively. The LOD and LOQ values were found to be 1.28µg/ml and 3.89µg/ml respectively for AUC method, and 1.34µg/ml and 4.06µg/ml respectively for first order derivative method. From all test results it can be says that the proposed methods can be employed for the routine analysis of acetazolamide from pharmaceutical dosage form. Results of validation parameters are summarised in table no. 6.

Conclusion:

The area under curve (AUC) and first order derivative methods are successfully developed and validated by ICH Q2 (R1) guidelines for analysis of acetazolamide from pharmaceutical tablet dosage form. As prior to present methods there was no methods were available for determination of acetazolamide by UV – Spectrophotometry method. So, these two methods can be employed for analysis of acetazolamide by UV – spectrophotometry.

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