INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.6 | Issue 3 | July - Sep -2017
Journal Home page: www.ijpar.com

Research article *Open Access*

Determination and validation of acetazolamide tablets 250mg by UV double beam spectrophotometry

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ABSTRACT

Analytical monitoring of a pharmaceutical product or of specific ingredients within the product is necessary to ensure its safety efficacy throughout all phases of its shelf life. Such monitoring is in accordance with the specifications elaborated during product development. Analytical validation is the corner stone of process validation without a proven measurement system it is impossible to confirm whether the manufacturing process has done what it purports to do. All new methods developed are validated. Determination of Acetazolamide in a fixed dosage form was carried out by UV Spectrophotometric method. The absorbance values were observed for different dilutions of drug at 263.00 nm and which are used for the dilution in Ethanol. This method obeys Beer's Lambert's Law in the concentration range of 1-5µg/ml. The results have been validated statistically and the recovery studies confirmed the accuracy of this proposed method.

Keywords: UV -Ultraviolet visible, µg -Micro gram, ml- Milliliter, nm- Nano meter, GIT -Gastro intestinal tract, R_S ..Resolution, CSF - Cerebro spinal fluid.

INTRODUCTION

The primary objective of validation is to form a basis for written procedures for production and process control which are designed to assure that the drug products have the identity, strength, quality and purity they purport rare represented to process. Quality, safety and efficacy must be designed and built into the products. Each step of the manufacturing process must be controlled to maximize the probability that the finished product meets all quality and design specifications.

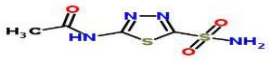
Analytical monitoring of a pharmaceutical product or of specific ingredients within the product is necessary to ensure its safety efficacy throughout all phases of its shelf life. Such monitoring is in accordance with the specifications elaborated during product development. Analytical validation is the corner stone of process validation without a proven measurement system it is

impossible to confirm whether the manufacturing process has done what it purports to do. All new methods developed are validated.

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According to ICH typical analytical performance characteristics that should be considered in the validation of the types of methods are

- Accuracy
- Precision
- Specificity
- Detection limit
- Quantization limit
- Linearity
- Range
- Robustness
- System suitability

DRUG PROFILE OF ACETAZOLAMIDE



IUPAC NAME: N-[5-Sulfomyl 1, 3, 4 thiadiazole 2 yl] acetamide
MOLECULAR FORMULA: C4H6N4O3S2

STRUCTURE METABOLISM

- Acetazolamide is a potent carbonic anhydrase inhibitors, effective in control of fluid secretion in the treatment of certain convulsive disorders.
- Acetazolamide is not a mercurial diuretic; rather it is a nonbacterioststic sulphonamides.
- EYE: It helps in the production of aqueous humour and reduces the intraocular pressure.
- CNS: It decreases the cerebral spinal fluid (CSF) formation and has antiepileptic action.

MACHANISM OF ACTION

The enzyme carbonic anhydrase is present in the kidney, gastric mucosa, pancreases, eyes, CNS and RBC.

It catalyses the following reaction:

$$H_2CO_3 \rightleftharpoons H_2O + CO_2$$

H_2CO_3 is then split in to H^+ and HCO_3^-Making H^+ available for Na^+ reabsorption.

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

The mechanism of diuresis involves proximal tubule of the kidney the enzyme carbonic anhydrous helps in the reabsorption of bicarbonate, sodium and chloride.

By inhibiting this enzyme these ions are excreted along with excess water, lowering blood pressure, intracranial pressure and intraocular pressure.

By excreting bicarbonate, the blood becomes acidic, causing compensatory hyperventilation with deep respiration increasing level of O_2 and decreasing level of CO_2 in blood.

MATERIALS AND METHODS

Drug sample

Acetazolamide tablets 250 mg.
Pure powdered Acetazolamide**.**

Chmicals and reagnts

Ethanol

Instruments used

PC Based Double Beam Spectrophotometer-2202, Mfd: Systronics.
PC Based Double Beam Spectrophotometer UV - 1800, Mfd:Shimadzu Sonicator Suction pump

METHODS

UV Spectrophotometric determination using Ethanol as a solvent.

Preparation of standard and test solution

10 mg of pure Acetazolamide drug sample was accurately weighed and transferred to 10 ml of standard volumetric flask. The drug was dissolved in Ethanol and finally the volume was made up to 10 ml with Ethanol. From the above solution take 0.1 ml solution and make up the volume with Ethanol up to 10 ml. From this solution 1 µg /ml to 5 µg/ml was prepared in standard flask.

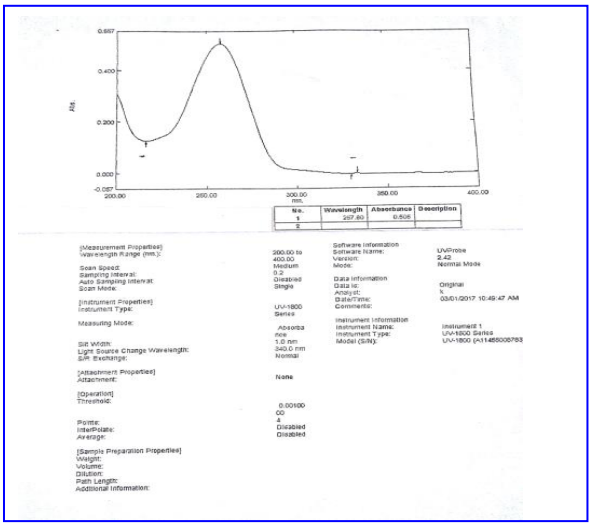
Assay, precision study, linearity.

Standard Graph Absorbance value

S. NO	CONCENTRATION (µg/ml)	ABSORBANCE AT 263 nm
1	0	0
2	1	0.26
3	2	0.44
4	3	0.63
5	4	0.80
6	5	0.99

λMax(maximum wavelength)

The absorption spectral data shows that the maximum absorbance was observed at 263 nm scanned between 200 to 400 nm



PREPARETION OF DRUG SOLUTION

Preparation of standard/sample Acetazolamide solution:

In a 10 ml volumetric flask, 10 mg of standard Acetazolamide was accurately weighed and dissolved in Ethanol the volume was made up to 10 ml with Ethanol to get concentration 1mg/ml. Quantity of tablet powder equivalent to 0.1 g of drug made up to 100 ml in Ethanol the volume was made up to 10 ml with Ethanol to get concentration 10µg/ml.

Determination of absorption maxima

In spectrometric analysis determination of absorption maxima is necessary for the method to be accurate and reproducible.

Instruments

PC Based Double Beam Spectrophotometer UV-1800, Mfd:Shimadzu.
PC Based Double Beam Spectrophotometer 2202, Mfd: systronics

Reagents and chemicals

Standard Acetazolamide stock solution (1mg/ml)
Ethanol

Procedure

Take the solution in the concentration range of 1,2,3,4,5µg/ml and blank without drug. The solution was scanned in the wavelength range of 200-400 nm.

Result

After scanning the absorption maxima of chromophore was found to be 263nm.

ACCURACY
ASSAY OF ACETAZOLAMIDE
PROCEDURE

Test solution

Weigh and powder 20 tablets. Weigh accurately quantity of the powder equivalent to 0.1gm of Acetazolamide 250 mg, add small quantity of Ethanol, shake well and sufficient Ethanol to produce 100 ml. Mix well sonicate and filter dilute 1ml of solution to 10 ml with ethanol and further dilute 1 ml of solution to 10 ml with Ethanol. Measure the absorbance of the resulting solution at about 263 nm, calculate the content of $C_4H_6N_4O_3S_2$. Taking

Standard solution

Weigh accurately quantity of the powder equivalent to 0.1gm of standard Acetazolamide powder with small quantity of Ethanol, shake well and sufficient Ethanol to produce 100 ml. Mix well sonicate and filter dilute 1ml of solution to 10 ml with ethanol and further dilute 1 ml of solution to 10 ml with Ethanol. Measure the absorbance of the resulting solution at about 263 nm, calculate the content of $C_4H_6N_4O_3S_2$. Taking

PRECISION
INTRA ASSAY PRECISION STUDY METHOD

UV spectrophotometric method

Instrument

PC based double beam UV spectrophotometer 1800, Mfr, systronic

Procedure

From the stock solution 100mg of pure acetazolamide 250 mg and dilute up to 100ml by using ethanol. From the solution 1 ml were taken in to 10 ml standard flask, prepare 2 and 4 µg/ml.

The percentage RSD of acetazolamide by the method of intra assay precision study was found to be
2µg/ml = 0.27
4µg/ml = 0.12

INTERMEDIATE OR INTRA ASSAY PRECISION STUDY

Method

UV spectrophotometric method.

Instrument

PC based double beam UV spectrophotometer 1800, - Mfr: systronics.

Procedure

From the stock solution of pure acetazolamide was diluted to 100mg in the 100ml with ethanol. From solution pipette out 2ml and 4ml of drug solution was taken in another two standard flasks and diluted up to 10 ml (2µg/ml &4µg/ml).

REPORT

The percentage RSD of acetazolamide by the method of inter assay precision study found to be

1st DAY

2µg/ml =0.12
4µg/ml =0.12

3rd DAY

2µg/ml =0.18
4µg/ml =0.11

6th DAY

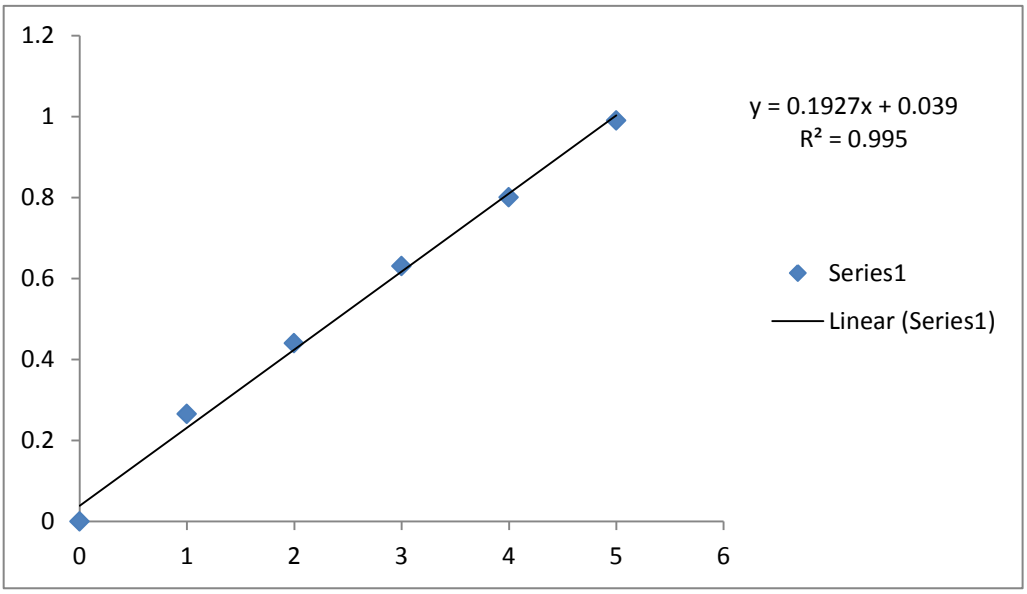
2µg/ml =1.2
4µg/ml = 1.8

9th DAY

2µg/ml =0.12
4µg/ml =0.11

LINEARITY AND RANGE

The linearity of the method was verified at 1 to 5µg/ml concentrations, but linearity was found to between 1 to 5µg/ml concentrations. The calibration graph were obtained by plotting the absorbance vs. the concentration data and were treated by linear regression analysis. The equation of calibration curve for acetazolamide obtained y=0.192x, the calibration curve was found to be linear in the above mentioned concentrations. The correlation coefficient (r^2) of determination was 0.995 Range from 20 to 140% of the test concentration



S.NO	CONCENTRATION µg/ml	ABSORBANCE
1	1	0.26
2	2	0.44
3	3	0.63
4	4	0.80
5	5	0.99

RUGGEDNESS

The Ruggedness of an analytical method is determined by aliquots from homogenous lots by analyst at different environment conditions that may differ but are also within the specific parameter of the assay the degree of reproducibility determined as function of the assay variables.

Mixed working reference standard solution and sample solution of Acetazolamide Were prepared by different analyst and on different days and the spectrum was recorded.

RESULT AND DISCUSSION

Literature survey cited a few spectrophotometers. HPTLC, HPLC, GC for the estimation of acetazolamide. The drug is assay by official method, with ethanol in U.S.P attempts were made to develop sensitive reproducible & economical method for the estimation of acetazolamide in tablet & bulk formulation.

Accuracy of the method was performed with concentration of 80% to 120% and percentage recovery was calculated and found to be the within the limits of 80% to 120% RSD is also less than 2.

Assay of method precision (intra-day precision) was evaluated by carrying out two independent assays of test sample of acetazolamide with 2µg/ml, 4µg/ml the intermediate precision of the method was also evaluated using five different analysts system & different days in the same laboratory the relative standard deviation (RSD) & assay values obtained by the analyst were respectively.

The linearity of the method was verified at 1-5µg/ml concentration. The calibration graphs were obtained by plotting the absorbance versus the concentration date & were treating by linear regression analysis. The equation of the calibration curve for acetazolamide obtained y=0.192x the calibration curve was found to be linear in the aforementioned concentration R^2= 0.995

Ruggedness was performed system to system analyst to analyst to study variability conducted on different uv systems four samples were prepared and each was analyzed as per test method. The relative standard deviation for Acetazolamide was found to be below 2 percent on the system and analyst

CONCLUSION

The development and validation of a new analytical method may therefore be an iterative process. Results of validation studies may indicate that a change in the procedure is necessary, which may then require revalidation. During each validation study, key method parameters are determined and the n used for all subsequent validation steps. To minimize repetitious studies and ensure that the validation data are generated under conditions equivalent to the final procedure, we recommend the following sequence of studies. The developed method was found to be simple sensitive, accurate, reproducible and can be used for routine quality analysis of acetazolamide 250 mg in bulk and pharmaceutical formulations.

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