Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RELATED SUBSTANCE METHOD FOR ACETAZOLAMIDE TABLETS

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ABSTRACT

Acetazolamide is the prototype of new class of antiepileptic drugs. Drugs which is carbonic anhydrase inhibitors used in mainly as an antiepileptic. The present work deals with development and validation of related substance method development through stability indicating of acetazolamide tablets, an adequate simple, precise, selective, economic High performance liquid chromatographic method has been developed then validated for identification and analysis purpose. Reversed phase chromatography was performed on a C-18 column with Phosphate buffer and acetonitrile in the ratio as mobile phase with a flow rate 1.0 ml/minute. 265nm wavelength shown adequate peak shape and height at retention time approximately 7.8 minutes. The % RSD of peak area response of acetazolamide in LOQ level concentrated shown less than 2%. The chromatographic method was validated in many parameters such as specificity, accuracy, precision, robustness, reproducibility according to ICH guidelines. Statistically found that method was qualify with all validation parameters on acetazolamide tablets. This method was suitable for routine analysis because proved that wide linearity range covered, sensitive, shorter retention time, simple mobile phase as well

INTRODUCTION

The RP-HPLC method developed for quantitative and qualitative analysis of Acetazolamide tablets was rapid, simple, accurate, precise and specific. Here conclude that this method is one of best method available in market because it is economically proved. Recovery study on tablet formulate ion gave accurate prediction of good relation between label claim and practical value.

Method is specific due to it shows no interference in analyte peak of any unknown or known compounds [1]. Method was validated as per USP acceptance criteria and ICH guidelines. Hence present validated method can be confidently utilize as an alternative method to reporting of result in routine practice or release of product report in quality control compliance acetazolamide is the prototype of new

class of antiepileptic drugs used for the prevention of high altitude pulmonary edema (HAPE), acute mountain sickness (AMS), high altitude cerebral edema (HACE), also commonly used as anti-epileptic [2]. Acetazolamide is good medicine due to its mechanism of action to inhibit carbonic anhydrase enzyme which increase the Concentration of respiratory alkalosis. These process facilitate the excretion of bicarbonate in the urine [3]. Acetazolamide here prevent high altitude disorders. ICH guideline internationally explained one thing that is impurity. Impurity is not an active pharmaceutical part. Impurity may reduce the purity of acetazolamide because it is a chemically related to acetazolamide, it may or may not also affect the action of main analyte [4]. Here we get that any extraneous part present in material is called as drug substance also has to be consider an impurity even it is biologically inert now a day's drug safety is the major concern due to mast pharmaceutical ingredients produced by organic synthesis. Impurity profile study in pharmaceutical proved its importance during safety attention on public and media domain. Recent updates on impurity profile from books and many journals address this aspect and available guideline of USFDA and other international authority [5]. At the time of organic chemical synthesis many type of components occur including trace amount of inorganics, organics compounds, and residual solvents. Some components which is present in final active pharmaceutical ingredients consider as impurity.

Commonly sources of impurity are from starting material, residual solvents, degradation product also occur in long term storage. [6-8]

MATERIAL AND METHOD

In RP-HPLC method, chromatographic separation was achieved on Zorbax SB, C-18, 250 × 4.6 mm; 5 micron) column using Buffer and Acetonitrile in the proportion of 90:10 v/v as the mobile phase with detection at 265 nm, Flow rate 1.0mL/min ,Column Temperature: 25°, Injection Volume: 25 μL, Diluent Acetonitrile :Water (10:90)v/v. Both the drugs were subjected to acid, alkali, oxidative, thermal and photolytic stress conditions individually whereas tablet formulation was % degradation found in Acid degradation, Base degradation and Peroxide degradation**.** Peak purity for Acetazolamide and all known impurities is passing in all degradation conditions. Mass balance achieved. [9-12]

Preparation of standard stock solution

Weigh accurately about 20.0 mg of Acetazolamide working standard and transfer in to 100 ml of volumetric flask. Add 40ml diluent, sonicate to dissolve and make up volume with diluent. This was standard stock solution having 200 μg/mL Pipette out 10 ml of this solution and transfer in to 100 ml of volumetric flask and dilute to volume with diluent. Further dilute 4 ml of this solution to 100 ml with diluents. This was standard solution having 0.8 μg/mL of Acetazolamide. [13-14]

Preparation of Test solution: (400ppm)

Weigh 20 tablets and crush finely. Weigh and transfer accurately the quantity of test sample equivalent to 40.0 mg of Acetazolamide in to 100 ml of volumetric flask. Add about 60 ml diluent, sonicate for 20 minutes with intermittent shaking (at temperature below 25ºC). Make up the volume with diluent and mix. Filter the solution with 0.45µ nylon filter. Discard the 5-6 ml of the filtrate and collect remaining filtrated solution.

Preparation of placebo solution

Weigh and transfer accurately the quantity of placebo powder equivalent to 40.0 mg of Acetazolamide in to 100 ml of volumetric flask. Add about 60 ml diluent, sonicate for 20 minutes with intermittent shaking (Maintain the sonicator temperature below 25ºC). Make up the volume with diluent and mix. Filter the solution with 0.45µ nylon filter. Discard the 5-6 ml of the filtrate and collect remaining filtrated solution. [15-16]

Preparation of impurity stock solution

Weigh and transfer 1.0 mg of each impurity (A, B, C, D, E & F) in individual 10 ml volumetric flask, dissolve and dilute with diluent.

Preparation of resolution Solution:

Weigh accurately about 40.0 mg of Acetazolamide working standard in to 100 ml of volumetric flask. Add about 30 ml of diluent and sonicate to dissolve. Add 0.8 ml of each impurity stock and dilute up to the mark with diluent.[17]

VALIDATION OF DEVELOPED HPLC METHOD

LINEARITY AND RANGE

The linearity of Acetazolamide and All Known impurity were found between LOQ to 150%. The calibration data is presented in Table and correlation coefficient and regression line equation analysis presented in Figure.

SPECIFICITY

Specificity is the term extent that analyte may be exist without interference from other related compound in a mixture. Specificity able to differentiate all possible impurities by applying forced stress testing. When in any chromatogram analyte peak not affected from other known or unknown impurity it indicates that chromatographic parameters good.

1. No peaks should be eluted at the retention time of Acetazolamide and all known impurities in blank and placebo solution.

2. Peak purity should be passing for Acetazolamide peak in standard solution.

3. No interference of any known impurity with the analyte peak.

4. Peak purity should be passing for Acetazolamide peak and all known impurity peaks. [18]

FORCED DEGRADATION STUDY

% Degradation of Acetazolamide and all known impurity in various stress condition are shown in table. From degradation study it was found that total impurity was marginally degraded in acidic,

oxidative and peroxide condition and stable in photolytic and thermal conditions. [19]

PRECISION METHOD PRECISION (REPEATABILITY)

Repeatability was determined by analyze in Prepared Resolution solution as per methodology and injected. Prepared Standard solution as per methodology and injected. Prepared six Sample solutions (Unspiked sample) as per methodology and injected. Prepared six sample solutions spiked with known impurities A at 0.2% each (viz., Impurity A, impurity B, Impurity C, Impurity D, Impurity E and Impurity F) of Test concentration & analyzed as per the methodology. [20]

CONCLUSION

The suitable chromatographic methods (RP-HPLC, HPTLC) were developed and validated for estimating a simple, economic, selective, and precise. HPLC method for stability indicating approach has been validated and developed for routine or specific analysis of acetazolamide tablets and its related compounds. Reversed phase chromatography was performed on a C-18 column with Acetonitrile: Phosphate Buffer in mobile phase with flow rate 1.0 ml/minute, spectra recording was performed on wavelength 265nm, occur adequate peak response and height at approximately 7.8 minutes retention time. The % RSD of peak area response of acetazolamide in LOQ level concentrated shown less than 5%. The chromatographic method was validated in many parameters such as specificity, accuracy, precision, robustness, reproducibility according to ICH guidelines. Statistically found that method was qualify with all validation parameters on acetazolamide tablets. This method was suitable for routine analysis because proved that wide linearity range covered, sensitive, shorter retention time, simple mobile phase as well. The pharmaceutical analyst plays in a major rule in assuring identity, safety, efficacy, purity, and quality of a drug product. New methods are now being developed with a great deal of consideration to worldwide harmonization. As a result, new products can be assured to have comparable quality and can be brought to international markets faster.

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EXPERIMENTAL TABLE AND FIGURE

Table No. 1: Preparation of Linearity levels

Linearity Level	Volume from Linearity stock solution in mL							Final Dilution with Diluent (mL)
	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F	Acetazolamide	
20%	0.4	0.4	0.4	0.4	0.4	0.4	0.8	100.0
50%	1.0	1.0	1.0	1.0	1.0	1.0	2.0	100.0
80%	1.6	1.6	1.6	1.6	1.6	1.6	3.2	100.0
100%	2.0	2.0	2.0	2.0	2.0	2.0	4.0	100.0
120%	2.4	2.4	2.4	2.4	2.4	2.4	4.8	100.0
150%	3.0	3.0	3.0	3.0	3.0	3.0	6.0	100.0

Table No. 2: Concentration (µg/mL)

Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F	Acetazolamide
0.053	0.057	0.111	0.127	0.043	0.134	0.069
0.175	0.163	0.171	0.169	0.172	0.179	0.161
0.438	0.408	0.428	0.424	0.430	0.448	0.404
0.700	0.652	0.684	0.678	0.687	0.716	0.646
0.875	0.815	0.855	0.847	0.859	0.895	0.807
1.050	0.978	1.026	1.016	1.031	1.074	0.969
1.313	1.223	1.283	1.271	1.289	1.343	1.211

Table No. 3: Average Area

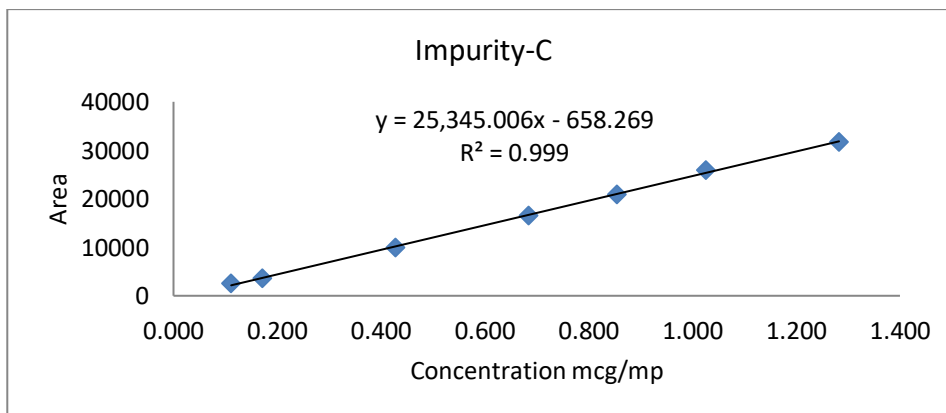| Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F	Acetazolamide
3478	2098	2466	5617	2513	7698	5240
11049	5982	3525	7379	8839	10209	11850
28061	14977	9944	18482	22347	24011	28314
46318	24435	16554	31221	35918	41474	46928
57988	29917	20884	38370	44850	51297	58652
72438	37571	25885	47575	56969	62572	71630
89395	46065	31643	57861	68841	76845	88366

Figure 1: Linearity plot for Acetazolamide
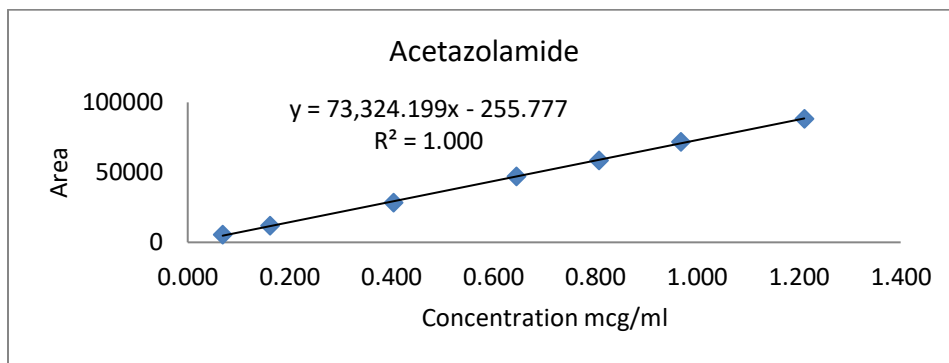
Figure2: Linearity plot for Impurity A
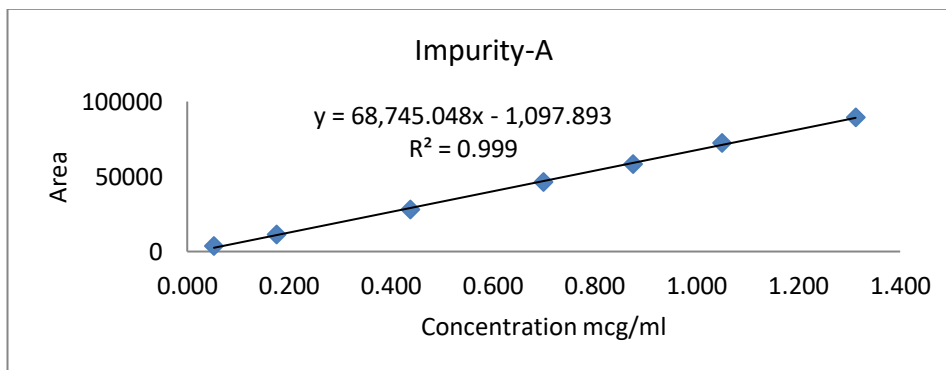
Figure 3: Linearity plot for Impurity B
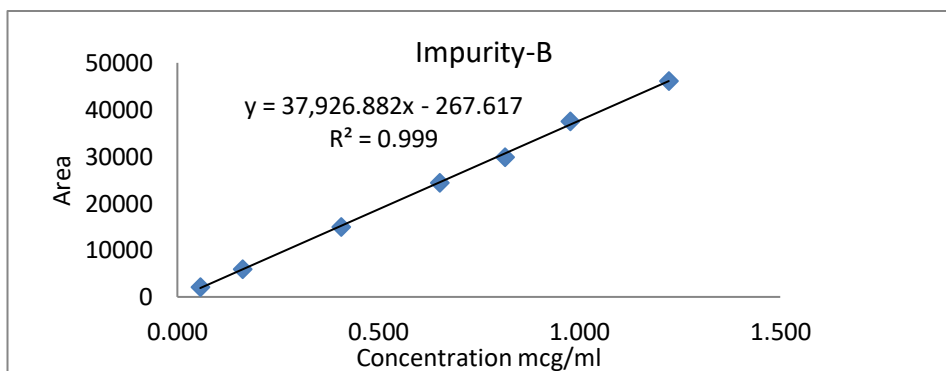
Figure 4: Linearity plot for Impurity C

Figure 5: Linearity plot for Impurity D

Impurity-D

$y = 46,322.023x - 488.227$
$R^2 = 0.999$
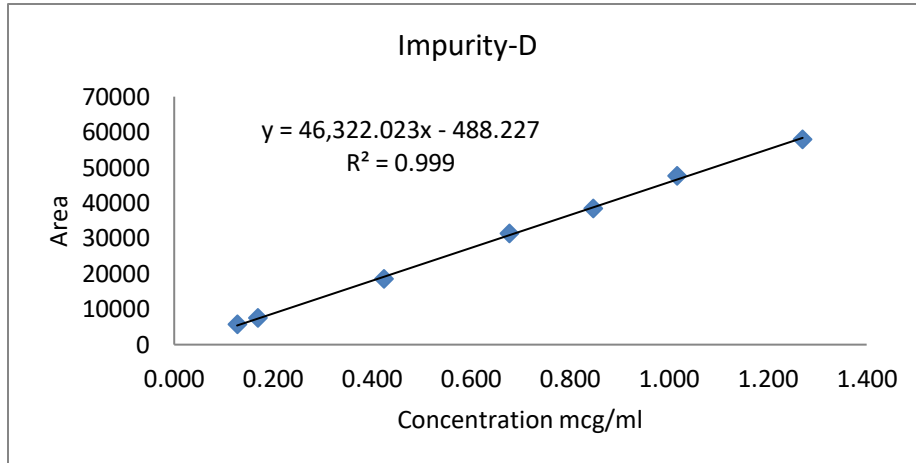
Figure 6: Linearity plot for Impurity E

Impurity-E

$y = 53,932.696x - 423.038$
$R^2 = 0.998$
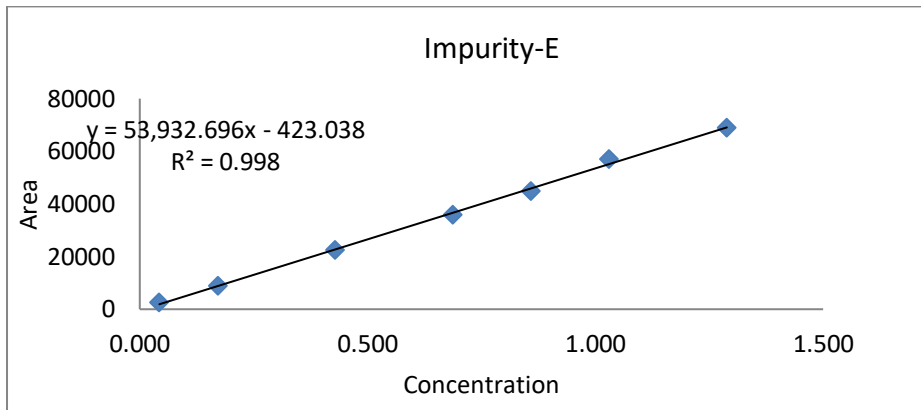
Figure 7: Linearity plot for Impurity F

Impurity-F

$y = 57,920.609x - 462.403$
$R^2 = 0.999$
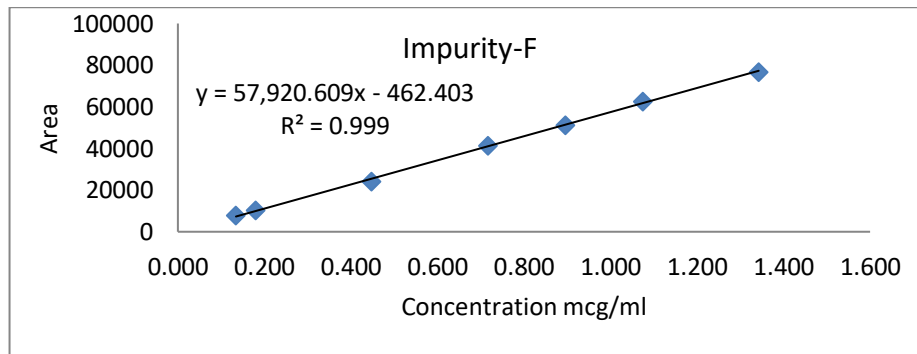
Accuracy and Recovery perform LOQ to 150% in triplicate preparation.

Table No. 4: Acceptance criteria

Spiked Level (%)	% Recovery
<0.03	60.0 to 140.0
0.03 to 0.05	70.0 to 130.0
0.05 to 0.10	80.0 to 120.0
>0.10	90.0 to 110.0

Table No. 5: PERCENTAGE OF RECOVERY

Level	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F	Acetazolamide
LOQ	105.8%	100.4%	88.3%	84.8%	87.3%	107.7%	113.2%
50%	99.3%	106.4%	92.2%	105.5%	106.2%	97.1%	-
100%	95.4%	99.7%	106.3%	105.8%	103.2%	100.8%	-
150%	106.0%	98.9%	101.1%	106.1%	101.4%	99.4%	-

Table No. 6: LOD AND LOQ

Impurity	RRT	RF	LOD (%)	LOQ (%)
Impurity E	0.38	1.52	0.004	0.014
Impurity D	0.42	1.47	0.002	0.007
Impurity B	0.64	2.07	0.003	0.011
Acetazolamide*	1.00	1.00	0.003	0.010
Impurity C	1.45	2.71	0.005	0.015
Impurity F	2.22	1.06	0.006	0.021
Impurity A	3.11	1.15	0.005	0.017

Table No. 7; Forced Degradation (Mass Balance)

Sr. No.	Condition	% Assay (A)	% Total impurities (B)	Total (A+B)	% Mass Balance
1	Untreated	98.7	0.445	99.145	NA
2	Acid Degradation	97.0	1.203	98.203	99.0
3	Base Degradation	94.4	1.061	98.461	99.3
4	Peroxide Degradation	96.1	1.123	97.223	98.1
5	Photolytic Degradation	100.1	0.162	100.262	101.1
6	Thermal Degradation	99.6	0.165	99.765	100.6

Table No. 8: Related substances compilation

Impurity	% RSD
Each Impurity from LOQ to 0.1 %	NMT 20.0
Each Impurity >0.1 %	NMT 15.0
Total Impurity	NMT 10.0

Table No. 9: Forced Degradation (Total impurity)

Sr. No.	Condition	% Impurity A	% Impurity B	% Impurity C	% Impurity D	% Impurity E	% Impurity F	%Single maximum unknown impurity	%Total Unknown impurities	%Total impurities
1	Untreated	NA	0.028	NA	0.046	0.015	0.056	0.012	0.300	0.445
2	Acid Degradation	NA	0.050	NA	1.068	0.011	0.053	0.021	0.021	1.203
3	Base Degradation	NA	0.034	NA	3.793	0.154	0.062	0.012	0.018	4.061
4	Peroxide Degradation	NA	0.279	NA	0.125	0.540	0.048	0.085	0.131	1.123
5	Photolytic Degradation	NA	0.030	NA	0.042	0.017	0.061	0.012	0.012	0.162
6	Thermal Degradation	NA	0.035	NA	0.046	0.015	0.056	0.013	0.013	0.165