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Synthesis, Characterization and Quantitative Estimation of Thiadiazole Impurity from Acetazolamide Bulk and its Marketed Formulations

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ABSTRACT

Impurity profiling study has been in limelight in the recent pharmaceutical scenario and its importance is increasing day-by-day. The toxicological and safety issues of impurity which are formed during the synthesis of drug, has emphasized need for impurity profiling. It can be predicted that the intermediate formed during synthesis, can act as an impurity by accompanying the desired final synthesized drug or drug product, thus creating hazardous health and safety issues. The present work is bonafide for synthesis, characterization and quantitation of Thiadiazole impurity, from Acetazolamide bulk and its marketed formulations. Acetazolamide drug and its thiadiazole process related intermediate, considered as impurity is been selected for the proposed study. The structure of the impurity and the bulk was confirmed by spectral study of IR, ^{1}H NMR and ^{13}C NMR. The new method was validated and developed using simple isocratic RP-HPLC for determination and quantification thiadiazole impurity from acetazolamide bulk and formulation. The method was validated according to ICH guidelines with respect to linearity, accuracy, precision and specificity. It can be conveniently used for quantitative determination of related compounds in acetazolamide bulk and formulation. The method was found to be specific, accurate, precise, rugged and robust and cam be used for routine analysis.

Key-words: *Acetazolamide, impurity, HPLC, validation.*

INTRODUCTION

During the manufacturing process of an active pharmaceutical molecule, many intermediates are formed during the process of manufacturing. These intermediates may account for the safety and efficacy issues of the pharmaceutical product. There are a lot of probabilities that such intermediates can execute superior toxicological or pharmacological properties as compared to the active pharmaceutical ingredients. These intermediates could be found in the formulation which would be due to the APIs or degraded moieties formed in the formulations under stressed conditions. Since such substances are formed during the process of manufacturing of APIs and formulation, which can cause safety and efficacy problems, they are accounted as process related impurities. The study regarding those impurities is called as impurity profiling. The Regulatory agencies are also emphasizing on the need for impurity profiling. It has also become a mandatory requirement in various pharmacopeias to include impurities present in APIs [1].

Thus the present research work is directed towards synthesis and characterization of the intermediates of a drug, which are its process related impurities and development of a validated analytical method for quantitation of synthesized impurities in the drug from its bulk and its formulation.

In this work, process related impurities are been focused. Acetazolamide has 6 reported impurities in British Pharmacopoeia 2010. They are designated as A, B, C, D, E, F, and G. Where A = N-(5-chloro-1,3,4-thiadiazol-2-yl) acetamide, B = N-(1,3,4-thiadiazol-2-yl) acetamide, C = N-(5-sulphanyl-1,3,4thiadiazol-2-yl) acetamide, D = 5-amino-1,3,4-thidiazole-2-sulphonamide, E = 5-acetamido-1,3,4-thiadiazole-2-sulphonic acid, F=N-[5-[(5-acetamido-1,3,4-thiadiazole-2yl)sulphonyl]sulphamoyl-1,3,4-thiadiazol-2-yl] acetamide, G=5-amino-1,3,4-thiadiazole-2-thiol [2] . Thus the G impurity with its chemical name 5-amino-1, 3, 4-thiadiazole -2-thiol was been selected for method development and quantitation in Acetazolamide bulk and its tablet and capsule formulations.

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EXPERIMENTAL

Materials, Reagents and Chemicals:

Acetazolamide bulk drug was obtained from Nakoda chemicals, Hyderabad and the dosage form were purchased from market. HPLC grade Acetonitrile and water were purchased from SD fine chem. Mumbai. Other chemicals were purchased from Modern scientific lab., Nashik and were of AR grade.

Melting points were determined by open capillary method and are uncorrected. The NMR spectra were recorded on sophisticated multinuclear FT-NMR Spectrometer model Advance-II (Bruker) using dimethylsulfoxide-d_6 as solvent and tetramethylsilane as internal standard for ^{1}H and ^{13}C NMR. IR spectra were recorded on Shimadzu FTIR-8400S spectrophotometer using KBr disc method.

Chromatographic conditions:

Chromatographic separation was performed with HPLC (SHIMADZU LC-20 AD) equipped with isocratic pump and manual injector (capacity 20µl). SPINCHROM chromatographic software was used for data acquisition. ARPC18 (250 mm X 4.6 mm), 5µ column (Phenomex) was used for analysis. Mobile phase comprising of Acetonitrile & water in ratio of 50:50 v/v was filtered through 0.45µ in membrane filter (Millipore) using vacuum pump and degassed by sonication with the sonicator (Sidilu, Bangalore). Throughout the run a flow rate 1ml/min was maintained. Column effluent was monitored at 317 nm with variable wavelength UV detector.

Synthesis of 5-Amino-1, 3, 4-Thiadiazole-2-Thiol:

A mixture of Potassium hydroxide (0.31g, 5.34mmoles) and Carbon disulphide (0.42g, 5.52mmoles) was dissolved in anhydrous ethanol 15ml. Latter it was followed by addition of Thiodemicarbazide (0.5g, 5.49mmoles) dissolved in anhydrous ethanol. The reaction mixture was stirred and heated to reflux for 8 hours. The ethanol was removed by evaporation in vacuum and the dissolved in water (50ml). This was slowly acidified with 5ml of Conc. Hydrochloric acid. The precipitate was filtered to produce the compound. The crude product was washed with cold water. This yellow solid was recrystallized out of ethanol to give white product. Expected Yield (0.52g, 71%), Melting Point: 230-232°C [3,4].

Preparation of standard solution:

100 ppm (100µg/ml) of standard solution of synthesized impurity; 5-amino-1,3,4- thiadiazole-2-thiol was prepared by dissolving into methanol: water (80:20). 10mg of standard synthesized impurity was dissolved in methanol: water (80:20) of 100. From this stock, 1 ml of solution was pipette out and diluted by the solvent up to 10 ml to prepare 10 ppm (10µg/ml) of solution. Of the 100 ppm solution, 0.1 ml was diluted by the solvent to 10 ml to form the solution of 10ppm (1µg/ml) and so on.

Preparation of stock solution of test samples:

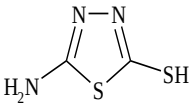
Stock solutions of bulk acetazolamide, tablet and capsule formulation of acetazolamide of 100 ppm in 100 ml volumetric flask were prepared. 10 mg of test samples were dissolved in 100 ml diluent. 1ml of this stock was diluted to 10 ml to prepare 10 ppm stock solution. For the tablet and capsule formulations, 20 tablets and 20 capsules were crushed respectively. The powder of this formulations equivalent to 10 mg of the drug was used to prepare the stock solutions. Further Dilutions of 1ppm, 2 ppm and so on, were prepared by taking 0.1ml, 0.2ml and so on of standard /test solution and diluting it to 10 ml. Validation experiment was performed to demonstrate system suitability, linearity, precision, accuracy study, ruggedness and robustness as per ICH guidelines [5, 6, 7].

RESULT AND DISCUSSION

Characterization of impurity:

The impurity was characterized by carrying out the UV spectra, IR spectra, ^{1}H-NMR and ^{13}C-NMR. Following are the recorded spectra. Table no. 01

Table No. 1: Analytical and physical properties of synthesized impurity

S. No.	Mole Formula And Str.	Mole Wt.	M.P. (^{0}C)	IR (cm^{-1})	^{1}H NMR (ppm)
01	$C_2H_3N_3S_2$ 	133	230-232	**3232, 3549(m)** N-H stretching (primary amines) **1612-1685(s-m)** N-H bending (primary amines) **2611-2553(w)** S-H stretching **1685-1612(v)** = C=N stretching **1311-1346(s)** C-N stretching **1091-1165(s)** C=S stretching **1091** N-N stretching	13.142 – 1H of SH 3.25 -2H of –NH_2

HPLC method validation:

At the commencement of the method validation phase, initially the required chromatographic conditions were optimized by practically carrying out various parameters like type of column, change in mobile phase, different mobile phase combinations, run time, use of diluent of preparing various dilutions, flow rate and column temperature. The column selected was C18 type with 5µ particle dimensions of column packing material. The HPLC run was carried out at ambient temperature conditions. The suitable wavelength of detection was decided by scanning the impurity dilution using UV, across 200-400nm. The wavelength of detection was found to be 317 nm. The mobile phase system of Acetonitrile: Water was selected according to the solubility and polarity differences between the solvents, peak symmetry and number of theoretical plates. Greater the HETP, greater will be the peak sharpness and lesser will be the peak asymmetry. The mobile phase composition of 50:50 of the acetonitrile: water system was selected according to the peak symmetry and number of theoretical plates.

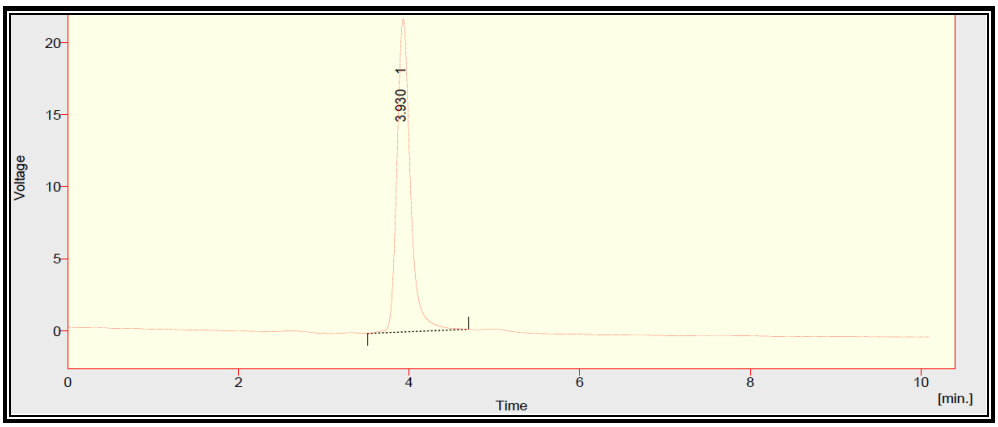


Fig. 1: Chromatogram of impurity

The following parameters like system suitability, linearity, accuracy, precision, ruggedness, robustness, limit of detection, limit of quantitation were calculated as follows:

Range: The range of the impurity was found to be 1-12µg/ml.

Linearity:

Dilutions of standard impurity in the range of 1-12 µg/ml were prepared by taking suitable aliquots of working standard solutions in different 10ml of volumetric flasks and diluting upto the mark with mobile phase. 20µl was injected from it each time into the column at flow rate of 0.8 ml/min. The standard from elute was monitored at 317 nm and corresponding chromatogram were obtained from these chromatograms peak area were calculated. A plot of peak area over concentration was constructed. Regression of the plot was computed by least square regression method. The experiment was performed 3 times and the mean was used for calculation.

Table No. 2: Linearity of impurity

Sr. no.	Concentration (ppm)	Area (milivolts) at 317nm
01	1	128.196
02	2	197.974
03	3	256.786
04	4	323.042
05	5	411.124
06	6	483.340

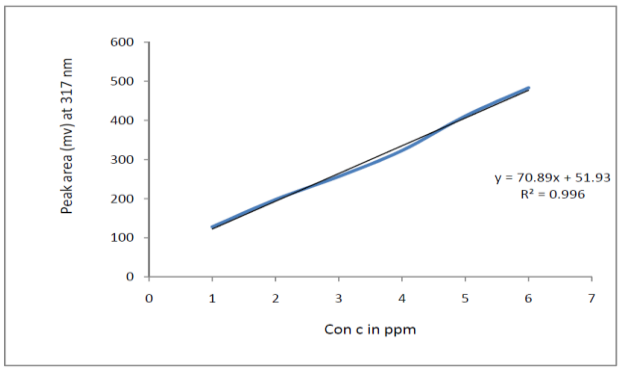


Fig. 2: Graph of linearity (Peak area Vs Concentration)

Precision:

Precision of analytical method was studied by multiple injection of homogenous sample. 7 replicates of 4 ppm solution were prepared and injected for precision at the same flow rate of 0.8ml/min .The intra-day and inter-day precision was used to study the variability of the method. The dilutions made for linearity were used for the intraday and inter day precision. SD and RSD were calculated for both.

Table No. 3: Precision by HPLC

S. No.	Concentration (ppm)	Peak Area (mV) at 317 nm	Mean	Standard Deviation	%RSD
01	4	323.042			
02	4	321.584			
03	4	326.014	323.4429	1.5159	0.4686
04	4	322.015			
05	4	323.246			
06	4	324.657			
07	4	323.542			

Ruggedness:

For the study of ruggedness intraday and interday reading of the dilutions of precision were calculated.

a. Intraday readings were taken after 4 hours:

Table No. 4: Results for intraday precision after 4 hrs

S. No.	Conc. (ppm)	0 hr at 317 nm	After 4 hrs at 317 nm	Mean	SD	% RSD
01	4	323.042	325.254	324.148	1.5641	0.4825
02	4	321.584	324.682	323.133	2.1906	0.6779
03	4	326.014	327.213	326.613	0.8478	0.2595
04	4	322.015	324.222	323.118	1.5605	0.4029
05	4	323.246	326.567	324.906	2.3483	0.7227
06	4	324.657	326.756	325.706	1.4842	0.4556
07	4	323.542	324.670	324.106	0.7976	0.2460
		Mean			1.5418	0.4753

b. Interday readings were taken after 24 hours:

Table No. 5: Results for interday precision after 24 hrs

S. No.	Conc. (ppm)	0 hr at 317 nm	After 24 hrs at 317	Mean	SD	% RSD
01	4	323.042	327.387	325.214	3.0723	0.9447
02	4	321.584	326.69	324.137	3.6104	1.1138
03	4	326.014	330.019	328.016	2.8319	0.8633
04	4	322.015	326.345	324.18	3.0617	0.9444
05	4	323.246	327.677	325.461	3.1331	0.9626
06	4	324.657	328.87	326.763	2.9790	0.9116
07	4	323.542	327.632	325.587	2.8206	0.8882
		Mean			3.0829	0.9470

c. Change in Analyst

Table No. 6: Results for ruggedness by change in analyst

S. No.	Conc. (ppm)	Analyst I	Analyst II	Mean	S.D	% R.S.D
		Peak Area (mV)				
01	1	128.196	127.126	127.661	0.7566	0.5926
02	2	197.974	195.854	196.914	1.4990	0.7612
03	3	256.786	254.564	255.675	1.5711	0.6145
04	4	323.042	321.022	322.032	1.4283	0.4435
05	5	411.124	410.119	410.6215	0.7106	0.1730
06	6	483.34	480.264	481.802	2.1750	0.4514
		Average		299.1176	1.3568	0.4536

Robustness:

Flow rate changed to 0.6 ml/s from 0.8 ml/s.

Table No. 7: Results for robustness by change in flow rate by HPLC

S. No.	Conc. (ppm)	Peak Area (mV) 0.8ml/s	Peak Area (mV) 0.6ml/s	Mean	S.D	% R.S.D
01	1	128.196	126.157	127.1765	1.4417	1.1336
02	2	197.974	196.157	197.0655	1.2848	0.6519
03	3	256.786	251.471	254.1285	3.7582	1.4788
04	4	323.042	319.00	321.132	2.7011	0.8411
05	5	411.124	410.453	410.7885	0.4744	0.1155
06	6	483.34	483.235	483.2875	0.0742	0.0153
		Average		298.9298	1.6224	0.7060

Accuracy:

Accuracy of the method was studied using the method of standard addition. Standard impurity solutions were added to the unknown bulk, tablet and capsule formulation of Acetazolamide. The percent recovery was determined at three different levels (50%, 100% and150%). Impurity content was determined and the percent recovery was calculated.

Table No. 8: Results for recovery study by HPLC

S. No.	Sample	% amount recovered			mean	SD	RSD
		50%	100%	150%			
01	**Bulk**	89.47	89.42	87.45	88.78	1.152	1.2976
02	**Tablet**	92.76	90.29	89.96	91.00333	1.53	1.6815
03	**Capsule**	94.09	90	89.09	91.33	2.663	2.6177

Limit of Detection:

LOD= 3.3*Standard deviation/ slope

LOD=0.0703

Limit of Quantitation:

LOQ= 10* Standard deviation/ slope

LOQ=0.213

System suitability parameters:

Table No. 9: System suitability parameters

S. No.	Conc. (ppm)	Asymmetry	Efficiency	Efficiency/length
01	01	1.9	4718	47177
02	02	0.8	4625	46252
03	03	0.84	4622	46224
04	04	1.4	4715	47156
05	05	0.18	4621	46211

QUANTITATION OF IMPURITY:

Quantitation of process-related impurity (5-amino-1,3,4-thiadiazole-2-thiol of acetazolamide) in bulk Acetazolamide, its tablet and capsule formulation was carried out.

Table No. 10: Quantitation of impurity in acetazolamide bulk and its tablet & capsule Formulations

S. No.	Bulk/ Formulations	Quantitation of 5-amino-1,3,4-Thiadiazole-2-thiol (%)
01	Bulk Acetazolamide	Negligible
02	Acetazolamide tablets	0.038%
03	Acetazolamide capsules	0.031%

CONCLUSION

The synthesis of a process-related impurity of Acetazolamide was successfully carried out by suitable synthetic procedure. Purification of impurity was carried out by column chromatography. Characterization was carried out by IR, ^{13}C NMR and ^{1}H-NMR. Based on the spectral data, the structure of impurity was characterized as 5-amino-1,3,4-thiadiazole-2-thiol (Impurity G of Acetazolamide), as reported in British Pharmacopoeia, 2011. A new method was developed and validated according to ICH guidelines with respect to specificity, precision, accuracy, linearity by using a simple isocratic RP-HPLC method. It can be conveniently used for quantitative determination of related substances in acetazolamide bulk and its formulations. This validated HPLC method was used for detection and quantitation of 5-amino-1,3,4-thiadiazole-2-thiol, a process related impurity of acetazolamide, from acetazolamide bulk and its marketed tablet and capsule formulations.

The stated thiadiazole impurity was found to be 0.038 % in acetazolamide tablet formulation, 0.031 % in capsule formulation and negligible amount was found in acetazolamide bulk drug. Thus, it can be concluded that 5-amino-1,3,4-thiadiazole- 2-thiol, a process-related impurity of acetazolamide was present in bulk and formulations, within the stated limit as per the ICH guidelines. The above method was found to be specific, accurate precise, rugged and robust and can be used for routine analysis.

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