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DETERMINATION OF RELATED SUBSTANCES IN HYDROCHLOROTHIAZIDE TABLETS BY RP HPLC

K. Swarna Priya*[1] and T. Rama Mohan Reddy[1]

[1*]Department of Pharmaceutical Analysis, CMR College of Pharmacy, Medchal, Kandlakoya, India.

ABSTRACT

Recently several methods have been developed for the determination of drugs and their impurities products by Reversed Phase liquid chromatography. The present paper describes about highly specific, linear, precise, rugged, accurate, robust and stability indicating RP-HPLC method for determination of related substances present in Hydrochlorothiazide tablets. Chromatographic separation for the separation of Hydrochlorothiazide and with Impurity-A by using Thermosil C18 column (4.5×150mm) 5.0µ, flow rate was 0.8ml/min, mobile phase ratio was 65:35% v/v methanol: water pH 3 (pH was adjusted with orthophosphoric acid), detection wavelength was 265nm. The analytical method validation was done according to ICH guidelines. The linearity study Hydrochlorothiazide and with Impurity-A was found in concentration range of 50µg-250µg and 5µg-25µg and correlation coefficient (r^2) was found to be 0.999 and 0.999. Hence the suggested RP-HPLC method can be used for routine analysis of Hydrochlorothiazide and with Impurity-A in API and Pharmaceutical dosage form.

KEYWORDS

Thermosil C18 column (4.0×150mm) 5µ, Hydrochlorothiazide, Impurity-A, RP-HPLC and Auto sampler.

Author for Correspondence:

Swarna Priya K,
Department of Pharmaceutical Analysis,
CMR College of Pharmacy,
Medchal, Kandlakoya, India.

Email: swarnapriya.k8@gmail.com

INTRODUCTON

Need for Impurity Characterization

Impurities that can influence the purity of drug substance or can be harmful to patients, it is important to segregate and characterize. Degradant which are obtained during stress testing are recognized. The presence of these impurities even in small amounts may impact the efficacy and safety of pharmaceutical dosage form.

Hydrochlorothiazide is used as antihypertensive agent (diuretic). Chemically; 6-chloro-1, 1-dioxo-3, 4-dihydro-2*H*-1, 2, 4-benzothiadiazine sulfonamide with molecular weight 297.739.

Impurity – A (Chlorothiazide)

Chemical name is 6-chloro-1, 1-dioxo-4H-1λ, 2, 4-benzothiadiazine-7-sulfonamide.

Molecular weight 295.723. Chemical structure of Impurity-A is shown below.

Aim

Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the analysis of Hydrochlorothiazide and with Impurity-A. The method will be validated.

Objective of the work

- The analytical method was developed for determination of related substances of *Hydrochlorothiazide* and with Impurity-A by RP-HPLC method by optimizing the chromatographic conditions.

MATERIAL AND METHODS

Assay

Assay preparation of the Hydrochlorothiazide and with Impurity-A standard and sample solution

Preparation of the Impurity-A sample solution

Sample solution preparation

10mg of Hydrochlorothiazide and 1mg Impurity-A tablet powder were accurately weighed and transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve the powder completely in the diluent and volume is made up to the mark with the same solvent (Stock solution). From the above solution pipette out 10ml solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

10mg Hydrochlorothiazide and 1 mg Impurity-A in working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate until the powder dissolves completely and solution is made up to mark with the same solvent (Stock solution). Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and dilution is made up to the mark using above diluent.

Assay calculation

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ standard}{dilution\ of\ sample} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where:

Avg.wt = average weight of tablets

P= Percentage purity of working standard

LC= Label Claim of Hydrochlorothiazide mg/ml.

ANALYTICAL METHOD VALIDATION

Validation parameters

- Specificity
- Linearity
- Range
- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Detection Limit
- Quantitation Limit
- Robustness

RESULTS AND DISCUSSION

The present investigation reported in the thesis was aimed to develop a new method development and validation for the simultaneous estimation of Hydrochlorothiazide and Impurity-A by RP-HPLC method. Literature reveals that there are no analytical methods reported for the simultaneous estimation Hydrochlorothiazide and Impurity-A by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Hydrochlorothiazide and Impurity-A in pharmaceutical dosage form.

Method Development

The wavelength was selected by dissolving the drug in mobile phase to get a concentration (10µg/ml). The above solution was scanned in U.V range from 200-400nm. The overlay spectrum of Hydrochlorothiazide and Impurity-A was obtained and the isosbestic point of Hydrochlorothiazide and Impurity-A showed absorbance's maxima at 265 nm. The spectrums are shown in below figures.

The chromatographic method development for the simultaneous estimation of Hydrochlorothiazide and Impurity-A were optimized by several trials for various parameters as different column, flow rate

and mobile phase, finally the following chromatographic method was selected for the separation and quantification of Hydrochlorothiazide and Impurity-A in API and pharmaceutical dosage form by RP-HPLC method.

Chromatographic trials for related substances of Hydrochlorothiazide and with Impurity-A by RP- HPLC

Column :	Thermosil C_{18} 4.5×150mm 5.0µm	
Column temperature	:	Ambient
Wavelength	:	265nm
Mobile phase ratio	:	65:35% v/v
methanol	:	water
Flow rate	:	0.8 min/ml
Auto sampler temperature	:	Ambient
Injection volume	:	20µl
Run time	:	6 minutes

Observation

The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

METHOD DEVELOPMENT RESULTS

System Suitability Results

- Tailing factor for the standard injection is 1.3
- Theoretical Plates for the standard injection is 4668.7

Assay Results

Weight of 10 tablets: 1.25 grams

Average Weight : 0.125grams

RESULTS

System Suitability Results

- Tailing factor for the standard injection is 1.3
- Theoretical Plates for the standard injection is 6090.3

Assay Results

The retention time of Hydrochlorothiazide and with Impurity-A was found to be 2.566mins and 3.417mins respectively. The system suitability parameters for Hydrochlorothiazide and with Impurity-A such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089, 1.2. Resolution was 6.0 the % purity Hydrochlorothiazide and with Impurity-A in pharmaceutical dosage form was found to be 99.24 and 101.04% respectively.

Linearity

The linearity study was performed for the concentration of 50 ppm to 250 ppm and 5ppm to 25 ppm level. Each level was injected into chromatographic system. The area obtained from each level was used for calculation.

The linearity study was performed for concentration range of 50µg-250µg and 5µg-25µg of Hydrochlorothiazide and with Impurity-A and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0. 999).

Accuracy

The accuracy study was performed for 50%, 100% and 150% for Hydrochlorothiazide with Impurity-A. Each level was injected thrice into chromatographic system. The area obtained was used for calculation of % recovery.

The accuracy study was performed for % recovery of Hydrochlorothiazide and with Impurity-A. The % recovery was found to be 99.71% and 99.47% respectively (NLT 98% and NMT 102%).

Precision

- Repeatability
- Intermediate Precision

The Method precision study was performed for the %RSD of Hydrochlorothiazide and with Impurity-A was found to be 0.82 and 0.86 (NMT 2).

The LOD was performed for Hydrochlorothiazide and with Impurity-A was found to be 5.016 and 2.382 respectively.

The LOQ was performed for Hydrochlorothiazide and with Impurity-A was found to be 1.327 and 5.014 respectively.

Robustness

The robustness was performed for the flow rate variations from 0.8ml/min to 1ml/min and1.2 ml/min mobile phase ratio variation from more organic phase to less organic phase ratio for Hydrochlorothiazide and with Impurity-A. The method found robust only in less flow condition and the method found robust even by change in the Mobile phase ±5%.

Chemicals and standards used

Table.No.1: List of chemicals and standards used

S.No	Chemicals	Manufacturer Name	Grade
1	Water	Merck	HPLC grade
2	Methanol	Merck	HPLC grade
3	Acetonitrile	Merck	HPLC grade
4	Ortho phosphoric acid	Merck	G.R
5	Potassium Dihydrogen phosphate	Merck	G.R
6	hydrochlorothiazide and Impurity-A	In – House	In- House

Instruments used

Table.No.2: List of instruments used

S.No	Instrument name	Model number	Soft ware	Manufacturers Name
1	HPLC-auto sampler –UV detector	Separation module2695, UV.detector2487	Empower-software version-2	Waters
2	U.V double beam spectrometer	UV 3000+	U.V win soft ware	Lab India
3	Digital weighing balance(sensitivity 5mg)	ER 200A	-	Ascoset
4	pH meter	AD 102U	-	ADWA
5	Sonicator	SE60US	-	Enertech

Table No.3: Details of Trail-5

S.No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	HCTZ	2.566	947124	157429	5105	1.3	1.32
2	Impurity-A	3.417	112541	13239	3788	1.4	

S.No	Name of compound	Amount taken	%purity
1	Hydrochlorothiazide	125	99.24
2	Impurity-A	126	101.04

Table No.4: Linearity Results for Hydrochlorothiazide

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	471543
2	II	100 ppm	956277
3	III	150 ppm	1494999
4	IV	200 ppm	1946124
5	V	250 ppm	2302139

Table No.5: Linearity Results for Impurity-A

S.No	Linearity Level	Concentration	Area
1	I	5ppm	116472
2	II	10ppm	273841
3	III	15ppm	392655
4	IV	20ppm	481541
5	V	25ppm	590567
Correlation Coefficient			0.999

Table No.6: Showing accuracy results for Hydrochlorothiazide

S.No	%Concentration (at specification level)	Average Area(n=3)	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
1	50%	470409	5	4.96	99.91%	99.71%
2	100%	967055	10	9.98	99.18%	
3	150%	1434836	15	15.02	99.60%	

Table No.7: Showing accuracy results for Impurity-A

S.No	%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
1	50%	126666	0.5	0.497	99.53%	99.47%
2	100%	267487	1.0	1.05	99.38%	
3	150%	381234	1.5	1.495	99.52%	

Table No.8: Showing %RSD results for Hydrochlorothiazide Peak Name: Hydrochlorothiazide

S.No	Peak name	RT	Area
1	Hydrochlorothiazide	2.755	952232
2	Hydrochlorothiazide	2.687	952087
3	Hydrochlorothiazide	2.632	963235
4	Hydrochlorothiazide	2.612	975952
5	Hydrochlorothiazide	2.616	927348
Mean			952576
Std.dev			452064
%RSD			0.86

Table No.9: Showing % RSD results for Impurity-A Peak name Impurity-A

S.No	Peak name	RT	Area
1	Impurity	3.616	232453
2	Impurity	3.634	236275
3	Impurity	3.460	237670
4	Impurity	3.446	233578
5	Impurity	3.437	238483
Mean			234987
Std.dev			22806.9
%RSD			0.82

Detection limit

Table No.10: Showing results for Limit of Detection

S.No	Drug name	Standard deviation(σ) of three injections	Slope(s)	LOD(µg)	S/N ratio blank to sample
1	Hydrochlorothiazide	0.577	11510	5.016	5:1
2	Impurity-A	1.527	6411	2.382	5:2

Quantification limit

Table No.11: Showing results for Limit of Quantitation

S.No	Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)	S/N ratio blank to sample
1	Hydrochlorothiazide	1.527	11510	5.014	5.1
2	Impurity-A	3.214	6411	1.327	5.3

Table No.12: Showing system suitability results for Hydrochlorothiazide

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5339	1.4
2	1	5105	1.3
3	1.2	5216	1.4

Table.No.13: Showing system suitability results for Impurity-A

S.No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7036	1.3
2	1	3788	1.2
3	1.2	6998	1. 3

Table No.14: Showing system suitability results for Hydrochlorothiazide

S.No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	6232	1.4
2	*Actual	4668	1.3
3	5 % more	6387	1.4

Table.No.15: Showing system suitability results for Impurity-A

S.No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	1.3
2	*Actual	6089	1.2
3	5 % more	4817	1.2

S.No	Validation Parameter	Result		Acceptance Criteria
1	System suitability	Tailing factor	Hydrochlorothiazide- 1.3 Impurity - 1.2	NMT 2.0 NLT 2000
		Theoretical plates	Hydrochlorothiazide- 5105 Impurity - 3788	
2	Specificity	The resolution between peaks due to impurities was found to be greater than 2, No interferences were observed		Demonstrated by the resolution between the peaks. No interference between the impurities and analyte peak.
3	Linearity	Correlation coefficient (r^2) value was found to be 0.999.		Correlation coefficient value (r^2) for the plot between concentration vs area of peak should NLT 0.999.
4	Accuracy	% mean recovery of Hydrochlorothiazide and impurity A was found to be 99.71% and 99.47% respectively.		Percentage recovery values should be 85-115% for 3 replicate injections at 3 concentrations.

5	Precision	The Method was found to be precise, the % RSD of Hydrochlorothiazide and Impurity-A was found to be 0.82 and 0.86	% RSD should not be more than 2
6	Limit of Detection	The LOD was found to be 5.016µg/mL and 2.382µg/mL for Hydrochlorothiazide and impurity A respectively	Signal to noise ratio should be 2 or 3:1
7	Limit of Quantitation	The LOQ was found to be 5.014µg/mL and for 1.327µg/mL Hydrochlorothiazide and impurity A respectively.	Signal to noise ratio should be 10
8	Robustness	The system suitability parameters were passed for all the conditions	The system suitability parameters should pass for all the conditions

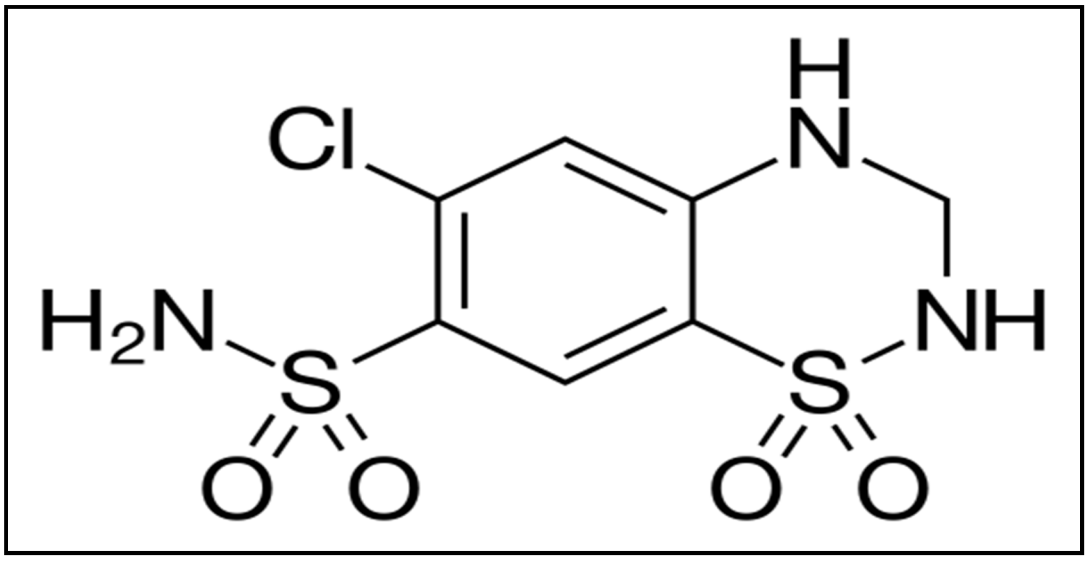


Figure No.1: Hydrochlorothiazide

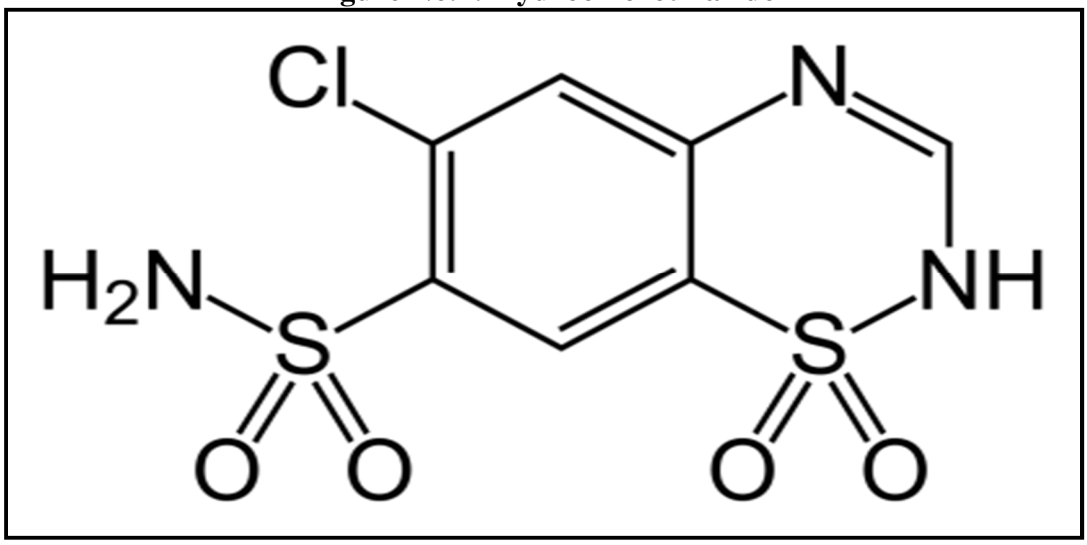


Figure No.2: Impurity-A

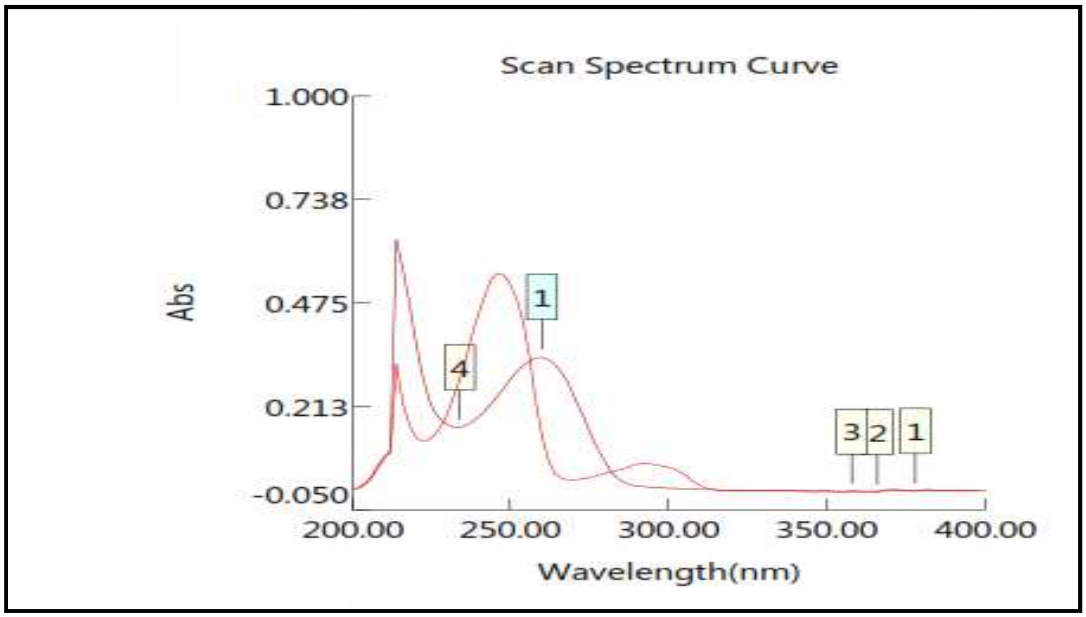
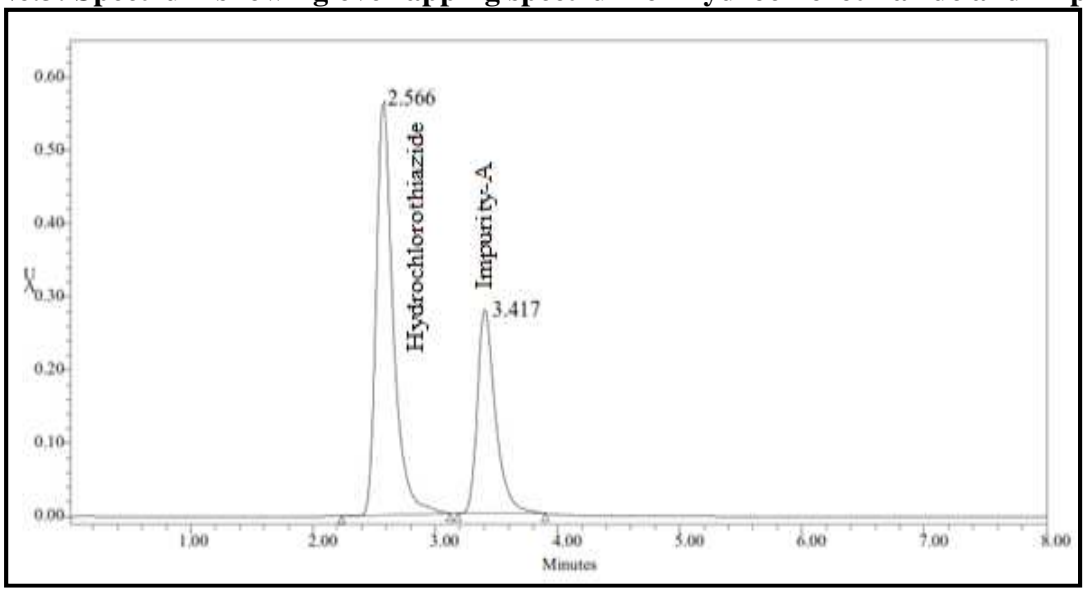


Figure No.3: Spectrum showing overlapping spectrum of Hydrochlorothiazide and Impurity-A



CONCLUSION

A new method was established for simultaneous estimation of Hydrochlorothiazide and with Impurity-A by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Hydrochlorothiazide and with Impurity-A. Hence the suggested RP-HPLC method can be used for routine analysis of Hydrochlorothiazide and with Impurity-A in API and Pharmaceutical dosage form

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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