Development of Method Furthermore Validation for Related Substances In Hydrochlorothiazide Injection With HPLC

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Abstract

A validated HPLC method is developed to determine Hydrochlorothiazide (HChT) in pharmaceutical formulations. Isocratic elution at a flow rate of 1.0 ml/min was employed on Zorbax C18 150 mm × 4.6 mm, 5µ, or similar for this chromatography analysis. For this analysis, the buffer solution is acetonitrile at 35 v/v and the buffer at 65 v/v, with a wave length of 272 nm. A sample of 20.0 µl is injected. The run time for the sample, placebo, system suitability, and sensitivity solutions is 240 minutes, and the diluted standard is 60 minutes. 1.30 minutes is the retention time for HChT. % R.S.D. is measured. The mean percentage recovery to HChT is found within the specification limit. This proposed process is validated as per the ICH rules and regulations. Thus, this proposed HPLC method should be successfully applied to routine quality control analysis of formulations. This method is simpler and more effective than other methods reported in the literature.

Keywords: *RP-HPLC Refractive index detector, HChT, flow rate, column, ICH Guidelines, USP reference.*

1. Introduction

The molecular formula of hydrochlorothiazide (HChT) is $C_7H_8ClN_3O_4S_2$, and its structure is shown in figure-1. HChT is a diuretic medication that is commonly used to treat high blood pressure and swelling caused by fluid buildup[1]. When compared to chlortalidone, HChT is ineffective in preventing heart stroke or heart attack[15]. Thiazides increase calcium reabsorption in a way that is unrelated to sodium transport[19]. It adheres for long-term protection of the lower peripheral vascular system[17]. HChT is administered to reduce peripheral vascular resistance[18]. It has been kept as one of the medicines on the essential drugs list, which is prescribed by WHO[4]. This drug is almost inexpensive[18]. In 2019, it is ranked 11th in the United States, with prescriptions totaling 38 million[3,16], and HChT is urged as initial monotherapy for those who have primary hypertension[5, 8-10, and 12]..

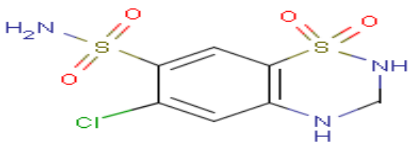

Figure 1. Structure of hydrochlorothiazide

This medication is prescribed to prevent osteopenia and hypoparathyroidism[11]. Nidhal S.M. et al.[14] used acetonitrile and water in a 50/50 ratio. At 30 °C, the injection volume is 20µL, the flow rate is 1.00 mL/min, and the wavelength is 272 nm. Authors are also put through their paces in a C_{18} Zorbax column (4.6 mm x250 mm, 5µm, Eclipse Plus). This method's validation produced very good precision (RSD% <1) and acceptable linearity ≥ 0.9978, as well as very low LOD and LOQ (0.5μ g/mL and 1.70 μg/mL, respectively; recovery is 99.93%. Nidhal M.S.M. et al.[13] used chromatographic conditions containing 60v/v acetonitrile and 40v/v water.

The column is a 250mm x 4.6mm 5µm ACE3-C18, the volume injected is 20µl, the flow rate is 1.00ml/min, and the wavelength is 226nm. Because of its high precision (RSD% < 1), acceptable linearity (R^2 ≥ 0.997), and low LOD and LOQ of 0.50 and 1.50µg/ml, respectively, the method is approved. On-pharma tablet dosage resulted in a 92% recovery rate. Jatin R.P. et al. (2006) used a C18 G column (150 mm x 4.6 mm, 5µm) with methanol and 0.10% formic acid in water as the mobile phase (85:15). The flow rate is 0.50 mL/min. Over the strength ranges of 2.0ng/mL, 170.0ng/mL for RAM, and 8.0ng/mL to 680.0ng/mL for HCTZ, the calibration curve has r(2) >0.99.Intra and inter day precisions are < to 15%, accuracy is ±15% at LOQ level as ±20%. Anand Rao et al., [2] described Zorbax C8 (150 mm x 4.6 mm, 3.5 µm), buffer (30 v/v) methanol (70 v/v) mobile phase. pH is listed as 3.0 by OPA. flow rate is 1.00 mL/min, and effluents are observed at 230 nm. HChT and Cesartancilexetil have retention times of 2.170 minutes and 7.280 minutes, respectively. Cesartancilexetil has a linearity to HChT of 25.0 mg/mL to 125.0 mg/mL, as well as 16.0 mg/mL to 80.0 mg/mL. Recovery to HChT and Cesartancilexetil are at 101.5% and 100.9%, respectively.

2. Material and Methods

Cosmosil C18 5 µm (15 cm x 4.6 mm) or its equivalent is utilized for this proposed chromatography analysis. From the local market, the reference sample of HChT is purchased. buffer, sodium phosphate monobasic, phosphoric acid Ammonium acetate, triethylamine, acetonitrile, and glacial acetic acid are procured as grade AR; furthermore, water is HPLC-grade. Separately dissolved 17.805 g of $Na_2HPO_4.2H_2O$ and 13.8 g of $NaH_2PO_4.H_2O$ in H_2O.Store the stock solutions at 4 °C for up to 6 minutes. Finally, for the obtained solution of 0.10 M sodium phosphate monobasic solution, whose pH is adjusted to 3 with phosphoric acid,To make a standard solution, place 100 mg of HChT in a 100.00 mL standard flask. After thoroughly shaking the contents, the required amount of methanol is added. Using methanol, dilute the obtained content from 10 mL to 10 mL. Finally, dilute by mobile phase 10 mL to 100 mL.0.45µm filter paper is used for filtering the sample.To make a sample solution, accurately weigh 208.00 mg of powdered tablet into a 100.00 mL standard flask. After thoroughly dissolving the contents to the required mark with methanol, Diluted by mobile phase from 5.00 mL to 100.00 mL. A stock solution of 1000 μg/mL of HChT is created by dissolving 0.10 gm of sample in 100.00 mL of acetonitrile. The obtained stock standard solution is kept in the refrigerator and then used to prepare solutions for working with different strengths.

3. Method Development

The peak wavelength for maximum absorbance in a 10 ppm solution of HChT is in the range of 272 nm, as shown in the HChT spectra. To identify the rate of flow, the mobile phase is changed from 0.50 mL/min to 1.50 mL/min to identify very good separation. Finally, the experiment revealed that a flow rate of 1 mL/min is best for eluting analytes.

4. Validation of Proposed Method Furthermore Requirements

System Suitability

Six replicate injections of the API working standard solution are passed through the method analysis. The percentage RSD to peak responses was calculated. The relative standard deviation (RSD) for peak responses caused by hydrochlorothiazide injections should be less than or equal to 2.0%. The analysis method necessitates six replicate injections of the working standard solution. This requirement, which is specified with system suitability, must be met by the analytical system. System suitability values for HChT area and %% RSD were noted in Table 1.

Table 1. System Suitability Results

Sample	Area of Hydrochlorothiazide	Injection No	HChT
1	748836	1	108529
2	748645	2	111658
3	749448	3	109187
4	748648	4	107848
5	748259	5	107043
6	748903	6	106967
Mean	748790	**Mean**	108782
% RSD	0.1	**% RSD**	1.3

Specificity

Analyzed parameter named specificity for this method after injecting a blank as diluent, preparing a placebo, solution for suitability system, diluted standard solution, sample preparation, and various impurities named A, B, C, D, and E into the chromatography system and recording retention times. Each and every product of degradation, as well as impurities, should be precisely resolved by the active compound peak as at least baseline resolution greater than 1.5.The threshold angle is less than the purity angle. No additional components were observed to co-elute by the HChT peak; solvent and placebo solutions do not contain components that may co-elute by the HChT peak; those that do are designated for analysis by Urirex K Tablets HPLC method HChT.

The HChT peak is not affected by UV exposure. No components were seen to co-elute with the peak of hydrochlorothiazide, and peak purity values denote that the HChT peak should be considered spectrally pure. There are peaks for the blank chromatogram, drug active, product, and drug active and product-UV stressed peaks. The peak purity for drug active, drug product, drug active-UV stressed, and drug productive-UV stressed for HChT are represented in Figures 2-5.

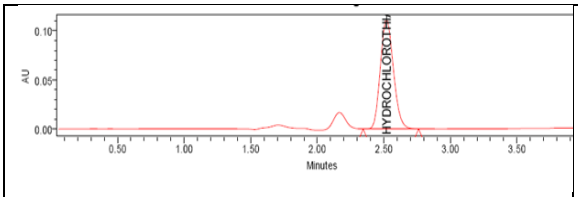

Figure 2: Chromatogram: Drug active – Peak by HChT eluted at 2.5 min.

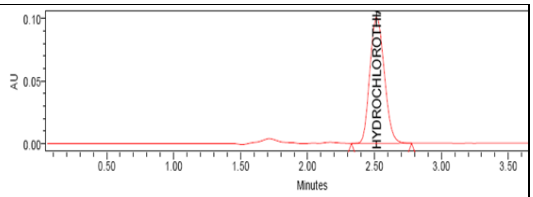

Figure 3: Chromatogram: Drug Active as UV stressed: Peak is mainly HChT elution at 2.5 min

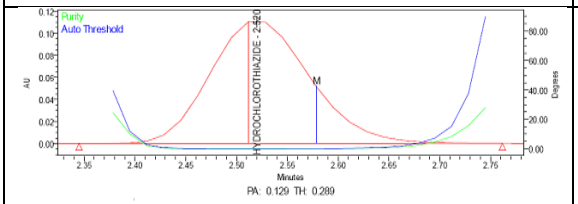

Figure 4: Peak purity: Drug Product Angle for Purity which is less to Threshold 0.125 < 0.357

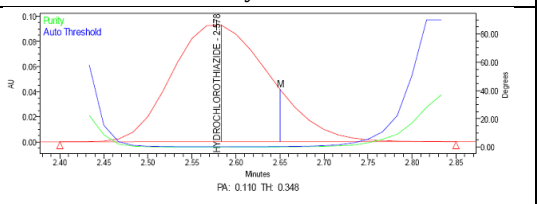

Figure 5: Peak purity: Drug Product UV Stressed Purity Angle < Threshold 0.110 < 0.348

5. Forced Degradation Studies:

If any other degradable compound or substance that does not meet the peaks of HChT is identified, forced degradation is studied for ratification of the assessment for stability or by its shelf life. These studies also aid in the identification of various degradation pathways, which may include oxidative, alkali solutions, acidic or neutral, and thermal. The authors investigated forced degradation studies using Sample Preparation for 4 mg/100 ml (200 ppm), Placebo Preparation for 4 mg/100 ml, Acid Stressed Sample, Alkali Stressed Sample, 3.0% w/v Hydrogen Peroxide Stressed Sample, Neutral Stressed Sample, UV light exposed sample, Sunlight exposed sample, and Thermal Stressed (Dry Heat) Sample. Finally, it is concluded that the purity obtained at the peak is the smallest compared to that obtained at the threshold. Values from forced degradation studies are shown in Table 2.

Table 2. Degradation chart for HChT

Parameter	Forced Degradation study											
	% Area											
Impurity Name	RT	RRT	Sample as such	1N HCl	0.1N HCl	1N NaOH	0.1 N NaOH	3.0%w/v H202	Neutral	Thermal	UV Light	Sun light
Angle of homogeneous			0.149	0.256	0.239	0.248	0.20	0.169	0.20	0.149	0.119	0.270
Purity Threshold			0.319	0.319	0.349	0.419	0.330	0.316	0.319	0.319	0.321	0.421
Chlorothiazide	17.139	2.892	-	-	-	-	-	0.06	-	-	-	0.19
Unknown -1	4.139	0.676	-	-	-	-	2.85	-	-	-	-	-
Unknown -2	6.649	1.120	-	0.19	0.50	-	0.54	0.65	0.74	0.54	-	-
Unknown -3	7.470	1.260	-	-	0.06	-	0.06	0.06	0.07	0.06	-	-
Unknown -4	7.519	1.270	-	-	-	0.13	-	-	-	-	-	-
Unknown -5	11.350	1.89	-	-	0.16	-	-	-	-	-	-	-
Unknown -6	12.068	2.029	-	-	0.63	-	-	-	-	-	-	-
Unknown -7	17.077	2.869	-	4.59	-	0.70	-	-	-	-	-	-
Unknown -8	25.659	4.320	-	-	-	-	-	-	-	-	-	0.19
Unknown -9	35.316	5.951	-	-	-	-	-	-	-	-	-	0.19

6. Precision:

System precision

Rt and area of total 6 measurements along with % RSD is measured. Injected system suitability HPLC. Taken % RSD to Rt along with area counts to HChT by using solution which is used for system suitability. It is obtained by values is finalized as Rt along with response to area should be regular this is finalized by %RSD which lies in among values

1.00% - 5.00%. Due to this case parameter of system precision reaches to required potentiality to validate method. Results are noted in Table 3.

Table 3. System precision Results

Sample	% Recovery
1	70
2	74
3	81
4	86
5	81
6	80
Mean	79

Method precision

According to recommendations, analysis is performed six times on a sample of HChT injection. Related substances were calculated for HChT for the preparation of the sample. Impurity is not detected. As a result, known quantities of are spiked in the preparation of a sample at a specific level. With these values, it is finally clear that this analysis is more precise, the recovery is good, and the values are within acceptable limits. Hence, this proposed process is more accurate. The values are represented in Table 4.

Table 4. HChT for 5mg/100ml

HChT 5mg/100ml		
% of Impurity		
Injection No.	HChT @ RRT:3.08	Combination of impurity's
1	0.0338	0.0339
2	0.0352	0.0355
3	0.0380	0.0380
4	0.0338	0.0350
5	0.0340	0.0340
6	0.0360	0.0360
Mean	0.0349	0.0342
%RSD	4.4	4.4

Intermediate Precision

% RSD is mainly due to HChT content to 06 samples should less than nor equal to 2.00%. Values of mean those are captured in repeatability, as well as intermediate precision won't varies to 3.0 %. Total 06 separate sample preparations for Urirex K Tablets are assayed with help of method of analysis. Specified parameters are changed from repeatability test. % RSD is mainly due to HChT content for samples are 1.00 %.

Intermediate precision mean of HChT deviates by obtained repeatability mean by 0.40%. The values obtained are represented in the Table 5.

Table 5. Repeatability and Intermediate precision

Sample number	Results in mg/tablet
	HChT
1	50.1
2	49.9
3	49.3
4	49.6
5	49.5
6	48.7
Mean	49.5
% RSD	1.0
Sample	Mean Results in mg/tablet
	HChT
Repeatability Intermediate Precision	49.2 49.5
Mean	49.4
% RSD	0.4

Repeatability

% RSD is mainly because the HChT content of six samples must be less than or equal to 2.0 % Six separate sample preparations of Urirex K Tablets are analyzed using the method of analysis. The % RSD is primarily due to HChT content in all six samples, which meets reproducibility requirements at 2.0%. Therefore, the, repeatability test passes for HChT. The results obtained are represented in Table 6.

Table 6. Repeatability Results

Sample number	Results in mg/tab
	HChT
1	48.5
2	49.2
3	50.3
4	49.0
5	48.4
6	49.6
Mean	49.2
% RSD	1.2

7. Linearity

Linearity was demonstrated with HChT, and impurities such as A, B, C, and D are also within the LOQ at 300% of the impurity specification limit. A plotted graph is shown for HChT, impurities A, B, C, D, and E, as well as strength, which is measured in PPM over the X-axis and area over the Y-axis. Correlation coefficient to HChT furthermore R square value is minimum to 0.995. The % intercept is below to 5.0 of response at 100 % specification area. Precision for maximum levels of %RSD is NMT 5.0. 05 solutions which contain 50, 75, 100, 125 as well as 150 % of HChT which may be relative to working strength 10 ppm is prepared after that passed as per method of analysis. A linear regression curve designed; along with R^2 as well as assessment values were computed. Figures 6-7 show the calibration curve, linearity, and residual plot for HChT.

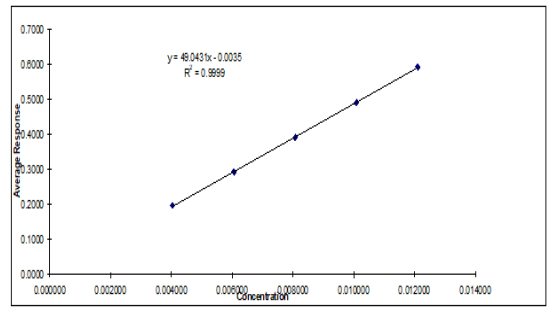

Figure 6. Calibration curve for HChT

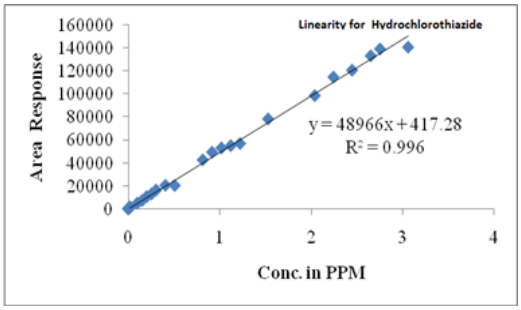

Figure 7. Linearity for HChT

8. Accuracy

Percentage recoveries to compounds those are active for each and every solution prepared were in 98.0 % – 102.0 % of actual amount. Solutions of sample are prepared by known strength HChT for result in strengths representing as 50, 75, 100, 125 and 150 % of HChT which is relative to working test strength. By accuracy values those are shown above, percentage recovery values to HChT satisfied acceptance criteria to accuracy across 50 % - 150 %. To HPLC assay methods, accuracy range varies in between 98.0 % - 102.0 %. Individual as well as mean recovery at each one furthermore every level is varying among 85% to 115% to known impurity. Individual furthermore mean recovery at each value lies as 80.0% to 120.0%. The values were tabulated in Table 7.

Table 7. Accuracy Results

Sample	Theoretical	Actual	% Recovery	% Recovery in terms of Average
50 %	0.9345	0.9183	98	98
50 %	0.9345	0.9168	98	
75 %	1.402	1.430	102	102
75 %	1.402	1.433	102	
100 %	1.869	1.923	103	

100 %	1.869	1.925	103	103
125 %	2.336	2.414	103	103
125 %	2.336	2.415	103	
150 %	2.804	2.894	103	103
150 %	2.804	2.893	103	

9. Robustness

By varying temperature of column ±5°C; change in rate of flow ±0.20ml/min; wavelength variation as ±5nm; organic variation as ±2.0%; Parameters of system suitability must obey all conditions. Total known impurities are separated in the sample barred by foreign substances by the HChT peak. The data presented above demonstrates that the processes of these related substances are resistant to small changes in method parameters. The obtained values are represented in Table 8.

Table 8. Results of Robustness

Change in rate of flow		Initial	-0.2 ml/min	+0.2 ml/min
The % RSD of HChT is NMT 5.0%		0.6mL/min	0.4mL/min	0.8mL/min
SYSTEM SUITABILITY PARAMETERS	HChT	1.2	0.7	0.4
	Imidazole acetic acid	0.6	0.2	0.3
	Imidazole	0.2	0.2	0.2
. **Change in Temperature(20°C±5°C)**		Initial	-5°C	+5°C
The % RSD of HChT NMT is 5.0%		20°C	15°C	25°C
SYSTEM SUITABILITY PARAMETERS	HChT	1.4	0.1	1.1
Change in Organic Phase (40±0.8mL) ACN		Initial	-2%	+2%
The % RSD of HChT is NMT 5.0%		39	38.1	40.7
SYSTEM SUITABILITY PARAMETERS	HChT	1.4	0.2	1.8
Change in wavelength(215±5nm)		Initial	-5nm	+5nm
The % RSD of HChT is NMT 5.0%		215	210	220
SYSTEM SUITABILITY PARAMETERS	HChT	1.4	1.2	0.6

10. Results and Discussion

For this process, the author used the Cosmosil C18 5 µm (15 cm x 4.6mm) Different mobile phase compositions were tried to assess RP-HPLC parameters. Finally, required separation along with a very good peak of symmetry is identified in the mixture of buffer 65 v/v and acetonitrile 35 v/v. The final report specifies a flow rate of 1.0 ml per minute because, when compared to other mixture peaks, this flow provided the best peak shape. The optimal wavelength was determined to be 272 nm. The approximate retention time is 2.5

minutes, and the run time is 4 minutes, which results in a better detector response to the drug. hydrochlorothiazide peak, which is more stable towards exposure to UV. There are no components that are seen to co-elute with the hydrochlorothiazide peak, and with the peak purity values indicated, the HCHT peak should be considered pure spectrally. The method employed is unique to the HChT assay in Urirex K Tablets. The % RSD of peak responses due to HChT must be less than or equal to 2.0% for six injections. R^2 for hydrochlorothiazide is 1.000. Plot is a straight line, and z falls within the specified limit of -1. The method is therefore linear within the specified range. According to the accuracy results, the percentage recovery values for hydrochlorothiazide meet the drug's acceptance criteria for accuracy in the 50%–150% range. The accuracy range for HPLC assay methods is 98.0–102.0 percent. All degradation products, if any, are well separated from each other and from the HCT peak. The theoretical plates for HChT peak from diluted standard solution are NLT 2000. The %RSD for all six injections of HChT peak from diluted standard solution is NMT 5.0, and the %RSD due to HChT content in six samples is 2.0%, which meets reproducibility requirements. Therefore, the repeatability test passes for HChT. For the samples, the RSD due to HChT content is 1.0%. HChT's intermediate precision mean deviates by 0.4% from the obtained repeatability mean. Based on the accuracy results, the Urirex K Tablets assay method has a range of 25–75 mg/tab of HChT, which represents 50%–150% of their working strength. The method for the assay of Urirex K Tablets does comply with requirements for linearity, method precision, and accuracy for HChT across a range of 50 to 150 percent. Therefore, the method is acceptable as valid. Finally, from the results obtained, it is concluded that this method provides better results when compared with other values.

Conclusion

For this research, the authors used the Cosmosil C18 5 µm (15 cm x 4.6 mm). Using buffer at 65 v/v and acetonitrile at 35 v/v, the required separation and peak symmetry are noted. The flow rate is 1.0 ml per minute. The optimum wavelength is 272 nm. The hydrochlorothiazide peak is stable toward UV exposure. There are no components seen to co-elute with the HChT peak, and peak purity values indicated that the HChT peak can therefore be considered spectrally pure. After six injections, the % RSD of the peak responses due to HChT is less than 2.0%. The R2 to HChT is 1.000. According to the accuracy results, the percentage recovery values for HChT satisfy the acceptance criteria for accuracy across the range of 50–150 percent. All degradation products are well separated by HChT. The precision of an analytical procedure expresses the degree of agreement among individual test results. For the samples, the RSD due to HChT content is 1.0%. HChT's intermediate precision mean deviates by 0.4% from the obtained repeatability mean. Based on the accuracy results, the Urirex K Tablets assay method has a range of 25-75 mg/tab of HChT, which represents 50-150 percent of their working strength. By using the above results, the authors conclude that this method provides better results when compared with other values.

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Conflicts of interest

None of the authors that worked on this project have any competing interests.

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