Available online at sjuoz.uoz.edu.krd

journals.uoz.edu.krd

Science Journal of University of Zakho

Vol. 12, No.3, pp.375–381 July-September, 2024

p-ISSN: 2663-628X
e-ISSN: 2663-6298

DEVELOPING AND VALIDATING A STABILITY-INDICATING UV-VIS NANODROP 2000C METHOD FOR THE SIMULTANEOUS DETERMINATION OF THE ANTI-HYPERTENSIVE DRUG HYDROCHLOROTHIAZIDE (HCTZ) IN BOTH BULK AND TABLET DOSAGE FORMS

Faroq O.Qasim [*a],Mohammed A. Hami [b], Nzar I. Yousif [c]

[a] Department of Horticulture, Technical College of Akre, Akre University for Applied Sciences, Kurdistan Region, Iraq- faroq.omer@auas.edu.krd

[b] Chemistry Department, College of Science, University of Zakho, Kurdistan-Region, Iraq- Mohammed.hami@uoz.edu.krd

[c] Department of Medical Laboratory Science, College of Science, Cihan University-Erbil, Kurdistan Region, Iraq. nzar.ibrahim@cihanuniversity.edu.iq

Received: 9 Jun., 2024 / Accepted:3 Aug., 2024 / Published: 15 Aug., 2024. https://doi.org/10.25271/sjuoz.2024.12.3.1324

ABSTRACT:

For the simultaneous determination of anti-hypertensive drugs Hydrochlorothiazide (HCTZ) in bulk and tablet dosage forms, a simple, specific, accurate, and precise stability-indicating UV-Vis Nanodrop 2000c method was developed and validated. The maximum absorption of Hydrochlorothiazide was revealed at 271 nm using methanol as a solvent. The validation results showed good linearity (R^2 = 0.9997) within the concentration range of 10-50µg/mL. The RSD revealed an acceptable value (less than 0.6 percent) in the precision study at three levels. 1.01 and 3.3g µg/mL were found to represent the LOD and LOQ, respectively. Locally and commercial tablets are Available Drug Products. Accuracy at three levels showed good recovery, more than 99% percent. The proposed method was used to analyse the stability of local and commercial tablets. The stability of their pharmaceuticals was investigated under acidic, oxidative, thermal, alkaline, and photolytic conditions in this research. In accordance with the guidelines set by the International Council for Harmonisation (ICH), the method was effectively validated. Subsequently, the validated method was employed to analyze commercially available pharmaceutical dosage forms.

KEYWORDS: UV-Vis Nanodrop 2000c, Validation, Hydrochlorothiazide, Stability Indicating, Hypertension.

1. INTRODUCTION

High blood pressure, scientifically named Hypertension, is a chronic medical condition characterised by increasing pressure within the arteries (Mills et al., 2016) (Zhou et al., 2021) (Qasim & Mohammed, 2021). High blood pressure is responsible for 45 per cent of deaths, as reported by the World Health Organization (Lloyd-Jones et al., 2009). Thiazide-related diuretics are still recommended as the initial treatment for all patients with Hypertension in certain countries, such as Australia, Canada, Europe, International/ASH, and JNCB, according to the latest guidelines (Lloyd-Jones et al., 2009).

Hydrochlorothiazide (HCTZ) can be used alone or in combination with an extensive list of over 40 other drugs, providing pharmacists with a wide range of options for patient care. These include Amiloride, Telmisartan, Aliskiren, Amlodipine, Atenolol, Candesartan, Cilazaprilat, Valsartan, Furosemide, Clonidine, Aspirin, Simvastatin, Losartan carboxylic acid, Ramiprilat, Irbesartan, Metoprolol, Quinaprilat, Losartan, Olmesartan, Doxazosin tartrate, Chlorthalidone, Enalapril, Nitrendipine, Dehydronitrendipine, Cilazapril, Quinapril, Fluvastatin, Triamterene, Benazepril, Fosinopril, Enalaprilat, Captopril disulfide, Nifedipine, Ramipril, Nebivolol, Labetalol, Salicylic acid, Lisinopril, Eprosartan, Reserpine, and more(Roberts, 2008).

Hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is a benzothiadiazide compound with a molecular weight of 297.74. It consists of a 3, 4-dihydro-2H-1, 2, 4-benzothiadiazine 1, 1-dioxide structure, with a chloro group added to 6 position and a sulphonamide linked to 7 position . HCTZ is quickly absorbed via the digestive system when ingested and can be found in urine within one hour. (Chakraborty et al., 2024). Figure 1 shows the structure of HCTZ.

Various Analytical Methods In the literature reviewing used for Determination of HCTZ Spectroscopic (Hemke et al., 2010)(Real et al., 2010). Chromatographic techniques include HPLC, IP-LC, TLC, and LC-MS (Mohammed & Mohammed, 2016)(Bhadresh et al., 2015)(Patel et al., 2014)(Ali et al., 2016)(Medoxomil, 2020)(Chakraborty et al., 2024)(Tsvetkova et al., 2015)(Patel & Patel, 2022), and Hyphenated techniques include LC-MS and UPLC(Bharathi et al., 2012)(Ongas et al., 2018)(Kaushik D, 2013)(Lahsini & Monser, 2015)(Devi & Bhavani, 2023).

Stability indication is one of the most essential steps in developing pharmaceutical products. Stability indicators are used to reveal how the quality of medical products changes over time in response to different environmental factors (Qasim & Mohammed, 2021) (Shaikh et al., 2020). Degradation of novel drug ingredients and derivatives under circumstances more difficult than accelerated conditions, such as acidic, alkaline, oxidative, photolytic, and thermal, is known as forced degradation studies (Chakraborty et al., 2018). The main purpose of a stability study is to generate the stability profile of a drug product so that the prediction of the shelf life of the product can be made before launching it into the market. In this context, the aim of this study was to develop and validate a stability-indicating method using UV-Vis Nanodrop 2000c for the quality control of pharmaceutical formulations and promoting benefits to public health and to compare its performance with established methods.

* Corresponding author

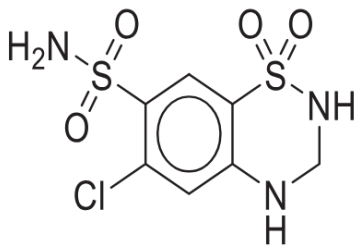

Figure 1: Chemical structure of HCTZ

2. METHODS AND MATERIALS

2.1 Chemicals

The Kurdish-Iraqi company Awamedica got the pharmaceutical active ingredient hydrochlorothiazide with 99% purity. Hydrogen peroxide (H_2O_2) came from Roth in Germany, while analytical grade reagents, including sodium hydroxide (NaOH) and hydrochloric acid (HCl), were purchased from Scharlau in Spain. Merck in Germany provided acetonitrile, water and methanol for high-pressure liquid chromatography (HPLC). Commercial tablets of HCT, AIWA and Hydrazine Awa were procured at random from local pharmacies.

2.2 Instrumentation

UV-Vis Nanodrop Thermo Scientific (2000C Micro volume) was used for these studies. A UV lamp (UVC-215 TS, 220-240v, 8W, 50/6 Hz) was utilized for the photodegradation investigation. The Voyager® was used as an analytical balance, Elmasonic P (100W, 80 KHz) was used as the water bath shaker, and the Lab Tech (LVO-2030) was used as an oven.

2.3 Standard stock preparation and working solutions

A 250 mg of pure drug of hydrochlorothiazide (HCTZ) was precisely weighed, and methanol was used as a solvent to dissolve the HCTZ and then transferred to a volumetric flask with a capacity of 250 mL to create a stock standard solution with 1000 ppm. After that, solvent was added to bring the overall volume up to the line. We kept this stock standard solution refrigerated and utilized it to create different concentrations of working solutions.

2.4 Method Optimization

Using UV-Vis Nanodrop 2000c spectrophotometer for the determination of the maximum wavelength (λmax) of HCTZ between 200 and 700 nm. A preliminary solubility study of HCTZ was also used with acetonitrile, water, ethanol, and methanol.

2.5 Method Validation

The International Conference on Harmonization (ICH) guidelines were followed in the validation of the nanodrop spectrophotometric technique with respect to system, linearity, accuracy, precision, specificity, LOD, LOQ, and robustness.

2.6 Forced Degradation Studies:

The stability of HCTZ in pharmaceutical formulations was investigated using several conditions, such as alkaline, oxidative, acidic, thermal, and photolytic, over a period of time.

2.6.1 Preparation of stock solution of formulation:

Accurately weighing ten tablets (2.5 g) of commercial products, each one containing 50 mg of pure HCTZ, they were then crushed. A quantity of 1.25 g of tablet powder, which is equal to 250 mg of HCT, was placed in a volumetric flask with a capacity of 250 mL. Then, 25 mL of solvent was added to the flask, and the mixture combination underwent sonication for a duration of 15 minutes. The solution was filtered with Whitman filter paper in a separate volumetric flask with a pore size of 0.2 µm. Subsequently, the volume was adjusted to the desired level with the solvent, resulting in the formation of a stock solution with a concentration of 1000 µg/mL of HCTZ in the drug products. Different concentrations of working solutions were generated using the formulation stock solution.

2.6.2 Blank solution preparation:

The blank solution was prepared by transferring 25 mL of each reagent used for degradation (including 2N, 4N, and 6N of HCl, NaOH, and 5, 10, and 15% of H_2O_2) to a 25 mL volumetric flask. The volume was then filled with methanol and heated at a temperature of 90 °C for 3 hours. An aliquot of 4 mL was taken from this solution and placed in a separate volumetric flask with a capacity of 25 mL at various time intervals. The aliquot was neutralized with the appropriate reagent, and then the volume was adjusted to the desired level using methanol. The absorbance was then estimated at 271 nm.

2.6.3 Acid degradation:

A 50-ppm solution of the pharmaceutical formulation was produced from the stock solution of the formulation. The test solution was prepared by putting 1 mL into a 50 mL volumetric flask. Then, 2.5 mL of HCl solution with concentrations of 2N, 4N, or 6N was added to the flask. The flask was equipped with a reflux assembly and heated in a water bath at a temperature of 90 °C for 3 hours. The solution was neutralized after refluxing using an equal concentration of NaOH and then diluted with methanol (HPLC grade) to a final volume of 50 mL. A 2.5 mL volume of the solution was extracted and then mixed with a solvent to get a solution with a concentration of 50 ppm.

2.6.4 Alkaline degradation:

The identical methodology outlined in section 2.6.3 was employed, substituting 2N, 4N, and 6N HCl with 2N, 4N, and 6N NaOH.

2.6.5 Oxidative degradation:

The identical methodology outlined in section 2.6.3 was employed, substituting 2N, 4N, and 6N HCl with 5, 10, and 15 H_2O_2.

2.6.6 Photolith degradation:

The drug's photodegradation was carried out by exposing it to sunlight, darkness, and ultraviolet (UV) light. The photodegradation HCTZ solutions were produced in methanol using a 1000 ppm stock solution. A volume of 2.5 mL of solution was extracted and then mixed with a solvent to get a solution with a concentration of 50 ppm. The UV lamp model is UVC-215 TS 8W, operating at a voltage range of 220-240v and a frequency of 50/60 Hz.

2.6.7 Thermal degradation:

The drug was subjected to heat degradation by exposing it to a temperature of 90°C for 72 hours. From these drugs, powder-prepared solutions of hydrochlorothiazide that had undergone thermal degradation were produced in methanol at a concentration of 1000 ppm. 2.5 mL of the solution was extracted and then mixed with a solvent to get a solution with a concentration of 50 ppm. Additionally, there is a solution consisting of one blank solution, a mixture of 2.5 mL of methanol diluted up to 50 mL, and a standard solution containing 50 ppm of HCTZ.

3. RESULT AND DISCUSSION

3.1 Method development

The quantitative determination of HCTZ pharmaceuticals was achieved through the improvement and validation of UV spectrophotometric method. The numerous analytical parameters, including precision, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, specificity, and Robustness, were determined in accordance with the International Conference on Harmonization's ICH Q2B criteria.

3.1.1 Determination and scanning of λ_{max}:

The overlain spectra of HCTZ revealed the maximum wavelength at 271 nm as given in figure2. Hence these λ_{max} was selected for simultaneous estimation of HCTZ.

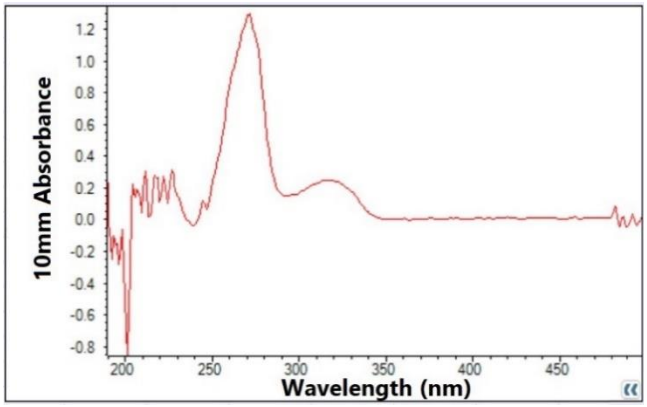

Figure 2: Spectrum of Hydrochlorothiazide 40 ppm

3.1.2 Types of solvent:

For this research, different solvents was used to select the maximum wavelength of HCTZ, such as Methanol, acetonitrile, water, and ethanol. The maximum wavelength was showed in methanol that is 1.28nm more than other solvents, and so on methanol were selected as a solvent.

3.2 Method Validation

Method validation is a process of the collection and evaluation of data to establish precise evidence that the analytical method is capable of producing quality products. The developed method was validated according to ICH guidelines for the precision, linearity, range, Sensitivity, LOD, LOQ, accuracy, specificity, and robustness.

3.2.1 Linearity and Range:

For determining the linearity correlation of the HCTZ drug, five different concentrations within the concentration range of 10-50 µg/mL were analyzed. Figure No.3 illustrates the regression equations for HCTZ, which were determined to be y = 0.032x - 0.0095 with correlation coefficients (R^2) of 0.9997.

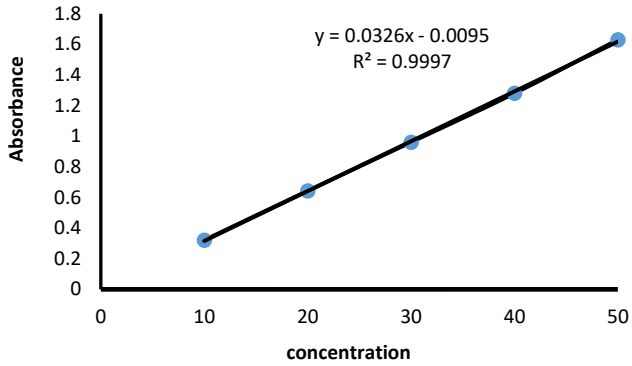

Figure 3: Calibration curve 10-50 µg/mL of Hydrochlorothiazide

3.2.2 Precision:

The method's precision was assessed by analyzing pure drug solutions at three distinct concentrations, with each determination performed three times. The relative standard deviations (RSD) were less than 0.6, indicating that the current method is precise, as shown in Table 1. The relative standard deviation values for intra-day, inter-day, and repeatable ranged from 0.12% to 0.72%. These results prove that the current method can used in routine days.

Table 1: Evaluation of Precision Study

Number of Samples	Intraday			Interday			repeatability		
	5	10	15	5	10	15	5	10	15
1	0.16	0.323	0.482	0.16	0.323	0.485	0.162	0.323	0.481
2	0.161	0.32	0.48	0.162	0.324	0.485	0.162	0.323	0.481
3	0.16	0.32	0.481	0.162	0.321	0.486	0.163	0.321	0.482
Mean (%)	0.16	0.32	0.48	0.16	0.32	0.49	0.16	0.32	0.48
SD	0.0006	0.0017	0.0010	0.0012	0.0015	0.0006	0.0006	0.0012	0.0006
% RSD	0.36	0.54	0.21	0.72	0.47	0.12	0.36	0.36	0.12

3.2.3 Accuracy (recovery method)

Evaluated the accuracy of the proposed method by analyzing a pure drug of HCTZ from excipients with known quantities of the drug. This resulted in solutions with concentrations of 5, 10, and 15 µg/mL for HCTZ, which correspond to 50, 100, and 150% of the expected analytical concentrations, respectively. The HCTZ values were obtained at concentrations of 5, 10.1, and 14.9 µg/mL, with relative standard deviations (RSDs) lower than 1.0%. The accuracy of the measurements was determined to be 100%, 100.17%, and 99.9% for the respective concentrations. These results demonstrate that the procedure was accurate within the targeted range.

Table 2: Evaluation of accuracy (Recovery) study.

Level of Recovery (%)	Added amount (mg)	(Abs.)	conc. Obtained	Recovery%	Statistical Analysis		
					Mean (%)	SD	%RSD
sample 1	5	0.159	4.97	99.38	100.00	0.63	0.63
	5	0.161	5.03	100.63			
	5	0.160	5.00	100.00			
sample 2	10	0.321	10.03	100.31	100.17	0.16	0.16
	10	0.320	10.00	100.00			
	10	0.321	10.02	100.21			
sample 3	15	0.478	14.94	99.58	99.93	0.32	0.32
	15	0.480	15.00	100.00			
	15	0.481	15.03	100.21			

3.2.4 Limit of detection (LOD) and limit of quantification (LOQ):

Determination of the limit of detection (LOD) and limit of quantification (LOQ). The limit of detection (LOD) of an analytical technique is the lowest concentration that can be detected and distinguished from zero but may not always be calculated as an exact measure. Whereas the limit of quantification (LOQ) is the lowest concentration of the analyte in a sample that can be quantitatively determined with suitable precision and accuracy(Qasim & Mohammed, 2021). LOD and LOQ were found using a linear regression model, as defined by the International Council for Harmonization (ICH). The LOD and LOQ were computed based on the slope and standard deviation of the intercept of the mean of three calibration curves. The limit of detection (LOD) and limit of quantification (LOQ) achieved for HCTZ were 1.01 and 3.3 µg/mL, respectively. The developed method was found to be sensitive, as seen in Table 3.

Table 3: LOD and LOQ data from the calibration curve.

Parameters	Value
Range	10-50 (µg/mL)
Regression eq.	y = 0.0326x - 0.0095
R^2	0.9997
Slope	0.0326
Intercept	-0.0095
SD	0.0110
LOD µg/mL	1.0145
LOQ µg/mL	3.3817

3.2.5 Specificity

An analytical method's specificity refers to its capacity to accurately measure the analyte's response even when other substances are present, such as contaminants, degradation products, and matrix. The analytical placebo solution, which included all excipients except HCTZ, was prepared according to the sample preparation procedure. To determine the impact of these additives, which are non-active substances, a combination of standard solutions and commercially available pharmaceutical formulations of HCTZ was investigated using the validated method. The method's specificity was further assessed to confirm the absence of interference products that result from forced degradation. The present method's specificity was found to be 99.99%, with RSD% lower than 0.45.

3.2.6 Robustness:

For the evaluation of the robustness study, different wavelengths were used to estimate the HCTZ drug. The RSD value was found to be less than 1%, as shown in Table 4; thus, it can be inferred that the developed method remains stable when using different wavelengths.

Table 4: Robustness data for change in wavelength (λ_{max}) from 269 to 273.

Number of the Samples	Abs. at 269 (nanometre)	Abs. at 270 (nanometre)	Abs. at 271 (nanometre)	Abs. at 272 (nanometre)	Abs. at 273 (nanometre)

1	0.159	0.158	0.159	0.158	0.158
2	0.159	0.157	0.161	0.159	0.16
3	0.158	0.16	0.16	0.158	0.158
4	0.16	0.157	0.161	0.159	0.16
5	0.157	0.156	0.161	0.161	0.157
Mean	0.159	0.158	0.160	0.159	0.159
SD	0.0011	0.0015	0.0009	0.0009	0.0013
% RSD	0.72	0.96	0.56	0.56	0.85

3.2.7 Applicability of Marketing Formulations

The improved method has been successfully implemented in the estimation HCTZ in marketing formulation. Recovery experiments were performed at three solutions of pure drug with two other tablet formulations, in which the sample stock solutions were spiked with pure and tablet formulation solutions containing 25 of the labelled amounts of drugs. The results are determined and summarized in Table 5. The mean (%) recovery was 99.58% and 100.04% for pure HCTZ, Hydrazide Awa, and HCT AIWA, respectively.

Table 5. Results of marketed formulation

Tablet formulation(brand)	Labelled amount	Amount found	Labelled amount (% Recovery)	RSD%
Standard HCT	25µg/ml	25.02	100.08	0.190
Hydrazide Awa	25µg/ml	24.94	99.75	0.375
HCT AIWA	25µg/ml	24.98	99.92	0.473

Each value is the mean of five observations

3.3 Stability Indicating:

The table (Table 6) illustrates the findings of force degradation tests conducted on HCTZ under various stress conditions, including acid, alkaline, oxidation, photolysis, and thermal degradation. The results are shown as the percentage of degradation of the active pharmaceutical ingredient (API).

The % degradation of both the drugs with pure HCTZ was found, as given in the Table 6. So, the proposed method for estimating Hydrochlorothiazide is very dependable and well-suited for regular analysis in pharmaceutical formulations.

Table 6. Results of forced degradation

Stress condition/ duration/ state		% Degradation			
		Standard HCT	Hydrazide Awa	HCT AIWA	Observation
Acidic	Acidic / 2N for three h	8.25	10.38	9.06	Degrade
	Acidic / 4N for three h	12.38	13.80	12.5	Degrade
	Acidic / 6N for three h	16.17	16.34	16.8	Degrade
Alkaline	Alkaline / 2N for three h	13.50	13.75	13.87	Degrade
	Alkaline / 4N for three h	23.2	24.12	23.41	Degrade
	Alkaline / 6N for three h	34.7	35.1	35	Degrade
Oxidative	Oxidative/ 5% H_2O_2 for three h	26.00	27.42	27.3	Degrade
	Oxidative/ 10% H_2O_2 for three h	45.28	45.9	44.9	Degrade
	Oxidative/ 15% H_2O_2 for three h	58.38	59.2	60.16	Degrade
Photo	Photo/three days / solid Sun	4.50	4.13	4.00	No change
	Photo/three days / solid Dark	1.23	1.89	1.61	No change
	Photo/three days / solid UV	1.2	2.6	2.23	No change
Thermal	90℃ for 72 hrs.	4.38	4.50	4.38	No change

4. COMPARISON OF THE PRESENT METHOD WITH OTHER METHODS IN THE LITERATURE:

A comparative analysis with other established methods, such as High-Performance Liquid Chromatography (HPLC), Ultra-performance liquid chromatography (UPLC), and traditional UV-Vis spectrophotometry, was conducted. The analysis highlighted the following advantages, limitations, and Rigidity of the UV-Vis Nanodrop 2000c method.

1- Advantages:

Nanodrop 2000c requires smaller sample volumes and provides rapid results, Lower operational costs due to reduced reagent and solvent use, simplified sample preparation and a user-friendly interface.

2- Limitation:

Limitation refers to the inherent constraints or restrictions that impact the scope, validity, or generalizability of the study's findings, Such as sensitivity: The Nanodrop 2000c have good sensitivity compared to LC methods and UV-Vis, as shown in Table 7.

3-**Rigidity:**

Rigidity refers to the strict adherence to specific protocols, methods, or procedures without allowing flexibility or deviations, such as:

- The method is validated rigorously following ICH guidelines, ensuring reliability and reproducibility.
- The method's specificity for HCTZ ensures minimal interference from excipients and degradation products.
- The degradation studies were conducted under controlled laboratory conditions, ensuring precise data.
- High precision (RSD less than 0.75%) indicates the method's reproducibility and repeatability.

Table 7: Comparative Analysis of Nanodrop 2000c with other established methods.

Method (system)	R^2	Precision (%RSD)	Recovery %	LOQ	LOD	Degradation %					Ref.
						Acid	Alkaline	Oxidative	photolith	thermal	
Nanodrop 2000c	0.999	Less than 0.75	100.03	3.3	1.0	Degrade	Degrade	Degrade	No change	No change	Present Method
HPLC	0.9996	Less than 1.5	98.39-100.94	0.32	0.10	-	-	-	-	-	(Vidyadhara et al., 2014)
UPLC	0.999	1.2	103.19	9.93	2.95	Degrade	No change	Degrade	No change	No change	(Devi and Bhavani, 2023)
LC method	0.9999	0.90	98.0 to 102.0	3.0	0.9	Degrade	Degrade	Degrade	No change	No change	(Menon et al., 2009)
UV-1601 Shimadzu	0.996	Less than 0.5	99.12	-	-	Degrade	No change	Degrade	-	Degrade	(Sayyed et al., 2015)
Shimadzu HPLC	0.999	1.15	100.45	0.070	0.023	Degrade	Degrade	Degrade	No change	No change	(Rane et al., 2010)
UHPLC	0.9903	0.72 ± 0.87	100.60 ± 0.26	3.19	1.05	-	-	-	-	-	(Oliveira et al., 2024)
Thermo Scientific UHPLC	0.9958	1.70	99.05-101.18	-	-	Degrade	Degrade	Degrade	Degrade	No change	(Proti et al., 2017)

CONCLUSION:

A UV spectroscopic method was developed and validated according to the guidelines set by the International Council for Harmonization (ICH). The validation process included assessing the method's linearity, precision, accuracy, repeatability, and stability indicating. All validation parameters were determined to be within the acceptable range as per the ICH recommendations. The method that was developed was effectively used to estimate the amount of HCTZ in specific commercial formulations. The established UV method may be determined to be precise, accurate, sensitive, and repeatable for quantitatively assessing the bulk and formulation of HCTZ.

Furthermore, the proposed method is a reliable stability-indicating method accurately capable of detecting HCTZ even in the presence of its degradation products in different stress conditions. It was observed that HCTZ is unstable under acidic, basic, and oxidizing conditions, while it is relatively more stable under photolytic and thermal conditions. The pharmaceutical industries can use the developed method for regular analysis of HCTZ.

REFERENCE:

Ali, T.A., Mohamed, G.G., Aglan, A.A. & Heakal, F.E.T. (2016). "RP-HPLC Stability-indicating Method for Estimation of Irbesartan and Hydrochlorothiazide in Bulk and Pharmaceutical Dosage Form", *Chinese Journal of Analytical Chemistry*, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Vol. 44 No. 1, pp. e1601–e1608.

Bharathi, D.V., Hotha, K.K., Chatki, P.K., Satyanarayana, V. & Venkateswarlu, V. (2012). "LC-MS/MS method for simultaneousestimationofcandesartanandhydrochlorothiazide in human plasma and its use in clinical pharmacokinetics", *Bioanalysis*, Vol. 4 No. 10, pp. 1195–1204.

Chakraborty, A., Jayaseelan, K., Rathinam, S., Kokilambigai, K.S. & Nethra, K. (2024). "Bioanalytical methods and

extraction techniques for the quantification of hydrochlorothiazideanditspharmaceutical combinations:Acomprehensivereview",*Acta Chromatographica*,No.0,available at:https://doi.org/10.1556/1326.2023.01187.

Devi, D.A. & Bhavani, P.G. (2023). "Development and validation of stability indicating UPLC method for the simultaneous estimation of triamterene and hydrochlorothiazide in combined dosage forms using quality by design approach", *Future Journal of Pharmaceutical Sciences*, Springer Berlin Heidelberg, Vol.9No. 1, available at:https://doi.org/10.1186/s43094-022-00438-0.

Hemke, A.T., Bhure, M. V., Chouhan, K.S., Gupta, K.R. and Wadodkar, S.G. (2010), "UV spectrophotometric determination of hydrochlorothiazide and olmesartan medoxomil in pharmaceutical formulation", *E-Journal of Chemistry*, Vol. 7 No. 4, pp. 1156–1161.

Kaushik D, A.P. (2013). "UPLC Method for Simultaneous Determination of Valsartan & Hydrochlorothiazide in Drug Products", *Journal of Chromatography & Separation Techniques*, Vol. 04 No. 05, available at:https://doi.org/10.4172/2157-7064.1000182.

Lahsini, R. and Monser, L. (2015). "Development of a new UPLC method for simultaneous determination of valsartan, amlodipine besylate, and hydrochlorothiazide pharmaceutical products", *Acta Chromatographica*, Vol. 27 No. 3, pp. 449–460.

Medoxomil, O. (2020). "Development and Validation of Rp-Hplc Method for Simultaneous Determination of a Combined Formulation of Development and Validation of Rp – Hplc Method for Simultaneous Determination of a Combined Formulation of Olmesartan Medoxomil&",No.June,availableat:https://doi.org/10.20959/wjpps20206-16468.

Menon, S.K., Panchal, J.G. & Patel, R. V. (2009). "Development and validation of a stability indicating LC method for the determination of faropenem in pharmaceutical formulations", *Chromatographia*, Vol. 69 No. 9–10, pp. 1013–1018.

Mohammed, N.S. & Mohammed, A.J. (2016). "Development and Validation of RP-HPLC Method for the Determination of Hydrochlorothiazide in Bulk Drug and Pharmaceutical Dosage Form", *Chromatography Research International*, Vol. 2016, pp. 1–7.

Oliveira, C.V. De, Peron, A., Rashid, A., Alonso, F., Fajardo, G., Lourenço, F.R., Franco, M., et al. (2024). "Validation , measurement uncertainty estimation and evaluation of UHPLC greenness for simultaneous determination of metoprolol tartrate and hydrochlorothiazide in binary tablet", available at:https://doi.org/10.1080/16583655.2024.2339010.

Ongas, M.O., Kokwaro, G., Oloo, F., Kirimi, C.K., Mkonje, A., Otieno, F., Muasya, C., et al. (2018), "LC–MS/MS Method for Quantitation of Hydrochlorothia-zide and Nifedipine in Human Plasma", *ABC Research Alert*, Vol. 6 No. 3, p. Kenya.

Patel, R.B. and Patel, M.R. (2022). "a Novel Validated Stability Indicating Analytical Hptlc Method for Quantitation of Hydrochlorothiazide and Lisinopril in Tablet Formulation", *Indian Drugs*, Vol. 59 No. 6, pp. 47–51.

Patel, U.M., Chokshi, A.B. and Desai, P.R. (2014), "Development and validation of RP-HPLC method for determination of hydrochlorothiazide, Olmesartan medoxomil and their related substances in combined tablet dosage form", *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 6 No. 9, pp. 318–323.

Proti, A., Jasmina, Š., Krmar, J. and Ze, M. (2017), "Multicriteria Optimization Methodology in Stability-Indicating Method Development of Cilazapril and Hydrochlorothiazide", Vol. 55 No. 6, pp. 625–637.

Qasim, F.O. and Mohammed, N.M.S. (2021), "A Nanodrop Spectrophotometric Method and Stability Indicating for DeterminationofAmlodipine Besylate in Pharmaceutical Formulations of Kurdistan of Iraq", *Science Journal of University of Zakho*, Vol. 9 No. 1, pp. 25–29.

Rane, V.P., Patil, K.R., Sangshetti, J.N., Yeole, R.D. and Shinde, D.B. (2010), "Stability Indicating LC Method for Simultaneous Determination of Irbesartan and Hydrochlorothiazide in Pharmaceutical Preparations", Vol. 48 No. August, pp. 595–600.

Real, F.J., Acero, J.L., Benitez, F.J., Roldán, G. and Fernández, L.C. (2010), "Oxidation of hydrochlorothiazide by UV radiation, hydroxyl radicals and ozone: Kinetics and elimination from water systems", *Chemical Engineering Journal*, Vol. 160 No. 1, pp. 72–78.

Sayyed, Z.M., Shinde, S.A., Chaware, V.J., Chaudhari, B.P., Biyani, K.R., Zuber, M. and Sayyed, M. (2015), "DevelopmentandValidation of UV-Spectrophotometric Method for Simultaneous Estimation of Amlodipine Besylate and Hydrochlorothiazide in Combined Dosage Form Including Stability Study", *J Pharm Sci Bioscientific Res. 2015*, Vol. 5 No. 5, pp. 487–493.

Tsvetkova, D.D., Obreshkova, D.P., Petkova, V.B., Pankova, S.A., Atanasov, P.J. and Kasnakova, P.S. (2015), "Simultaneousdeterminationofvalsartanand hydrochlorothiazideintabletsbythin-layer chromatography- densitometric method.", *World JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES*, Vol. 4 No. 4, pp. 1–11.

Vidyadhara, S., Sasidhar, R.L.C., Rao, B.V., Tejaswi, K. and Reshma, M. (2014), "Method development and validation for simultaneous estimation of olmesartan medoxomil and hydrochlorothiazide by RP-HPLC", *Oriental Journal of Chemistry*, Vol. 30 No. 1, pp. 195–201.

Roberts, B. H. (2008). Treating and Beating Heart Disease: A Consumer's Guide to Cardiac Medicines: A Consumer's Guide to Cardiac Medicines. Jones & Bartlett Learning.