Research Article

Development and Validation of RP-HPLC Method for the Determination of Hydrochlorothiazide in Bulk Drug and Pharmaceutical Dosage Form

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An HPLC-PDA method was developed and validated for the determination of hydrochlorothiazide in bulk and pharmaceutical formulation. The method was optimized selecting chromatographic conditions of 50 : 50 acetonitrile : water, Inertsil® column (ODS-3 250 mm × 4.6 mm 5 μm), 20 μL injection volume, flow rate of 1 mL/min at ambient temperature (30°C), and 272 nm. Another column of C₁₈Zorbax® (Eclipse Plus, 4.6 × 250 mm, 5 μm) was tested showing no big difference in the method results. The method was validated giving good precision (RSD% < 1), acceptable linearity ($R^2 \geq 0.9978$), and low LOD and LOQ (0.5 and 1.7 μg/mL, resp.) on both columns. Successful application on pharmaceutical dosage tablet form gave high recovery of 99.93%. The method was compared with official BP and other reported methods. The proposed method is economic, simple, and rapid and hence can be employed for routine analysis in quality control laboratories.

1. Introduction

Hydrochlorothiazide chemical name is 2H-1,2,4-Benzothiadiazine-7-sulfonamide,6-chloro-3,4-dihydro-,1,1-dioxide (Figure 1). It is slightly soluble in water and sparingly soluble in acetonitrile. Hydrochlorothiazide belongs to a class of drugs called thiazide diuretics antihypertensive [1]. Hydrochlorothiazide binds to and inhibits the enzyme carbonic anhydrase. It is frequently used alone or in combination with other medications for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, hypoparathyroidism, and edema and prevention of kidney stones and used in the treatment of osteoporosis [2].

An extensive literature survey was carried out and found that there are few analytical methods for the determination of hydrochlorothiazide alone or in its combination with other drugs in pharmaceutical preparations including spectrophotometry [3, 4], flow injection [5], TLC [6], and HPLC [7–9]. A recent study involved a review of developing methods using these analytical techniques for estimation of

hydrochlorothiazide in various biological samples with focus on sample pretreatment methods and separation methods [10].

In this study, an attempt was made to develop new HPLC method for hydrochlorothiazide estimation in bulk and pharmaceutical formulation with good precision, accuracy, linearity, and reproducibility using two columns C₁₈Inertsil® ODS-3 and C₁₈Zorbax Eclipse Plus.

Nowadays, HPLC columns packed with a stationary phase of C₁₈-bonded silica are produced by many manufacturers in different parts of the world for the separation of many organic compounds. C₁₈ columns from different manufacturers, but with the same bonded phase, may provide different separation selectivity for many separation components [11]. Even differences between columns provided by the same manufacturers have been reported [12].

2. Materials and Methods

2.1. HPLC System. Analytical HPLC system comprised a Flexar autosampler injector-no Peltier, Flexar Binary pump,

FIGURE 1: The chemical structure of hydrochlorothiazide.

Inertsil (ODS-3 250 mm $\times$ 4.6 mm, 5 μ m siloxane), and a Flexar/S275/S200EP Photo Diode Array Detector coupled with chromera® version 3.4.0.5712 software. Flexar column oven was connected with a system to control the column temperature. Column of Zorbax® Eclipse Plus (4.6 $\times$ 250 mm, 5 μ m) was used as well.

2.2. Chemicals. Pharmaceutically pure sample of HCTZ drug was obtained from Awamedica Company (in Erbil city, Kurdistan region of Iraq). Commercial tablet of HCTZ (100 mg) was procured from the local drug market. Acetonitrile and water were HPLC from merck KGaA, 64271 Darmstadt, Germany.

2.2.1. Preparation the Mobile Phase. The mobile phase of HPLC analysis was prepared from organic solvent of acetonitrile and water (v:v%) (50%, 60%, and 70% of ACN). The mobile phase was degassed using an ultrasonic bath (100 W, 80 KHz, Elmasonic P, Germany.).

2.2.2. Stock and Working Solutions. Stock solution of 1000 μ g/mL of HCTZ was prepared by dissolving 0.1 g of sample in 100 mL of acetonitrile. This stock standard solution was stored in a refrigerator and used to prepare the working solutions at different concentrations.

2.2.3. Sample Solution Preparation. Ten tablets were crushed to fine powder. An accurately weighed portion of the powder (equivalent to 20 μ g/mL) was taken and dissolved in 100 mL of acetonitrile.

2.3. Determination of Maximum Absorption of HCTZ. A UV-Visible Double beam Spectrophotometer (200–700 nm, Lambda 25, Perkin Elmer, USA) was used to measure the wavelength ($\lambda_{\max}$) at maximum absorbance for HCTZ in acetonitrile in the range 200–400 nm analysing 10 μ g/mL standard solution of HCTZ.

2.4. Development and Optimization of the HPLC Method

2.4.1. The Effect of Different Strengths of the Mobile Phase. To construct a basic background for developing an HPLC method with fast analysis for the determination of HCTZ, the effect of the different concentrations of the mobile phase was investigated. Isocratic elution run was employed by analysing solution of 10 μ g/mL at different strengths of the mobile phase (50%, 60%, and 70% (v/v)) of ACN. The chromatographic conditions were set using a 20 μ L injection volume, flow rate

of 1.0 mL/min, column oven at ambient temperature (30°C), and UV detection at a wavelength of 272 nm.

2.4.2. The Effect of Injection Volume. The effect of injection volume was examined as parameter that may affect peak elution during method development. Three injection volumes were tested including 10, 20, and 30 μ L using solution of 10 μ g/mL at 50% ACN mobile phase.

2.4.3. The Effect of Column Temperature. Three different temperatures of 40°C, 35°C, and ambient temperature (30°C) were tested to study the effect of the column oven temperature analysing 10 μ g/mL of HCTZ at 50% ACN mobile phase.

2.4.4. The Effect of Different Columns. The effect of the different column brands on the optimization of HCTZ peak was investigated using two columns C₁₈Inertsil ODS-3 (4.6 $\times$ 250 mm, 5 μ m) and C₁₈Zorbax Eclipse Plus (4.6 $\times$ 250 mm, 5 μ m).

2.5. Method Validation

2.5.1. Assessment of the Precision of Method. The precision of the HPLC analytical method was estimated by repeated injections ($n = 5$) of 5 μ g/mL solution of HCTZ using two columns (C₁₈Inertsil column and C₁₈Zorbax column) calculating relative standard deviation (RSD%) of the peak area of HCTZ on each column.

2.5.2. Linearity Assessment. Linearity of the calibration curve was determined at two ranges (2–10 and 10–30 μ g/mL) preparing five different concentrations at each range. Linearity was determined using two columns (C₁₈Inertsil and C₁₈Zorbax columns). Aliquots of these solutions (20 μ L) were injected into the analytical HPLC system and the detector response (peak area) was plotted against the nominated concentration to generate the calibration curve. Using Excel® software, from the regression line, the coefficient of determination (R^2) was obtained to statistically assess the linear relationship.

2.5.3. Limit of Detection and Limit of Quantification. The limit of detection (LOD) and limit of quantification (LOQ) of HCTZ in solution were calculated using two columns based on the data from the lower range of the calibration curve [13]. The calibration curve at low levels of HCTZ was used to statistically estimate the HCTZ peak area, which was significantly different from the blank peak area. The equation used to estimate this peak area was

$$\text{LOD peak area} = y_B + 3s_B, \quad (1)$$

where y_B is the blank peak area and s_B is the standard deviation of the blank peak area. In practice, the value of y_B and s_B can be estimated from the calibration graph at the lower range by using values from the regression analysis of variance. Therefore, the value of y_B was replaced by the intercept value of the regression equation of the calibration graph of HCTZ at the lower range of 2–10 μ g/mL. Similarly,

the term s_B was replaced by the residual standard deviation, which is the square root of the error mean square. Both terms were estimated using the regression function in Excel software. The statistical data consist of the intercept, slope, and standard deviation of the regression line (SD); after calculating the LOD and the LOQ peak area depending on the above information of the regression line, the LOD and the LOQ were determined according to the following equations:

$$\text{LOD Peak area} = \text{intercept} + 3 \text{ SD},$$

$$\text{LOD} = \frac{(\text{LOD Peak area} - \text{intercept})}{\text{slope}}, \quad (2)$$

$$\text{LOQ Peak area} = \text{intercept} + 10 \text{ SD},$$

$$\text{LOQ} = \frac{(\text{LOQ Peak area} - \text{intercept})}{\text{slope}}.$$

2.5.4. Application of the Method to Dosage Form. The accuracy of proposed method was ascertained on the basis of average recovery % to determine HCTZ in its pharmaceutical preparation as tablets (equivalent to 20 $\mu\text{g/mL}$) by triplicate injections under the same chromatographic conditions of the proposed method. The recovery was calculated according to the following:

$$\text{Recovery \%} = \frac{(\text{Recovered amount} \times 100)}{\text{Injected amount}}. \quad (3)$$

3. Results and Discussion

3.1. Determination of Maximum Absorption of HCTZ. In HPLC-UV methods, selecting a wavelength that is not close to the λ_{max} of the studied compound can produce low sensitivity and nonlinearity measurements of response. Therefore, all the measurements should be determined at the λ_{max} of the studied compound to obtain a high response for its peak. Using solution of HCTZ prepared in acetonitrile solvent showed strong UV absorbance at the optimum wavelength of 272.07 nm as shown in Figure 2. Reviewing the literature, the obtained wavelength is confirmed in different studies [4, 14–16].

3.2. Development and Optimization of the HPLC Method. The basic chromatographic conditions were selected after testing different parameters that influence the HPLC analysis of HCTZ, such as the strength of mobile phase, injection volume, column temperature, and different column manufacturers, as follows.

3.2.1. The Effect of Different Strengths of the Mobile Phase. Under isocratic chromatographic conditions using mobile phase of mixture ACN and H_2O and Inertsil column (ODS-3 250 mm $\times$ 4.6 mm, 5 μm siloxane), different ratios (50%, 60%, and 70% ACN) were tested. The HPLC chromatograms in Figure 3 show the analysis of 10 $\mu\text{g/mL}$ solution of HCTZ at different strengths of the mobile phase.

FIGURE 2: UV-spectrum of HCTZ.

TABLE 1: The effect of different mobile phase strengths on the peak area and retention time of HCTZ.

Mobile phase (ACN : H_2O)	t_r (min)	Peak area
50 : 50	3.37	384763
60 : 40	3.14	386386
70 : 30	3.01	390123

The results presented in Figure 3 and Table 1 show good shape of eluted peak of HCTZ at all concentrations of the mobile phase and no disturbance in the baseline is observable along the chromatographic record. A little decreasing in retention time and increasing of peak area of HCTZ occur within increasing of mobile phase (50%, 60%, and 70%) of ACN.

From economic point of view, to avoid waste of ACN solvent, 50% of ACN was selected as concentration of the mobile phase in this method with approximately 3.5 min retention time of HCTZ peak and 5 min chromatographic runtime. Taking into account that the column hold-up is about 2.5 mL for these columns (25 $\times$ 0.46 cm), so it is advisable to use the weakest eluent.

3.2.2. The Effect of Injection Volume. Three injection volumes of 10, 20, and 30 μL were examined through injecting 10 $\mu\text{g/mL}$ of HCTZ. The results shown in Table 2 indicated that the retention time shows small decreasing and the peak area increases with the increase of the injection volume. Injecting a very large volume, the column can be overloaded, which will lead to peak broadening, most often peak fronting, or, in some cases, peak tailing [17, 18]. This problem limits the injection volume to less than 25 μL for columns with 0.46 cm internal diameter [11]. In this study, the autosampler was set up for a 20 μL injection volume to overcome the probability of peak distortion.

3.2.3. The Effect of Column Temperature. The effect of column temperature was studied testing three different temperatures: 30 (ambient), 35, and 40°C. The results show that there was no big effect on eluted peak of HCTZ showing nearly close retention times ($\sim$ 3.5 min) and peak areas. Therefore, column oven was set at ambient temperature (30°C) in this study.

FIGURE 3: Chromatogram of HCTZ (10 µg/mL) with mobile phase of ACN : H₂O at (a) 50 : 50, (b) 60 : 40, and (c) 70 : 30 (v/v).

TABLE 2: Effect of injection volume on peak area and retention time of HCTZ peak.

Injection volume (µL)	t_r (min)	Peak area
10	3.41	192007
20	3.36	384602
30	3.33	574737

3.2.4. The Effect of Column. To compare the efficiency of C₁₈ Inertsil column (ODS-3 250 mm × 4.6 mm, 5 µm siloxane), another column C₁₈ Zorbax (Eclipse Plus, 4.6 × 250 mm, 5 µm) was tested. Figure 4 shows the chromatograms of injection solution of 10 µg/mL of HCTZ on the two columns; under the HPLC conditions they showed a little difference in t_r values.

As shown in Figure 4, a narrow and symmetrical peak was obtained on both columns but with a small decreasing in the retention time with little decreasing in the peak area of HCTZ using tested column of Zorbax compared with Inertsil column. A possible explanation for this might be due to the age of the column; Inertsil column is used from long time compared with Zorbax column.

3.3. Method of Validation. Validation of the analytical method prior to determining HCTZ in dosage sample was done by examining such parameters of precision, linearity, and LOD and LOQ using two columns as follows.

3.3.1. Assessment of the Precision of Analytical Method. Repeatability of the method was checked by replicate injections of 5 µg/mL of the solution for five times on the same day as intraday precision study of HCTZ using two

FIGURE 4: Chromatogram of 10 µg/mL of HCTZ using (a) Inertsil column and (b) Zorbax column.

columns. %RSD values were found to be 0.53 and 0.62 on the Inertsil and Zorbax columns, respectively, indicating a good precision of the HPLC method.

3.3.2. Linearity of the Calibration Curve. Linearity of the calibration curve was determined at two ranges of concentrations (2.0–10 and 10–30 µg/mL) of HCTZ using two columns. The values of coefficient of determination (R^2) were found to be

TABLE 3: Comparison among the proposed method and other HPLC methods in the literature applied to HCTZ.

Method	Detector	Column	Mobile phase	Flow rate (mL/min)	Detection wavelength	t_r (min)	Recovery %	Linearity	LOD	LOQ
B P [19]	UV	ODS C_{18} , (100 × 4.6 mm) 3 μ m	MeOH : H ₂ O (50 : 50 v/v)	0.8	224 nm	8	—	—	—	—
Ref [8]	UV	Hypersil (ODS 250 × 4.6 mm, 5 μ m)	ACN : Buffer	1.0	215 nm	3.0371	100	20–120 μ g/mL (R^2 = 0.9980)	0.644 μ g/mL	1.951 μ g/mL
Ref [20]	UV	Hypersil ODS C_{18} (250 × 4.6 mm, 5 μ m)	MeOH : Buffer (60 : 40 v/v)	1.0	270 nm	2	100.12	60–140 μ g/mL (R^2 = 0.9990)	10 μ g/mL	15 μ g/mL
Ref [21]	DAD	Hypersil ODS C_{18} (100 × 4.0 mm, 3 μ m)	ACN : Buffer (18 : 72 v/v)	0.3	220 nm	6	94–100.2	2–50 μ g/mL (R^2 = 0.9998)	—	2 μ g/mL
Ref [22]	UV	Phenomenex ODS2, C_{18} , (250 × 4.6 mm, 5 μ m)	TEA : ACN : MeOH (50 : 25 : 25 v/v)	2.0	235 nm	2.183	99	200–300 μ g/mL (R^2 = 0.9996)	3.40 μ g/mL	10.31 μ g/mL
Ref [23]	UV-Vis	Phenomenex C_{18} (150 × 4.6 mm, 5 μ m)	MeOH : orthophosphoric acid (50 : 50 v/v)	0.6	271 nm	3.49	101.12	1–10 μ g/mL (R^2 = 0.9872)	0.05 μ g/mL	0.5 μ g/mL
Ref [24]	UV	ODS C_{18} (250 × 4.6 mm × 5 μ m)	ACN : Buffer (50 : 50 v/v)	1.0	226 nm	2.9	94–112	80–120 μ g/mL (R^2 = 0.999)	3.3 μ g/mL	10 μ g/mL
Ref [25]	PDA	Inertsil ODS3 (250 × 4.6 mm, 5 μ m)	ACN : Buffer (20 : 80 v/v)	1.0	220 nm	7.6	94–105	5–25 μ g/mL (R^2 = 0.996)	0.007 μ g/mL	0.022 μ g/mL
Ref [26]	UV	Xterra (250 × 4.6 mm, 5 μ m)	ACN : Buffer (gradient elution)	1.5	265 nm	—	100–102	0.00625–0.15 μ g/mL (R^2 = 0.9999)	0.008 μ g/mL	0.075 μ g/mL
Ref [27]	UV-Vis	Phenomenex Luna C_{18} (150 × 4.6 mm, 5 μ m)	ACN : MeOH : buffer (20 : 50 : 30 v/v)	1.0	250 nm	3.34	100.2	1–10 μ g/mL (R^2 = 0.9997)	0.164 μ g/mL	0.5 μ g/mL
Ref [28]	PDA	Xterra ODS C_{18} (4.6 × 150 × 5 μ m)	H ₂ O : MeOH (50 : 50 v/v)	0.8	230 nm	2.442	100–102	50–150 μ g/mL (R^2 = 0.999)	0.000003 μ g	0.0001 μ g
Ref [29]	UV-Vis	Zorbax C_{18} (4.6 × 250 mm, 5 μ m)	Buffer : ACN : TEA (95 : 5 : 0.1 v/v)	0.9	280 nm	4.489	101.17 ± 1	1.25–25 μ g/mL (R^2 = 0.9992)	1.012 μ g/mL	3.067 μ g/mL
Ref [30]	UV-Vis	Phenomenex Gemini C_{18} (250 × 4.6 mm × 5 μ m)	Buffer : MeOH : ACN (30 : 35 : 35 v/v)	1.5	240 nm	2.2	98.99	20–30 μ g/mL (R^2 = 0.9980)	54.13 μ g/mL	164.03 μ g/mL
Ref [31]	UV	Hypersil BDS, (250 × 4.6 mm, 5 μ m)	ACN : TEA (40 : 60 v/v)	1.0	228 nm	3.3	100 ± 2	6.25–37.5 μ g/mL (R^2 = 0.9999)	0.075 μ g/mL	0.224 μ g/mL
Ref [32]	PDA	Phenomenex Gemini C_{18} (250 × 4.6 mm, 5 μ m)	ACN : Buffer (70 : 30 v/v)	1.0	254 nm	4.25	98.65	2.5–12.5 μ g/mL (R^2 = 0.9995)	5.0 ng/mL	15 ng/mL
Ref [33]	PDA	Hypersil BDS C_{18} (250 × 4.6 mm, 5 μ m)	MeOH/ACN (70 : 30 v/v)	1.0	254 nm	3.4	100	20–80 μ g/mL (R^2 = 0.999)	0.13 μ g/mL	0.05 μ g/mL
Present study	PDA	Inertsil ODS3 C_{18} , (4.6 × 250 mm, 5 μ m)	ACN : H ₂ O (50 : 50 v/v)	1.0	272 nm	3.5	99.93	2–30 μ g/mL (R^2 ≥ 0.9978)	0.5 μ g/mL	1.7 μ g/mL

acceptable ≥ 0.9978 showing good linearity over the ranges studied of the assay method on both columns.

3.3.3. LOD and LOQ Determination. The LOD and LOQ were determined for the analytical method using two columns by the statistical data of calibration curve at low level of the concentrations. The results obtained showed values of LOD and LOQ of 0.5 and 1.7 $\mu\text{g/mL}$, respectively, and these values are close for both columns.

3.3.4. Application of the Method to Dosage Form. The proposed method was applied successfully for the determination of the HCTZ drug in its pharmaceutical dosage. This application was done using Inertsil column. The recovery calculated was equal to 99.93% with RSD% of 0.89 ($n = 3$) indicating that the proposed method is applicable for the determination of HCTZ in pharmaceutical dosage form.

3.4. Comparison with Other Methods. In reviewing the literature, the present method for the determination of HCTZ was compared with other methods as shown in Table 3. Under the chromatographic conditions applied in the present method using a new Perkin Elmer HPLC system coupled with PDA detector, the proposed method shows short retention time and run time with high recovery of HCTZ.

4. Conclusion

An RP-HPLC method coupled with PDA detector was developed and validated for the determination of HCTZ in bulk drug and pharmaceutical dosage form. The selected chromatographic conditions were 50 : 50 acetonitrile : water as a mobile phase, C_{18} Inertsil ODS-3 column (4.6×250 mm, $5 \mu\text{m}$), and $20 \mu\text{L}$ injection volumes with flow rate of 1 mL/min applied at ambient temperature (30°C) and 272 nm wavelength. Testing another column of C_{18} Zorbax (Eclipse Plus, 4.6×250 mm, $5 \mu\text{m}$), which has same type of stationary phase of siloxane as C_{18} Inertsil but with different manufacture, shows no big difference in the method results. Validation parameters indicated a good precision and linearity with low values of LOD and LOQ (approximately 0.5 and 1.5 $\mu\text{g/mL}$, resp.). The application of the proposed method on pharmaceutical dosage of tablet gave recovery of 99.93%. The proposed method is economic, simple, and rapid and hence can be employed for routine analysis in quality control laboratories.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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