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Application of High Performance Liquid Chromatography to the Analysis of Hydrochlorothiazide in Bulk and Pharmaceutical Formulations

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Abstract

Hydrochlorothiazide is a common blood pressure medicine. To analysis of it as pure form and in pharmaceutical formulations we used isocratic HPLC techniques supplied with a Octadecyl silane (ODS or C_{18}) column (4.5mm x 250 mm, 5µm) as stationary phase and (Methanol/Acetonitrile/Water) in the ratio of [75: 15:10 v/v/v/] as mobile phase at (25 Cº). The Hydrochlorothiazide was detected at 270 nanometer and a flow rate was at 1.0 $ml.min^{-1}$. This method was validated (Linearity, Accuracy, Precision, LOD and LOQ) and all results that obtained refer to accepted within ICH Guidelines.

Keywords: High performance liquid chromatography; Pharmaceutical formulations; Hydrochlorothiazide;

Introduction:

Hydrochlorothiazide (Fig.1) is a medication used to treat Hypertension. It belongs to a class of medications called diuretics, also known as water pills. Hydrochlorothiazide helps the body eliminate fluid overload. This drug comes in capsule and tablet forms. Common side effects of Hydrochlorothiazide involve repeated urination, dizziness and weakness.[1-3]

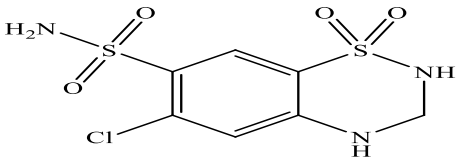


Fig. (1): Chemical structures of hydrochlorothiazide

Several techniques are available to analysis of hydrochlorothiazide using HPLC [4-19], HPTLC [20] , spectrophotometry [21-26] , TLC [27], voltammetry [28], GC [29], flow injection [30], polarography [31].

Materials and Methods:

Instruments:

The liquid chromatographic technique development was achieved on a HPLC Model Agilent -1200 (USA), equipped with a quaternary pump, mobile phase degasser, column thermostat regulator and UV sensor. A separation Chromatographic was achieved by a Octadecyl

silane (ODS or C_{18}) column with dimensions of 250 × 4.6 mm and porous silica particle size of 5 µm. with UV detection at 270 nm. The mobile phase consisted of a mixture of MeOH: ACN: H_2O (75:15:10 v/v/v) adjusted with dilute H_3PO_4 to a pH of 3. The sample was run at 1 ml /min flow rate. The volume of injection was 10 µl at 25 C° with 5 min run time. Table 1. illustrates all Instrumental conditions.

Table (1): Instrumental Conditions

Item	Conditions
Instrument	High performance liquid chromatography (HPLC)
Chromatographic technique	Reverse phase Chromatography
Stationary phase	Octadecyl silane (ODS or C_{18}) (250 mm × 4.6mm,5 µm)
Mobile phase	MeOH: ACN: H_2O (75:15:10 v/v/v)
pH	3
UV detection (nm)	270
Flow rate (ml/min)	1.0
Temp. (C°)	25
Inject volume of sample (µL)	10

Reagents:

The reference samples of Hydrochlorothiazide was supplied by SDI drug industries (Samarra - Iraq)**.** Hydrochlorothiazide tablet (Hydrothiazide 25mg) was manufactured by Ibn Hayyan pharmaceuticals -Syria. Methanol, acetonitrile, water and phosphoric acid that used for preparation of mobile phase were HPLC GRADE.

Preparations:

1- Hydrochlorothiazide stock solution (1000 µg/ml):

Equivalent to 25 mg of pure Hydrochlorothiazide, in a 25 ml volumetric flask. Added 15 ml of methanol, sonicate for 10 min to dissolve. Diluted with the same solvent to the flask mark then passes through a filter having a 0.45 µm or finer porosity. For a working solution, another dilution was done by methanol.

2- Hydrochlorothiazide sample solution (1000 µg/ml):

Equivalent to 25 mg of hydrochlorothiazide, from finely crushed tablets to a 25 ml volumetric flask. Added 15 ml of methanol shake by mechanical ways for 10 min then sonicate for 5 min. Diluted with the same solvent to the mark and filtered. For working solution, another dilution is done by methanol.

Results and discussion:

Detection wavelength estimation:

25 µg/ml of Hydrochlorothiazide was dissolved in methanol and scanned by spectrophotometer (200-400 nm.).This solution was given three different absorbance at (226 nm, 270 nm and 318 nm) (Fig. 2). We chose the beak at 270 nm. because it was sharp.

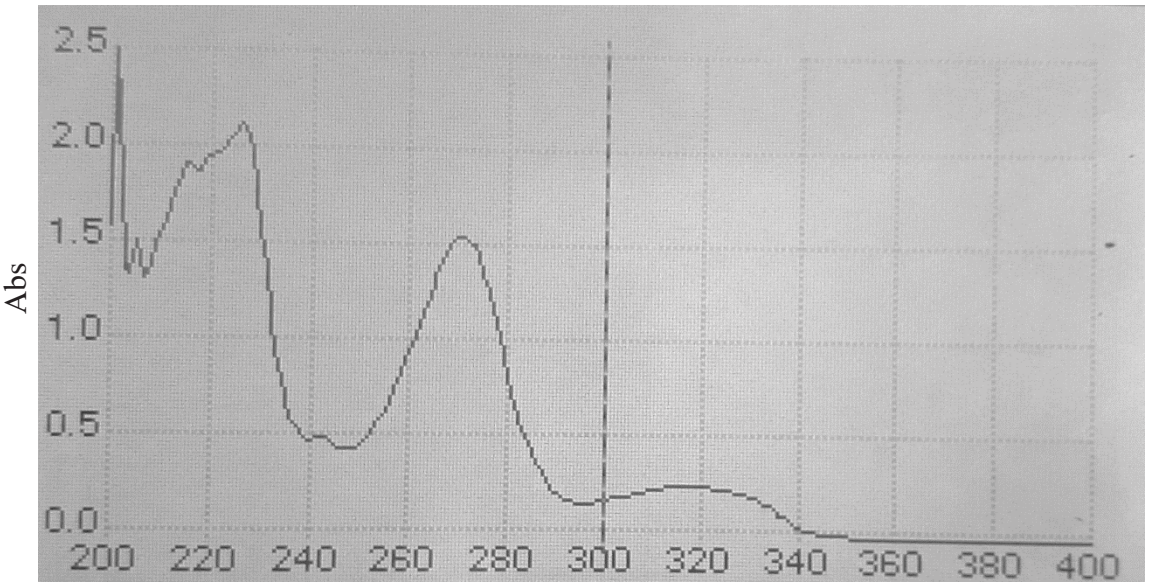
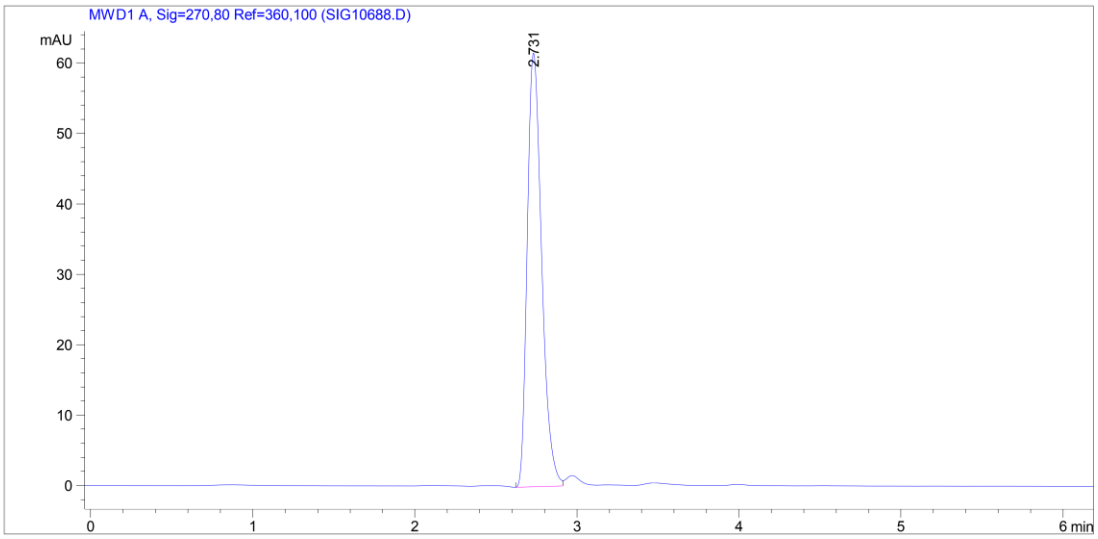


Fig. (2): UV-spectrum of Hydrochlorothiazide

Assay of Hydrochlorothiazide (Tablets):

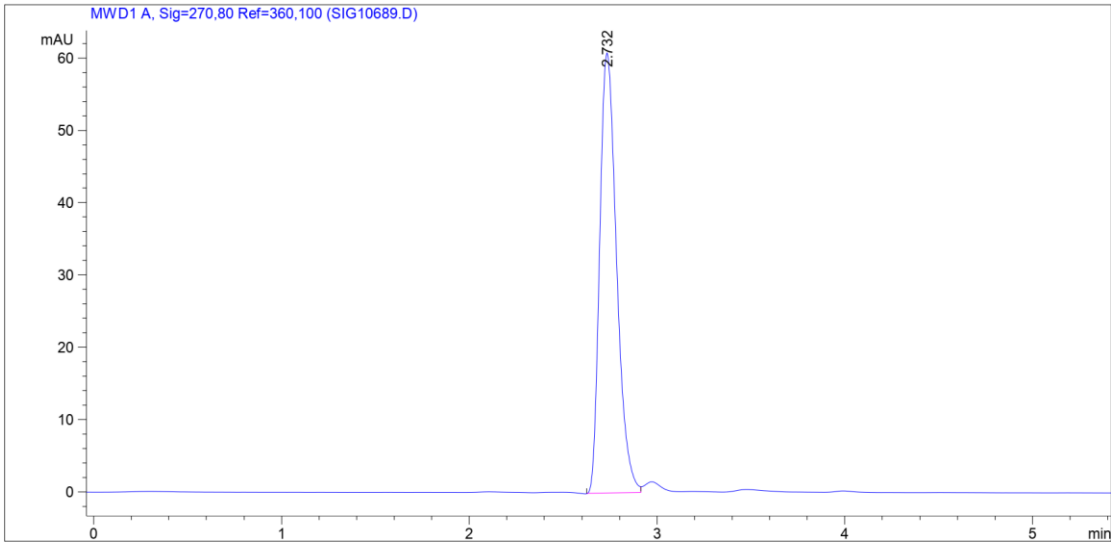
Hydrochlorothiazide (AQUAZIDE 25 mg) can be analyzed by HPLC technique. The chromatographic factors for the analysis are as Instrumental Conditions.

The chromatographic procedure may be performed using Octadecyl silane (ODS or C_{18}) column as the stationary phase and the mobile phase above aforesaid with a flow rate of 1.0 ml/min and a detection wavelength of 270 nanometer. Perform the HPLC assay by solutions in the methanol containing (25µg/ml) of Hydrochlorothiazide standard and (25µg/ml) of Hydrochlorothiazide (AQUAZIDE 25 mg) tablet (sample), 10 µl of each solution was injected. Calculate the contentment of Hydrochlorothiazide tablets from the peak zones and by using the stated content of the same in Hydrochlorothiazide standard. The chromatogram is shown on the (Fig. 3, 4).



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.731	BV	0.0959	380.52911	61.75525	100.0000

Fig. (3): Chromatogram of Hydrochlorothiazide standard (25µg/ml)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.732	BV	0.0987	380.22891	60.98191	100.0000

Fig. (4): Chromatogram of Hydrochlorothiazide tablets (25µg/ml)

Validation of the proposed method:[32]

1- Linearity:

10 µl of 1-50 µg/ml of pure Hydrochlorothiazide in methanol was injected as above Instrumental Conditions. A graph of concentration of Hydrochlorothiazide versus peak area was plotted, slope of regression line, y-intercept and correlation coefficient was detailed (Fig. 5). The result data shows that the correlation coefficient is (R^2= 0.998), hence the data shows that the method is linear.

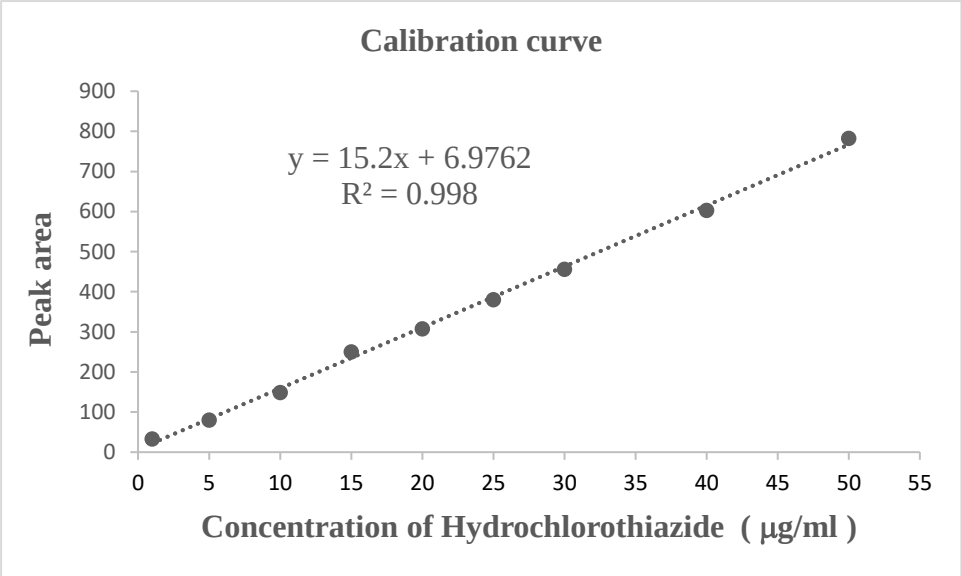


Fig. (5): Linearity graph of Hydrochlorothiazide

2- Precision:

25 µg/ml of pure Hydrochlorothiazide in methanol was tested six time to investigate the precision by using above Instrumental Conditions. The obtained data of Relative Standard Deviation (RSD, %) for this method was 0.206% and this refer to good precision of suggested study as mentioned by ICH directions (Table 2).

Table (2) : Precision test parameters for Hydrochlorothiazide

Sample Number	Area [mAU*s]
1	380.593
2	381.381
3	382.029
4	380.529
5	380.228
6	379.908
Mean	380.778
Standard Deviation	0.785
%RSD	0.206

3- Accuracy:

Table (3) displays accuracy test parameters for Hydrochlorothiazide through studying the recoveries of drug by using the standard addition way. Identified amounts of standard solutions of Hydrochlorothiazide (50%, 100%, and 150%) were added to pre-quantified sample solutions of drug formulation.

Table (3): Accuracy test parameters for Hydrochlorothiazide

Level %	Concentration of Hydrochlorothiazide Before µg/ml	Concentration of Hydrochlorothiazide* recovered µg/ml	Recovery %
50	10	8.9	98.0
100	20	19.9	99.7
150	30	29.6	98.6

*Average of 3 tests

The result shows that the recovery lies between (98.0-99.7 %), confirming good accuracy of the method.

4- Detection limit and quantitation limit:

1-10 µg/ml of Hydrochlorothiazide in methanol was used to reading the sensitivity of the suggested study as stated below:

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

σ = The Standard Deviation of Y intercept.

S = The Slope of Calibration Curve.

The results of detection limit (LOD) and quantitation limit (LOQ) that obtained refer to be 0.27 μg/ml and 0.84 μg/ml respectively, which indicates that suggested study is sensitive.

Conclusion:

From the result obtained it can be deduced that the method is fast, precise, specific and accurate to successfully determined Hydrochlorothiazide in tablet formulation and the way can be employed for program quality control of the drug.

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