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# Determination of Hydrochlorothiazide and Enalapril Maleate in Tablet Formulations by Reversed-Phase HPLC

**Idress F. AL-MOMANI**
*Chemistry Department, Yarmouk University,*
*Irbid-JORDAN*

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A high-performance liquid chromatographic procedure is presented for the determination of hydrochlorothiazide (HCT) and enalapril maleate (EM) in pharmaceutical tablets. An aliquot of the sample is dissolved in 15% acetonitrile (ACN) containing thiophylline as an internal standard and chromatographed on a Supelcosil LC-8 (5 $\mu$m), (150 mm $\times$ 4.6 mm i.d.) column. The mobile phase was 3.0 mM tetrabutylammoniumhydrogen sulfate (TBAHS) in ACN/water/triethylamine (TEA), (14, 85.6, 6.4 V/V) adjusted to pH 4.1 by glacial acetic acid. The detection was at 220 nm. The method was tested for linearity, accuracy, recovery, and specificity.

**Key Words:** HPLC, reverse phase, hydrochlorothiazide, enalapril maleate, stability, analysis.

## Introduction

Hydrochlorothiazide (HCT) is a thiazide diuretic which reduces the resorption of electrolytes and consequently of water. Enalapril maleate (EM) is a derivative of 2 amino acids, L-alanine and L-proline, and is an antihypertensive and a vasodilator in congestive heart failure. The 2 drugs are used in association in the treatment of hypertension[1].

Several methods are available in the literature for the determination of HCT and EM. Most of these methods are for the determination of either HCT or EM separately. For instance, HCT has been determined in urine samples and tablet formulation by HPLC[2−3] and by non-aqueous titration[4]. Enalapril maleate has been analyzed in pharmaceutical combinations containing 0.5-0.1% methylcellulose by extraction to acetonitrile and injecting the extracts to HPLC[5]. However, few methods are present in the literature for the simultaneous determination of EM and HCT. Enalapril maleate and HCT were determined simultaneously in pharmaceutical tablets using first-derivative ultraviolet spectrophotometry, with zero-crossing and peak-to-base measurement methods[6] and by HPLC with either programmable UV-detectors at 2 different wavelengths or with electrochemical detection[6−7]. In many cases, the direct UV/VIS methods lack specificity, because many compounds my absorb in the UV region and they will interfere if they are not separated. Other methods described in the literature[6−7] are based upon the use of either a programmable UV-detector or

the use of electrochemical detectors which are sophisticated, or not readily available detectors. This paper describes a reversed-phase HPLC method for the simultaneous determination of HCT and EM in commercial tablets. The method is specific and simple. The method was applied successfully to commercial products and proved to be free of interference from excipients normally used in tablet formulation. It was also validated for precision and accuracy.

# Experimental

## Apparatus

The apparatus employed was a Du Pont 8800 pump equipped with a 10-$\mu$L manual Rheodyne loop-injector, a Du Pont UV variable wavelength detector, and a Spectra-Physics (SP-4100) integrator. A reversed-phase Supelcosil LC-8 (5$\mu$), (150 mm $\times$ 4.6 mm i.d.) column was used.

## Chemicals

Generally, all chemicals used were the purest grade available and were used as received without further purification. Acetonitrile HPLC-grade (99.8%) (May & Baker), methanol (99.5%) (Riedel-deHaen), glacial acetic acid (99%) (Koch-Light), triethylamine (TEA) (99%) (Chambrian Chemicals) and tetrabutylammoniumhydrogen sulfate (TBAHS) (99%) (Fluka) were used. The active ingredients hydrochlorothiazide, enalapril maleate, and the internal standard thiophylline were obtained from USP and BP as reference standards. The water used was always distilled and deionized. Commercial tablets were purchased locally.

## Chromatographic Conditions

The mobile phase consists of 3.0 mM TBAHS in ACN/$H_2O$/TEA (14 : 85.6 : 0.4 V/V) solution, and the pH was adjusted to 4.1 by glacial acetic acid. The mobile phase was always filtered using 0.45 $\mu$m membrane filters and was degassed by vacuum prior to use. The sample solutions were also filtered using 0.45 $\mu$m membrane filters. The flow rate was 2 mL/min. The chart speed was 0.5 cm/min. The wavelength was 220 nm and the sensitivity was set at 0.08 AUFS.

## Preparation of the standard solutions:

**Internal standard solution:** The internal standard solution was prepared by dissolving 200 mg of theophylline in 1 L of 15% acetonitrile.

**Standard solution for linearity:** Standard stock solutions, containing 100 mg EM and 80 mg HCT, were prepared in 50 mL of the internal standard solution. The following concentrations in the internal standard solution were prepared by dilution: 0.1, 0.20, 0.30, 0.40, 0.50 and 0.60 mg/mL for EM and 0.08, 0.16, 0.24, 0.32, 0.48 and 0.54 for mg/mL for HCT.

**Preparation of the sample solutions:** Twenty tablets (1 tablet if content uniformity was to be determined) were weighed and powdered. Accurately weighed portions of the powder (each equivalent to the weight of 1 tablet) were placed in 50 mL volumetric flasks. Each sample was sonicated for 10 minutes with 30 mL of the internal standard solution. Samples were further diluted with the internal standard solution to the required concentration. The solutions were filtered through 0.45 $\mu$m membrane filters.

**Percent recovery study:** This study was performed on synthetic mixtures identical to the pharmaceutical formulations. Synthetic mixtures were spiked with known amounts of EM and HCT spanning the range 25-150% of the expected assay values. The resulting mixtures were assayed and the results obtained were compared with the expected one.

**Assay method:** Equal volumes (10 $\mu$L) and approximately equal concentrations of standard and sample solutions were injected into the HPLC and chromatographed under the conditions described above. The standard and the sample solutions contained the same concentration of the internal standard. The quantity of each component injected was always within the linearity range.

## Results and Discussion

The chromatograms shown in Figure 1 indicate the possibility of separating HCT and EM using theophylline as the internal standard. The chromatograms are free of interference from excipients normally used in tablet formulation. No significant changes in sample retention and column efficiency were observed for a long period of time (several months). The total elution time was less than 4 minutes.

To determine the linearity of the detector response, calibration standard solutions of HCT and EM were prepared as described in the text. A plot of peak area ratio versus amount injected was found to be linear in the range of 0.8-5.0 $\mu$g for HCT and 1.0-6.0 $\mu$g for EM with a correlation coefficient of 0.999 or better. The detection limits based on a signal-to-noise ratio of 3 were 36 ng for HCT and 24 ng for EM as determined by diluting the standard solutions and injecting 10 $\mu$L of each solution into the column.

The recovery of HCT and EM for synthetic mixtures was calculated using different concentrations for each. The recoveries ranged from 101.5 to 97.9% for EM and 97.2 to 101.0 for HCT (Table 1). In all cases excellent recoveries and reproducible results of the peak area were obtained. The selectivity of the method is validated by the fact that no interference due to excipients was detected in the chromatograms produced (Figure 1).

**Table 1.** Percent Recovery of HCT and EM From Spiked Placebo Samples

| EM | | HCT | |
|---|---|---|---|
| mg added/T | %Recovery* | mg added/T | %Recovery* |
| 30.00 | 100.8±1.3 | 18.00 | 99.4±1.4 |
| 25.00 | 100.7±1.2 | 15.00 | 98.4±0.7 |
| 20.00 | 100.2±0.8 | 12.00 | 98.0±1.5 |
| 15.00 | 101.5±1.5 | 9.00 | 97.2±2.1 |
| 10.00 | 100.7±2.5 | 6.00 | 101.2±1.2 |
| 5.00 | 97.9±1.8 | 3.00 | 98.7±0.7 |

*Mean ± RSD for 5 determinations.

The results of analysis of the commercial tablets (Table 2) indicate that the developed assay can be used for the quantitation of HCT and EM in commercial tablets. The closeness of the results to the label claim support the accuracy of the method. Replicate analysis of a single lot of angiozide tablets (n = 10) on 3 days gave precision (expressed as the relative standard deviation) of less than 1.8% for both HCT and EM, which support the precision of the method. The specificity of the method is further confirmed by the results of the content uniformity test of HCT and EM, which was performed on commercial tablets (Table

3). The results of content uniformity show compliance to the specificity of tablets and support the specificity of this method.

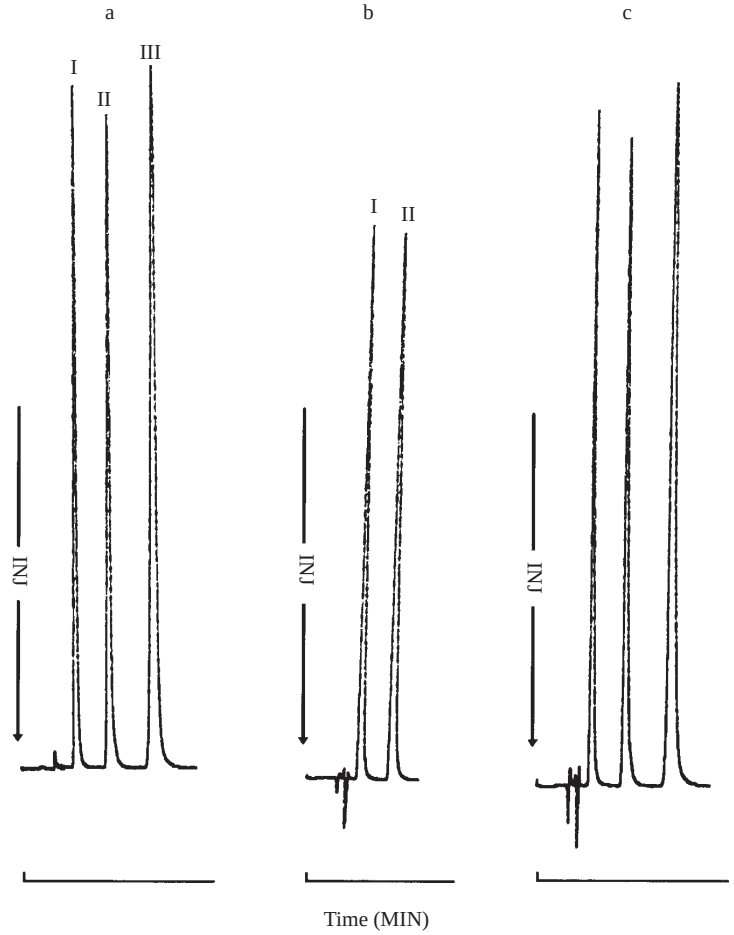


**Figure 1.**

a) A typical chromatogram of a standard 10 $\mu$L injection containing 3.0 $\mu$g enalapril maleate ($II, t_R = 2.18$ min), 5.0 $\mu$g hydrochlorothiazide ($III, t_R = 3.10$ min) in the internal standard ($I, t_R = 1.56$ min).

b) A chromatogram for 1 tablet of renitic drug in 50 mL internal standard.

c) A chromatogram for 1 tablet angiozide in 50 mL internal standard.

The validity of the method is further confirmed by analyzing 2 drugs (Angiozide and Renitec) for HCT using the method described by Hitscherich et al.[3]. The mobile phase was a 15:85 mixture of $CH_3$: 0.05M ammonium phosphate. According to the t- and F-tests, there were no significant differences between the results obtained by the proposed method and the method described by Hitscherich at p = 0.05, demonstrating that the proposed method is as accurate and precise as the reported methods.

A stability study was performed on pure HCT and EM powder by placing samples in dark bottles in a glycerin-water bath at 70 °C for 1 month. Samples were taken at 5-day intervals and assayed. No significant loss in concentrations of HCT and EM was observed throughout the study.

**Table 2.** Assay Results for HCT and EM in Commercial Tablets

| Product | Label Claim (mg/T) | | Percent Found ± RSD[a] | |
|---|---|---|---|---|
| | HCT | EM | HCT | EM |
| Angiozide[b] | 12.5 | 20 | 98.3±1.4 | 99.1±1.3 |
| | | | 101.2±1.8 | 100.5±1.6 |
| | | | 97.8±2.3 | 98.4±1.9 |
| Renitic[c] | | 20 | | 100.4±1.5 |
| | | | | 100.2±1.2 |
| | | | | 100.1±0.6 |
| Vaseretic[d] | | 10 | | 99.7±0.8 |
| | | | | 101.9±2.9 |
| | | | | 102.7±0.8 |

[a] Meant ± RSD for 5 determinations.
[b] Lot 950612 (The Jordanian Pharm. Manufacturing Co. LTD., Naor, Jordan).
[c] Lot 313990 L (Merk Sharp & Dohm, Haarlem-Netherlands).
[d] Lot R8502 (Merk Sharp & Dohm, Haarlem-Netherlands).

**Table 3.** Content Uniformity in Commercial Products[a]

| Tablet No. | Percent Label Claim Found | | | |
|---|---|---|---|---|
| | Renitic Tablets | Angiozide Tablets | | Vaseretic Tablets |
| | EM | EM | HCT | EM |
| 1 | 98.9 | 98.2 | 97.8 | 100.0 |
| 2 | 100.1 | 101.6 | 100.6 | 96.7 |
| 3 | 100.3 | 102.4 | 101.2 | 99.3 |
| 4 | 97.0 | 98.8 | 98.9 | 99.6 |
| 5 | 100.2 | 102.4 | 103.6 | 101.8 |
| 6 | 99.9 | 101.9 | 101.3 | 99.8 |
| 7 | 100.2 | 99.7 | 101.8 | 101.3 |
| 8 | 100.2 | 98.3 | 97.1 | 101.5 |
| 9 | 99.7 | 101.8 | 101.1 | 97.6 |
| 10 | 100.3 | 102.0 | 102.2 | 101.5 |
| Mean | 99.7 | 100.7 | 100.6 | 99.9 |
| RSD | 1.02 | 1.7 | 2.0 | 1.7 |

[a] The same lots indicated in Table 2.

In conclusion, the HPLC assay described here has been shown to be accurate, precise, rapid, and easy to perform. In addition, the proposed method can be applied to the quality control analysis of EM either alone or in combination with HCT without interferences from the additives and excipients normally used in tablet formulations. The method was successfully applied to analyze EM and HCT in commercially available products.

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