Simultaneous Determination of Hydrochlorothiazide and Triamterene in Capsule Formulations by High-Performance Liquid Chromatography

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Abstract □ A stability-indicating, high-performance liquid chromatographic method is presented for the simultaneous determination of hydrochlorothiazide and triamterene in two-component capsule formulations. An aliquot of the sample is dissolved in a mixture of acetonitrile and aqueous acetic acid, mixed with *m*-hydroxyacetophenone as an internal standard, and chromatographed on octadecylsilane bonded to microparticulate silica using 80% acetate buffer (pH 5.0, 0.004 *M*)–15% acetonitrile–5% methanol as the mobile phase. The relative standard deviations are ±1.1–1.6% for hydrochlorothiazide and ±1.7–2.2% for triamterene for two formulations. Recoveries of the two drugs added to placebos ranged from 98.4 to 101.7%.

Keyphrases □ Hydrochlorothiazide—simultaneous determination by high-performance liquid chromatography with triamterene, capsule formulations □ Triamterene—simultaneous determination by high-performance liquid chromatography with hydrochlorothiazide, capsule formulations □ High-performance liquid chromatography—simultaneous determination of hydrochlorothiazide and triamterene, capsule formulations

A combination dosage form of triamterene and hydrochlorothiazide is indicated in the treatment and management of edema and hypertension. Several methods are available for the assay of hydrochlorothiazide, alone (1–3) or in combination with other drugs such as methyldopa (4), hydralazine (5), reserpine (5, 6), and furosemide (7), and other diuretic–antihypertensive drugs of the thiazide family (8). These methods range from nonspecific approaches such as titrimetry, spectrophotometry, and polarography to specific determinations by high-performance liquid chromatography (HPLC). Triamterene has been assayed by absorption spectroscopy, fluorescence, and TLC fluorometry (9–11).

While separation of triamterene and hydrochlorothiazide from other diuretic–antihypertensive drugs by HPLC was reported (12, 13), no details are available for the specific assay of the two drugs in a dosage form. Furthermore, there are no indications in the literature whether the HPLC separations obtained are specific for the intact drug substances only. A direct method for the simultaneous determination of triamterene and hydrochlorothiazide, used to study the dissolution rate of a tablet dosage form (14), also does not differentiate between the drugs and their respective degradation products.

This paper reports a simple, rapid, and precise stability-indicating assay for triamterene and hydrochlorothiazide in capsule formulation by HPLC.

EXPERIMENTAL

Instrumentation—The chromatographic system was equipped with a dual-piston reciprocating pump[1], a universal injector[2], and a variable-wavelength detector[3]. The separation was performed on a 30-cm × 4-mm i.d. column containing microparticulate-bonded (10-μm) octadecylsilane material[4]. The chromatographic peaks were electronically integrated and recorded[5].

Mobile Phase—A solution of 20 ml of 0.2 *M* sodium acetate[6] adjusted to pH 5.0 with acetic acid was mixed with 780 ml of water, 150 ml of acetonitrile[7], and 50 ml of methanol[7]. The solution was filtered, degassed under vacuum, and used as the mobile phase.

Internal Standard Solution—A solution of *m*-hydroxyacetophenone[8] (3 mg/ml) in acetronitrile–water (1:1) was used as the internal standard.

Standard Preparation—A stock solution of hydrochlorothiazide was prepared using 100 mg of USP reference standard dissolved in 200 ml of acetonitrile. Stock triamterene solution was prepared by adding 25 ml of acetonitrile, followed by 4 ml of acetic acid, to 100 mg of USP reference standard, slurrying well, dissolving, and diluting to 100 ml with water.

A working standard solution was prepared by mixing 5 ml of each stock solution with 10 ml of the internal standard solution and diluting to 100 ml with water.

Sample Preparation—An aliquot of a powder blend from 10 capsules of a commercial formulation[9], equivalent to 50 mg of hydrochlorothiazide and 100 mg of triamterene, was slurried with 25 ml of acetonitrile followed by 4 ml of acetic acid. The mixture was shaken well for 5 min, mixed with 10 ml of internal standard solution, dissolved, and diluted to 100 ml with water. A portion of the solution was filtered through a polycarbonate membrane[10] using a filter assembly[11] and was analyzed.

Analysis—The chromatographic conditions used for the analysis were; flow rate, 2 ml/min; detector, 273 nm, 0.1 aufs; injection volume, 40 μl; and temperature, ambient.

Quantitative determinations were made by comparison of the peak area ratios of hydrochlorothiazide and triamterene to the *m*-hydroxyacetophenone from a sample injection to the corresponding area ratios from a standard injection.

RESULTS AND DISCUSSION

The HPLC system described by Honigberg *et al.* (13) provided excellent separation of hydrochlorothiazide and triamterene in a commercial formulation[9]. However, when formulations degraded by acid and alkali reflux were examined, a degradation product of triamterene eluted on the shoulder of the hydrochlorothiazide peak. Therefore, the mobile phase was modified to provide for adequate resolution of the various components of degraded formulations.

Figure 1 shows a typical chromatogram from the analysis of a formulation. The detection wavelength of 273 nm was chosen so that the peak area ratios of both drugs to the internal standard were close to unity.

Triamterene is only slightly soluble in water and forms insoluble complex salts with mineral acids (15). The complexes formed with acetic acid are reportedly (15) more soluble than the inorganic acid complexes. The use of acetic acid and the order of addition of the solvents are critical in the rapid dissolution of the formulation.

Hydrochlorothiazide is susceptible to both acid and alkaline hydrolysis and yields formaldehyde and 4-amino-6-chloro-*m*-benzenedisulfonamide

[1] Model 6000A, Waters Associates, Milford, Mass.
[2] Model U6K, Waters Associates, Milford, Mass.
[3] Model SF 770 Spectroflow monitor, Schoeffel, Westwood, N.J.
[4] μBondapak C_{18}, Waters Associates, Milford, Mass.
[5] Model 3385A automation system, Hewlett-Packard, Avondale, Pa.
[6] Analytical reagent, Mallinckrodt, St. Louis, Mo.
[7] Distilled in glass, Burdick & Jackson, Muskegon, Mich.
[8] Aldrich Chemical Co., Milwaukee, Wis.
[9] Dyazide®, Smith Kline and French Laboratories, Philadelphia, Pa.
[10] Nuclepore Corp., Pleasanton, Calif.
[11] Swinnex, Millipore Corp., Bedford, Mass.

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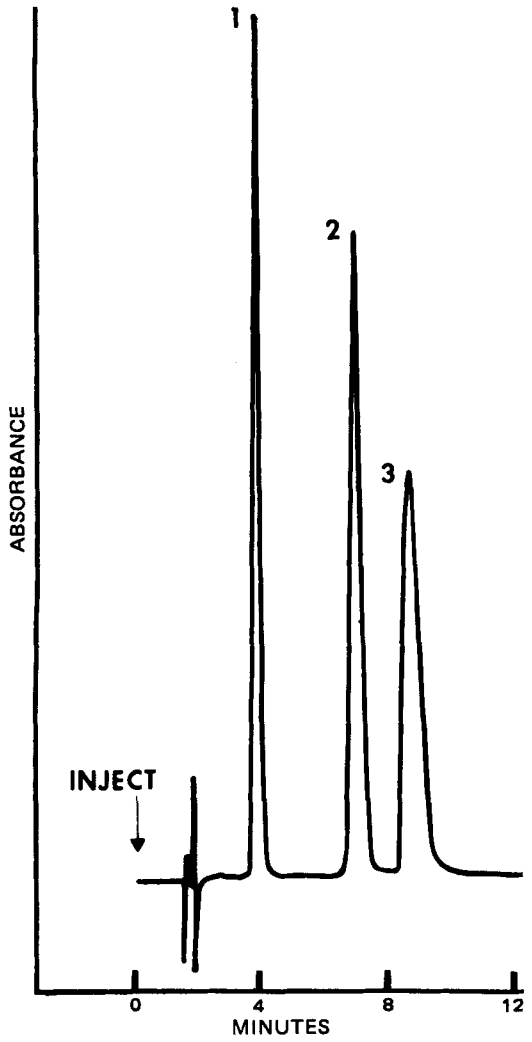


Figure 1—*Chromatogram of typical commercial capsule formulation. Key: 1, hydrochlorothiazide; 2, m-hydroxyacetophenone; and 3, triamterene.*

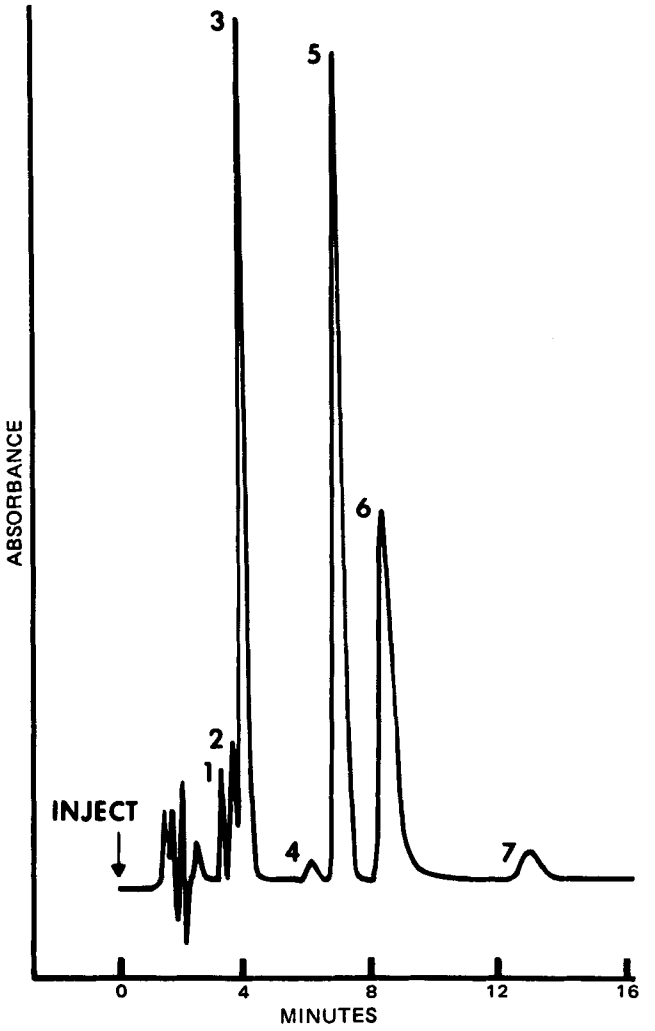
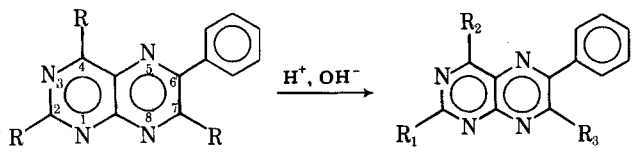


Figure 2—*Chromatogram of acid- and alkali-degraded commercial formulation. Key: 1, 4-amino-6-chloro-m-benzenedisulfonamide; 2, 4, and 7, degradation products of triamterene; 3, hydrochlorothiazide; 5, m-hydroxyacetophenone; and 6, triamterene.*

on degradation (16, 17). Although the degradation products of triamterene are not described in the literature, hydrolysis with 5 *N* HCl reportedly produced the 4-hydroxy and 7-hydroxy analogs of triamterene (18). Samples of the formulation were refluxed in 1 *N* HCl, 1 *N* NaOH, and water to observe the hydrolytic effects and resultant changes in the chromatogram. In a separate experiment, triamterene alone was subjected to similar hydrolytic stress for 3 hr. No degradation of triamterene was observed on water reflux, although total degradation resulted on base reflux and ~50% degradation occurred under acid reflux.

Figure 2 shows a chromatogram obtained from the injection of a mixture of acid- and alkali-degraded formulation. Alkali reflux of triamterene produced predominantly peak 7 with minor amounts of peak 2. Acid reflux resulted in larger amounts of peaks 2 and 4 with a trace of peak 7. The refluxed solutions were filtered and neutralized. Upon neutralization, precipitates formed in both the acid and base hydrolyzates. These precipitates were analyzed by HPLC, mass spectrometry, and spectrophotometry. By mass spectrometry, both precipitates showed *m/z* 254 but with different fragmentation patterns, suggesting that they were positional isomers of the hydroxy analogs of triamterene (Scheme I).



triamterene: R = NH_2
peak 7 analog: R_1 = OH, R_2 = R_3 = NH_2 in alkali
peak 4 analog: R_2 = OH, R_1 = R_3 = NH_2 in acid
peak 2 analog: R_3 = OH, R_1 = R_2 = NH_2 in acid

Scheme I

Based on the spectral properties in acidic and basic media and comparison to the synthetic hydroxypteridines prepared by Spickett and Timmis (18), peak 7 (the major alkaline degradation product) was identified as 4,7-diamino-2-hydroxy-6-phenylpteridine and peak 4 was identified as 2,7-diamino-4-hydroxy-6-phenylpteridine. Peak 2 probably is the 7-hydroxy analog isolated (18) upon acid hydrolysis of 2,4,7-triamino-6-phenylpteridine. Since peak 2 elutes earlier, the compound may have higher solubility than the 4- and 2-hydroxy compounds and is not precipitated on neutralization of the hydrolyzates. The major degradation pathways are represented in Scheme I.

The mass spectra of the identified compounds are in agreement with the proposed assignments. Since all components are adequately resolved from each other and the internal standard, the method indicates the

Table I—HPLC Analysis of Hydrochlorothiazide–Triamterene Capsule Formulations

Parameter	Hydrochlorothiazide		Triamterene	
	Product I[a]	Product II[b]	Product I[a]	Product II[b]
Number of analyses	9	9	9	9
Milligrams per capsule (mean)	25.8	25.5	51.0	51.3
RSD, %	±1.6	±1.1	±2.2	±1.7

[a] Dyazide®, Smith Kline and French Laboratories, Philadelphia, Pa. [b] Abbott Laboratories, North Chicago, Ill.

stability of the drugs in the formulation. None of these degradation products was detected in a formulation subjected to high-temperature stability testing.

The linearity of the detector response was established for hydrochlorothiazide in the range of 15–30 mg/capsule (r = 1.000, y-intercept = 0.002) and for triamterene in the range of 30–70 mg/capsule (r = 1.000, y-intercept = 0.04). Standard addition–recovery experiments performed in two different placebos at various drug levels showed recoveries of 98.4–101.7% for hydrochlorothiazide (n = 12, CV = ±1.2%) and 99.5–102.0% for triamterene (n = 12, CV = ±0.9%).

The reproducibility of the method was demonstrated by performing replicate analyses on a commercial formulation[9] and another experimental formulation with different excipients. The statistical data generated from these analyses are presented in Table I.

In conclusion, HPLC provides a rapid and precise method for the quantitative determination of hydrochlorothiazide and triamterene in a combination oral dosage form. The method is sensitive to the degradation products of these drugs and is free from interference due to excipients in the products examined.

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Test for Reproducibility of Metered-Dose Aerosol Valves for Pharmaceutical Solutions

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Abstract □ Seven independent testing sites received metered-dose aerosols containing a standard test solution to assess a newly designed protocol for studying the reproducibility of metered-dose aerosol valves. In accordance with the rather small sampling protocol design, the amount of product dispensed per actuation was measured over the life of the aerosol at designated regions of actuation. A statistical analysis of the data collected at each testing site clearly indicated that the testing protocol is sufficiently reliable and workable for assessing the reproducibility of valve delivery for a given lot of metered-dose valves.

Keyphrases □ Aerosols—testing procedure for establishing the reproducibility of metered-dose valves □ Inhalation products—testing procedure for establishing the reproducibility of metered-dose aerosol valves □ Valve delivery—metered-dose aerosols, testing procedure for establishing reproducibility

Test methods, including specific gravity, net contents, vapor pressure, moisture content, spray patterns, and particulate-size determinations, have been established for determining the acceptance of metered-dose aerosol valves for pharmaceutical use (1–4). Reproducibility of the dosage delivered may be determined by assay techniques which establish the amount of active (5). Although USP XX (6) includes metered-dose inhalation products in the monographs and establishes some standards with which these metered-dose products must comply, little has been done in establishing a uniform test procedure for all metered-dose products.

The Aerosol Specification Committee[1] elected to establish a simple and uniform test method for metered-dose aerosol valves by examining the uniformity of valve delivery (amount of solution delivered by the valve) within a valve and between individual valves in any given lot. The procedure outlined in this study provides a means for manufacturers to assess the reproducibility of valve delivery for a given lot of metered-dose valves throughout the life of the product.

EXPERIMENTAL

Aerosol Test Solutions—Pharmaceutical test solutions were prepared by taking 0.10% by weight of isopropyl myristate[2] and adding it to a mixture of 24.90% by weight of trichloromonofluoromethane (Propellant

[1] Of the Industrial Pharmacy Technology Section, Academy of Pharmaceutical Sciences, American Pharmaceutical Association.

[2] Givaudan Corp., Clifton, N.J.

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