Analysis of Captopril and Hydrochlorothiazide Combination Tablet Formulations by Liquid Chromatography

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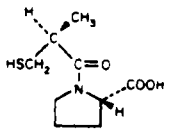
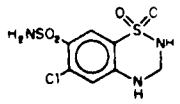
Abstract □ A reverse-phase, high-performance liquid chromatographic (HPLC) procedure was developed for the simultaneous assay of captopril and hydrochlorothiazide in a combination tablet formulation. Gradient elution was used to quantify these two drugs, as well as the oxidized form of captopril, the disulfide. Tablets were extracted with methanol and, after centrifugation, were chromatographed. Initially, a methanol–0.05% aqueous phosphoric acid (25:75, v/v) solution was pumped at 2 mL/min into a phenyl column. After 8 min, the flow rate was increased to 4.5 mL/min and the methanol content of the mobile phase was increased to 45% to elute the disulfide. Detection was at 210 nm. Linearity and repeatability of all constituents were satisfactory. The hydrolytic degradation product of hydrochlorothiazide, 4-amino-6-chloro-1,3-benzene disulfonamide (also called the disulfonamide), could be resolved in test solutions but was not visible in chromatograms of tablets carried through the gradient procedure even after storage at elevated temperatures for prolonged time periods prior to assay. The method can be automated.

Keyphrases □ Captopril—analysis by HPLC, combination with hydrochlorothiazide, tablet formulations □ Hydrochlorothiazide—analysis by HPLC, combination with captopril, tablet formulations

Captopril (I), an antihypertensive agent (1), has been formulated with the diuretic hydrochlorothiazide (II). An assay was needed to quantify these two drugs in a combination tablet formulation (2) together with two degradation products, the disulfide (IV) of captopril and the disulfonamide of hydrochlorothiazide [4-amino-6-chloro-1,3-benzene disulfonamide (III)]. High-performance liquid chromatography (HPLC) has the potential for assaying all four compounds simultaneously. Such an assay should elute the isomeric forms of captopril (3) as one peak, as described in a previously developed assay for captopril bulk material (4). HPLC assays were previously developed for hydrochlorothiazide in tablets (5, 6) and in body tissues (7, 8). The method described below is stability indicating and amenable to automation.



I



II

EXPERIMENTAL

Materials—Captopril[1], the disulfide[1] of captopril, and hydrochlorothiazide[1] were used as received. Water was double-distilled and stored in glass. The disulfonamide[2], HPLC-grade methanol[3], and ACS reagent-grade phosphoric acid[4] were obtained commercially.

Apparatus—A gradient system[5] capable of both flow-rate and mobile-phase composition adjustments was connected to a 50 × 0.2-cm saturator column packed with 37-μm silica. Next in sequence were an inlet filter[6] (2-μm pore size) and a precision loop injector[7] (nominal volume 20 μL), attached to a reverse-phase (medium polarity) phenyl column[8], 5 or 10 μm, 250–300 mm × 4.6 mm i.d., with >7000 theoretical plates per column. The column temperature was maintained slightly above ambient (30°C) by a thermostatically controlled heater[9], to avoid variation in retention time. A variable-wavelength detector[10] set to 210 nm was used. The 20- or 25-cm two-channel strip-chart recorder[11] had input compatible with the detector output. One recorder channel was adjusted to trace the captopril and hydrochlorothiazide peaks on scale. The other channel was set to be ~40 times more sensitive to detect possible impurities. For automated unattended analysis, an autosampler[12] was used in conjunction with a laboratory computer system[13]. Calculations are based on peak area or peak height, which appear to be interchangeable.

Mobile Phase and Gradient Program—At time zero, a mobile phase consisting of methanol–0.05% phosphoric acid in water (25:75, v/v) was pumped at 2 mL/min. After 8 min, the flow rate was increased to 4.5 mL/min and the methanol content to 45%. After 15 min, the methanol content was returned to 25%, and after 20 min, the flow rate was also returned to the original 2 mL/min.

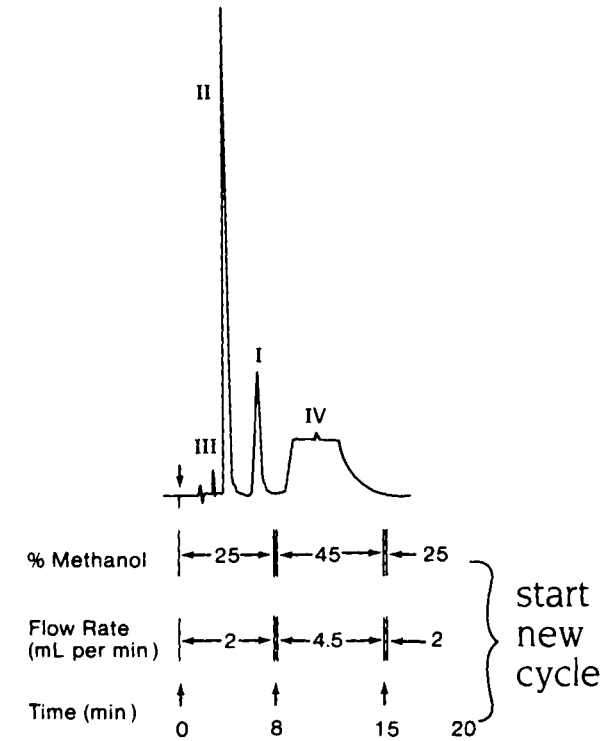


Figure 1—*HPLC of mixed standards for analyzing captopril (I) and hydrochlorothiazide (II) combination tablets, also including the disulfonamide (III) and disulfide (IV). Gradient conditions are appropriately noted, using methanol and 0.05% aqueous phosphoric acid. Detection was at 210 nm (0.16 AUFS) using a phenyl column. For captopril, typical asymmetry factor is 1.25, and number of theoretical plates is 470 (manufacturers' specifications are for >7000 theoretical plates/column using their test systems). Tailing of both standards and samples gradually appears with prolonged use of the column.*

[1] E. R. Squibb House Standard.
[2] Aldrich.
[3] Baker.
[4] Fisher.
[5] Beckman.
[6] Rheodyne Model 7362.
[7] Rheodyne Model 7010.
[8] Waters Associates or E.S. Industries or Waters columns repacked by Analytical Sciences, Inc.
[9] Bioanalytical Systems LC-22 and LC-23.
[10] Schoeffel 770 or 773 or Perkin-Elmer LC-75 or LC-85.
[11] Kipp and Zonen or Linear Instruments.
[12] Perkin-Elmer Model 420B or ISS-100.
[13] Hewlett-Packard 3356.

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Table I—Gradient and Isocratic Systems for Captopril and Hydrochlorothiazide

Captopril (Theory: 50 mg/tablet)			Hydrochlorothiazide (Theory: 25 mg/tablet)		
Gradient	Isocratic	Δ	Gradient	Isocratic	Δ
52.8	50.8	+2.0	24.9	25.1	−0.2
50.8	51.6	−0.8	25.1	24.8	+0.3
49.5	50.1	−0.6	24.6	24.8	−0.2
49.5	50.9	−1.4	24.6	24.3	+0.3
50.8	50.5	+0.3	24.3	24.3	—
50.1	51.6	−1.5	24.6	24.8	−0.2
51.3	50.1	+1.2	24.7	24.0	+0.7
49.6	49.3	+0.3	23.9	23.8	+0.1
52.0	51.9	+0.1	24.3	24.5	−0.2
50.0	50.3	−0.3	24.4	24.6	−0.2
Mean 50.6	50.7	−0.7	24.5	24.5	+0.4
RSD 2.2%	1.6%		1.5%	1.6%	

Tablet Analyses—Ten formulations were assayed: 12.5, 25, 37.5, 50, and 100 mg of captopril with either 15 or 25 mg of hydrochlorothiazide. For content uniformity, the intact, weighed tablet was extracted with methanol; Twenty-five milliliters of methanol was added to a 12.5-mg captopril/15-mg hydrochlorothiazide combination tablet, and 40 mL of methanol was used to extract all other potencies. For the assay of the drug content of a tablet, 10–20 tablets were ground and the weight of powder equivalent to one tablet was extracted with methanol. After 20 min of ultrasonication, the suspensions were shaken vigorously and then centrifuged at 1500 rpm (1200×*g*).

A mixed standard was prepared consisting of captopril and hydrochlorothiazide equivalent in concentration to the potency of the tablets being analyzed and 0.1% disulfonamide. A second standard containing 0.6% disulfide is necessary since captopril normally contains traces of this reversible oxidation product.

Assay—The column was initially equilibrated by passing 100 mL of methanol 0.5% aqueous phosphoric acid (1:3) through it. Prior to the first injection, the gradient program was activated. After the first cycle, the mixed captopril—hydrochlorothiazide disulfonamide standard was injected twice. All subsequent samples and standards (including the disulfide) were then injected into the column. No daily column cleaning was necessary.

System Suitability Test—The resolution of the mixed standard constituents was as follows: disulfonamide from void volume, ≫1.4; disulfonamide from hydrochlorothiazide, ≫1.4; hydrochlorothiazide from captopril, >2; and captopril from disulfide, >2. Resolution was calculated using $R_S = 2(t_2 - t_1)/(W_1 + W_2)$, where R_S is resolution, t_1 and t_2 are the retention times or distances, and W_1 and W_2 are the respective peak widths of tangents drawn to the baseline. Responses from the initial injections of standard are usually within 1.5% of each other, indicating continuation of the assay.

RESULTS AND DISCUSSION

The gradient HPLC system was developed to quantify captopril, its reversible oxidized form, hydrochlorothiazide, and its possible hydrolytic product, the disulfonamide. Figure 1 illustrates the separation of all four constituents. The baseline aberration occurring after the captopril peak is due to a change in the refractive index of the mobile phase as its composition varies. The disulfide peak is clearly visible above the plateau. (Detectors are available with autozero capability which can electronically correct for such shifts. Alternatively, we successfully used a laboratory data acquisition system[13] to make such a baseline correction.) Separation of all four constituents indicates selectivity of the HPLC system and is the basis of a system suitability test.

Precision of the system (9) was determined by means of six injections of mixed standards to give the following relative standard deviations: disulfonamide, 1.2%; hydrochlorothiazide, 0.5%; captopril, 1.9%; and disulfide, 3.5%. The disulfide value is high due to a combination of long retention time and low (0.6%) concentration. The precision of the method was determined by pooling 10 tablets and then analyzing six tablet weights carried through the recommended procedure. The relative standard deviations are as follows: hydrochlorothiazide, 0.86%; captopril, 1.1%; and disulfide, 4.8%.

The response *versus* concentration curves from 10 to 200% of the concentrations of the standards were found to be linear for all four substances, with calculated correlation coefficients of ≥0.99 for all substances except the disulfide, which was 0.98. Recoveries of captopril and hydrochlorothiazide added to placebo formulation were 100.5% ($n = 5$, $RSD = 0.8$) and 98.7% ($n = 5$, $RSD = 0.8\%$) respectively. No interfering peaks were noted after extracting and injecting placebo tablets. At least six phenyl columns from two vendors[8] have been used, indicating ruggedness.

The gradient assay was verified by using an isocratic HPLC system which could quantitate captopril, hydrochlorothiazide, and the disulfide. This assay used methanol–0.05% aqueous phosphoric acid (55:45, v/v) and a 15%-loaded octadecylsilane column. This system failed to resolve the disulfonamide from the void volume; however, since no disulfonamide appears to be present in any sample studied, it is suitable for verifying the gradient system. Individual tablets of 50 mg of captopril and 25 mg of hydrochlorothiazide were extracted with methanol and simultaneously injected onto the recommended gradient and alternative isocratic systems. The results (Table I) show that the gradient and isocratic systems give similar results for captopril and hydrochlorothiazide, indicating the statistical reliability of the gradient system. The disulfide contents of 0.3% are identical using both systems. No change in strength was observed after storage for 52 weeks at temperatures between ambient and 40°C for these or any other formulations. No other extraneous peaks were detected.

Verification of the absence of disulfonamide was by TLC[14]. The disulfonamide limit of detection by TLC is ~0.01%. However, the compendial (10) colorimetric assay gave values of 0.2–0.4%. Kinetic studies (11) showed these values to be an artifact of the colorimetric method and indicate that this HPLC method has additional utility by replacing an erroneous assay.

For routine assays of the disulfonamide, hydrochlorothiazide, and captopril (but omitting the disulfide), the mobile phase used in the first 8 min of the gradient assay can be used. In this second isocratic system, the disulfide slowly elutes from the column as a broad peak that is unnoticeable because disulfide contents are very low. The first and last chromatograms from a series of repetitive injections are superimposable, indicating that the slow elution of disulfide does not affect the system. The remainder of the chromatogram is similar to Fig. 1, except for the lack of the disulfide plateau region.

In summary, an assay using gradient HPLC has been developed for the active constituents and possible impurities in captopril–hydrochlorothiazide combination tablets. This multiconstituent assay is selective, amenable to automation, and stability indicating.

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[14] Henry Roberts, personal communication.