DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 12(5), 691-700 (1986)

QUANTITATION OF HYDROCHLOROTHIAZIDE IN COMBINATION WITH METHYLDOPA AND PROPRANOLOL HYDROCHLORIDE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A stability indicating assay method based on high-performance liquid chromatography has been developed for the quantitation of hydrochlorothiazide in combination with methyldopa and propranolol hydrochloride. The method is accurate, reproducible and precise with an average percent relative standard deviation of 1.3. The method can also be used for the quantitation of the only known impurity/decomposition product (4-amino, 6 chloro, 1,3 benzenedisulfonamide) in hydrochlorothiazide. For the complete separation of methyldopa from hydrochlorothiazide, a counterion, 1-heptanesulfonic acid sodium was added to the mobile phase to increase the retention time of methyldopa. A 4-5 minutes time to extract hydrochlorothiazide from tablets appears to be satisfactory.

0363-9045/86/1205-0691$3.50/0

BACKGROUND

Hydrochlorothiazide is one of the most commonly used diuretics. It is often mixed with methyldopa and propranolol hydrochloride. The USP-NF method[1] for the quantitation of hydrochlorothiazide is very tedious. It requires preparation of silicious earth column, subsequent extraction with sodium hydroxide and UV spectroscopy. Three high-performance liquid chromatography (HPLC) methods have been reported[2-4]. The authors of these reports[2-4] did not analyze hydrochlorothiazide in the presence of methyldopa and propranolol hydrochloride. The purpose of these investigations was to develop a method for the quantitation of hydrochlorothiazide in combination with methyldopa and propranolol hydrochloride which can also be used for contents uniformity.

MATERIALS AND METHODS

Materials: All chemicals and reagents were USP, NF or ACS grade and were used without further purification. Hydrochlorothiazide[5] (I), methyldopa[6] (II), propranolol hydrochloride[7] (III) and 4-amino, 6 chloro, 1,3 benzenedisulfonamide[8] (IV) were used as received. A high-performance liquid chromatograph[9], equipped with a multiple wavelength detector[10], a recorder[11] and a digital integrator[12] was used. A nonpolar column[13] (30 cm long x 4 mm i.d.) consisting of a monomolecular layer of octadecyltrichlorosilane permanently bonded to silica gel was used.

Chromatographic Conditions: Two mobile phases were used: a. contained 7% (V/V) methanol, 0.5% (V/V) of glacial acetic acid and 0.02M ammonium acetate in water and b. contained 10% (V/V) of meth-

anol, 0.5% (V/V) of glacial acetic acid and 0.002M 1-heptanesulfonic acid sodium[14] salt (the counterion) in water. The flow rate was 2.0 ml/minute, the temperature was ambient and the chart speed was 30.5 cm/hr. The UV detector was set at 273 nm (265 nm when determining IV in powder of I) and the sensitivity was 0.04 (0.02 for powder of hydrochlorothiazide). The wavelength was 270 nm for mobile phase b.

Methods: The stock solutions of hydrochlorothiazide (1.0 mg/ml) and salicylamide (5.0 mg/ml) in methanol were prepared fresh daily. A standard solution was prepared by mixing 2.0 ml (1.0 ml for mobile phase b) of the stock solution of I with 3.0 ml (2.0 ml for mobile phase b) of the stock solution of salicylamide and bringing the volume to 50.0 ml with water. Solutions of other concentrations were prepared as needed. A standard solution of IV was prepared by dissolving 4.0 mg in 5 ml of methanol and bringing the volume to 100.0 ml with water.

Assay Solution: Ten tablets (one if content uniformity was to be determined) were ground to a fine powder. A quantity of the powder representing 10.0 mg of hydrochlorothiazide was accurately weighed and mixed with 40 ml of methanol. The mixture was stirred for 4-5 minutes, brought to volume (50.0 ml) with methanol and filtered. First 15 ml of the filtrate was rejected and then collected for further dilution. A 10.0 ml (5.0 ml for mobile phase b) quantity of the filtrate was mixed with 3.0 ml (2.0 ml for mobile phase b) of the stock solution of salicylamide and brought to volume (50.0 ml) with water.

To determine the concentration of 4-amino, 6 chloro, 1,3 dibenzenedisulfonamide (IV) in hydrochlorothiazide powder, a 40.0 ml quantity of the stock solution of I was diluted to 100.0 ml with water.

A 20.0 µl aliquot of the assay solution was injected into the chromatograph using the described conditions. For comparison, an identical volume of the standard solution was injected after the assay solution eluted.

Calculations: The results were calculated using:

$$\frac{(Ph)_a}{(Ph)_s} \times 100 = \text{Percent of the label claim}$$

where $(Ph)_a$ is ratio of the peak heights (hydrochlorothiazide/salicylamide) of the assay solution and $(Ph)_s$ is that of the standard solution of an identical concentration. Preliminary investigations indicated that concentrations versus ratio of peak heights were linear between 0.3 to 0.9 µg of I when using mobile phase a and 0.2 to 0.6 µg of I when using mobile phase b.

Decomposition of Hydrochlorothiazide - A 20.0 mg quantity of I was added to 0.5 g of sodium hydroxide pellets and 30 ml of water in a 150 ml beaker. The mixture was heated to boiling on a hot plate for 1-1/2 hours (more water was added as needed), and cooled. The pH was adjusted[15] to about 4 with ∿1N HCl and the mixture was diluted with water to an appropriate concentration for assay.

RESULTS AND DISCUSSION

The results indicate (Table 1, Figures 1-3) that HPLC method can be used for the quantitation of hydrochlorothiazide in tablets

TABLE 1 - Hydrochlorothiazide Assay Results

Contents of the Tablet/Synthetic Mixture	Percent of the Label Claim Found Using Mobile Phase a	Mobile Phase b
Hydrochlorothiazide (I) 50 mg-Tablets	100.1	99.8
Methyldopa 250 mg and 25 mg of I-Tablets	99.4	99.6
Propranolol HCl 40 mg and 25 mg of I-Tablets	99.2	--[a]
Propranolol HCl 60 mg and 20 mg of I-Synthetic Mixture	99.8	--[a]
Methyldopa 500 mg and 50 mg of I-Synthetic Mixture	101.2	100.0
Content Uniformity of Tablets Containing 250 mg of Methyldopa and 25 mg of I		
Tablet 1	--[b]	98.6
Tablet 2	--[b]	99.2
Tablet 3	--[b]	98.1
Tablet 4	--[b]	102.4
Tablet 5	--[b]	99.2
Tablet 6	--[b]	101.7
Tablet 7	--[b]	100.9
Tablet 8	--[b]	102.1
Tablet 9	--[b]	97.9
Tablet 10	--[b]	98.9
Content Uniformity of Tablets Containing 40 mg of Propranolol HCl and 25 mg of I		
Tablet 1	97.8	--[a]
Tablet 2	100.9	--[a]
Tablet 3	102.5	--[a]
Tablet 4	98.2	--[a]
Tablet 5	98.2	--[a]
Tablet 6	102.0	--[a]
Tablet 7	99.5	--[a]

[a]Not determined using mobile phase b.
[b]Not determined using mobile phase a.

in combination with methyldopa and propranolol hydrochloride. The method is accurate (Table 1), reproducible and precise. The percent relative standard deviations based on 5 readings were 1.5 for mobile phase a and 1.2 for mobile phase b.

Using mobile phase a which was similar to the one reported in the literature[2], there was no interference from propranolol hydrochloride (Figure 1) which did not elute even in 12 minutes. How-

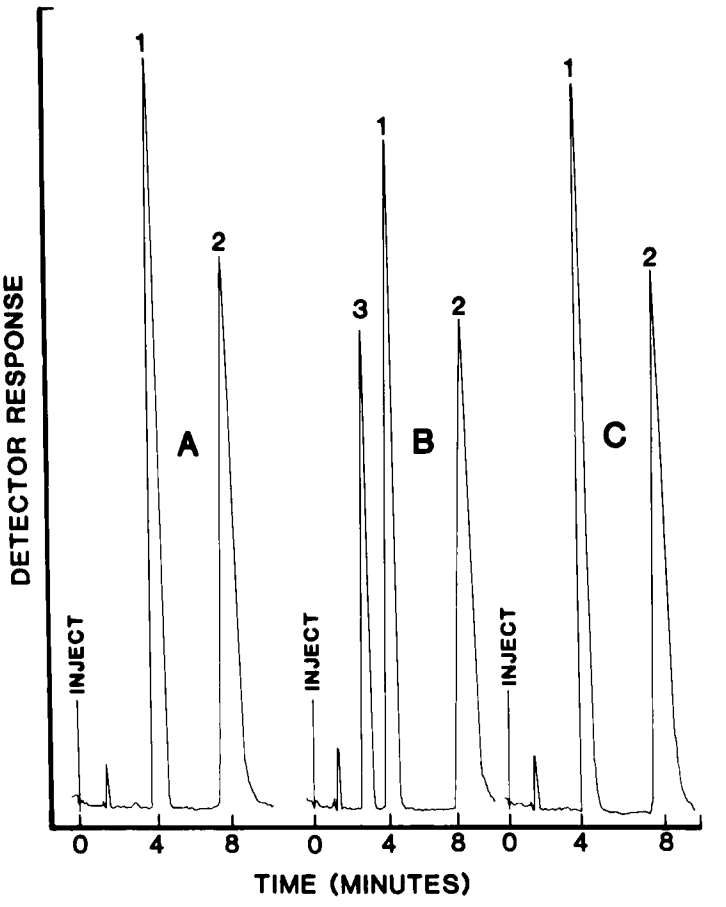

FIGURE 1

Sample chromatograms using mobile phase a. Peaks 1-3 are from hydrochlorothiazide, salicylamide (the internal standard) and 4-amino, 6 chloro, 1,3 benzenedisulfonamide (the common impurity and the decomposition product in I), respectively. Chromatogram A is from a standard solution; B from a standard solution with an equal volume of standard solution of IV; and C from a tablet containing propranolol hydrochloride. For chromatographic conditions, see text.

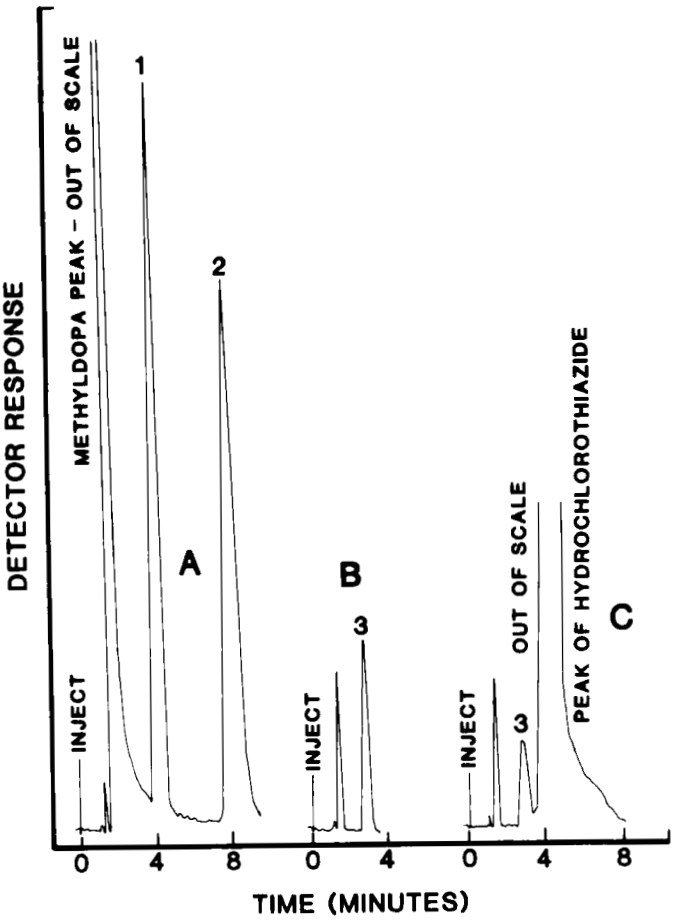

FIGURE 2

Sample chromatograms using mobile phase a. Peaks 1-3 are from hydrochlorothiazide, salicylamide and 4-amino, 6 chloro, 1,3 benzenedisulfonamide, respectively. Chromatogram A is from a tablet with methyldopa; B from a 4.0 µg/ml solution of IV (at 265 nm and sensitivity of 0.02) and C from a 400 µg/ml solution of I for the determination of IV. For chromatographic conditions, see text.

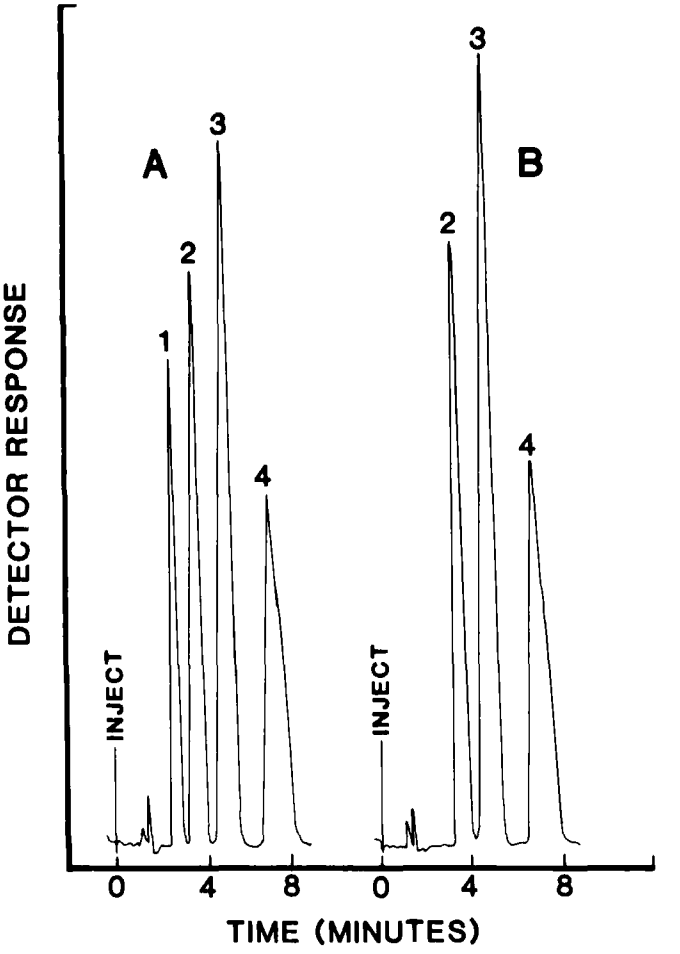

FIGURE 3

Sample chromatograms using mobile phase b. Peaks 1-4 are from 4-amino, 6 chloro, 1,3 benzenedisulfonamide, hydrochlorothiazide, methyldopa and salicylamide, respectively. Chromatgram A is from a solution containing 20.0 µg/ml each of I and IV and 200 µg/ml each of methyldopa and salicylamide and B from a tablet of I with methyldopa (same as in Figure 2). For chromatographic conditions, see text.

ever, the separation of methyldopa from hydrochlorothiazide was not complete (Figure 2A), therefore, it was necessary to modify the mobile phase for complete separation. Mobile phase b which contained 1-heptanesulfonic acid sodium salt (the counterion) was developed in order to increase the retention time of methyldopa without affecting the retention time of hydrochlorothiazide. The percentage of methanol was increased in order to shorten the assay time. Originally, hydrochlorothiazide was quantified at 273 nm but later on it was determined that any wavelength between 265-273 nm could be used. However, to quantify 4 amino, 6, chloro, 1,3 benzenedisulfonamide (IV), the only known impurity and the decomposition products[2] in I, the sensitivity was maximum at 265 nm. With mobile phase b, it was possible to separate methyldopa from hydrochlorothiazide (Figure 3) completely. With both mobile phases, there was no interference from the only known impurity/decomposition product of hydrochlorothiazide i.e. IV (Figures 2-3).

The USP method[1] for the quantitation of IV in powder/tablets is tedious and time consuming. With HPLC method, it was possible to determine the concentration of IV in the powder (Figure 2 B-C) using mobile phase a. The powder contained 0.45% of IV which is within the USP limits of 1%.

The decomposed solution (see Materials and Methods) contained only 28.5% of intact drug and there was a new peak in the chromatogram with the same retention time as of IV. Therefore, the method is stability-indicating as has already been reported in the literature[2].

A 4-5 minutes of extraction time for hydrochlorothiazide from tablets appears to be satisfactory versus 35 minutes as recommended[2] in the literature.

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