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QUANTITATION OF SPIRONOLACTONE IN THE PRESENCE OF CANRENONE USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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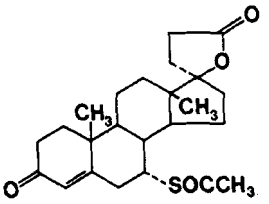
ABSTRACT

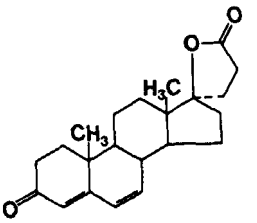
Two stability-indicating high-performance liquid chromatography methods have been developed to quantify spironolactone in the presence of its product(s) of decomposition. Both methods are accurate and precise with percent relative standard deviation of 0.7 based on 5 readings using microC_{18} column and 0.9 using microphenyl column. By using different wavelengths, the sensitivity of the methods to quantify spironolactone or canrenone (the major product of decomposition of spironolactone) can be increased many times. Spironolactone appears to be relatively stable between pH values of about 3.4-5.2. In the acidic pH, the decomposition is slower than on the basic side of the pH. Even at pH 7.3, the decomposition was about 3.1 times faster than at pH 2.3.

INTRODUCTION

Spironolactone (Figure 1) is extensively used in medicine as a diuretic. The major product of decomposition of spironolactone is a structurally related compound, canrenone (Figure 1). It is important that an analytical chemist be able to separate these two. The USP-NF method (1) which is based on HPLC is not stability-indicating since



(I)



(II)

Figure 1 Structures of spironolactone (I) and canrenone (II).

both spironolactone and canrenone elute out at the same time. Furthermore, the method recommends the use of a mobile phase whose pH is ~8.2 with microC_{18} column. It is well known (2), that this pH buffer destroys the costly reverse phase columns very fast. No internal standard has been recommended by USP-NF. There is no justification for using the USP-NF method to quantify spironolactone due to the high pH value of the mobile phase.

Gupta and Ghanekar reported (3) an HPLC method for the quantitation of spironolactone using microCN column and 40% aqueous ethanol as the mobile phase. The wavelength was 254 nm. Although new peaks were recorded in the chromatograms of the decomposed samples, the method was never tried in the presence of canrenone, the major pro-

duct of decomposition of spironolactone. The present study indicated that canrenone does interfere with this method. Williamson reported (4) the determination of canrenone in pharmaceutical dosage forms using a silica column and nonpolar solvent as the mobile phase. The author never tried this method in the presence of spironolactone. This method is based on normal phase HPLC. The columns in a normal phase chromatography usually get deactivated very fast. Another method to quantify althiazide in the presence of spironolactone has been reported (5). In this method, the authors used microC_{18} column with 50% (V/V) of acetonitrile in water as the mobile phase. In our laboratory this mobile phase did not separate spironolactone from canrenone using microC_{18} column.

The purpose of these investigations was to develop stability-indicating HPLC assay methods for the quantitation of spironolactone (I) in the presence of canrenone (II), the major product of decomposition of I. The method was required in order to develop a stable oral liquid dosage form of spironolactone.

METHODOLOGY

Chemicals and Reagents - All the chemicals and reagents were either USP-NF or ACS grade and used without further purification. The powders of spironolactone and canrenone were generously supplied by G.D. Searle & Co. and used as received.

High-Performance Liquid Chromatography System - A Waters ALC 202 liquid chromatograph equipped with a multiple wavelength detector (Spectroflow 770, Applied Biosystems), a recorder (Omniscribe 5213-12, Houston Instruments, Austin, TX) and an injector (Rheodyne model

7125) was used. Either a microbondapakC_{18} column or microbondapak phenyl column (30 cm x 3.9 mm i.d.) was the stationary phase. The mobile phase contained 63% methanol (V/V) in 0.01M KH_2PO_4 aqueous buffer solution with C_{18} column. The flow rate was 2.0 ml/min and the sensitivity was set 0.04 or 0.1 at the wavelengths of 238, 254 or 283 nm. The chart speed was 30.5 cm/hr and the temperature was ambient. With phenyl column, the mobile phase was 39% solution of acetonitrile in 0.01M KH_2PO_4 aqueous buffer. The flow rate was 2.2 ml/min at 254 nm (0.04 AUFS).

Preparation of Spironolactone Solutions for Stability Studies - Samples containing 0.25 mg/ml of spironolactone in aqueous ethanol (20% V/V) buffers (0.05 M phosphate) of varying pH values (between 2.3 to 9.7) were prepared. The ionic strength was adjusted to 0.2 with potassium chloride. After the initial data (physical appearances, pH values and assays), the solutions were stored at 40° ($\pm$ 1) and reassayed at the appropriate intervals.

Preparation of Stock and Standard Solutions of Spironolactone (I), Canrenone (II) and Methyltestosterone (III) - The stock solutions of spironolactone and canrenone (1.25 mg/ml) in methanol or ethanol were prepared fresh every week. These solutions were diluted further with water (5 in 25) to obtain the stock solutions containing 0.25 mg/ml of I and II. The stock solution of methyltestosterone (the internal standard) was prepared by dissolving 100 mg of the drug in enough methanol to make 100 ml. The most commonly used standard solution was prepared by mixing 5.0 ml of the stock solution of I (0.25 mg/ml) with 2.5 ml of the stock solution of II (0.25 mg/ml) and 1.5 ml of

the stock solution of III and diluting the mixture with 0.01M phosphate buffer in water containing 25% (V/V) of either acetonitrile or methanol (the diluting solvent). The solutions containing other concentrations of either I and II or I with III were prepared as needed using the above procedure.

Preparation of Assay Samples - A 5.0 ml quantity of the assay sample (8.0 ml with phenyl column) was mixed with 1.5 ml (0.6 ml with phenyl column) quantity of the stock solution of III (the internal standard) and brought to volume (25 ml) with diluting solvent (see above).

Decomposition of Spironolactone and Canrenone Under Drastic Conditions - A 5.0 ml quantity of the stock solution of I or II (0.25 mg/ml, separately) were mixed with either 1 ml of ∿1N H_2SO_4 or 1 ml of ∿1N NaOH solution in a 150 ml beaker. Ten ml of water was added and the mixture was heated to boiling using a hot plate. More water was added (if needed) to prevent splashing. After 15 minutes, the mixture was cooled, neutralized with either 1N H_2SO_4 or 1N NaOH solution and brought to volume (25 ml for I and 50 ml for II) using the diluting solvent (see above). The mixture was filtered (Whatman #1, qualitative) if required, first 10-15 ml of the filtrate was rejected and then some clear filtrate was collected for analysis. No internal standard was added in order to detect all the peaks from the decomposition products.

Assay Procedure - A 20 µl quantity of the assay sample was injected into the chromatograph using the conditions described. For purpose of comparison, the standard solution (containing identical concentrations of the drug based on the label claim and the internal standard) was injected after the assay eluted.

Calculations - Since preliminary investigations indicated that the ratio of the peak heights (drug/internal standard) were directly related to the concentrations of the drug (range tested ± 50% of the standard solution concentration), the results were calculated using a simple equation:

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

where $(R_{ph})_a$ is the ratio of the peak heights (drug/internal standard) of the assay and $(R_{ph})_s$ that of the standard. In the case of solutions decomposed under drastic conditions (see above), the results were estimated by comparing the peak heights of the drug (I or II) of the assay with the standard containing same quantity of the drug based on the label claim since no internal standard was added in order to detect new peak(s) in the chromatograms.

RESULTS AND DISCUSSION

USP-NF Assay Method - Using the USP-NF method (1) for the quantitation of spironolactone, the authors were unable to separate canrenone (the major product of decomposition of spironolactone) from spironolactone. Furthermore, the USP-NF method (1) recommends the use of a mobile phase whose pH is 8.2 which can destroy the costly microC_{18} column very fast (2). The authors first tried to use acetonitrile (as recommended in the USP-NF) in 0.01M KH_2PO_4 in water (pH ∿5.9) instead of pH 8.2 buffer, this did not separate canrenone from spironolactone either. It was not surprising since both compounds (Figure 1) appear to be neutral, therefore, the change in the pH value of the mobile phase did not affect the retention time of

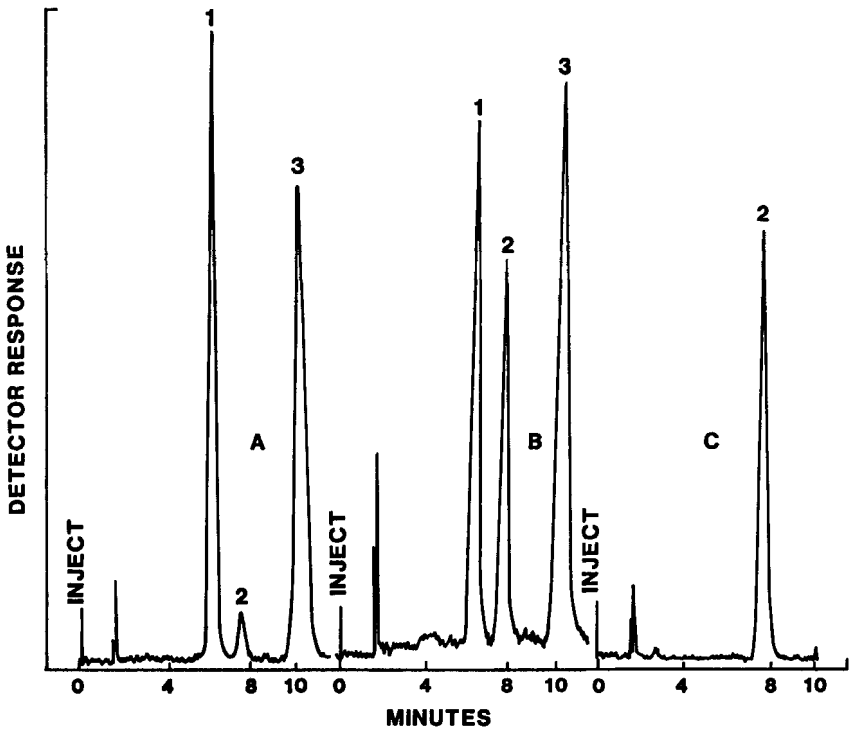


Figure 2 Sample chromatograms using microC_{18} column. All the chromatograms are from a standard solution. Peaks 1-3 are from spironolactone, canrenone, and methyltestosterone (the internal standard), respectively. Chromatogram A was developed at 238 nm (AUFS 0.1), B at 254 nm (AUFS 0.04) and C at 283 nm (AUFS 0.1).

either one. This mobile phase was also tried with microCN column but without success.

HPLC Method Using MicroC_{18} Column - The mobile phase containing 63% methanol (V/V) in 0.01M KH_2PO_4 in water (pH ~6.1) was used as the mobile phase with microC_{18} column as the stationary phase. This mobile phase separated spironolactone (peak 1 in Figure 2) from

canrenone (peak 2 in Figure 2). With this system, progesterone which was developed as an internal standard, had a very long retention time and therefore, was not useful. Another internal standard (methyltestosterone) had to be developed (peak 3 in Figure 2). The developed method to quantify spironolactone is accurate and precise with percent relative standard deviation of 0.7 based on 5 readings. It separated not only canrenone from spironolactone but also an additional product of decomposition (peak 4 in Figure 3). Obviously, this unidentified product is more polar than spironolactone or canrenone since it eluted right after the solvent peak.

Use of Various Wavelengths and Sensitivity Settings With MicroC$_{18}$ Column - The maximum wavelength of absorption for spironolactone is 238 nm and for canrenone 283 (6). The USP-NF recommends the use of 254 nm. In these investigations, it was determined that to quantify both I and II at the same time, the best wavelength is 254 nm and a sensitivity of 0.04 (Figure 2B). At this wavelength, one μg of spironolactone and 0.5 μg of canrenone and 1.2 μg of methyltestosterone (the internal standard) were injected to develop the sample chromatogram (Figure 2B). Furthermore, the ratio of peak heights for both sprironolactone and canrenone were related to the concentrations when ± 50% of the above quantities were injected. However, if more sensitivity for spironolactone is required, the wavelength of 238 nm should be preferred where the peak is ∿3 times higher (Figure 2A). It must be pointed out that chromatogram 2A was developed using the same standard solution as chromatogram 2B except that the sensitivity had to be decreased by a factor of 2.5. On the

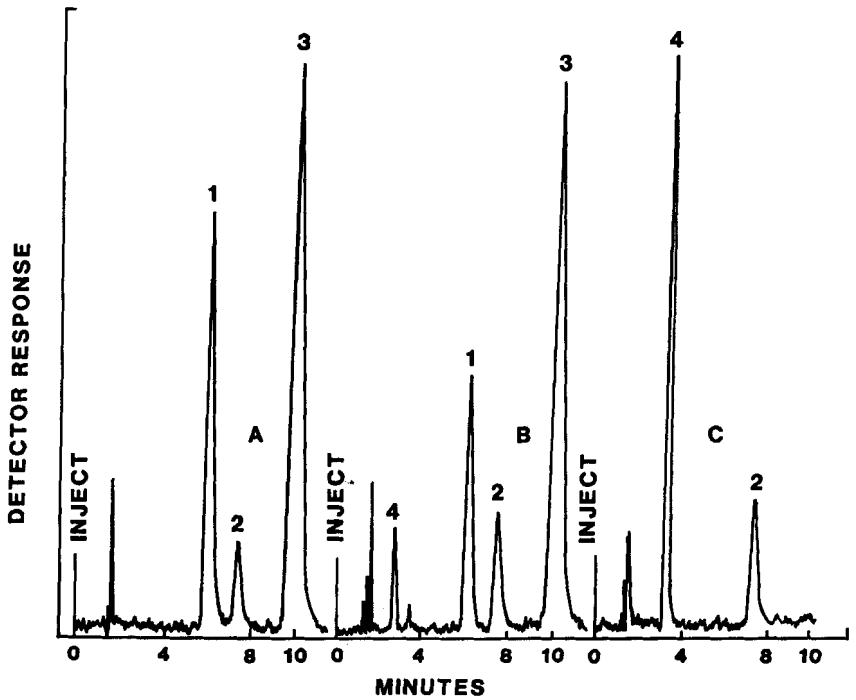


Figure 3 Sample chromatograms developed at 254 nm (0.04 AUFS) using microC_{18} column. Peaks 1-4 are from spironolactone, canrenone, methyltestosterone and unidentified product of decomposition, respectively. Chromatogram A is from a 26 day old solution (pH 2.3, stored at 40°); B from a 25 day old solution (pH 7.3, stored at 40°) and C from a spironolactone solution decomposed using sodium hydroxide (see text). For chromatographic conditions, see text.

other hand, there was a loss of sensitivity in the assay method for canrenone at 238 nm versus 254 nm by a factor of about 3.3. If more sensitivity is desired to quantify canrenone, the maximum wavelength of absorption of 283 nm should be preferred (Figure 2C). Chromatogram 2C was developed using the same standard solution as for chromatogram 2B except that sensitivity had to be decreased by 2.5 to prevent the peak of canrenone from going out of scale. At this wavelength, peaks of canrenone were ∿2.7 times higher than at 254 nm. Furthermore, spironolactone did not absorb any light at this wavelength which means it could never interfere in the assay method. However, the usual problem in practice is to prevent the interference of canrenone (the major decomposition product) in the assay method for spironolactone. If it is necessary to quantify low levels of canrenone, such as when working with solid dosage forms, this wavelength should be preferred. In these investigations, the authors were able to quantify 0.02 µg of canrenone at 283 nm (AUFS 0.04) with an error of ± 3.5%.

Analysis of Synthetic Mixtures Using MicroC_{18} Column - The synthetic mixtures containing 50 µg/ml of spironolactone and 5 or 10 µg/ml quantities of II were assayed at 254 nm and 283 nm for canrenone. The recoveries of I and II at both the wavelengths were excellent (Table 1). Therefore, the use of 283 nm for canrenone is not necessary unless the levels are too low. If 283 nm was desired, a new internal standard may have to be developed since methyltestosterone had poor absorption at this wavelength. Many times, the internal standard is not used such as in the USP-NF HPLC methods for a number

of drugs including the presently recommended method for spironolactone (1).

Decomposition Under Drastic Conditions (For Assays, MicroC_{18} Column Was Used) - The spironolactone stock solution which was decomposed using base showed almost zero percent of intact drug and had changed to canrenone (~20%) and the unidentified product of decomposition (peak 4 in Figure 3). The decomposed solution of canrenone using sodium hydroxide showed 17% canrenone intact and an unidentified new peak (same as peak 4 in Figure 3) in the chromatogram. The acid decomposed solution of spironolactone showed 46.6% spironolactone intact, 19.7% canrenone and the balance changing to unidentified product (same as peak 4 in Figure 3). The acid decomposed canrenone solution had 87% of compound intact and only 13% had changed to the unidentified product of decomposition (peak 4, Figure 3). It is apparent that the decomposition of spironolactone is a series reaction (spironolactone $\xrightarrow{k_1}$ canrenone $\xrightarrow{k_2}$ unidentified product) or a combination of series and side reaction since some of the spironolactone may be changing directly also to the unidentified product. It has been suggested that the lactone would hydrolyze reversibly in acid and form a carboxylate in base.

When determining percent of canrenone from the decomposed spironolactone sample, the quantity of canrenone found was multiplied by 1.2237 to determine the quantity of spironolactone in the form of canrenone. This quantity was then changed to percent of spironolactone in the form of canrenone.

Assay Method Using Microphenyl Column - Later on in these investigations, a stability-indicating assay method using phenyl column was also developed. The mobile phase contained 39% acetonitrile in 0.01M KH_2PO_4 aqueous buffer. This method also appears to be accurate and precise with percent relative standard deviation of 0.9 based on 5 readings. With this method, the order of elution was altogether different than with microC_{18} column (Figure 4). For example, methyltestosterone (the internal standard) eluted first, followed by canrenone and then spironolactone. There was no interference from the unidentified product of decomposition (Figure 3, peak 4) in this procedure. The advantage of using microphenyl column with acetonitrile is that the pressure on the column is low (about half of that with 63% methanol and microC_{18} column) which prolongs the life of the costly columns. The authors are now using these chromatographic conditions for the rest of the preformulation studies.

Stability of Buffered Solutions Using Micro C_{18} Columns - The solutions of pH values above 7.3 had completely decomposed in 3 weeks. The solutions of pH values between 3.4 to 5.2 did not decompose in 3 weeks. Therefore, the data of only 2 solutions (pH 2.3 and 7.3) have been included in Table 1. At pH 7.3 the decomposition was faster than at pH 2.3. At both pH values, the first-order law was followed with K_{obs} values of 0.0274^{-1} at pH 7.3 and 0.008 day^{-1} at pH 2.3. The sample chromatogram at pH 2.3 (Figure 3A) indicated that only canrenone was present after decomposition. However, at pH 7.3 another peak (peak 4 in Figure 3B) from an unidentified product

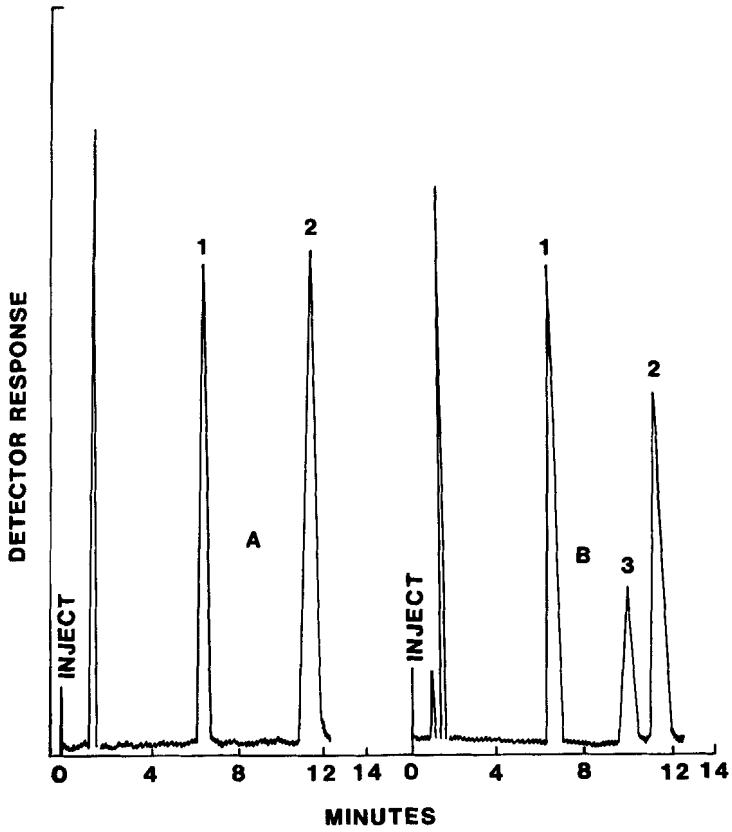


Figure 4 Sample chromatograms using microphenyl column. Peaks 1-3 are from methyltestosterone (the internal standard), spironolactone and canrenone, respectively. Chromatogram A is from a standard solution and B from a 126 day old solution (storage temperature 40°, pH 4.4 using 0.16M phosphate-citrate buffer). Only 69.5% of spironolactone was left intact. For chromatographic conditions, see text.

TABLE I
Assay Results Using MicroC_{18} Column

Sample	Percent of the Claim Found (AUFS 0.04) Spironolactone 254 nm	Canrenone at 254 nm	Canrenone at 283 nm
Spironolactone Decomposed with Acid	46.6	19.7	20.6
Spironolactone Decomposed with Base	0	20.4	21.0
Canrenone Decomposed with Acid	-[a]	-[b]	87 (AUFS 0.1)
Canrenone Decomposed with Base	-[a]	-[b]	17
Synthetic Sample 1 Spironolactone 50 µg/ml Canrenone 2.5 µg/ml	98.0	103.2	101.0
Synthetic Sample 2 Spironolactone 50 µg/ml Canrenone 5 µg/ml	98.5	101.8	100.1
Synthetic Sample 3 Canrenone 1 µg/ml	-[a]	-[b]	103.5
pH 2.3 (26 day old samples)	80.5	13.4	12.7
pH 3.4-5.2 (21 day old samples)	No significant decomposition was found[c].		
pH 7.3 (25 day old samples)	46.6	19.7	17.5
pH 8.4-9.7 (21 day old samples)	Samples had completely decomposed.		

[a]Not applicable, that is spironolactone was not present in the solution.
[b]Not determined at this wavelength.
[c]At pH 6.3, the loss in concentration in 21 day was ∿7%.

of decomposition was also present. This is the same peak as peak 4 in chromatogram C (Figure 3). Apparently spironolactone is very stable between pH values of ∿3.4-5.2, less so below pH 3.4 and very unstable above pH 6.2. Further details of the stability studies of these solutions and others are in progress and will be presented in a separate communication. The purpose of the present study was to develop good stability-indicating assay methods which could be used to study the stability of spironolactone in the yet to be developed oral liquid dosage form and a dermatological cream.

REFERENCES

1. Anonymous, The United States Pharmacopeia 21st Rev., The National Formulary 16th Rev., The United States Pharmacopeial Convention, 1985, p. 981.
2. Microbondapak, Care and Use Manual, Waters Associates, 1988, p. 15.
3. V.D. Gupta and A.G. Ghanekar, J. Pharm. Sci., 67, 889 (1978).
4. D.E. Williamson, Ibid., 65, 138 (1976).
5. F. de Croo, W.V. Bossche and P. de Moerloose, J. Chromatogr., 329, 422 (1985).
6. J.L. Sutter and E.P.K. Lau in Analytical Profiles of Drug Substances, Vol. 4, K. Florey, Ed., Academic Press, NY 1975, pp. 431-51.