Drug Development and Industrial Pharmacy, 24(2), 101–107 (1998)

RESEARCH PAPER

An Improved High-Performance Liquid Chromatography Assay for Spironolactone Analysis

Kenneth S. Alexander,[1,*] Shyam Sundar K. S. Vangala,[1,†] and David Dollimore[1,2]

[1]*College of Pharmacy and*
[2]*Department of Chemistry, The University of Toledo, 2801 W. Bancroft St., Toledo, Ohio 43606*

ABSTRACT

This study prepared an extemporaneously formulated liquid suspension dosage form (5 mg/ml) from commercially available 25 mg tablets. Stability-indicating HPLC assay procedures were established and utilized to analyze the concentration of the drug. The method proved to be a simple model since it does not contain a buffer system. The mobile phase used was the same as that suggested by the manufacturer for the storage of the column. Therefore, the solvent system saves analytical processing time, since it does not require a change in the mobile phase before or after the analysis. The analytical method has been shown to be stability-indicating. The results have shown that there is no interference from any of the degradation products obtained from stressing spironolactone by heat and extremes in pH or with the internal standard, hydrocortisone 21-acetate.

INTRODUCTION

Many drugs have assay procedures available which are either cumbersome or difficult to reproduce. The USP only admits new assay procedures which have proven themselves over time. Researchers are usually obliged to develop methods which are not currently recognized as being the official assay method of the compendium. The present study was been done to address the stability of extemporaneously prepared oral liquid dosage forms for spironolactone [17-hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone acetate (1)].

The specific objectives of the study were (*a*) formulate a liquid oral dosage form for spironolactone. The procedure for making the product must be simple enough

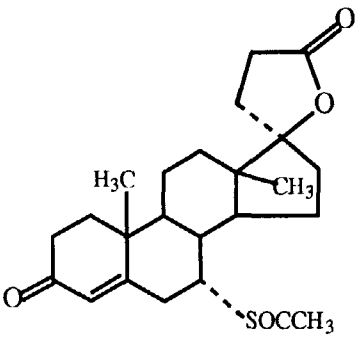
*To whom correspondence should be addressed.
†Currently in the Ph.D. Program at the University of Wisconsin.

to be done in a pharmacy; (*b*) modify or develop an assay method for the quantitative analysis of the drug in the formulation with the assay method being stability-indicating; and (*c*) investigate the chemical stability of the drug in the formulation using accelerated stability studies.

To perform the investigation, a quantitative analytical method is required. The analytical procedure should have the ability to distinctively determine the parent compound without interference from the degradation products so as to be stability-indicating (2). This is most often tested by subjecting the drug solution to stress conditions. The stress is applied by changing the pH of the solution to extremes and heating. The precision of this assay procedure will be evaluated in the following ways: the correlation coefficient of the calibration curve obtained from the assay procedure will be not less than 0.950; and the percent relative standard deviation of the readings obtained from 16 identical concentrations will be not more than 2.00.

According to *Remington's Pharmaceutical Sciences* (3), spironolactone is a steroid that acts as a competitive antagonist of the potent endogenous mineral-corticosteroid, aldosterone. Spironolactone, by blocking the sodium-retaining effects of aldosterone on the distal convoluted tubule, corrects one of the most important mechanisms responsible for the production of edema.

Spironolactone is a light cream-colored to light tan crystalline powder. It has a faint to mild mercaptan-like odor. It is stable in air. It is freely soluble to chloroform, soluble in alcohol, and slightly soluble in fixed oils (3). It is practically insoluble in water at 25°C with a solubility of 0.028 mg/ml (4). The formula for spironolactone is as follows:



Stability of spironolactone in solution has been previously studied (4–7). The pH profile of spironolactone is V-shaped. It shows a maximum stability at pH 4.5. It has been shown that phosphate and citrate buffers catalyze the degradation of spironolactone. The ionic strength does not effect the stability of the drug. Spironolactone has been shown to undergo degradation with an apparent first-order rate. Its activation energy has been reported to be 18.9 kcal/mole (79 kJ/mole) at pH 4.3 (5).

ANALYTICAL PROCESS

It is necessary to have a stability-indicating assay procedure to quantitatively analyze spironolactone in the dosage form. Canrenone is the major decomposition product of spironolactone. Canrenone is structurally related to spironolactone and, hence, its separation from spironolactone is essential (8). Sadana et al. (9) provided a chromatographic method for the simultaneous determination of spironolactone and furosemide in pharmaceutical dosage forms. However, this method has not been shown to be stability-indicating. De Croo et al. (10) reported a stability-indicating HPLC assay procedure which uses acetonitrile:water (1:1 v/v) as a mobile phase with a micro C18 column. This method was replicated in our laboratory, however, the method did not separate canrenone from spironolactone. Pramar et al. (8) also reported the same result with this method. These deviations may be due to the use of different brands of micro C18 columns. Gupta et al. (11) reported a stability-indicating assay procedure using a micro CN column and 40% aqueous ethanol as the mobile phase. However, Pramar et al. (8) also reported that this method does not separate canrenone from spironolactone and have thus reported two methods. The first method uses a micro C18 column with a 63% methanol (v/v) in 0.01 M phosphate aqueous buffer as the mobile phase with a flow rate of 2.0 ml/min. The second method uses a micro phenyl column with 39% acetonitrile (v/v) in 0.01 M phosphate aqueous buffer. The first method was selected for our stability studies. However, it was felt that the buffer in the mobile phase was unnecessary, since both spironolactone and canrenone appear to be neutral and hence their retention times are not affected by pH (8). It is also known that phosphate buffers are insoluble in methanol. This can result in the precipitation of the phosphate buffer within the column when the mobile phase contains a high methanol concentration for the analysis. To avoid this phenomenon phosphate buffer was removed after each analysis with filtered and degassed distilled water. The first method was adapted with modifications for the stability studies. The modified method does not contain a buffer system. The mobile phase has 66:34

methanol:water (v/v) and a flow rate of 1.3 ml/min. Hydrocortisone 21-acetate was used as the internal standard. The advantage of this method is that the micro C18 columns are usually stored in 66% (v/v) methanol:water (12). The micro C18 column used in the study carried the recommendation of the manufacturer for storage in this same solvent (13). This process results in the avoidance of a mobile phase change before and after the analysis and saves analytical processing time.

METHODOLOGY

Reagents and Chemicals

All reagents used were either USP, NF, or ACS grade. HPLC grade methanol (lot no. 925439) was obtained from Fisher Scientific Co., Fairlawn, NJ. Hydrocortisone 21-acetate (lot no. 87C-0442) was obtained from Sigma Chemical Co., St. Louis, MO. Spironolactone (lot no. J0356) and canrenone were generously supplied by G.D. Searle & Co., Skokie, IL.

HPLC Instrumentation and Conditions

The HPLC system consisted of a Beckman 110B solvent delivery system; a Beckman system organizer injector fitted with 20 μl loop; a model 420 system controller programmer (Beckman Instruments Inc., San Ramon, CA); a Knauer spectrophotometric UV-detector (number 731.7100000) from Knauer Corp., Berlin, Germany; and a Shimadzu C-R3A Chromatopac Integrator (Shimadzu Corp., Kyoto, Japan). The column was a Supelcosil LC-18 (25 cm × 4.6 mm i.d. with 5 μm particles).

The flow rate for the mobile phase was set at 1.3 ml/min. The column pressure varied between 1500 and 2000 psi. The detector was operated at 254 nm and 0.04 AUFS. The analytical column was at ambient temperature for all separations. The chart speed was maintained at 10 mm/min.

The mobile phase for the HPLC assay of spironolactone was prepared according to the following procedure. A 660-ml volume of methanol was transferred into a graduated cylinder. It was made up to 1 liter with distilled water. The solution was filtered through a 0.45-μm membrane filtration system (Millipore Corp., Milford, MA) to remove any particulate matter. The mobile phase was degassed with nitrogen before use.

The internal standard stock solution was prepared using a 50-mg portion of hydrocortisone 21-acetate quantitatively weighted on a Mettler balance and transferred into a 100-ml volumetric flask. The volume was made up to 100 ml with methanol to give a final concentration of 0.5 mg/ml hydrocortisone 21-acetate internal standard stock solution.

Calibration Curve and Assay Precision

Spironolactone stock solution was prepared by accurately weighing out a 50-mg portion of spironolactone and dissolving it in 100 ml of methanol in a volumetric flask to give a final concentration of 0.5 mg/ml spironolactone stock solution.

The calibration plot for the spironolactone HPLC method was obtained using the following procedure. A 1-ml aliquot of internal standard stock solution was transferred to each of seven 50-mL volumetric flasks. A 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 ml portion of spironolactone stock solution was transferred to each of seven volumetric flasks. The volume was made up to 50 ml with 66% (v/v) methanol in water. About 5 ml of each of these solutions was filtered through a 0.2-μm filter. About 50 μl of each solution was injected onto the column fitted with a 20-μl injector loop. Each solution was injected thrice. The peak areas for spironolactone and the internal standard were recorded. Peak area ratios were plotted on the *y*-axis against the concentration of spironolactone in the final dilution on the *x*-axis. The equation obtained by linear regression was used to calculate the spironolactone concentration in the spironolactone suspension samples.

The precision of the HPLC assay procedure was tested by the following procedure. Precision of the assay was determined by injecting identical samples four times each day over a 4-day period. The detector response was compared and the percent relative standard deviation for the area under the curve (AUC) ratios were calculated for between and within days.

Stability-Indicating Nature of the Assay

The stability-indicating nature of the spironolactone HPLC assay procedure was tested using the following procedure. The drug was subjected to extreme acidic and alkaline conditions while being heated to promote possible degradation. A 1.5-ml portion of spironolactone stock solution in methanol was added to both 1 ml of 1 M HCl and 1 ml of 1 M NaOH. Both of these solutions were made up to 15 ml and boiled for 30 min. The resulting solutions were cooled to room temperature and neutralized using HCl and NaOH solutions. They were

then made up to 25 ml with distilled water. Samples for chromatographic injection were prepared by filtering the final diluted samples through a 0.2-μm filter. Since canrenone is the major product of decomposition for spironolactone, a solution containing spironolactone and canrenone was prepared and injected to test for the separation of both the compounds.

Precision of the Sampling Assay

The precision of the HPLC sampling method was obtained using the following procedure. Five samples were prepared from the suspension and 50 μml of each were injected thrice onto the chromatographic column. The average of the area under the curve ratios for the three injections were calculated. From this average, the standard deviation for the sampling procedure was calculated.

RESULTS AND DISCUSSION

When a solution containing spironolactone and hydrocortisone 21-acetate was injected onto the chromatographic column, a clear separation of these compounds occurred as seen in Fig. 1. A sample prepared from the suspension showed that there was no interference from any of the ingredients in the suspension as seen in Fig. 2. The retention times for hydrocortisone 21-acetate and spironolactone were 8.0 and 9.5 min, respectively.

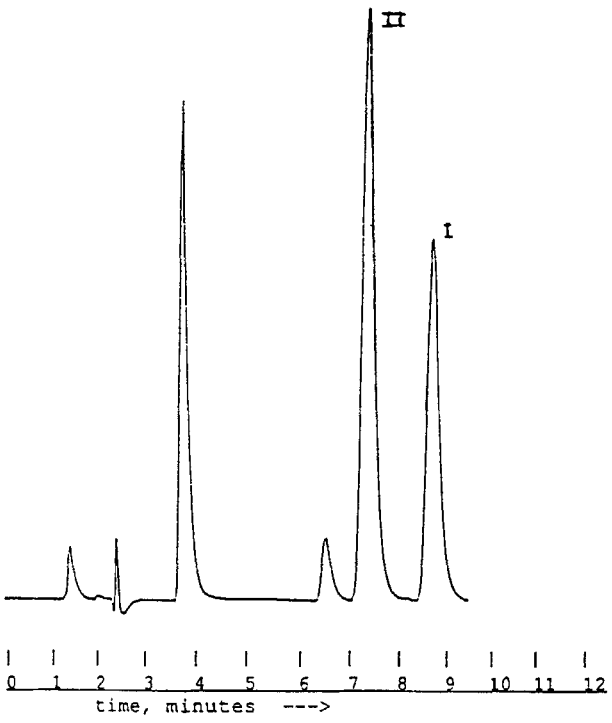


Figure 1. Chromatogram showing separation of spironolactone (I) from the internal standard, hydrocortisone 21-acetate (II).

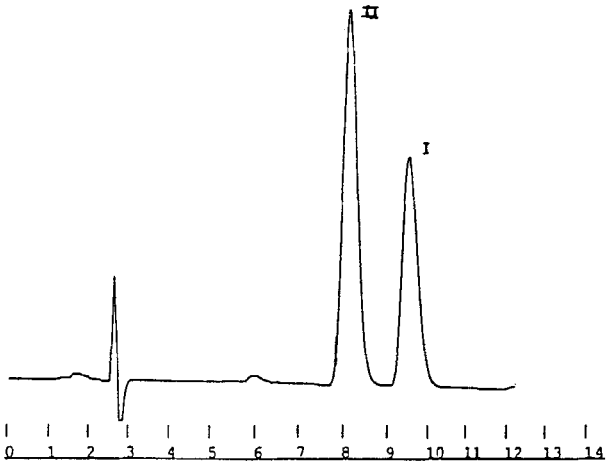


Figure 2. Chromatogram showing separation of both spironolactone (I) and the internal standard, hydrocortisone 21-acetate (II), from the ingredients of the suspension formulation.

The calibration plot providing the area under the curve ratios for spironolactone and the internal standard, hydrocortisone 21-acetate, on the *y*-axis against concentration of spironolactone on the *x*-axis showed an excellent coefficient of correlation of 1.000. The data for the calibration plot are given in Table 1.

The percent relative standard deviation of the area under the curve ratios for within and between days was obtained by injecting an identical sample four times on each of 4 days and the results are presented in Table 2. The percent relative standard deviations for within day and between days was calculated and found to be 0.996% and 1.45%, respectively.

Table 1

Data for the Calibration Plot for the Spironolactone HPLC Assay

Concentration (μg/ml)	Area Under the Curve Ratio (AUC)
4	0.3603
6	0.5413
8	0.7377
10	0.9188
12	1.0942
14	1.2739
16	1.4794

Equation obtained from calibration plot: AUC ratio $= 9.7357 \times 10^{-3} + 9.2482 \times 10^{-2} \times$ (μg/ml).

Table 2

Precision of the Spironolactone HPLC Assay

AUC (Day 1)	AUC (Day 2)	AUC (Day 3)	AUC (Day 4)
0.834	0.832	0.830	0.815
0.821	0.845	0.837	0.840
0.817	0.845	0.837	0.836
0.829	-	0.837	0.844

Standard deviation for within days = 8.30119×10^{-3}
Standard deviation for between days = 1.20833×10^{-2}
% Relative standard deviation of within days = 0.996%.
% Relative standard deviation between days = 1.45%.

The percent relative standard deviation of the area under the curve determined from five samples prepared from the spironolactone suspension was found to be 1.4%, as given in Table 3. From these data the percent standard error of the mean of three samples was calculated to be 0.799%. This percent standard error gives the estimated standard deviation for the means of three samples. Since one sample is taken from each of the three bottles containing the formulation, and the mean of these three samples was utilized in the calculations, the percent standard error for the mean of three samples has been calculated.

The stability-indicating nature of the HPLC assay was determined by subjecting spironolactone to either extreme acidic or alkaline conditions and heating. The spironolactone solution stressed under alkaline conditions showed a zero potency for spironolactone and a peak at 4.8 min, as seen in Fig. 3. This solution was spiked with the internal standard stock solution and again injected onto the HPLC column. This showed a complete separation between the internal standard and the degradation peak, as seen in Fig. 4. No interference

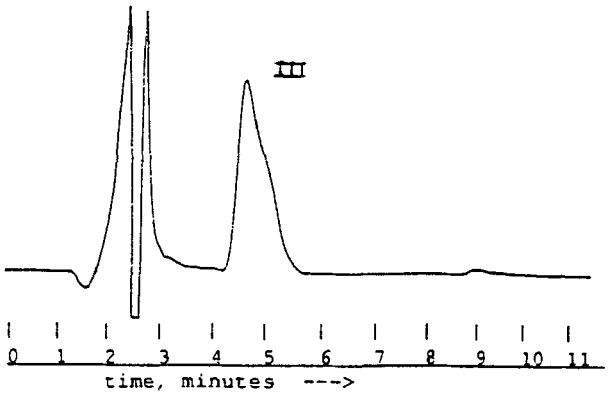


Figure 3. Chromatogram for the spironolactone sample stressed under basic conditions showing zero potency for spironolactone and a peak for a degradation product (III) at 4.8 min.

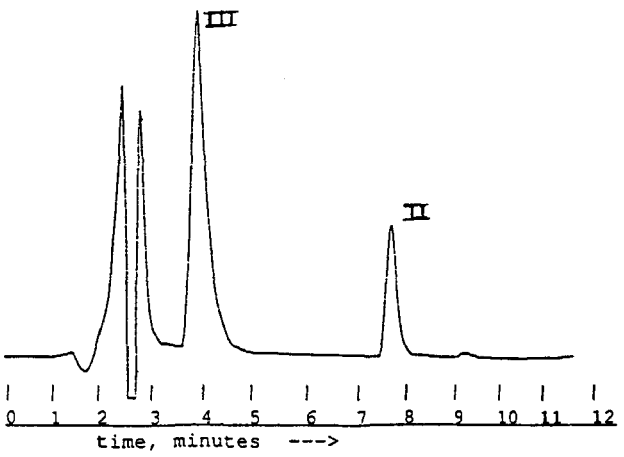


Figure 4. Chromatogram for the spironolactone sample stressed under basic conditions and spiked with the internal standard, hydrocortisone 1-acetate (II), demonstrating clear separation between the degradation product (III) and the internal standard.

Table 3

Precision of the Sampling Method for Spironolactone in the Formulation

	Area Under the Curve Ratios			
Sample No.	Injection No. 1	Injection No. 2	Injection No. 3	Mean
1	0.8642	0.8606	0.8203	0.8484
2	0.8762	0.8721	0.8828	0.8770
3	0.8618	0.8626	0.8618	0.8621
4	0.8765	0.8772	0.8817	0.8785
5	0.8744	0.8661	0.8681	0.8695

Standard deviation = 0.012, mean = 0.8671.
% Relative standard deviation = 1.4%.
Standard error for means of three samples (n = 3) = $0.012\ n^{1/2} = 6.928 \times 10^{-3}$.
% Relative standard error for means of three samples = 0.799.

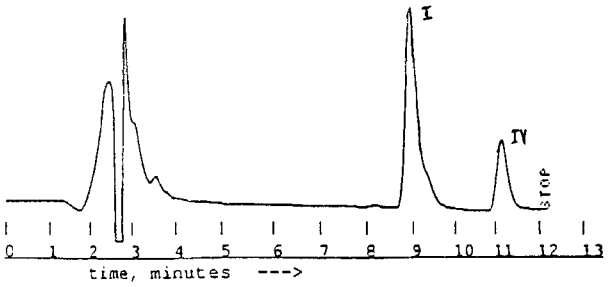


Figure 5. Chromatogram for the spironolactone sample stressed under acidic conditions showing a peak for spironolactone (I) and the degradation product (IV).

from the degradation peak with spironolactone is expected since the retention time of the drug is more than that for the internal standard. The spironolactone solution stressed under acidic conditions showed a peak for the drug and a peak at 11.8 min, as seen in Fig. 5. The retention time for the degradation peak is the same as that of the canrenone and hence the degradation product might be canrenone. Both the peaks showed complete separation. Since canrenone is a known major degradation compound, a solution containing spironolactone and canrenone was injected onto the column, as seen in Fig. 6. The resultant chromatogram showed complete separation between the drug and canrenone. The above results demonstrate that the HPLC assay procedure is stability-indicating.

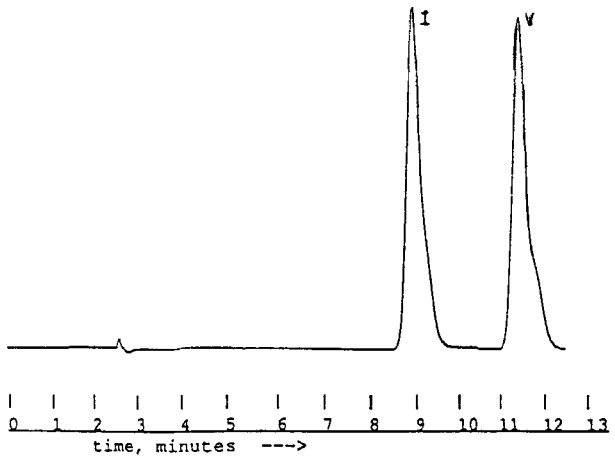


Figure 6. Chromatogram resulting from a solution containing spironolactone (I) and a known degradation product, canrenone (V), demonstrating complete separation of these products by the assay method.

CONCLUSIONS

The investigation has developed a modified stability-indicating HPLC procedure for the quantitation of spironolactone in dosage forms. This method proved to be a simple model since it does not contain a buffer system. The mobile phase used was the same as that suggested by the manufacturer for the storage of the column. Therefore, the process saves analytical processing time, since it does not require a change in the mobile phase before or after the analysis. It may also be possible to maximize column life by using the mobile phase suggested by the manufacturer to store the column. The calibration plot obtained using this method of analysis has resulted in a coefficient of correlation of 1.000. The percent relative standard deviations for within day and between days for the HPLC assay were calculated to be 0.996% and 1.45%, respectively, demonstrating good precision of the assay. The analytical method has been shown to be stability-indicating through the analysis of samples stressed under either acidic or basic conditions with applied heat. The results have shown that there is no interference from any of the degradation products with either spironolactone or its internal standard, hydrocortisone 21-acetate. The assay has also shown that there is good separation between spironolactone and its major degradation product, canrenone.

ACKNOWLEDGMENT

The authors gratefully acknowledge the generosity of G.D. Searle & Co., Skokie, IL, for the support of this project by their donation of spironolactone and canrenone used to verify the assay procedure.

REFERENCES

1. A. R. Gennaro, ed., *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 941.
2. L. Lachman, H. A. Lieberman, and J. L. Koenig, *The Theory and Practice of Industrial Pharmacy*, 3rd ed., Lea and Febiger, Philadelphia, 1986, p. 849.
3. A. R. Gennaro, ed., *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 942.
4. G. M. Peterson, M. F. Meaney, C. A. Reid, and G. R. Taylor, Stability of extemporaneously prepared mixtures of metoprolol and spironolactone, Aust. J. Hosp. Pharm., 19(6), 344 (1989).

5. Y. Pramar and V. D. Gupta, Preformulation studies of spironolactone: effect of pH, two buffer species, ionic strength, and temperature on stability, J. Pharm. Sci., 80(6), 551 (1991).
6. V. D. Gupta, C. W. Gibbs, and A. G. Ghanekar, Stability of pediatric liquid dosage forms of ethacrynic acid, indomethacin, methyldopate hydrochloride, prednisone and spironolactone, Am. J. Hosp. Pharm., 35(11), 1382 (1978).
7. L. K. Mathur and A. Wickman, Stability of extemporaneously compounded spironolactone suspensions, Am. J. Hosp. Pharm., 46(10), 2040 (1989).
8. Y. Pramar, V. D. Gupta, and T. Zerai, Quantitation of spironolactone in the presence of canrenone using high performance liquid chromatography, Drug Dev. Ind. Pharm., 17(5), 747 (1991).
9. G. S. Sadana and A. B. Ghogare, Simultaneous determination of spironolactone and furosimide in pharmaceutical dosage forms by HPLC, Ind. J. Pharm. Sci., 52(9), 244 (1990).
10. F. De Croo, W. V. D. Bossche, and P. D. Moerloose, Simultaneous determination of althiazide and spironolactone in tablets by high performance liquid chromatography, J. Chromatogr., 329, 422 (1985).
11. V. D. Gupta, and A. G. Ghanekar, Stability-indicating methods for quantitative determination of spironolactone using high pressure liquid chromatography and blue tetrazolium reaction, J. Pharm. Sci., 67(6), 889 (1978).
12. A. Martin, J. Swarbrick, and A. Cammarata, *Physical Pharmacy*, 3rd ed., Lea and Febiger, Philadelphia, 1983, p. 392.
13. Supelco, *How to Install and Care for Your New HPLC Column*, Supelco Inc., 1987.