Table II—R_f Values and Color Reactions for Methadyl Acetate, Nor-methadyl Acetate, 6-(Dimethylamino)-4,4-diphenyl-3-heptanol, and Methadone Using LQD Plates[a]

Compound	R_f	Color
Methadyl acetate	0.75	Pinkish purple
Nor-methadyl acetate	0.64	Pinkish purple
6-(Dimethylamino)-4,4-diphenyl-3-heptanol	0.72	Purple
Methadone	0.68	Pinkish purple

[a] Solvent System B was used, the spray reagent was iodoplatinate, and the sensitivity was 0.1 μg.

Table III—R_f Values and Color Reactions for Methadyl Acetate, Nor-methadyl Acetate, 6-(Dimethylamino)-4,4-diphenyl-3-heptanol, and Methadone Using LQ6D Plates[a]

Compound	R_f	Color
Methadyl acetate	0.75	Pinkish purple
Nor-methadyl acetate	0.40	Pinkish purple
6-(Dimethylamino)-4,4-diphenyl-3-heptanol	0.68	Purple
Methadone	0.62	Pinkish purple[b]

[a] Solvent System B was used, the spray reagent was iodoplatinate, and the sensitivity was 0.1 μg. [b] Pinkish purple changed to brownish purple after a few minutes.

amino)-4,4-diphenyl-3-heptanol are the same metabolites found in human urine (12).

The identity of methadyl acetate, nor-methadyl acetate, and 6-(dimethylamino)-4,4-diphenyl-3-heptanol was confirmed by GLC, using 3% OV-1 on Shimalite W, 80–100 mesh[6]. The column temperature was 230°, and helium was the carrier gas (60-ml/min flow rate). The retention times for methadyl acetate, nor-methadyl acetate, 6-(dimethylamino)-4,4-diphenyl-3-heptanol, and methadone were 5.0, 4.8, 5.8, and 4.4 min, respectively. Methadone, a structurally related compound, was included for comparison because it is used along with methadyl acetate in opiate addicts.

Of the three different TLC plates used, LQ6D was the best in separating the methadyl acetate, nor-methadyl acetate, 6-(dimethylamino)-4,4-diphenyl-3-heptanol, and methadone. Iodoplatinate proved to be a better spraying reagent than iodine.

[6] American Instrument Co., Atlanta, Ga.

REFERENCES

(1) R. E. Billings and R. E. McMahon, *Fed. Proc., Abstr. 57 Annual meeting*, **32,** 764 Abstr.(1973).

(2) J. H. Jaffe and E. C. Senay, *J. Amer. Med. Ass.*, **216,** 1303(1971).

(3) A. Zaks, M. Fink, and A. M. Freedman, *ibid.*, **220,** 811(1972).

(4) R. E. Billings, R. Booher, S. Smits, A. Pohland, and R. E. McMahon, *J. Med. Chem.*, **16,** 305(1973).

(5) R. E. Billings, R. E. McMahon, and D. A. Blake, *Life Sci.*, **14,** 1437(1974).

(6) C. Y. Sung and E. L. Way, *J. Pharmacol. Exp. Ther.*, **110,** 260(1954).

(7) R. F. Kaiko and C. E. Inturrisi, *J. Chromatogr.*, **82,** 315(1973).

(8) R. F. Kaiko and C. E. Inturrisi, *Fed. Proc.*, **32,** 764 Abstr.(1973).

(9) A. G. Gornall, C. F. Bardawill, and M. M. David, *J. Biol. Chem.*, **177,** 751(1949).

(10) I. Nash, *Biochem. J.*, **55,** 416(1966).

(11) K. K. Kaistha and J. H. Jaffe, *J. Pharm. Sci.*, **61,** 679(1972).

(12) S. E. Smits and R. Booher, *Fed. Proc.*, **32,** 764 Abstr.(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 25, 1974, from the *School of Pharmacy, Auburn University, Auburn, AL 36830*

Accepted for publication April 7, 1975.

[x] To whom inquiries should be directed.

High-Speed Liquid Chromatographic Determination of Canrenone in Pharmaceutical Dosage Forms

DAVID E. WILLIAMSON

Abstract □ Canrenone can be determined by high-speed liquid chromatography in pharmaceutical dosage forms without interference from common excipients or degradation products. This stability-indicating assay, using *o*-nitroaniline as the internal standard, is rapid and accurate.

Keyphrases □ Canrenone—analysis, high-speed liquid chromatography, pharmaceutical dosage forms □ High-speed liquid chromatography—analysis, canrenone, pharmaceutical dosage forms

Canrenone (I), 17-hydroxy-3-oxo-17α-pregna-4,6-diene-21-carboxylic acid γ-lactone, is a steroid that is an aldosterone antagonist and diuretic (1). Its synthesis was reported previously (2, 3).

Previous methods of analysis in biological fluids involved GC and fluorometric analyses (4–6). The fluorometric analysis involved the conversion of canrenone, a dienone, to a trienone using sulfuric acid (62% v/v). Since the major acid degradation product of canrenone is a trienone (II), this procedure could not be used as a stability-indicating assay for pharmaceutical dosage forms. Attempts by this author to use GC were not successful.

Recently, high-speed liquid chromatography

Table I—Standard Additions

Milligrams of Canrenone Added beyond 50 mg/25 μl	Found, mg/25 ml	Total Recovery, %
0.00	97.3	100.0
31.02	128.5	100.1
54.34	151.2	99.7

Table II—Precision and Accuracy

Trial	Canrenone Found, mg[a]	Percent Label Claim
1	49.1	98.2
2	48.9	97.8
3	49.5	99.0
4	49.5	99.0
5	49.1	98.2
6	48.9	97.8
7	49.3	98.7
8	49.4	98.7
9	49.1	98.2
10	49.0	98.1
	49.2 ± 0.23 mg/dose	$98.4 \pm 0.45\%$
Relative standard deviation	0.47%	0.46%

[a] Theory: 50 mg of canrenone.

(HSLC) has found wide usage in the analysis of steroids (7–12). This article describes the application of HSLC to the analysis of canrenone in pharmaceutical dosage forms.

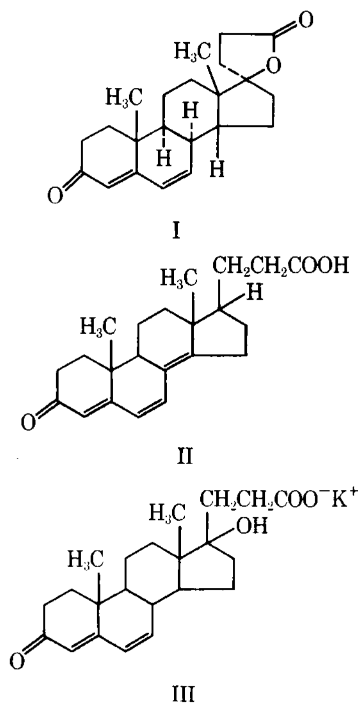
EXPERIMENTAL

Apparatus—A high-pressure liquid chromatograph[1] equipped with a UV detector (254 nm) capable of operating at inlet pressures up to 1000 psig was used.

Column—The column was purchased from the instrument manufacturer[1] and was a 1-m × 2.1-mm i.d. × 0.6-cm o.d., precision bore, stainless steel tube, packed with Sil-X, an adsorption material[2].

Reagents—Heptane, 2-propanol, and methanol were reagent grade and required no further purification. *o*-Nitroaniline[3] and canrenone[4] were reference quality and were used without further purification.

2-Propanol in Heptane (4% v/v)—Dilute 20.0 ml of 2-propanol to 500 ml with heptane.



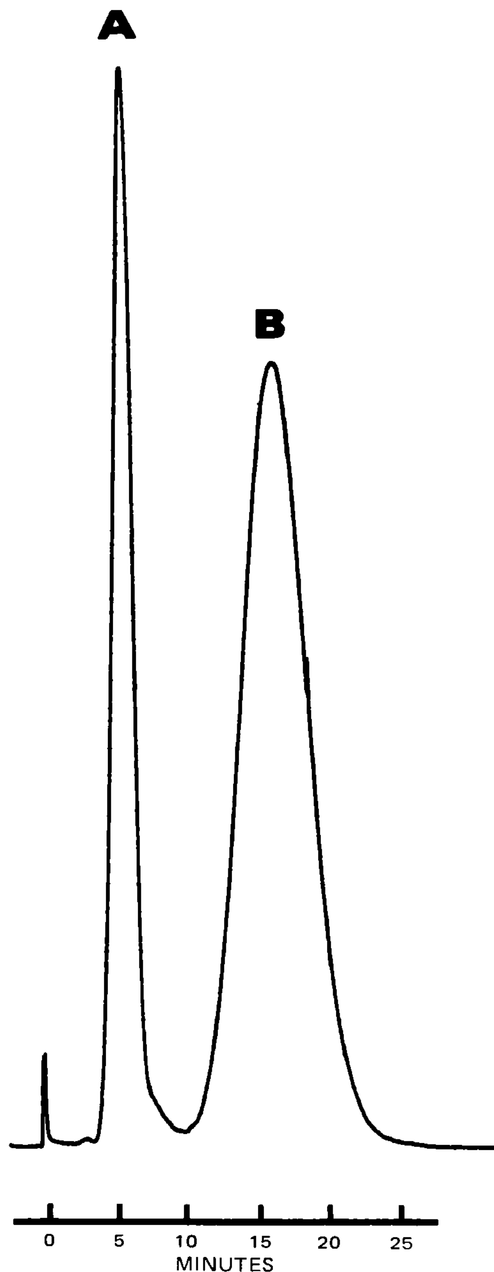


Figure 1—*High-speed liquid chromatogram of a sample preparation with internal standard. Key: A, o-nitroaniline, internal standard; and B, canrenone.*

Internal Standard Solution—Dissolve ~1.0 g of *o*-nitroaniline in 100 ml of methanol to yield a final concentration of ~10 mg/ml.

Standard Reference Solution—Accurately weigh about 125 mg of canrenone into a 25-ml volumetric flask containing ~10 ml of methanol and shake to dissolve. Pipet 5.0 ml of the internal standard solution into the flask and dilute to volume with methanol.

Sample Preparation—Weigh accurately an amount of powder equivalent to 125 mg of canrenone and transfer it to a 25-ml volumetric flask. Add about 10 ml of methanol, pipet in 5.0 ml of the internal standard solution, and shake for 15 min before diluting to volume with methanol. Withdraw about 10 ml of this suspension, using a 10-ml glass syringe equipped with a Teflon needle on a Kel-F-Hub. Filter the suspension through a filter holder[5] containing a silver membrane[6] filter and collect the filtrate in a 10-ml

[1] DuPont 820, E. I. du Pont de Nemours and Co., Wilmington, Del.
[2] Perkin-Elmer, Norwalk, Conn.
[3] Highest Purity No. 646, Eastman Kodak Co., Rochester, N.Y.
[4] Abbott Laboratories, North Chicago, Ill., and Specia Paris, Paris, France.

[5] Swinnex-25 SX00-025-00, Millipore Corp., Bedford, Mass.
[6] FM-25 (0.45 μm), Selas Flotronic, Spring House, Pa.

ground-glass-stoppered flask. This solution is then ready to be chromatographed.

Chromatographic Procedure—The mobile phase was 4% 2-propanol in heptane, the temperature was ambient, and the solvent flow was ~1 ml/min (at an inlet pressure of 1000 psig). The precision photometer detector (254 nm) was set at an attenuation of ×16.

Samples and standards of 3 μl (~15 μg of canrenone) were injected with the flow stopped. A standard was injected for every five sample injections, and the peak heights of canrenone and the internal standard were measured.

Calculations—The concentration of canrenone in milligrams per dose is calculated using:

$$\text{mg/dose canrenone} = F \times \frac{H_1}{H_2} \times \frac{\text{average dose weight (mg)}}{\text{weight sample used (mg)}} \quad \text{(Eq. 1)}$$

$$\text{\% label claim} = \frac{\text{mg/dose found}}{\text{mg/dose theory}} \times 100 \quad \text{(Eq. 2)}$$

where H_1 and H_2 are the peak heights of canrenone and internal standard in the sample, respectively.

The response factor, F, is the ratio of the internal standard peak heights to the canrenone peak height in the standard reference solution multiplied by the weight of canrenone in the standard.

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram of a typical sample containing the internal standard. The major degradation products, II and III, do not interfere with the analysis. Compound III does not elute but is retained on the column. Compound II elutes at ~32 min if present. Canrenone elutes at ~15 min, and the internal standard elutes at ~5 min. Methanol was chosen as the solvent for drug extraction due to the insolubility of common excipients.

The linearity of response has been shown for canrenone pharmaceutical formulations containing 40–60 mg/dose. Table I shows the results of standard addition experiments when the theory was 50 mg/dose.

The precision of the method was determined from 10 replicate determinations of canrenone in 50-mg/dose pharmaceutical formulations performed over 2 days (Table II). Peak height rather than peak area was found to yield better results on this analysis, because the pumping system on the chromatographic apparatus caused a recorder spike which interfered with digital integrator calculations.

This method is also applicable to single-tablet and capsule analysis if the attenuation of the UV detector and the internal standard concentrations are adjusted appropriately.

REFERENCES

(1) "Merck Index," 8th ed., Merck and Co., Rahway, N.J., 1968, p. 802.

(2) J. A. Cella and R. C. Twirt, *J. Org. Chem.*, **24**, 1109(1959).

(3) J. A. Cella (to G. D. Searle and Co.), U.S. pat. 2,900,383 (1959).

(4) W. Sadee, S. Riegelman, and S. C. Jones, *J. Pharm. Sci.*, **61**, 1129(1972).

(5) W. Sadee, M. Dagcioglu, and S. Riegelman, *ibid.*, **61**, 1126(1972).

(6) J. Chamberlain, *J. Chromatogr. Sci.*, **55**, 249(1971).

(7) S. Siggia and R. A. Dishman, *Anal. Chem.*, **42**, 1223(1970).

(8) K. Lotscher and H. Kern, *Chimia*, **27**, 348(1973).

(9) J. C. Touchsto and W. Wortmann, *J. Chromatogr.*, **76**, 244(1973).

(10) R. A. Henry, J. A. Schmit, and J. F. Dieckman, *J. Chromatogr. Sci.*, **9**, 513(1971).

(11) J. F. K. Huber, J. A. R. Hulsman, and C. A. M. Meyers, *J. Chromatogr.*, **62**, 79(1971).

(12) F. A. Fitzpatrick, S. Siggia, and J. Dingman, *Anal. Chem.*, **44**, 2211(1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 30, 1975, from *Analytical Services & Methods Development, Hospital Products Division, Abbott Laboratories, North Chicago, IL 60064*

Accepted for publication April 15, 1975.

The author thanks James Raihle and Victor Papendick for assistance.

Time-Dependent Change in Renal Clearance of Bethanidine in Humans

A. N. CHREMOS [*], D. SHEN [‡], M. GIBALDI [‡x], J. D. PROCTOR [§], and J. H. NEWMAN [§]

Abstract □ Blood levels and urinary excretion rates of bethanidine were determined in three normal human subjects following oral administration of a single dose of the drug. The postabsorptive decline of blood concentration with time was noticeably slower than the corresponding decline in the urinary excretion rate. The discrepancy can be attributed to a continual decrease in the renal clearance of bethanidine throughout the study. Therefore, pharmacokinetic modeling of urinary excretion data alone would lead to erroneous conclusions concerning the persistence of drug in the blood.

Keyphrases □ Bethanidine—time-dependent change in renal clearance and blood levels, humans □ Excretion, urinary—rate of clearance of bethanidine, compared to blood levels, humans □ Antihypertensives—bethanidine, renal clearance compared to blood levels, humans

A previous study (1) on the pharmacokinetics of bethanidine in hypertensive patients found that the renal clearance of unchanged bethanidine fluctuated greatly in the initial 6 hr following a single 25-mg iv dose. The study also revealed that the elimination of bethanidine in humans occurs almost exclusively *via* renal excretion. Hence, a more extensive study on the renal clearance of the drug was undertaken.

EXPERIMENTAL

Three healthy male volunteers, 65–75 kg, were selected. Their