Journal of Chromatography, 354 (1986) 367–373
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 283

SIMULTANEOUS QUANTITATIVE DETERMINATION OF BUTIZIDE, POTASSIUM CANRENOATE AND CANRENONE IN TABLETS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(Received October 11th, 1985)

SUMMARY

A procedure for the simultaneous quantitative determination and selective identification of potassium canrenoate and butizide is proposed. The amount of canrenone, the degradation product of potassium canrenoate, is also determined. Elution of these compounds is investigated on LiChrosorb RP-18 and RP-8 columns. The analysis time was shorter on a RP-8 column while the specific identification was still possible. Therefore, the LiChrosorb RP-8 column was chosen. The eluent consisted of an acetonitrile–0.05 *M* phosphate buffer, pH 4 (45:55) mixture. Quantitative determination was performed at the absorption maximum of each compound: 286 nm for potassium canrenoate and for canrenone, 271 nm for butizide. This change in wavelength is performed automatically by the computer that controls the spectrophotometric diode array detector.

INTRODUCTION

Potassium canrenoate (KCAN; a potassium-sparing diuretic) and butizide (BUT; a thiazide diuretic) are often administered together for the treatment of hypertension. Canrenone (CAN), the degradation compound of KCAN, still preserves some diuretic activity. Therefore, the amount of CAN is also analysed.

Until now, there has been no method available for the simultaneous quantitative determination of those compounds. BUT has been analysed by a spectrophotometric method[1]. The determination of KCAN is not described in the literature. CAN has been determined by several methods[2–5]. This paper describes the development of the simultaneous determination of KCAN, BUT and CAN. The accent is put on analysis time and selectivity. In a previous paper, the elution of thiazide, potassium-sparing and loop diuretics was investigated on a LiChrosorb RP-18 column[6]. Starting from these data, the separation of these compounds was optimized with regard to efficiency, speed and selectivity.

0021-9673/86/$03.50

EXPERIMENTAL

Apparatus

An SP 8770 isocratic pump (Spectra Physics, Darmstadt, F.R.G.) was used, equipped with a HP 1040A UV spectrophotometric detector (Hewlett-Packard, Palo Alto, CA, U.S.A.), a HP 85 computer, a HP 82901M flexible disc drive and a HP 3390A integrator. The HP 1040A UV detector contains a photodiode array. This detector can follow up to eight different wavelengths at the same time. The wavelength selection can be changed automatically by the program of the HP 85 computer. This is used for changing the wavelength from 286 to 271 nm for the detection of KCAN and BUT, respectively. CAN, if present in the sample, was measured at 286 nm.

The eluent was filtered through a 5-μm filter and degassed with helium. A Valco six-port injection valve with a 10-μl sample loop was used. The temperature of the column was thermostatted with a water bath.

Chromatographic procedure

LiChrosorb RP-18 and RP-8 columns (Chrompack, Middelburg, The Netherlands) of the same dimensions were used. The chromatographic conditions are listed in Table I. Unless otherwise specified, these conditions were used. The eluents were prepared by mixing the stated volume percentages.

Chemicals and reagents

All reagents were of analytical grade. Acetonitrile was HPLC grade. The diuretics were obtained from different pharmaceutical firms: KCAN (Searle), CAN (Searle), BUT (Boehringer Pharma), hydroflumethiazide (HFM) (Squibb), ethacrynic acid (ETA) (Merck, Sharp & Dohme) and polythiazide (PT) (Pfizer). KCAN and BUT were analysed in Canrenide® (Prospa) at a label claim of 50 and 5 mg per tablet, respectively.

Determination of potassium canrenoate, butizide and canrenone

Internal standard solutions. Two methanolic standard solutions containing 15 mg/ml HFM and 1.2 mg/ml PT were prepared.

Standard solutions. Three different methanolic standard solutions were prepared containing 2.5 mg/ml KCAN, 1 mg/ml BUT and 0.5 mg/ml CAN. A concentrated working standard solution was prepared by diluting 20 ml of the KCAN solution, 5 ml of the BUT solution, 3 ml of the CAN solution, 5 ml of the HFM

TABLE I

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC CONDITIONS

Column	LiChrosorb RP-8, 5 μm, 150 × 4.6 mm I.D.
Eluent	Acetonitrile–0.05 *M* phosphate buffer, pH 4 (45:55)
Flow-rate	1 ml/min
Temperature	25°C
Detector	UV detector, wavelength 286 nm for KCAN and for CAN, 271 nm for BUT
Recorder	Chart speed 0.5 cm/min
Sample loop	10 μl

internal standard solution and 5 ml of the PT internal standard solution to 50 ml with methanol. The working standard solution was prepared by diluting this concentrated solution ten-fold with an acetonitrile–water (1:1) mixture.

Sample preparation. An amount of ground tablet powder corresponding to 50 mg of KCAN and 5 mg of BUT was weighed into a 50-ml volumetric flask. A 5-ml volume of each internal standard solution was added and the mixture was diluted to 50 ml with methanol. This solution was stirred for 10 min and filtered. The filtrate was diluted ten-fold with an acetonitrile–water (1:1) mixture, and refiltered.

RESULTS AND DISCUSSION

Optimization of the chromatographic parameters

The purpose of this paper was to develop a specific, fast and reproducible determination of BUT, KCAN and CAN. The chromatographic behaviour of these diuretics, together with some other thiazide, potassium-sparing and loop diuretics was investigated on a LiChrosorb RP-18 stationary phase[6]. In an acetonitrile–water (40:60) mixture, BUT eluted within a reasonable analysis time ($k' = 2.6$). KCAN eluted close to the column dead-volume. The retention of KCAN can be altered by changing the pH of the eluent, while the retention of BUT remains constant. Therefore the pH of the phosphate buffer was acidified to pH 3. This resulted in an increased retention of KCAN. However, CAN, the degradation product of KCAN, eluted rather late from the column ($k' = 10.5$). 4-Amino-6-chlorobenzene-1,3-disulphonamide, the hydrolysis product of BUT, eluted close to the column dead-volume. As we also wanted a quick determination of the amount of CAN present in the tablets, we needed a system where CAN eluted faster from the column. On a LiChrosorb RP-8 column (Table II), CAN eluted somewhat faster compared to the LiChrosorb RP-18 column. Therefore, we decided to use a LiChrosorb RP-8 column.

A further increase of the acetonitrile content of the eluent (up to 45%) and the phosphate buffer at pH 4 resulted in a good compromise between selectivity and speed (Table II and Fig. 1). Indeed, BUT is separated from other thiazide diuretics

TABLE II

k' VALUES OF KCAN, BUT AND THEIR DEGRADATION COMPOUNDS

Eluent A: acetonitrile–0.05 *M* phosphate buffer, pH 3 (40:60); eluent B: acetonitrile–0.05 *M* phosphate buffer, pH 4 (45:55).

Compound	*Column*		
	LiChrosorb RP-18	*LiChrosorb RP-8*	
	Eluent A	*Eluent A*	*Eluent B*
4-Amino-6-chlorobenzene-1,3-disulphonamide	0.42	0.58	0.38
KCAN	2.1	2.4	1.3
BUT	2.7	3.0	1.9
CAN	10.5	9.3	5.0

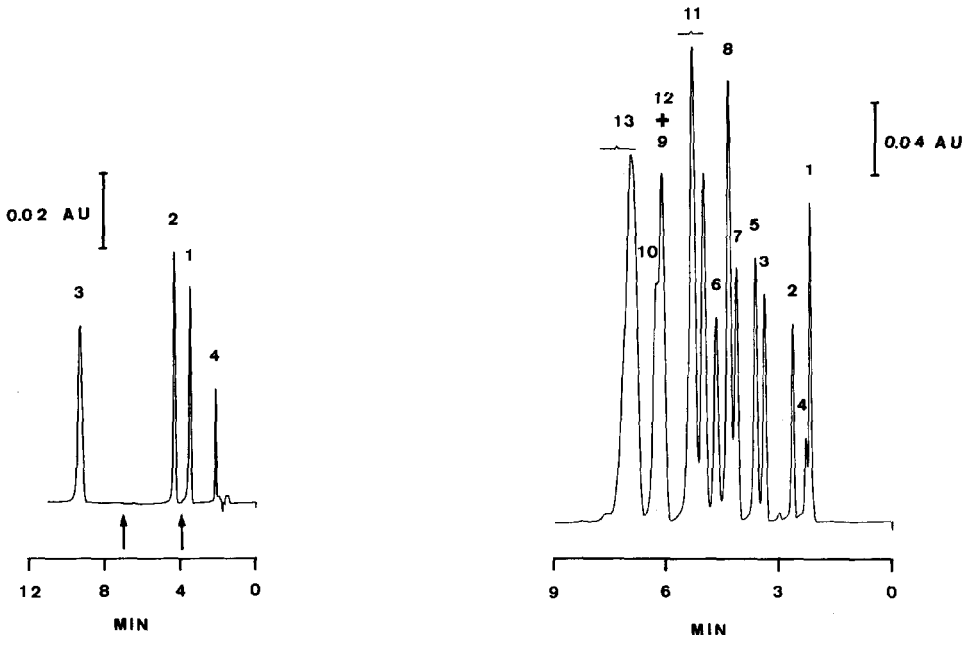

Fig. 1. Separation of KCAN (1) and BUT (2) from their respective degradation compounds: CAN (3) and 4-amino-6-chlorobenzene-1,3-disulphonamide (4) on a LiChrosorb RP-8 column. Mobile phase: acetonitrile–0.05 *M* phosphate buffer, pH 4 (45:55). The arrows indicate the time when the wavelength was changed (Table I).

Fig. 2. Selective identification of BUT. BUT (8) is separated from other thiazide diuretics: hydrochlorothiazide (1), hydroflumethiazide (2), trichlormethiazide (3), chlorthalidone (4), methylclothiazide (5), epithiazide (6), althiazide (7), polythiazide (9), bendroflumethiazide (10), cyclothiazide (11), cyclopenthiazide (12) and mebutizide (13). Chromatographic conditions: see Table I. Detection wavelength: 275 nm.

(Fig. 2). With higher amounts of acetonitrile (50%) in the eluent, a decrease in selectivity occured: co-elution of BUT and epithiazide. At pH 3, KCAN cannot be differentiated from furosemide, a loop diuretic, on the RP-8 column. The influence of eluent pH was investigated on a LiChrosorb RP-18 column[6]. On increasing the pH from 3 to 4, the k' value of furosemide decreased to a larger extent than the k' value of KCAN. This resulted in a greater selectivity between the two compounds. As we expected a similar behaviour on the LiChrosorb RP-8 column, we increased the pH to 4. Indeed, at this pH, KCAN can be separated from some other currently used potassium-sparing and loop diuretics (Fig. 3).

Quantitative determination

KCAN and BUT have different UV absorption maxima: 286 nm and 271 nm, respectively. Measurements carried out at the absorption maximum are more selective and more reproducible. We therefore decided to change the wavelength from 286 to 271 nm after KCAN eluted from the column. This change was performed automatically by the program of the HP 85 computer that controls the spectrophotometric diode array detector.

As the concentration of KCAN and BUT in the tablets differed by a factor of

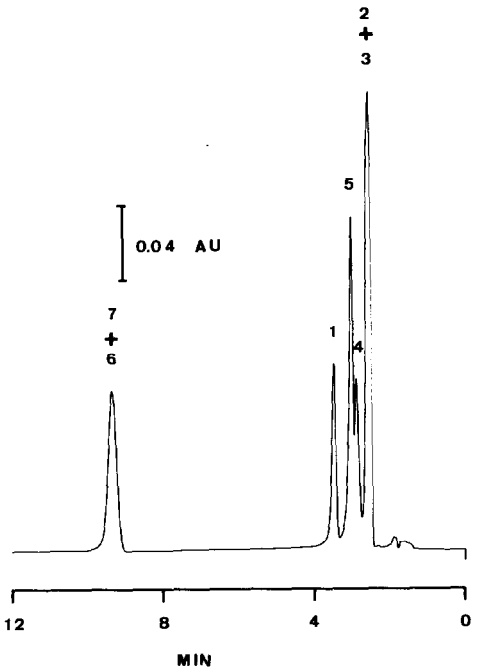

Fig. 3. Selective identification of KCAN. KCAN (1) is separated from: amiloride hydrochloride (2), furosemide (3), triamterene (4), ethacrynic acid (5), spironolactone (6) and CAN (7). Chromatographic conditions: see Table I. Detection wavelength: 275 nm.

10, we selected two internal standards: one for each compound. We selected ETA and PT, respectively, according to their structure relationship with KCAN and BUT and according to their elution behaviour[6]. Indeed, ETA contains a carboxylic acid function, as does KCAN. However, aqueous solutions containing both compounds were not stable: ETA, an acidic compound, catalysed the degradation of KCAN to CAN[7]. Thus, acidic compounds could not be used as internal standard. We therefore selected HFM as internal standard for KCAN. These solutions were stable.

The proposed method was linear for KCAN (r = 1.0000) and for BUT (r = 1.0000) over a concentration range of 50–150% of the amount of drugs normally present in the tablets.

TABLE III

DETECTION LIMIT (SIGNAL-TO-NOISE RATIO = 4)

Compound	*Detection limit (ng injected)*	*Wavelength (nm)*
KCAN	2	286
BUT	2	271
CAN	3	286
4-Amino-6-chlorobenzene-1,3-disulphonamide	6	286

TABLE IV

RECOVERY OF KCAN AND BUT FROM SYNTHETIC TABLET MIXTURES

Recovery (% of amount added)	
KCAN	*BUT*
99.8	99.1
99.9	100.3
100.0	100.0
100.0	99.7
99.2	100.1
99.8*	99.8*
0.34**	0.47**

* Average (%).
** Relative standard deviation (%).

TABLE V

RESULTS OF THE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF KCAN AND BUT IN TABLETS

Compound	*% Found of the label chain*	*R.S.D. (%)*	*n*
KCAN	101.3	1.1	8
BUT	96.9	1.2	8

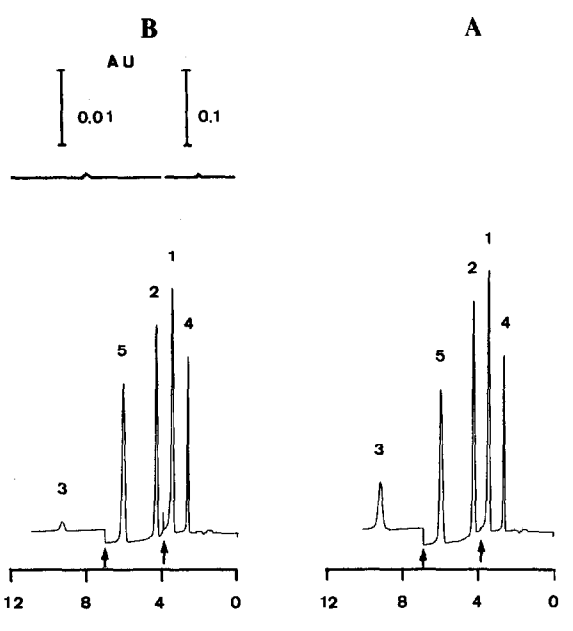

Fig. 4. Chromatogram of the simultaneous determination of KCAN (1), BUT (2) and CAN (3). HFM (4) and PT (5) are the internal standards. Chromatographic conditions: see Table I. A = Standard sample; B = canrenide sample.

The sensitivity of the proposed method is listed in Table III. Small amounts of CAN and 4-amino-6-chlorobenzene-1,3-disulphonamide can be determined.

The drugs were extracted from the pharmaceutical preparation with methanol. Table IV lists the results of the standard-addition recovery experiments on artificial mixtures at various drug levels. A recovery of 99.8% (relative standard deviation = 0.34%) for KCAN and 99.8% (relative standard deviation = 0.47%) for BUT was obtained.

The reproducibility of the method was investigated by performing several analyses on a commercial formulation. These results are shown in Table V. These data indicate that the proposed method is reliable and reproducible. Fig. 4 shows a chromatogram of the determination of KCAN, BUT and CAN: baseline separations were obtained.

CAN, the degradation product of KCAN, may be present in the tablet up to 3% of the amount of KCAN in the tablet. This corresponds to 1.5 mg of CAN per tablet. This maximum concentration is shown in the standard mixture in Fig. 4. The concentration of CAN in this tablet formulation was far below the maximum concentration: 0.25 mg of CAN per tablet.

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