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Note

Simultaneous determination of althiazide and spironolactone in tablets by high-performance liquid chromatography

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Althiazide, a thiazide diuretic, and spironolactone, a potassium-sparing diuretic, are often administered together for the treatment of hypertension. There is no method available for the simultaneous determination of these two compounds. Spironolactone has been determined by high-performance liquid chromatography (HPLC)[1] and thin-layer spectrofluorimetry[2]. Moskalyk *et al.*[3] developed an HPLC method for the determination of polythiazide; the conditions used can be adapted to other thiazide diuretics such as althiazide. However, the column they employed, diphenyldichlorosilane bonded to a pellicular packing, was not very efficient.

This paper describes the development of an HPLC method using a LiChrosorb C_{18} reversed-phase column for the simultaneous determination of the two compounds. We started from our previous chromatographic data[4], which enabled us to select an eluent consisting of acetonitrile–water (1:1) as a good compromise between selectivity and analysis time. The proposed method is selective, no interference being observed from 4-amino-6-chlorobenzene-1,3-disulphonamide, the hydrolysis product of althiazide, or from canrenone or canrenoic acid, degradation products of spironolactone[5,6]. Further, the method is specific: althiazide can be distinguished from other thiazide diuretics. Also, the analysis time is reduced to a minimum: a simple extraction with methanol and a chromatographic separation can be completed within 8 min.

EXPERIMENTAL

Apparatus

An SP 8770 isocratic pump (Spectra-Physics, Darmstadt, F.R.G.), equipped with an HP 1040A UV spectrophotometric detector (Hewlett-Packard, Palo Alto, CA, U.S.A.), an HP 85 computer, an HP 820901M flexible disc drive and an HP 3390A integrator, is used. The HP 1040A UV detector contains a photodiode array and can follow up to eight 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 271 to 238 nm for the detection of althiazide (ALT) and spironolactone (SPIR), respectively.

The eluents were filtered through a 5-μm filter and degassed with helium. A

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Valco six-port injection valve with a 10-μl sample loop was used. The temperature of the column was thermostated with a water-bath.

Chromatographic procedure

A LiChrosorb C_{18} reversed-phase column (Chrompack, Middelburg, The Netherlands) was used. The chromatographic conditions are listed in Table I. Unless specified otherwise, these conditions were used. The eluents were prepared by mixing the stated volume percentages of the components.

TABLE I

HPLC CONDITIONS

Column: LiChrosorb RP C_{18}, 5 μm (150 × 4.6 mm I.D.)
Eluent: acetonitrile-water (1:1)
Flow-rate: 1 ml/min
Temperature: 25°C
UV detector:
wavelength 271 nm for ALT and other thiazide diuretics;
wavelength 238 nm for SPIR and CAN
Recorder chart speed: 1 cm/min
Sample loop: 10 μl

Reagents and chemicals

All reagents were of analytical-reagent grade, except acetonitrile (HPLC grade). The diuretics were obtained from different pharmaceutical companies: althiazide (Searle), polythiazide (Pfizer), bendroflumethiazide (Squibb), cyclopenthiazide (Ciba-Geigy), spironolactone (Searle) and canrenone (Searle). Canrenoic acid was prepared from potassium canrenoate (Searle). An aqueous solution of potassium canrenoate was acidified and than filtered. The precipitated canrenoic acid was washed with 0.02 *M* hydrochloric acid and dried under reduced pressure. Althiazide and spironolactone were analysed in Aldactazine (Searle) at label claims of 15 and 25 mg, respectively.

Determination of althiazide and spironolactone

Internal standard solution. A methanolic solution of polythiazide (PT) (4 mg/ml) was used.

Standard solutions. About 50 mg of SPIR and 30 mg of ALT were weighed accurately in a 100-ml volumetric flask, 10.0 ml of internal standard solution were added and the mixture was diluted to 100 ml with methanol. A working standard solution was prepared by diluting 5.0 ml of this stock standard solution to 25 ml with acetonitrile–water (1:1).

Sample preparation. An amount of ground tablet powder corresponding to 15 mg of ALT and 25 mg of SPIR was weighed into a 50-ml volumetric flask, 5.0 ml of internal standard solution were added and the mixture was diluted to 50 ml with methanol. The mixture was stirred for 10 min and centrifugated at 3000 *g* for 5 min. A 5.0-ml volume of the supernatant was diluted to 25 ml with acetonitrile–water (1:1).

RESULTS AND DISCUSSION

Optimization of the mobile phase

To determine ALT and SPIR, we selected a mobile phase that is able to effect their separation with the necessary selectivity, efficiency and speed. The two diuretics should be separated from their degradation products (4-amino-6-chlorobenzene-1,3-disulphonamide, the hydrolysis product of ALT; canrenone and canrenoic acid, degradation products of SPIR) and from an unidentified impurity sometimes present in ALT. The separation of ALT from other thiazide diuretics enhances the selectivity of the system. We investigated the chromatographic behaviour of the thiazide, loop and potassium-sparing diuretics on a LiChrosorb C_{18} reversed-phase column[4]. Different organic modifiers (methanol, acetonitrile and tetrahydrofuran) were compared with respect to selectivity for the diuretics.

The retention data of ALT, SPIR, their degradation products and the impurity in ALT are summarized in Table II. Using an eluent consisting of methanol–water (45:55), ALT was eluted with a retention time of 5.2 min and with the necessary selectivity, ALT being separated from the other thiazide diuretics[4]. However, SPIR was eluted too slowly from the column. Increasing the methanol content of the eluent resulted in faster elution of SPIR, but the selectivity of ALT towards the other thiazide diuretics decreased[4]. We increased the eluotropic strength of this eluent by adding tetrahydrofuran: up to a concentration of 10% of tetrahydrofuran in a methanol–water (45:55) eluent, ALT was still separated from the other thiazide diuretics. The retention time of SPIR decreased from 50 to 8.8 min. However, the impurity sometimes present in ALT was no longer separated from ALT. We tried a third organic modifier, acetonitrile. Using an eluent consisting of acetonitrile–water (1:1), an excellent separation of all compounds was obtained within a reasonable time (Fig. 1) and with the necessary selectivity, ALT being separated from other thiazide diuretics. This eluent was therefore selected for the quantitative determination.

TABLE II

RETENTION TIMES OF THE INVESTIGATED DIURETICS, THEIR DEGRADATION PRODUCTS AND POSSIBLE IMPURITIES WITH DIFFERENT ELUENTS ON A C_{18} REVERSED-PHASE COLUMN

Compound	*Retention time (min)*		
	CH_3OH–H_2O (45:55)	*CH_3OH–H_2O (45:55) containing 10% THF*	*CH_3CN–H_2O (1:1)*
4-Amino-6-chlorobenzene-1,3-disulphonamide	1.70	1.91	1.95
ALT	5.17	4.38	3.10
Impurity in ALT	8.13	4.48	3.44
SPIR	50	8.82	7.24
CAN	55	9.95	8.01
Canrenoic acid	–	–	2.45

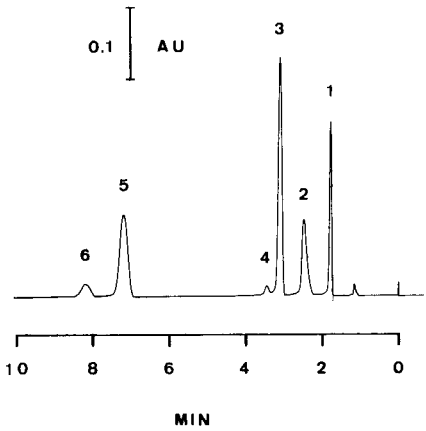

Fig. 1. Separation of ALT (3) and SPIR (5) from 4-amino-6-chlorobenzene-1,3-disulphonamide (1), canrenoic acid (2), an impurity in ALT (4) and canrenone (6). For chromatographic conditions, see Table I.

Quantitative determination

ALT and SPIR have different UV absorption maxima, 271 and 238 nm, respectively. As the measurements are most reproducible at the absorption maximum, the wavelength of the detector was automatically changed after 6.2 min from 271 to 238 nm by the program of the computer. Relative standard deviations of 0.37 and 0.81% were obtained for ALT and SPIR, respectively, for fourteen consecutive injections of the same sample solution.

We selected a thiazide diuretic, similar in structure to ALT, as an internal standard. Polythiazide (PT), bendroflumethiazide (BHF) and cyclopenthiazide (CPT) were all eluted between ALT and SPIR and could be used as internal standards. The thiazide diuretics are subject to hydrolysis in aqueous solutions, and PT was more stable than BHF and CPT. We therefore selected PT as the internal standard. ALT is also subject to hydrolysis, but no degradation was observed during the analysis involved. SPIR is stable in aqueous solutions, 98% being retained after storage for 6 days at 65°C[1].

The linearity of the detector response was investigated for ALT in the range 1.5–38 mg per tablet (r = 1.0000) and for SPIR in the range 2.5–63 mg per tablet (r = 1.0000).

The sensitivity of the proposed method is given in Table III. Small amounts

TABLE III

DETECTION LIMITS

Compound	*Detection limit (ng injected)*	*Wavelength (nm)*
4-Amino-6-chlorobenzene-1,3-disulphonamide	0.6	271
ALT	0.8	271
SPIR	2	238
CAN	1	288
	15	238

TABLE IV

RECOVERIES OF ALTHIAZIDE AND SPIRONOLACTONE

Recovery (%)	
Althiazide	*Spironolactone*
99.08	98.53
100.35	99.64
98.18	97.46
98.88	99.98
98.88	101.21
98.47	99.24
Mean: 98.97	99.34
R.S.D.: 0.76	1.29

TABLE V

RESULTS OF THE DETERMINATION OF ALTHIAZIDE AND SPIRONOLACTONE IN TABLETS

Batch No.	*Althiazide*		*Spironolactone*	
	% of label claim found	*R.S.D. (%)*	*% of label claim found*	*R.S.D. (%)*
1	99.53	0.46 (n = 6)	98.70	0.47 (n = 6)
2	101.54	(n = 2)	98.41	(n = 2)

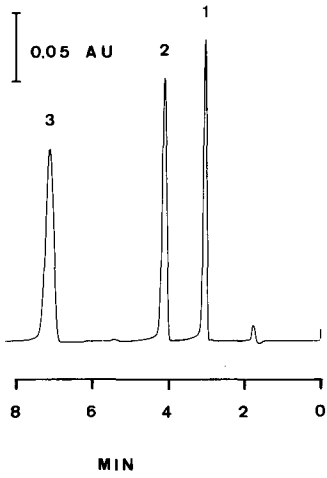

Fig. 2. Determination of ALT (1) and SPIR (3) in tablets. PT (2) is the internal standard. For chromatographic conditions, see Table I.

of canrenone and 4-amino-6-chlorobenzene-1,3-disulphonamide can be detected.

The drugs are extracted from the pharmaceutical preparation with methanol. Table IV lists the results of standard additions recovery experiments on artificial mixtures at various drug levels. The recoveries ranged from 98.2 to 100.4% for ALT and from 97.5 to 101.2% for SPIR.

Table V gives the results of the determination of ALT and SPIR in two different batches of tablets. The reproducibility of the method was investigated by performing different analyses on one batch of tablets; relative standard deviations of 0.46 and 0.47% were obtained for ALT and SPIR, respectively. Fig. 2 shows a chromatogram for the determination of both compounds in tablets; baseline separations were obtained within a reasonable analysis time.

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REFERENCES

1 V. Das Gupta and G. Ghanekar, *J. Pharm. Sci.*, 67 (1978) 889.
2 P. J. Van der Merwe, D. G. Müller and E. Clark, *J. Chromatogr.*, 171 (1979) 519.
3 R. E. Moskalyk, R. A. Locock, L. G. Chatten, A. M. Veltman and M. F. Bielech, *J. Pharm. Sci.*, 64 (1975) 1407.
4 F. De Croo, W. Van den Bossche and P. De Moerloose, *J. Chromatogr.*, 325 (1985) 395.
5 W. Stüber, E. Mutschler and D. Steinbach, *Arch. Pharm. (Weinheim)*, 314 (1981) 148.
6 N. Gochman and C. L. Gantt, *J. Pharmacol. Exp. Ther.*, 135 (1962) 312.