Journal of Chromatography, 349 (1985) 301–304
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 689

Note

Influence of the chain length of chemically bonded phases on the behaviour of several thiazide, potassium-sparing and loop diuretics in high-performance liquid chromatography

F. DE CROO*, W. VAN DEN BOSSCHE and P. DE MOERLOOSE
Department of Pharmaceutical Chemistry and Drug Quality Control, State University of Ghent, Harelbekestraat 72, B-9000-Ghent (Belgium)

Thiazide, potassium-sparing and loop diuretics have been analysed by different techniques: paper and thin-layer chromatography[1,2] and high-performance liquid chromatography (HPLC)[3–6]. Most of these publications considered only some diuretics. We have investigated both thiazide, potassium-sparing and loop diuretics on a reversed-phase (RP) LiChrosorb C_{18} column[7]. In order to investigate the selectivity and speed of analysis, other stationary phases have now been used: LiChrosorb RP C_8 and C_2.

EXPERIMENTAL

Apparatus

A SP 8770 isocratic pump (Spectra-Physics, Darmstadt, F.R.G.) was equipped with an HP 1040A UV spectrophotometric detector (Hewlett-Packard, Palo Alto, CA, U.S.A.), an HP 85 computer, an HP 82901M flexible disc drive and an HP 3390A integrator. The detector contained a photodiode array.

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 column was thermostatted with a water-bath.

Chromatographic procedure

LiChrosorb RP C_{18}, C_8 and C_2 columns (Chrompack, Middelburg, The Netherlands) of the same dimensions were used. The chromatographic conditions listed in Table I were used, unless specified otherwise. The organic solvent–water ratios of the eluents are given as volume ratios. The acetonitrile–buffer eluents were prepared by mixing the stated volume percentages. The molarity and pH of the buffer refer to the water phase.

Chemicals and reagents

All reagents were of analytical grade quality; acetonitrile was of HPLC grade quality. The diuretics are listed in Table II. The chemical structures of these diuretics are given in ref. 7.

0021-9673/85/$03.30

TABLE I

HPLC CONDITIONS

Column	LiChrosorb RP C_{18}, C_8 or C_2, 5 μm, 150 × 4.6 mm I.D.
Eluent	See text or figures
Flow-rate	1 ml/min
Temperature	25°C
Detector	UV, 275 nm for the diuretics, 238 nm for spironolactone and canrenone
Recorder	Chart speed: 0.5 or 1 cm/min
Sample loop	10 μl

RESULTS AND DISCUSSION

Thiazide, potassium-sparing and loop diuretics have been analysed on a LiChrosorb RP C_{18} stationary phase[7]. An acetonitrile–water (40:60) eluent was used to separate the thiazide diuretics. The loop and the potassium-sparing diuretics were separated with acetonitrile–phosphate buffer (pH 3) (40:60) as eluent.

Possible changes in the selectivity and elution characteristics were tested on other alkyl stationary phases. The retention characteristics of the diuretics were tested on commercially available LiChrosorb columns: RP C_{18}, C_8 and C_2. Some authors have already dealt with this problem[8–10]. Berendsen and De Galan[8] found that both the capacity factor and the selectivity increase up to a certain alkyl chain length, the

TABLE II

DIURETICS STUDIED

*No.**	*Diuretic*	*Origin*
1	Hydrochlorothiazide	Merck, Sharp & Dohme
2	Hydroflumethiazide	Squibb & Sohns
3	Trichloromethiazide	Essex
4	Chlorthalidone	Ciba Geigy
5	Methylclothiazide	Abbott
6	Epithiazide	R.I.T.
7	Althiazide	Searle
8	Butizide	Boehringer farma
9	Polythiazide	Pfizer
10	Bendroflumethiazide	Squibb & Sohns
11	Cyclothiazide	Eli Lilly
12	Cyclopenthiazide	Ciba Geigy
13	Mebutizide	CCP Thyssen
14	Furosemide	Hoechst
15	Ethacrynic acid	Merck, Sharp & Dohme
16	Potassium Canrenoate	Searle
17	Triamterene	R.I.T.
18	Amiloride hydrochloride	Merck, Sharp & Dohme
19	Spironolactone	Searle
20	Canrenone	Searle

* The numbers of the diuretics are indicated in the text in parentheses.

critical chain length, after which they remain constant. Engelhardt and Ahr[9] mentioned that the increase in selectivity was observed when the stationary phase was wetted by the eluent. However, we observed a decrease in k' for nearly all thiazide diuretics with increasing chain length (Fig. 1). The k' values of the acidic and neutral potassium-sparing and loop diuretics showed a slight increase, except for furosemide (14) (Fig. 2). We observed a marked increase in retention only for the basic drugs amiloride hydrochloride (18) and triamterene (17).

This abnormal behaviour of the thiazide diuretics prompted us to check the properties of the commercially available LiChrosorb RP materials. LiChrosorb RP C_{18} and C_8 are made of 100-Å silica gel, whereas LiChrosorb RP C_2 is made of 60-Å silica gel. The specific surface areas of these packings amounted to 125, 250 and 350 m^2/g respectively[11]. The higher specific surface area of the RP C_2 phase might explain this abnormal behaviour. Goldberg[10] also investigated different commercially available RP stationary phases, among them LiChrosorb RP C_{18}, C_8 and C_2. The k' values of compounds of a wide polarity range, containing neutral, basic and acidic compounds, were compared: the values increased with increasing chain length; however, the increase observed was much smaller than the increase found by Berendsen and De Galan. Those workers used laboratory-prepared bonded phases on 10-μm, 100-Å silica particles, whereas Goldberg used commercially available phases. However, none of the compounds tested by Goldberg showed a "decrease" in k'. Probably the greater accessibility of the silanol groups on the RP C_2 phase plays an important rôle in this abnormal retention behaviour of the thiazide diuretics. The importance of the "accessibility" of the silanol groups has been discussed previously[12,13]. Bidlingmeyer *et al.*[12] found that the "accessibility" of the silanol groups plays an im-

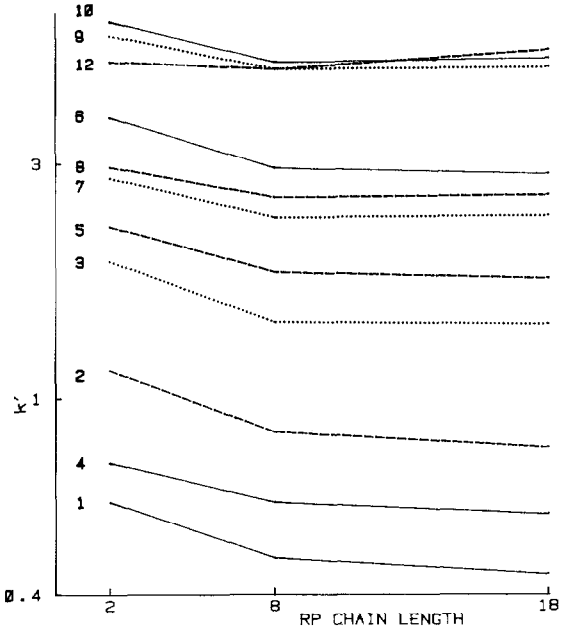

Fig. 1. Capacity factor of some thiazide diuretics as a function of RP chain length. Eluent: acetonitrile–water (40:60). Chromatographic conditions as in Table I. Key to diuretics as in Table II.

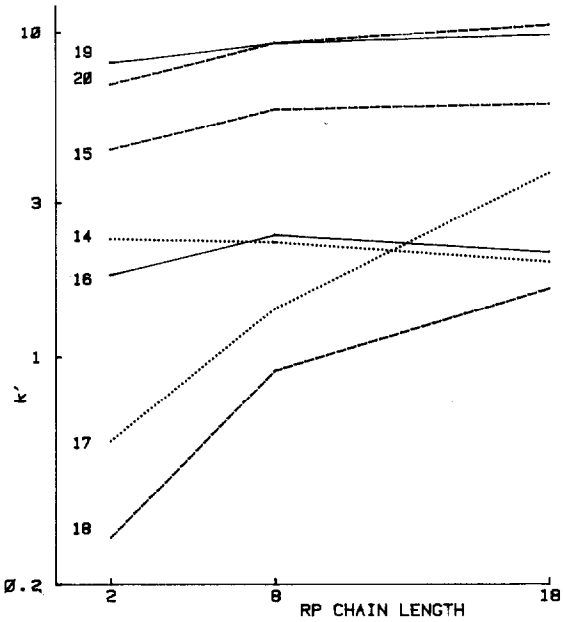

Fig. 2. Capacity factor of some potassium-sparing and loop diuretics as a function of RP chain length. Eluent: acetonitrile–0.05 *M* phosphate buffer (pH 3) (40:60). Other details as in Fig. 1.

portant rôle in the peak symmetry, and not the presence or absence of silanol groups. Kohler and Kirkland[13] found that not only the concentration of residual silanol groups, but also their type and availability largely determine the behaviour of bonded-phase columns.

When the stationary phase is changed, only small differences in selectivity are observed for the thiazide diuretics that have an analogous structure (Fig. 1) but a reverse elution order is observed for cyclopenthiazide (12). On the other hand, with the potassium-sparing and loop diuretics that do have different chemical structures, great differences in selectivity are observed when the stationary phase is changed (Fig. 2).

REFERENCES

1 J. B. Pilsbury and J. V. Jackson, *J. Pharm. Pharmacol.*, 18 (1966) 713.
2 D. Sohn, J. Simon, M. A. Hanna, G. Ghali and R. Tolba, *J. Chromatogr.*, 87 (1973) 570.
3 V. P. Shah, J. Lee and V. K. Prasad, *Anal. Lett.*, 15 (1982) 529.
4 P. A. Tisdall, T. P. Moyer and J. P. Anhalt, *Clin. Chem.*, 26 (1980) 702.
5 I. L. Honigberg, J. T. Stewart, A. P. Smith and D. W. Hester, *J. Pharm. Sci.*, 64 (1975) 1201.
6 R. E. Moskalyk, R. A. Locock, L. C. Chatten, A. M. Veltman and M. F. Bielech, *J. Pharm. Sci.*, 64 (1975) 1407.
7 F. De Croo, W. Van den Bossche and P. De Moerloose, *J. Chromatogr.*, 325 (1985) 395.
8 G. E. Berendsen and L. de Galan, *J. Chromatogr.*, 196 (1980) 21.
9 H., Engelhardt and G. Ahr, *Chromatographia*, 14 (1981) 227.
10 A. P. Goldberg, *Anal. Chem.*, 54 (1982) 342.
11 *Preparations for HPLC*, Merck, Darmstadt, 1979, p. 11.
12 B. A. Bidlingmeyer, J. K. Del Rios, J. Korpi and J. N. Little, *Anal. Chem.*, 54 (1982) 442.
13 J. Kohler and J. J. Kirkland, *9th International Symposium on Column Liquid Chromatography, 1985, Edinburgh*, CEP Consultants, Edinburgh, 1985, p. 254.