Journal of Chromatography, 117 (1976) 81–86

CHROM. 8725

PARTITION HIGH-PRESSURE LIQUID-CHROMATOGRAPHIC SYSTEMS FOR THE SEPARATION OF DIGITALIS GLYCOSIDES OF THE CARDENOLIDE GROUP

W. LINDNER

Institute of Pharmaceutical Chemistry, University of Graz, Graz (Austria)

and

R. W. FREI*

Analytical Research and Development, Pharmaceutical Department, Sandoz Ltd., 4002 Basel (Switzerland)

(Received September 4th, 1975)

SUMMARY

Several multi-component liquid–liquid systems have been investigated on silica gel SI-60 supports of particle size 10 μm. By using two solvent systems, it was possible to separate 14 digitalis glycosides, ranging from genins of relatively low polarity to the highly polar deacetyl-lanatosides. Solvents with good ultraviolet transparency at 220 nm ($\lambda_{max.}$ for the butenolide ring) were chosen in order to improve the sensitivity of detection. The technique should also permit the determination of by-products and degradation products of these drug substances. Detection limits are as low as 15 ng for a 5-μl injection, and separation times vary between 4 and 20 min. The reproducibility of the retention times and the baseline separations attainable make the systems suitable for quantitative work.

INTRODUCTION

This particular group of cardiac glycosides is of pharmaceutical importance. Analytical methods currently in routine use are based on thin-layer chromatographic procedures[1], and studies carried out with gas–liquid chromatography[2,3] after derivatization with different types of silylation reagents have met with only limited success. High-pressure liquid chromatography (HPLC) has for some years shown its value in pharmaceutical analysis for compounds of low vapour pressure and/or poor thermal stability; also, its reproducibility makes it a valuable tool for routine quantitative analysis.

Little has yet been published on the HPLC separation of cardenolides[4]. In work carried out by our group[5] over the last two years, use has been made of chloro-

* To whom correspondence should be addressed.

form–methanol mixtures and silica gel supports for this purpose. This procedure is satisfactory for the analysis of these drug substances in many dosage forms, but it is not very sensitive, as these mixtures absorb strongly at wavelengths shorter than 240 nm, and the absorption maximum of the compounds (butenolide ring) is around 220 nm.

In the work described here, we attempted to introduce multi-component systems with good ultraviolet (UV) transparency around 220 nm; with the resulting gain in sensitivity, it was hoped to use HPLC also for determining by-products and degradation components in pharmaceutical dosage forms.

EXPERIMENTAL

Reagents

The cardiac glycosides (Table I) were obtained from Sandoz (Basel, Switzerland). The chromatographic solvents (Table II) were of analytical grade from Fluka (Buchs, Switzerland) and Merck (Darmstadt, G.F.R.). The HPLC adsorbent LiChrosorb SI-60 of particle size 10 μm (Merck) was used.

TABLE I

MAIN DIGITALIS GLYCOSIDES OF THE CARDENOLIDE GROUP

Glycoside	*Aglycone*	*Sugar**
Lanatoside A	Digitoxigenin	dx-dx-dx(ac)-gl
Purpureaglycoside A (deacetyl-lanatoside A)	Digitoxigenin	dx-dx-dx-gl
Acetyldigitoxin	Digitoxigenin	dx-dx-dx(ac)
Digitoxin	Digitoxigenin	dx-dx-dx
Lanatoside B	Gitoxigenin	dx-dx-dx(ac)-gl
Purpureaglycoside B (deacetyl-lanatoside B)	Gitoxigenin	dx-dx-dx-gl
Gitoxin	Gitoxigenin	dx-dx-dx
Lanatoside C	Digoxigenin	dx-dx-dx(ac)-gl
Deacetyl-lanatoside C	Digoxigenin	dx-dx-dx-gl
Acetyldigoxin	Digoxigenin	dx-dx-dx(ac)
Digoxin	Digoxigenin	dx-dx-dx
Lanatoside D	Diginatigenin	dx-dx-dx(ac)-gl
Diginatin	Diginatigenin	dx-dx-dx
Lanatoside E	Gitaloxigenin	dx-dx-dx(ac)-gl
Glucogitaloxin	Gitaloxigenin	dx-dx-dx-gl
Gitaloxin	Gitaloxigenin	dx-dx-dx

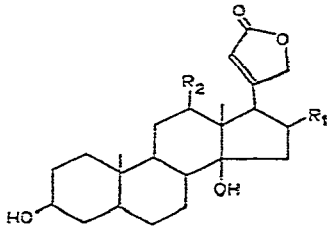
* The following abbreviations are used: dx = digitoxose; gl = glucose; ac = acetyl group.

Apparatus

All chromatographic separations were carried out on a Hewlett-Packard liquid chromatograph (Model 1010 A), with a UV detector (monochromator H-P Model DSF 1000). Separations were effected on stainless-steel columns of 25 cm × 3.0 mm I.D., packed by the equal-density procedure described earlier[6]. For injection, 5-μl syringes (SGE) were used in conjunction with the Hewlett-Packard injector or a septum-injection device.

RESULTS AND DISCUSSION

From Fig. 1 it can be seen that, for example, digoxigenin and gitoxigenin differ only by the position of one hydroxyl group; the same is true for gitoxin and digoxin and for lanatosides B and C (see also Table I). The major separation problem is therefore encountered with these pairs.



$R_1 = R_2 = H$	Digitoxigenin
$R_1 = OH \quad R_2 = H$	Gitoxigenin
$R_1 = H \quad R_2 = OH$	Digoxigenin
$R_1 = R_2 = OH$	Diginatigenin
$R_1 = O\text{-}CHO \quad R_2 = H$	Gitaloxigenin

Fig. 1. General structure of the aglycone segment of the digitalis glycosides.

Separations were attempted with the solvent systems in Table II; the composition of these mixtures comes close to the mixing gap (phase diagram).

According to Hesse[7], one can assume that a demixing effect takes place on the surface of the adsorbent, the polar component being adsorbed on the active sites and the non-polar component acting as a mobile phase. The stability of the partition chromatographic environment thus created can be quite good. It is for this reason that we classify the systems discussed in this paper as liquid–liquid (predominantly partition) systems.

A detection wavelength of 225 $\pm$ 10 nm has been used in this work. Retention data are summarized in Table II and Fig. 2. Separation was achieved by varying the type and concentration of the alcohol component (see Table II). For a quantitative evaluation of peak areas, a resolution (R) of approximately 1.5 (baseline separation of gaussian peaks) is desirable; with peak height measurements, an R value of 1 is sufficient.

Separations of this quality can be achieved primarily with *tert.*-butanol and *n*-pentanol; the other alcohols tested are too polar. An increase in the concentration of acetonitrile by 50% will result in a decrease in the retention time (t_R) by 50%, but also in a loss of resolution by 25%. The water content also exerts a strong influence on t_R. Since water is the most polar component in our systems, it will be adsorbed and act as stationary phase. An increase in the concentration of the polar stationary phase would mean larger t_R values; this indeed was observed (see Fig. 2).

Actual separations achieved by using the optimum solvent systems for glycoside groups of different polarities are shown in Figs. 3–5. The separations in all three examples are sufficiently good and reproducible to permit quantitative work.

CONCLUSIONS

The systems proposed in this paper permit the separation of all the cardiac glycosides investigated (Table I). By choosing solvents with good UV transparency,

TABLE II

SOLVENT SYSTEMS INVESTIGATED AND INFLUENCE ON RETENTION TIME AND RESOLUTION

Solvent system	*Mixing proportions*	*Retention time (cm/min)*			*Resolution (R)*	
		Digoxigenin	*Digoxin*	*Lanatoside C*	*Gitoxin–digoxin*	*Lanatoside B–lanatoside C*
(A) Methanol–isopropanol–acetonitrile–hexane–water	400:350:200:1000:10	–	–	5,9*	–	0.21
(B) Isopropanol–acetonitrile–hexane–water	230:115:445:8.6	–	–	8.2**	–	0.53
(C) *n*-Butanol–acetonitrile–heptane–water	230:100:700:10	–	3.5*	20.2**	0.56	0.62
(D) *tert.*-Butanol–acetonitrile–heptane–water	(a) 220:70:800:10	–	10.0***	–	1.86	–
	(b) 204:93:712:10.4	–	3.6***	9.6***	1.36	1.51
(E) *n*-Pentanol–acetonitrile–iso-octane–water	(a) 270:93:660:9.3	–	3.8*	14.1*	0.57	0.83
	(b) 230:100:700:10	–	5.2**	–	1.17	–
	(c) 175:60:620:10	3.7*	10.4*	–	1.49	–
				22.7§	–	1.48
	(d) 175:60:620:6	–	8.2*	41.0*	1.29	–
				19.2§	–	1.23

* Flow-rate, 1.3 ml/min.
** Flow-rate, 1.4 ml/min.
*** Flow-rate, 2.2 ml/min.
§ Flow-rate, 2.5 ml/min.

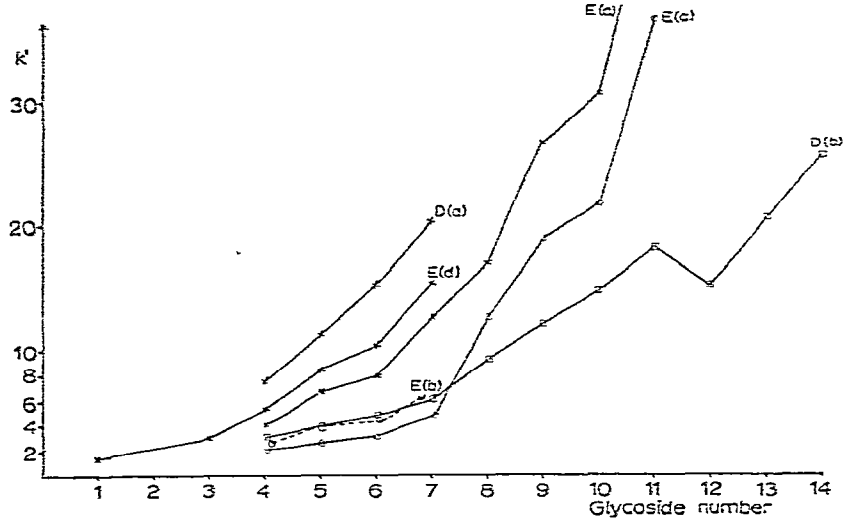


Fig. 2. Capacity coefficients (k') of 14 digitalis glycosides as a function of the solvent system used (for solvent-system abbreviations see Table II). Peak numbers for the compounds investigated: 1 = digitoxigenin; 2 = gitoxigenin; 3 = digoxigenin; 4 = digitoxin; 5 = gitoxin; 6 = digoxin; 7 = diginatin; 8 = lanatoside A; 9 = lanatoside B; 10 = lanatoside C; 11 = lanatoside D; 12 = deacetyl-lanatoside A; 13 = deacetyl-lanatoside B; 14 = deacetyl-lanatoside C.

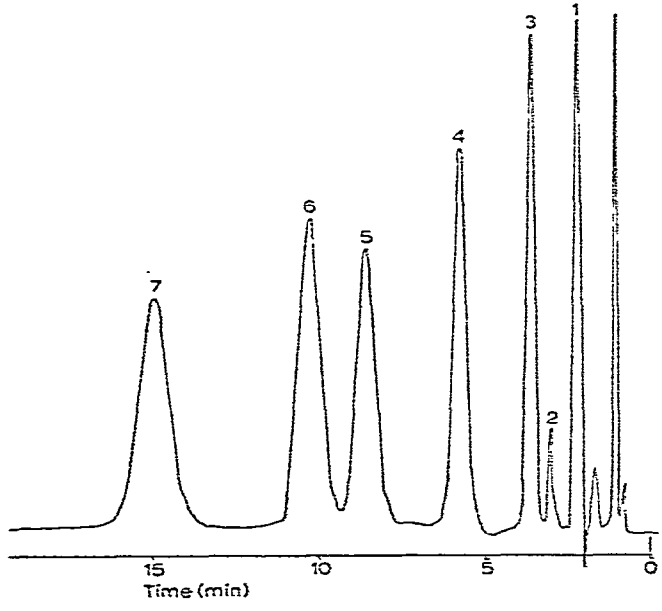
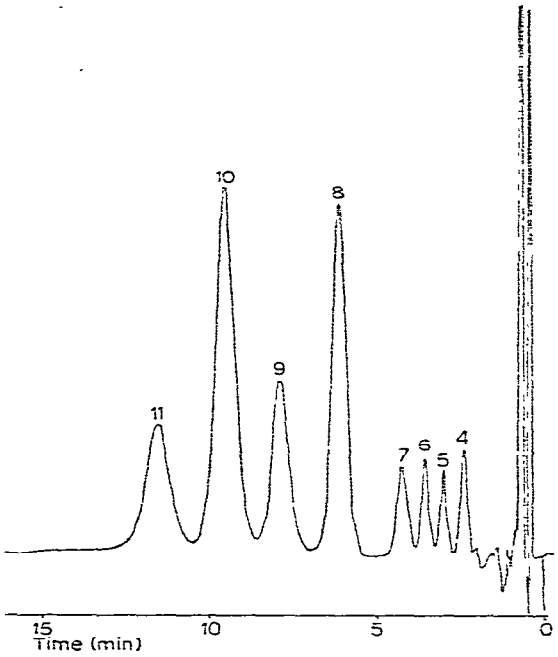


Fig. 3. Separation of digitalis compounds of lower polarity on a column (25 cm × 3 mm I.D.) of LiChrosorb SI-60 (particle size 10 μm). Mobile phase: *n*-pentanol–acetonitrile–isooctane–water (175:60:620:10); flow-rate, 1.3 ml/min; chart speed, 1 cm/min; detection, 220 nm; sample volume, 5 μl. For peak identification see Fig. 2.



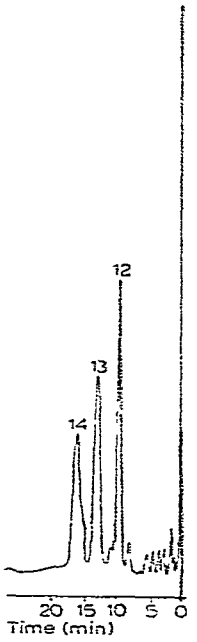


Fig. 4. Separation of digitalis glycosides of medium polarity. Mobile phase: *tert.*-butanol–acetonitrile–heptane–water (204:93:712:10.4); flow-rate, 2.2 ml/min; other conditions as in Fig. 3. For peak identification see Fig. 2.

Fig. 5. Separation of digitalis glycosides of high polarity. Conditions as in Fig. 4 except for chart speed (0.2 cm/min) and flow-rate (2.8 ml/min). For peak identification see Fig. 2.

it is possible to work close to the absorption maximum (220 nm), and the corresponding gain in sensitivity permits the determination of by-products and degradation products of the drug substances. Detection limits at a signal-to-noise ratio of 3:1 are 15 ng for digitoxigenin and 25 ng for digitoxin for a 5-μl injection. The separation times vary between 4 and 20 min, and the proposed systems are suitable for quantitative evaluation of the components.

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