Journal of Chromatography, 133 (1977) 412–414

CHROM. 9756

Note

Chromatographic separation of androgens, estrogens and progestogens on hydroxyalkoxypropyl-Sephadex (Lipidex®)

R. J. FAIRCLOUGH, M. A. RABJOHNS and A. J. PETERSON

New Zealand Ministry of Agriculture and Fisheries, Research Division, Ruakura Agricultural Research Centre, Hamilton (New Zealand)

(Received September 28th, 1976)

Purification of steroids by column chromatography on Sephadex LH-20 is a useful adjunct to the analysis of steroids by competitive protein binding[1,2] or by radioimmunoassay[3]. Advantages of its use include low and consistent assay blanks, good reproducibility of elution volumes and good recoveries[4]. Recent studies[4] indicate that non-polar steroids can be separated more effectively on hydroxyalkoxypropyl-Sephadex (Lipidex®). Apter *et al.*[5] have reported a method for separating non-polar steroids on relatively long columns of Lipidex (32 cm) before their analysis by radioimmunoassay. These columns, however, required external pressure for adequate elution rates and no attempt was made to separate both non-polar and polar steroids. The present paper reports a simple chromatographic system for the separation of a number of steroids of widely differing polarities using microcolumns of Lipidex.

EXPERIMENTAL

[1α,2α-^{3}H]Progesterone (58 Ci/mmole), [4-^{14}C]testosterone (57 Ci/mmole), [1α,2α-^{3}H]5α-androstan-17β-ol-3-one (5α-dihydrotestosterone, 47 Ci/mmole), [4-^{14}C]androst-5-en-3β-ol-17-one (dehydroepiandrosterone, 52 Ci/mmole), [7-^{3}H] Androst-4-en-3,17-dione (11 Ci/mmole), 17α-hydroxy-[7α-^{3}H]progesterone (10 Ci/mmole), [6,7-^{3}H]estrone (42 Ci/mmole), [1,2-^{3}H]cortisol (45 Ci/mmole) and [6,7-^{3}H] estradiol (45 Ci/mmole) were purchased from the Radiochemical Centre (Amersham, Great Britain) and were checked for purity on Lipidex before use. Non-radioactive steroids 20α-hydroxy-pregn-4-en-3,17-dione (20α-hydroxyprogesterone) and pregn-5-en-3, 17-diol-20-one (17α-hydroxypregnenolone) were obtained from Steraloids (Pawling, N.J., U.S.A.). Hydroxyalkoxypropyl-Sephadex was purchased from Packard (Zürich, Switzerland). Analar-grade solvents chloroform (BDH, Poole, Great Britain) and *n*-hexane (May & Baker, Dagenham, Great Britain) were refractionated from a Vigreux column before use.

Lipidex was allowed to swell overnight in *n*-hexane–chloroform (9:1) and then poured into columns (8 cm × 0.4 cm I.D.) fitted with a glass reservoir (25-ml capacity) and a plug of glass wool. Columns were packed afresh each day. Radioactive steroids were applied to the columns in two 100-μl portions of hexane–chloroform (90:10). The columns were eluted first with 14 ml of this solvent then successively with 6 ml of hexane–chloroform (40:60), 5 ml of the 50:50 mixture and 10 ml of the 40:60 mixture.

One-millilitre fractions of the column eluate were collected into vials, evaporated to dryness, and then counted in a toluene-based scintillation fluid. Two or three labelled steroids were chromatographed simultaneously on the columns if they showed good separation or if steroids with different labels were used. The fractions were counted in a Nuclear Chicago scintillation counter. The radio-inert steroids (500 ng) were chromatographed together on one column and the elution pattern was determined by gas–liquid partition chromatography using a flame-ionization detector.

The chromatograph was fitted with a 2 m × 5 mm I.D. silanized glass column containing 3% silicone DC QF1 (Applied Science Labs., State College, Pa., U.S.A.) on Gas-Chrom Q at 260°. Quantification was achieved by the addition of a progesterone standard to each fraction before analysis.

RESULTS AND DISCUSSION

Resolution of most of the steroids studied was satisfactory although for certain steroids separation was incomplete (Fig. 1). Some of the steroids that eluted together from these columns (*e.g.* dihydrotestosterone and dehydroepiandrosterone) can be separated on longer columns of Lipidex[6], but this may not be necessary for the radioimmunoassay of these compounds since specific antisera are now available. The technique allows the rapid separation of a wide range of steroids in small volumes of eluent. All steroids up to and including cortisol are eluted in 2.5 h. Recoveries of labelled steroids are greater than 85%, which compares favourably with values reported by others[6]. It is concluded that this chromatographic technique, when used in conjunction with highly specific steroid antisera, offers a simple method for the purification and subsequent radioimmunoassay of a number of steroids from one biological sample. The Lipidex columns may also be used to obtain a preliminary separation of radioactive products in *in vivo* and *in vitro* incubation studies.

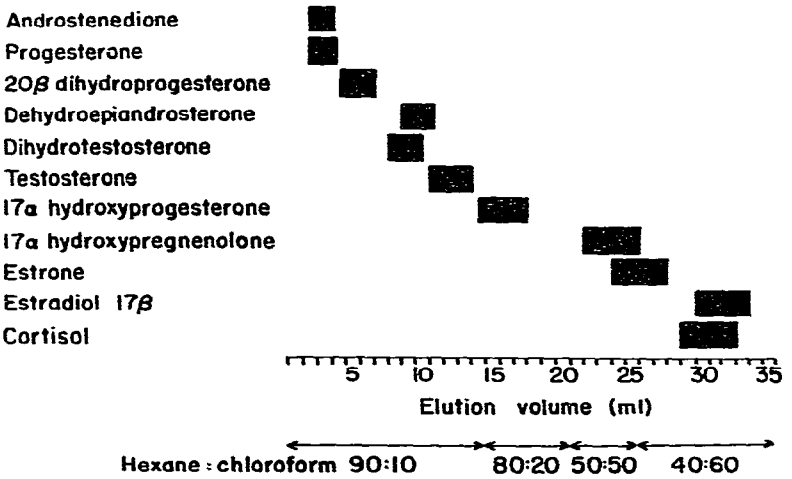

Fig. 1. Elution pattern of steroids on hydroxyalkoxypropyl-Sephadex (Lipidex).

ACKNOWLEDGEMENT

We thank Dr. E. Payne for advice on the gas–liquid partition chromatography.

REFERENCES

1 B. E. P. Murphy, *Acta Endocrinol. (Copenhagen), Suppl.*, 147 (1970) 37.
2 T. Holmdahl and J. Sjövall, *Steroids*, 18 (1971) 69.
3 C.-H. Wu and L. E. Lundy, *Steroids*, 18 (1971) 91.
4 E. Nyström and J. Sjövall, *Methods Enzymol.*, 35 (1975) 378.
5 D. Apter, O. Jänne, P. Karvonem and R. Vihko, *Clin. Chem.*, 22 (1976) 32.
6 O. Jänne, D. Apter and R. Vihko, *J. Steroid Biochem.*, 5 (1974) 155.