Notes

The separation of oestrogens by horizontal thin-layer chromatography

The technique of separating several oestrogens by thin-layer ascending chromatography has been described by LISBOA AND DICZFALUSY[1] and LUISI *et al.*[2], and has since found extensive application.

The authors found that horizontal chromatography on silica gel layers improved the separation of oestrogens, both in mixtures of pure substances and in extracts of biological fluids.

The advantages of this technique are speedy development with high resolving power, and better preparative purification with continuous development. REISERT AND SCHUMACHER[3] have used a similar technique for the fractionation of urinary 17-ketosteroids.

Table I lists both R_F values and the R_{Oe} values relative to oestrone, of the several oestrogens investigated. The solvent system is cyclohexane–ethyl acetate (35:65, v/v).

TABLE I

Compound	R_F ± *S.D.*		R_{Oe} ± *S.D.*	
Oestrone	0.87	0.03	1.00	—
17β-Oestradiol	0.73	0.03	0.83	0.03
16-*epi*-Oestriol	0.45	0.02	0.52	0.02
Oestriol	0.17	0.01	0.20	0.01

Experimental

A suspension of silica gel G (7731, Merck) was applied on glass plates (20 cm × 20 cm) using a Desaga thin-layer applicator, calibrated to give a 250 μ layer according to LUISI *et al.*[2]. The resulting plates were dried in air and activated at 110° in an oven. Small amounts of steroids (0.5 to 5 μg) in absolute ethanol (0.5–5 μl) were spotted on the starting line, approximately 1.5 cm from the bottom edge of the plate. Care was taken not to apply steroids within 2 cm of the side edges because the reproducibility of chromatographic mobilities was considerably poorer in this portion of the plates.

The plates were developed, at room temperature, by the horizontal technique using the B.N.-Kammer apparatus (Desaga, Heidelberg); development time is about 20 min. The solvent front was marked and the plates were air-dried and then heated, for 10 min, in an oven at 60°.

The chromatoplates were then sprayed with a freshly-prepared solution of BARTON's[4] reagent or with 2,4-dinitrophenylhydrazine 0.5 % in alcoholic sulphuric acid 10 % v/v.

Different proportions of the present solvent system and other solvent systems were investigated and no appreciable improvements noted.

The purification of oestrogens contained in urinary extracts has also been attempted and is still being studied.

This work was carried out under a grant from the Consiglio Nazionale delle Ricerche (Rome).

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Received February 27th, 1964

A method for automatic packing of Sephadex columns

Various methods have been investigated and used for packing Sephadex columns. One, described by FLODIN[1], gave good results with Sephadex G-25 and G-50. Another packing method, useful for all Sephadex types, was later developed by the same author[2]. In this latter method, the extension tube with the Sephadex suspension, connected to the column to be packed, has to be emptied of the supernatant as soon as it is depleted of gel and refilled with new suspension. This must be repeated until the desired bed length has been obtained. The packing of large columns with this method would be very time consuming. Furthermore, after several fractionations, the flow in such large columns may cease altogether so that frequently repacking is necessary.

In this note an automatic packing method is described which does not require continuous service during the packing process. This method can also be used for column materials other than Sephadex, although it is especially suitable for this material.

Method

A schematic diagram of the packing setup is shown in Fig. 1. The column is carefully mounted vertically and the outlet is closed. It is filled with the desired liquid and a small amount of Sephadex suspension is added. When a layer of 2–5 cm has formed[2] the system is closed, as shown in Fig. 1, and the outlet is opened. From the flask with the Sephadex suspension under stirring, the gel grains are entrained by the liquid which is siphoned through the PVC tubing into the column.

The packing rate depends upon the siphoning head between the level of the Sephadex suspension in the flask and the bottom of the column and also upon the