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SHORT COMMUNICATION

DETERMINATION OF ESTROGENS BY GAS–LIQUID CHROMATOGRAPHY WITH AN OPEN TUBULAR COLUMN

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INTRODUCTION

Only a few methods for the estimation of low titer urinary estrogens are reliable. Interfering substances have been reported with colorimetric methods [1] spectro-fluorometric methods [2] radioimmunoassay [3] and gas chromatographic methods [4]. We have made several gas chromatographic analyses on packed columns (PC) without success. Extensive purification would be necessary to ensure sufficient specificity. Mass fragmentography actually provides the highest specificity, but we have been looking for a less sophisticated method, which can separate estrogens from the most frequently interfering compounds on an open tubular column (OTC).

EXPERIMENTAL

The glass OTC was coated with SE 30 liquid phase, according to the procedure of Bouche and Verzele[5]. The parameters are listed in Table 1. In order to have reasonable analysis times, we used high values for $\bar{u}$ ($\bar{u}$ = 100 cm./sec at 200°C; $\bar{u}$ = 85 cm./sec at 250°C). A solid injector, described by Ros[6], was fitted to a Carlo Erba Fractovap model G1 gas chromatograph with an oven temperature controlled by a PT model programmer and a flame ionization detector.

Qualitative analysis

Open tubular columns differ from packed columns through their B_0 and β values [7]. The former have higher β values and thus overloading occurs with smaller sample sizes. The B_0 value is also higher for OTC and thus for the same number of theoretical plates, the time of analysis is shorter.

We have tried several temperature programs (Fig. 1). We found higher apparent efficiency [8] and better peak symmetry with programs 2 and 3 than with the isothermal operation 1. Program 2 has been selected for urinary extracts because the peak width remains almost constant. The separation of 12 estrogens in a mixture of pure compounds as their TMSi derivatives has been studied. Retention indices

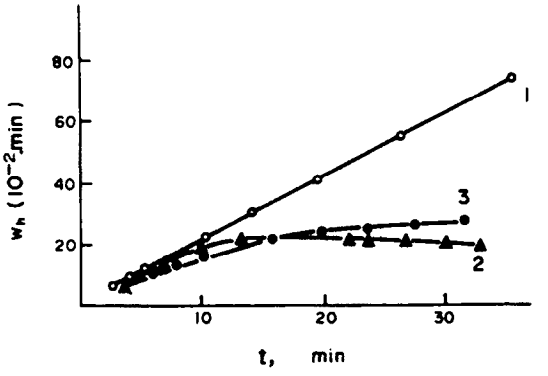

Fig. 1. Comparison of peak widths at half height for a series of *n*-alkanes (24 to 32 carbon atoms). 1—Isothermal program 220°C; 2—temperature program, 10 min at 200°C, 200–250°C, 2·2°C/min; 3—temperature program, 200–250°C, 1·3°C/min.

Table 1. Parameters of the open tubular column

Parameters	Symbols	Values
Column length	L	20 m
Internal diameter	d	0·25 mm
Specific permeability	B_0	195×10^{-7} cm^2
Compressibility correction factor	j	0·83
Column temperature	T_c	200°C
Average linear gas velocity	$\bar{u}$	37 cm/s
Average liquid film thickness	d_f	0·33 μm
Phase ratio [7]	β	192
Capacity ratio of *n*-eicosane	k	5·4
Height equivalent of a theoretical plate	HETP	0·85 mm
Theoretical minimum value of the HETP curve [7]	HETP = min	0·212 mm
Coating efficiency [7]		25%
Separation number	$SN = \frac{t_{24} - t_{22}}{w_{h_{24}} - w_{h_{22}}}$	19

t_{22}, t_{24}—Retention times for the *n*-alkanes with 22 and 24 carbon atoms.
w_h—Peak width at half height.

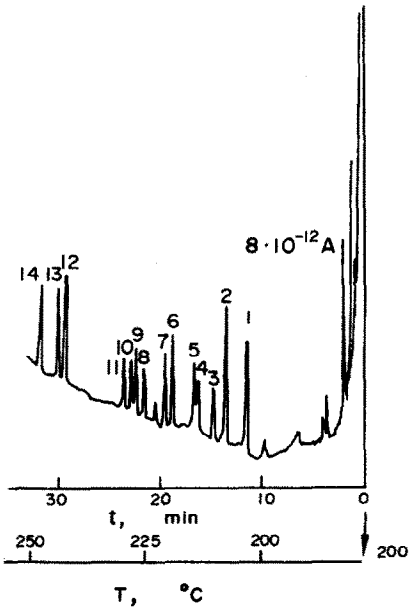

Fig. 2. Separation of 12 estrogens from a mixture of reference compounds as TMSi derivatives. Amounts expressed as underivatized compound 1—estrone (8 ng); 2—estradiol-17α (4 ng); 3—estradiol-17β (4 ng); 4—2-methoxyestrone (4 ng); 5—16-oxoestradiol (4 ng); 6—16α-hydroxyestrone (4 ng); 7—2-methoxyestradiol (4 ng); 8—17-epiestriol (1 ng); 9—16,17-epiestriol (2 ng); 10—estriol (2 ng); 11—16-epiestriol (2 ng); 12—cholesterol (4 ng); 13—15α-hydroxyestriol (4 ng); 14—octacosanol (4 ng).

(I) with program 1 have been calculated according to Kovats' equation [9]

$$I_T^{(A)}(x) = 100\left[\frac{\log V_N(x) - \log V_N(z)}{\log V_N(z+1) - \log V_N(z)} + Z\right]$$

where: I = isothermal retention index; T = column temperature; A = liquid phase; x = compound; z and $z + 1$ = n-alkanes with z and $z + 1$ carbon atoms respectively; and V_N = net retention volume of carrier gas.

In order to investigate the reliability of the program 2, we have calculated the reproducibility of relative retention times (RRT) (Table 2). Methylene unit (MU) values [11] are also calculated for this program where there is a linear relationship between the retention time and the number of carbon atoms of n-alkanes. Neutral steroids have slightly lower MU values on PC when compared to values obtained on OTC [12]. We found generally a reverse effect with phenolic steroids.

Quantitative analysis

For the simultaneous estimation of E_1, E_2 and E_3 preliminary trials were made using 100 ml of urine previously purified according to Scholler *et al.*[13]. An additional purification was made on a Sephadex LH 20 column (0·9 × 21 cm – LH 20 Sigma Chemical (U.S.A.) with 2-propanol (Merck) for residue determination as an eluent. Recovery was between 91 and 95%. Octacosanol (2 μg) was added as

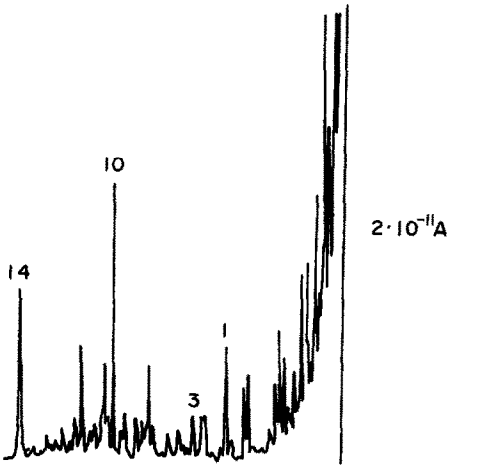

Fig. 3. Urinary extract = Women treated With Sexovid® [Bis-(p-acetoxyphenyl) cyclohexylidenemethane). Identified peaks: see Fig. 2.

Table 2. RRT (n-dotriacontane), MU and I for 12 estrogens, cholesterol and octacosanol TMS derivatives. Program no. 1—five determinations; program no. 2—seven determinations

Trivial names	OTC Program 1 $\bar{I}$	S_I	PC MU	OTC Program 2 MU	$\overline{RRT}$	CV_{RRT}
Estrone	2577·3	0·9	26·15	25·50	0·346	0·23
Estradiol-17α	2615*		26·43	26·01	0·405	
Estradiol-17β	2653·4	0·3	26·83	26·34	0·439	0·30
2-Methoxyestrone	2697*			26·80	0·490	0·29
16-Oxoestradiol	2712·0	1·3	26·92	26·89	0·499	0·28
16α-Hydroxyestrone	2761·6	1·2	27·75	27·48	0·562	0·30
2-Methoxyestradiol	2781·9	0·9	27·73	27·69	0·585	0·27
17-Epiestriol	2834·4	0·3	28·53	28·27	0·646	0·31
16,17-Epiestriol	2860·7	0·5	28·15	28·53	0·672	0·22
Estriol	2873·7	1·1	28·70	28·65	0·685	0·22
16-Epiestriol	2899*		28·90	28·90	0·710	0·20
Cholesterol	3072·4	0·8		30·73	0·885	0·10
15α-Hydroxyestriol	3096*			30·97	0·908	
Octacosanol (internal standard for urine extract)	3155·4	0·5		31·54	0·959	0·08

$\overline{RRT}$—average RRT.
CV_{RRT}—coefficent of variation of RRT.
$\bar{I}$—average I.
S_I—standard deviation of I.
PC—packed column SE_{30} 1%, 1·50 m × 3 mm, 220°C. Isothermal (Ref. [10]).
* Approximation due to incomplete separation.

an internal standard before the preparation of TMSi derivatives. The fraction of the extract applied on the top of OTC varies from 4/100 to 4/1000.

The precision (CV%) for nine duplicate urines (2–50 μg/l for each estrogen) is less than 5%.

The accuracy of the method has been assessed by adding known amounts of E_1, E_2 and E_3 to the urine samples before Sephadex purification. The following linear regression equations have been calculated.

$$E_1: y = (1{\cdot}00 \pm 0{\cdot}03)x + (0{\cdot}16 \pm 0{\cdot}21)$$
$$E_2: y = (0{\cdot}97 \pm 0{\cdot}04)x + (0{\cdot}09 \pm 0{\cdot}14)$$
$$E_3: y = (0{\cdot}96 \pm 0{\cdot}04)x + (0{\cdot}14 \pm 0{\cdot}26)$$

The coefficients of variation estimated from residual variances are respectively 4·5, 5·0 and 5·1%. (Mean values.)

Urinary blanks (unpubescent male children) gave no signal at the retention time of E_1, E_2 and E_3 at an electrometer sensitivity of $8.\ 10^{-12}$ A.

CONCLUSION

The results obtained with several urines containing known interfering substances, demonstrate that the method with temperature program 2 is sufficiently reproducible and allows quantitative determination.

Further quantitative data will be required to test the method as a whole and the gas chromatograph-mass spectrometer combination with an OTC will be a valuable tool to check specificity.

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