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Note

Determination of precise retention indices of steroids using an all-glass solid injection system

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Accurate and precise retention index data of steroids are required for their identification and the determination of physicochemical properties. The experimental factors that affect the determination of precise retention are the accuracy of the carrier gas pressure, column temperature and the method of "dead time" determination.

The use of a precise pressure controller has minimized the effect of pressure fluctuations on retention¹, and temperature variations have been decreased to 0.2°C by placing the column inside an aluminium block². Methods of "dead time" determination have been summarized by Kaiser³ and Wainwright and Haken⁴. Ezretz and Vigdergauz⁵ estimated the error in the retention index due to the solubility of gases used for measuring the "dead time".

Since the introduction of gaseous samples by all-glass solid injection systems is not possible, the "dead time" has to be determined indirectly. The three equidistant *n*-alkanes method⁶ may result in considerable errors due to extrapolation through more than twenty carbon atoms when steroid samples are used. The solvent front or *n*-pentane peak maxima, although used extensively, are limited to analyses at high column temperatures^{7,8}.

The present paper describes the determination of precise retention indices of steroid derivatives, directly by introducing methane through a solid injection system for "dead time" measurement, and indirectly by converting arithmetic or linear retention indices, I_A , into retention indices, I , by the method developed in this laboratory⁹.

EXPERIMENTAL

A Pyrex glass capillary column (30 m × 0.25 mm) was deactivated with 1% benzyltriphenylphosphonium chloride solution in methylene chloride and coated by the static procedure⁷ with 0.5% (w/w) SE-30 in *n*-hexane.

A self assembled gas chromatograph and a flame ionization detector with well controlled pneumatics (Becker, Delft, The Netherlands) was fitted vertically with an all-glass solid injector. Shrinkable PTFE tubing was used to connect the glass capillary column which was housed inside an aluminium block designed by Krupcik *et*

*al.*². Temperature gradients of 0.5, 0.6 and 0.7°C inside and 6.5, 7.0 and 7.5°C outside the aluminium block were observed, when the column oven was maintained at 221, 231 and 241°C respectively. The inlet pressure of the nitrogen carrier gas was maintained within 0.005 atm by using two fine pressure regulators in series.

In order to introduce methane and other *n*-alkane (C₅–C₈) vapours directly, a Swagelok T-joint with a self sealing septum was connected to the carrier line prior to the solid injector.

A mixture of *n*-alkanes (C₂₂–C₃₀) dissolved in *n*-hexane and methoxime trimethylsilyl (MO-TMS) derivatives of steroids, prepared as described elsewhere⁸, were injected. The retention data measured were averages from three injections.

RESULTS AND DISCUSSION

Accurate retention indices of steroids can be determined either directly by using adjusted retentions from methane injection or indirectly by unadjusted retentions from an arithmetic index system⁹.

Direct methane injection

In order to determine the methane retention time using the solid injection system, a T-joint was attached as mentioned earlier. The vapours of lower *n*-alkanes (C₅–C₈) and methane were injected individually in the T-joint and the retention times of alkanes and methane (*t*_{CH₄}) were measured. Liquid *n*-alkanes (C₅–C₈) were injected using the solid needle after reduction in volume by evaporation, in order that the respective peaks fitted on the scale. The difference between the retention times of the vapour and liquid of each alkane was measured and found to be constant (say *t*_c). Therefore, the column "dead time" *i.e.*, the retention time of methane injected using a solid needle, can be calculated: dead time = *t*_{CH₄} – *t*_c. The "dead time" determined in this way was found to be 165, 174 and 183 sec at 221, 231 and 241°C respectively and the retention indices for some standard steroids derivatives are given in Table I.

Indirect arithmetic index

It has been demonstrated^{9,10} that linear or arithmetic retention indices can be measured more accurately than retention indices. The arithmetic retention indices for MO-TMS derivatives of steroids were calculated using the non-adjusted retention times (see Table I). In order to determine the retention indices from arithmetic indices, the graphical and analytical method developed by Mitra⁹ has been utilized. The mathematical relation employed is

$$\frac{I_A - 100n}{100} = \frac{\sigma^{(I - 100n)100} - 1}{\sigma - 1} \quad (1)$$

where

$$\sigma = \frac{t_{R_{n+2}} - t_{R_{n+1}}}{t_{R_{n+1}} - t_{R_n}} = \frac{t_{R_{n+1}} - t_{R_n}}{t_{R_n} - t_{R_{n-1}}}$$

Here *t*_{R_{n+2}}, *t*_{R_{n+1}}, *t*_{R_n} and *t*_{R_{n-1}} are the unadjusted retention times of the standard *n*-alkanes of carbon number, *n*, between which the components are eluted.

TABLE I

RETENTION INDEX DATA OF MO-TMS DERIVATIVES OF STEROIDS ON AN SE-30 GLASS CAPILLARY AT VARIOUS TEMPERATURES

Compound	Retention time (sec) at		Arithmetic indices of				Retention indices from methane at				Retention indices from I_4 at				
	221°C	231°C	241°C	221°C	231°C	241°C	221°C	231°C	241°C	221°C	231°C	241°C	221°C	231°C	241°C
Methane	165.0	174.0	183.0	100.0	—	—	100.0	—	—	100.0	—	—	—	—	—
<i>n</i> -C ₂₄	759.0	574.0	459.5	2400.0	—	—	2400.0	—	—	2400.0	—	—	—	—	—
Androsterone	909.5	690.0	560.5	2473.4	2487.9	2509.8	2476.2	2489.3	2511.2	2476.1	2489.2	2511.2	2489.2	2511.2	2511.2
Etiocholanolone	947.0	712.0	574.5	2491.7	2503.5	2521.7	2492.7	2504.0	2524.3	2492.1	2504.7	2524.1	2504.7	2524.1	2524.1
<i>n</i> -C ₂₅	964.0	706.0	549.0	2500.0	—	—	2500.0	—	—	2500.0	—	—	—	—	—
Dehydro-epiandrosterone	1080.5	804.5	641.5	2542.4	2557.3	2578.7	2546.0	2560.6	2580.2	2546.2	2560.6	2580.1	2560.6	2580.1	2580.1
<i>n</i> -C ₂₆	1239.0	878.0	666.5	2600.0	—	—	2600.0	—	—	2600.0	—	—	—	—	—
Pregnanolone	1338.0	962.5	746.5	2626.8	2637.1	2652.5	2629.8	2640.4	2656.0	2629.1	2640.3	2655.6	2640.3	2655.6	2655.6
Testosterone	1363.5	990.0	772.5	2633.6	2649.1	2669.5	2637.0	2652.6	2672.4	2636.9	2652.4	2672.2	2652.4	2672.2	2672.2
Estradiol	1460.0	1040.5	798.5	2659.7	2671.3	2686.6	2663.2	2674.0	2688.1	2662.9	2674.1	2688.0	2674.1	2688.0	2688.0
11- β -Hydroxyetiocholanolone	1607.5	1133.0	861.5	2699.6	2708.9	2721.3	2700.0	2710.2	2723.8	2700.0	2710.2	2723.4	2710.2	2723.4	2723.4
<i>n</i> -C ₂₇	1609.0	1106.0	819.0	2700.0	—	—	2700.0	—	—	2700.0	—	—	—	—	—
<i>Allo</i> -Pregnenediol	1919.5	1334.0	1001.5	2762.2	2775.5	2791.4	2765.6	2777.9	2792.7	2765.6	2778.0	2792.4	2778.0	2792.4	2792.4
Pregnenediol	2005.0	1378.5	1025.0	2779.4	2790.2	2802.3	2781.6	2791.4	2803.1	2781.7	2791.3	2802.6	2791.3	2802.6	2802.6
<i>n</i> -C ₂₈	2108.0	1408.0	1018.0	2800.0	—	—	2800.0	—	—	2800.0	—	—	—	—	—
Estriol	2678.0	1784.0	1290.5	2884.9	2894.2	2903.7	2886.7	2894.9	2904.2	2886.6	2894.6	2904.3	2894.6	2904.3	2904.3
<i>n</i> -C ₂₉	2799.0	1807.0	1278.0	2900.0	—	—	2900.0	—	—	2900.0	—	—	—	—	—
<i>n</i> -C ₃₀	3690.0	2337.0	1616.5	3000.0	—	—	3000.0	—	—	3000.0	—	—	—	—	—

The σ values determined for all the sets of alkanes are generally found to be constant for a given stationary phase and column temperature. In this case, the average σ values were found to be 1.349, 1.325 and 1.304 at 221, 231 and 241°C on SE-30, and have been used for the calculation of retention index values (see Table I).

The retention index values determined by using the solvent front peak as "dead time" were comparable with those obtained by the direct and indirect methods as the column temperatures were quite high.

In conclusion, the measurement of the arithmetic retention indices of steroid derivatives when working with a solid injection system and lower column temperature yields precise and reproducible results, and has the added advantage of giving accurate retention index values, indirectly, if needed.

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