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**Short Communication**

# Separation and identification of unsaturated steroids by combined gas chromatography–mass spectrometry with Silanox-type glass open tubular columns

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A combined gas chromatograph–mass spectrometer equipped with packed columns is a uniquely powerful tool for the simultaneous separation and identification of the components of complex organic mixtures. After some earlier difficulties, a number of investigators have reported procedures for the preparation of reproducible, thermostable glass open tubular chromatographic columns[1–4]. Injectors which conform to the critical requirements of these columns have been developed[5,6] and the impact of these has led to a burgeoning of applications of these columns to biochemical problems. Several successful techniques for the connection of open tubular columns to mass spectrometers have demonstrated a significantly enhanced performance of the combined instrument in the analysis of complex mixtures[7,8]. Advances in analytical techniques based on glass open tubular columns have been reported in a recent symposium[9]. We report the application of Silanox-type glass open tubular columns to the gas chromatographic–mass spectrometric (GC–MS) analysis of two mixtures of steroids differing in respect of stereochemistry and/or unsaturation within the steroid nucleus.

## EXPERIMENTAL

Open tubular glass spirals were drawn from 6-mm light-walled Pyrex glass tubing in a device constructed according to the design of Desty *et al.*[10] to an internal diameter of 0.5 ± 0.01 mm. The column blanks were inactivated by a gas phase silanization method[11] and were coated with 6–10-$\mu$m Silanox® (Cabot Corp., Billericia, Mass., U.S.A.), a finely divided silylated fumed silicon dioxide, and OV-1 methyl silicone stationary phase (Applied Science Labs., State College, Pa., U.S.A.) by the method of German and Horning[2].

These columns were installed in an LKB 9000 gas chromatograph–mass spectrometer (LKB, Stockholm-Bromma, Sweden) with a "falling needle" type dry injector device[6]. The discrepancy between column effluent flow-rate (4–6 ml/min) and the flow requirement for optimum performance of the two-stage stainless steel jet-orifice separator (30 ml/min) was overcome by the addition of gas to the effluent at the column outlet. This was accomplished without significant reduction in chromatographic efficiency by means of a "make-up" device which added the extra gas to the column effluent in a laminar fashion[12]. The carrier gas (helium) flow-rate through the

column was maintained at 5 ml/min, and the supplementary flow of helium at approximately 25 ml/min. MS conditions were: separator and source temperature, 270°; filament emission current, 70 $\mu$A; electron energy, 70 eV.

Packed-column GC was carried out on a Perkin-Elmer Model 881 gas chromatograph equipped with a flame ionization detector. A 9-ft. glass spiral column of 3.5 mm I.D. was packed with 1% OV-1 coated on 100–120 mesh Gas-Chrom Q (Applied Science Labs.) and the carrier gas (nitrogen) flow-rate was 40 ml/min.

Reference materials were obtained from the following sources: $\Delta^5$-androsten-3$\beta$-ol and 5$\alpha$-androstan-3$\beta$-ol, Medical Research Council Steroid Reference Collection (Westfield College, London, Great Britain); $\Delta^{5,16}$-androstadien-3$\beta$-ol, $\Delta^{16}$,5$\alpha$-androsten-3$\alpha$-ol, $\Delta^{16}$,5$\alpha$-androsten-3$\beta$-ol and $\Delta^{16}$,5$\beta$-androsten-3$\alpha$-ol. Dr. G. F. Woods, Organon Research Labs. (Newhouse, Great Britain): 5$\alpha$-androstan-3$\alpha$-ol. Ikapharm (Ramat-Gan, Israel); $\Delta^2$, $\Delta^3$ and $\Delta^{8(9)}$-5$\alpha$-cholestenes and $\Delta^4$- and $\Delta^5$-cholestene: Dr. P. Bladon (University of Strathclyde, Glasgow, Great Britain).

Trimethylsilyl ethers were prepared in neat bis(trimethylsilyl)acetamide at 65° for 30 min, and excess reagent was removed under a stream of dry nitrogen. Solutions for analysis were in nanograde hexane at a concentration of 1 $\mu$g/$\mu$l.

## DISCUSSION

Fig. 1A illustrates the separation of a six-component mixture consisting of $\Delta^{16}$,5$\alpha$-androsten-3$\alpha$-ol (a), $\Delta^{16}$,5$\beta$-androsten-3$\alpha$-ol (b), $\Delta^{5,16}$-androstadien-3$\beta$-ol (c), $\Delta^{16}$,5$\alpha$-androsten-3$\beta$-ol (d), $\Delta^5$-androsten-3$\beta$-ol (e) and 5$\alpha$-androstan-3$\beta$-ol (f), as their trimethylsilyl ethers. As expected, the 3$\alpha$/3$\beta$ hydroxy epimers (a, d) and the 5$\alpha$/5$\beta$

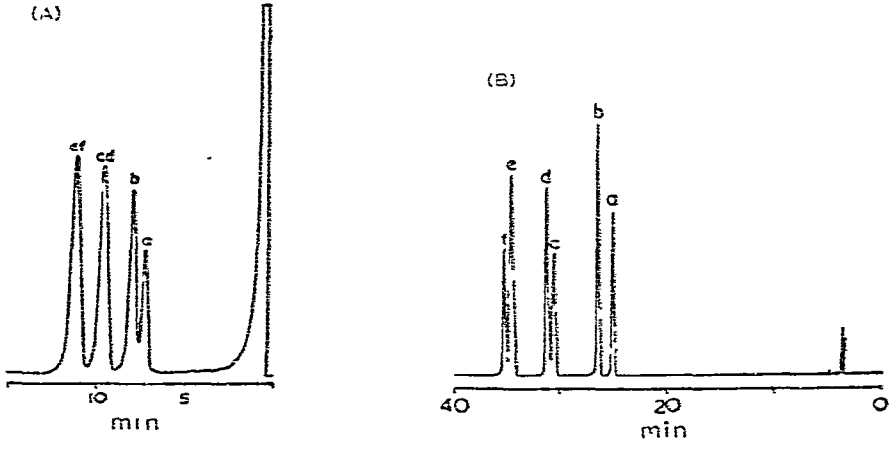


Fig. 1. Packed column chromatogram with a 9-ft. column packed with 1% OV-1 (A), and total ionization current chromatogram with a 50-m glass open tubular column coated with OV-1 over Silanox (B) of a synthetic mixture consisting of the trimethylsilyl ethers of the following compounds: a = $\Delta^{16}$,5$\alpha$-androsten-3$\alpha$-ol ($I$ = 2223); b = $\Delta^{16}$,5$\beta$-androsten-3$\alpha$-ol ($I$ = 2277); c = $\Delta^{5,16}$-androstadien-3$\beta$-ol ($I$ = 2290); d = $\Delta^{16}$,5$\alpha$-androsten-3$\beta$-ol ($I$ = 2296); e = $\Delta^5$-androsten-3$\beta$-ol ($I$ = 2330); f = 5$\alpha$-androstan-3$\beta$-ol ($I$ = 2335) $I$ = Kováts retention indices for the $C_{19}$ alcohol trimethylsilyl ethers on the open tubular column at 230°. Conditions: (A), carrier gas (nitrogen) flow-rate, 40 ml/min; column temperature, 170°; flame ionization detection; (B), carrier gas (helium) flow-rate, 5 ml/min; column temperature, 230°.

epimers (a, b) were well separated by packed-column chromatography. However, the 3$\beta$-hydroxy-$\Delta^{16}$ and ring D saturated $\Delta^5$/5$\alpha$ pairs (c, d and e, f respectively) were eluted as single unresolved peaks. Fig. 1B illustrates the marked improvement in chromatographic resolution afforded by a 50-m glass open tubular column. Allsix components were well resolved, and mass spectra obtained at the apex of each component corresponded closely to those recorded for the individual derivatives.

This model separation has relevance for the analytical characterisation of the $\Delta^{16}$-steroids that occur as porcine pheromones[13,14].

Fig. 2 represents the separation of two mixtures of closely related cholestenes. The dry injection system, whereby samples are introduced without solvent, obviates the need for isolation of the spectrometer ion source during analysis. The undisturbed baseline also permits the use of intercalated injections (as in Fig. 2) affording efficient use of instrument time. An additional convenience is the option of loading the injector with a number of separate solutions, removing the solvent and injecting the combined sample.

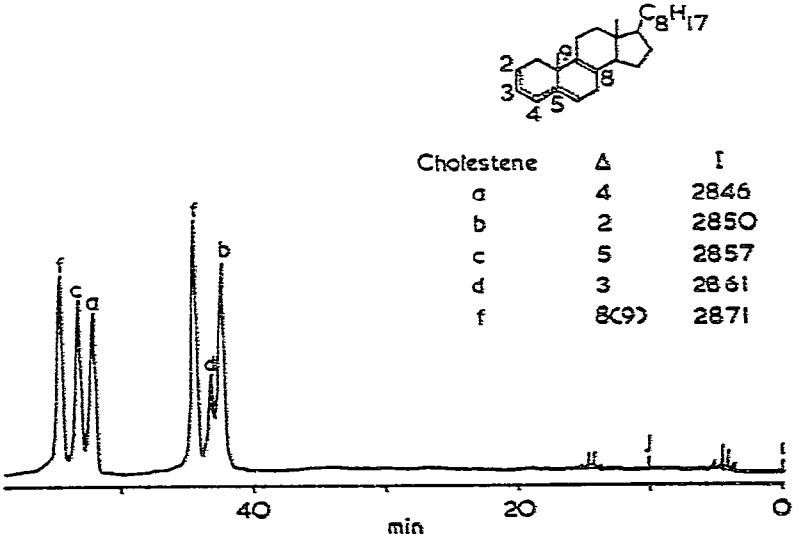


Fig 2. Total ionization chromatogram with 50-m glass open tubular column coated with OV-1 over Silanox of two successive injections (at i and j). a = Cholest-4-ene; b = 5$\alpha$-cholest-2-ene; c = cholest-5-ene; d = 5$\alpha$-cholest-3-ene; f = 5$\alpha$-cholest-8(9)-ene. Carrier gas (helium) flow-rate, 5 ml/min; column temperature, 240°.

The mixtures in Fig. 2 represent difficult chromatographic separations which with the use of conventional packed columns would result in largely unresolved peaks. The facility demonstrated here is being applied in the analysis of mixtures of natural steroids.

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