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CHROMATOGRAPHY OF STEROLS, ALKALOIDS AND OTHER DRUGS USING STEAM AS THE MOBILE PHASE

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SUMMARY

The chromatographic separation of steroids, alkaloids and other drugs with water vapour as the mobile phase has been studied. It is shown that this technique facilitates the analysis and reduces the retention times. The determination of the substances in low concentrations in aqueous solutions or dispersions also appears to be possible.

INTRODUCTION

Gas–liquid chromatography is now an important technique in the analysis of steroids, alkaloids and other drugs, both for estimating the purity of products and for studying their chemistry and metabolism[1]. The last application involves particularly difficult determinations of drugs in low concentrations in aqueous solutions, such as biological media.

The high polarity, extremely low vapour pressure and low chemical stability of these substances creates many difficulties in gas chromatographic (GC) determinations. In many instances, their conversion into more volatile derivatives such as trimethylsilyl or acetyl is necessary[1–3]. The GC of free steroids and alkaloids containing amino-groups is often difficult because of tailing, which decreases drastically the resolution and reduces the overall sensitivity of the chromatographic system[4–7]. The thorough deactivation of the solid support is necessary in circumstances that considerably complicate the GC analysis. Substantial decreases in retention times and improvement of peak shapes can be achieved by the use of steam as the mobile phase[8–11], and the chromatography of free sterols, alkaloids and some other pharmaceuticals has been accomplished by this means in the present work. Selected sterols (cholesterol and sitosterol), some steroidal terpenoids (lanosterol), steroid genin (diosgenin), alkaloids of the atropine, morphine and pyrrolizidine series together with some widely used drugs (pyrazole and aromatic amino acid derivatives) were studied.

EXPERIMENTAL

A home-made all-glass chromatograph with a flame ionization detector and

TABLE I

RETENTION INDICES OF STEROID COMPOUNDS ON AN XE-60 COLUMN AT 230°

Compound	*Peak*	*Retention index*	
		Using water vapour	*Using nitrogen*
Ergosterin	I	2867	2670
	II	3074	2757
Cholesterol		3024	3100
Diosgenin		3148	3150
Stigmasterol		3163	2990
β-Sitosterol	I	3140	—
	II	3235	3000
Lanosterol	I	3163	3120
	II	3201	—
Phytosterol	I	3113	3016
	II	3139	3120
	III	3198	—

a steam generator was used. Glass columns (1.3 m × 4 mm I.D.) were packed with 1% (w/w) polydimethylsiloxane SCTV-K or 1% (w/w) polar silicone XE-60 on 60–75 or 75–90 mesh Chromaton N. The temperature of the low-polar column was 230°, while the high-polar column was operated at 175–260°. The inlet pressure of water vapour was 0.5–1.5 atm. Materials were injected as chloroform solutions or as dilute aqueous solutions and suspensions.

RESULTS AND DISCUSSION

Steroid compounds

Retention indices of the steroids studied are listed in Table I, together with the retention indices obtained with nitrogen as carrier gas under similar conditions. Typical chromatograms are shown in Figs. 1 and 2.

The results show that the utilization of steam as the mobile phase allows the retention time to be reduced considerably and the peak shape to be improved. For

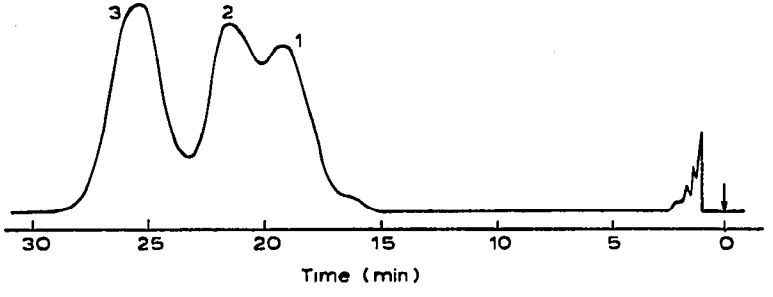

Fig. 1. Chromatogram of phytosterol. Glass column (1.3 m × 0.4 mm I.D.). Liquid phase: polydimethylsiloxane SCTV-K. Temperature: 230°. Inlet steam pressure: 0.5 atm. Retention indices: 1, 3113; 2, 3139; 3, 3198.

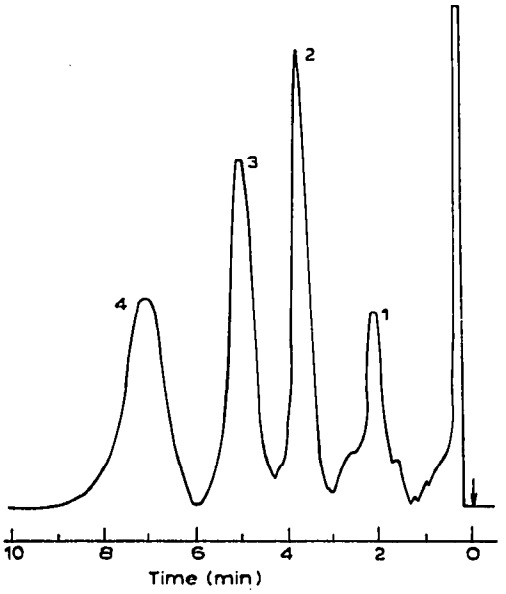

Fig. 2. Chromatogram of sterols. Glass column (2 m × 0.4 mm I.D.). Liquid phase: XE-60. Temperature: 230°. Inlet steam pressure: 0.5 atm. 1 = Ergosterin; 2 = cholesterol; 3 = stigmasterol; 4 = diosgenin.

three compounds studied, β-sitosterol, phytosterol and lanosterol, considerably altered chromatograms were obtained when nitrogen was replaced with steam (Table I).

Lanosterol and β-sitosterol give two clear peaks, but phytosterol gives three peaks, possibly as a result of better separation in water vapour, as the products extracted from natural sources are reported to be non-homogeneous[12].

The retention indices of steroid species in steam and nitrogen differ substan-

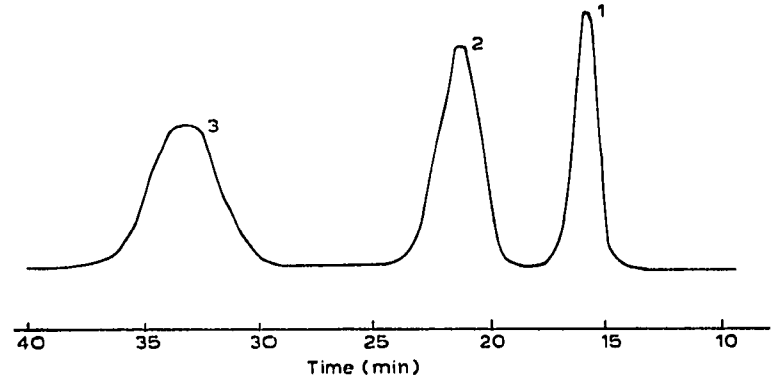

Fig. 3. Chromatogram of alkaloids. Glass column (1.5 m × 0.4 mm I.D.). Liquid phase: XE-60. Temperature: 185°. Inlet steam pressure: 0.5 atm. 1 = Platiphylline hydrotartrate; 2 = codeine hydrochloride; 3 = ethylmorphine hydrochloride.

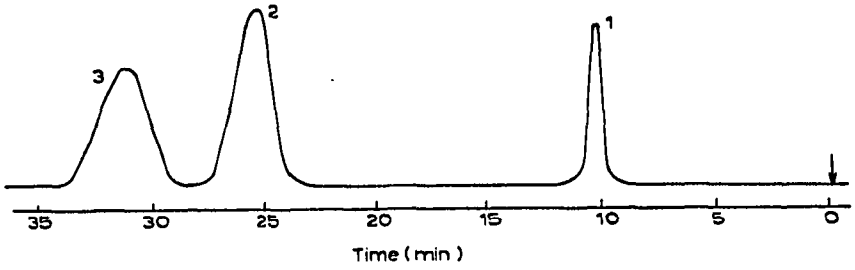

Fig. 4. Chromatogram of atropine alkaloids. Glass column (1.5 m × 0.4 mm I.D.). Liquid phase: XE-60. Temperature: 190°. Inlet steam pressure: 0.5 atm. 1 = Homatropine hydrobromide; 2 = tropacine; 3 = scopolamine hydrobromide.

tially, for example by 100–150 units in the case of cholesterol and diosgenin, and the elution order is changed.

The use of the more polar silicone liquid phase XE-60, containing cyano groups, gives a large decrease in retention time and a considerable improvement in separation when steam is used (Fig. 2). The column containing XE-60 was stable for at least 6–8 months when water vapour was used.

Alkaloids and other drugs

Symmetrical peaks were obtained when derivatives of morphine, atropine and

TABLE II

RETENTION INDICES OF MORPHINE ALKALOIDS ON AN XE-60 COLUMN AT 190°

Compound	*Retention index*	
	Using water vapour	*Using nitrogen*
Morphine	3061	—
Ethylmorphine hydrochloride	2761	2943
Codeine	2748	2939
Codeine phosphate	2732	2922

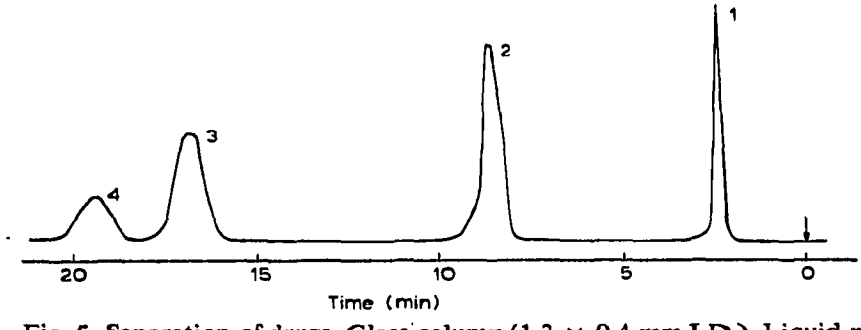

Fig. 5. Separation of drugs. Glass column (1.3 × 0.4 mm I.D.). Liquid phase: XE-60. Temperature: 180°. Inlet steam pressure: 0.5 atm. 1 = Anestesine; 2 = novocaine; 3 = amidopyrine; 4 = butadione.

pyrrolizidine were chromatographed in water vapour. The chromatographic separation was usually accomplished in 5–15 min at moderate column temperatures (Figs. 3 and 4). The retention indices of morphine alkaloids obtained on an XE-60 column are listed in Table II. There were no difficulties in the chromatographic determination of other drugs under similar conditions (pyrazole derivatives, aromatic amino acid esters, amidopyrine, butadione, anestesine, novocaine and others) (Fig. 5).

Satisfactory chromatograms were obtained by injecting both the free bases and the salts of species to be analyzed (platiphylline hydrotartrate, homatropine hydrobromide, scopolamine hydrobromide, codeine hydrochloride, codeine phosphate and ethylmorphine hydrochloride).

CONCLUSIONS

The compounds studied were mostly injected in the form of dilute aqueous solutions or dispersions of concentration 0.1–1 %. No baseline disturbance after the injection of such samples was observed. It can be concluded that the chromatographic technique described might be of considerable use for the determination of drugs in aqueous solutions and biological liquids.

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