Notes

Collection of steroids eluted from a gas chromatography column

Since the detection mechanisms of gas chromatography are non-specific, identification of peaks is desirable, especially when establishing reliability of analytical methods. There have been few reports of quantitative collection of steroids from analytical gas chromatography columns[1–3]. This report describes a means of quantitatively collecting microgram amounts of components eluted by the effluent gas stream. The technique was initially developed to aid in characterising the "pregnanediol" peak of the procedure of Cox[4]. Its usefulness for other steroids is also discussed.

Gas chromatography

The gas chromatograph was a Perkin-Elmer model 801 with a hydrogen flame ionisation detector. Nitrogen, dry and free of oxygen, was used as carrier gas. Columns of length 6 ft. and internal diameter 2 mm were glass, with an integral glass injection area. They were silanised and packed to a length of 165 cm. Both the solid support, Gas Chrom Q 60/80 mesh, and the stationary phase, neopentylglycol adipate (NGA), were obtained from Applied Science Laboratories. The support was coated with 0.5 % NGA by filtration[5]. This concentration was determined by weighing the packing before and after exhaustive extraction with acetone in a Soxhlet apparatus. The column was conditioned with nitrogen flow rate 10 ml/min, by increasing the temperature from ambient to 235° at the rate of 1.4°/min and then heating at 235° for 30 ± 2 h. Operating conditions were: injector 255°, column 205–220°, detector 220° and carrier gas flow rate 30–50 ml/min. All steroids were injected in 1 μl volumes.

Experimental and results

The column effluent was collected with the apparatus shown in Fig. 1. A stream splitter was fitted to the column exit, so that 22 % of the gas stream passed to the detector and 78 % to the outside. This splitting ratio was determined before and after

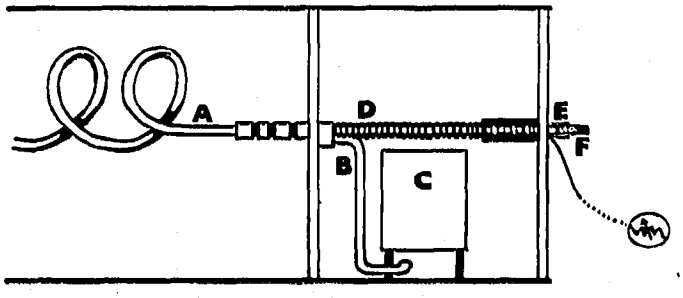

Fig. 1. Diagram of the apparatus used to collect the effluent gas. (A) Exit of column; (B) column effluent passing to detector; (C) flame ionisation detector; (D) stream splitter with heating tape wound around it; (E) teflon tubing connecting stream splitter exit to collection tube; (F) collection tube with potassium bromide at proximal end and fibre glass plug at distal end.

installation of the splitter and collection tube by measurements of (a) carrier gas flow rate through the detector and at the exit of the collection tube attached to the splitter, and (b) peak heights of standards. In this way, any resistance to gas flow caused by the potassium bromide was accounted for. The splitter was heated electrically to about 230° and insulated with asbestos.

Silanised glass tubing of internal diameter 2 mm was cut into lengths of 8–10 mm. These tubes were plugged at one end with silanised fibre glass and the open ends were filled loosely with 4 mg potassium bromide (Merck, spectroscopic grade), which had been previously dried for 48 h at 130°. Pieces of teflon tubing, length 15 mm and internal diameter 3 mm were attached to the potassium bromide and so that about 5 mm covered the glass. The assembled tubes were kept in an oven at 130°, until immediately before collection. This pre-drying process is essential for successful collection.

To collect the effluent corresponding to the pregnanediol peak, a tube was attached with the teflon tubing to the splitter exit about 20 sec before emergence of the peak. It was held in contact with the splitter until the descending side of the peak had reached base-line. The time delay between appearance of the component in the tube and the recorder response was negligible.

Recovery of eluted pregnanediol was determined by transferring the potassium bromide to centrifuge tubes. Benzene (2 ml) was added, together with benzene (3 ml) used to rinse the glass tubing. Distilled water (2 ml) was then added to dissolve the potassium bromide and after shaking the tube, it was centrifuged at 3,000 r.p.m. for 5 min. Gas chromatography of the evaporated benzene supernatant enabled calculation of the amount of pregnanediol recovered. When a correction was made for the stream splitter, recoveries of 89–96 % with a mean of 94 % ± 7.1 (S.D.) were obtained for eight collections of 0.21 μg pregnanediol.

The relationship between concentration of sample and percentage recovery was examined. Injection of large amounts of sample resulted in distortion of peak shape (Fig. 2). When sample size exceeded 6 μg pregnanediol, retention times and peak base widths increased, while column performance (theoretical plate number) decreased. For a 40 μg sample, retention time had increased by 11 % and peak base width by 170 %, while column performance had decreased by 82 %. It would be of interest to know whether these effects reflect column or detector overloading.

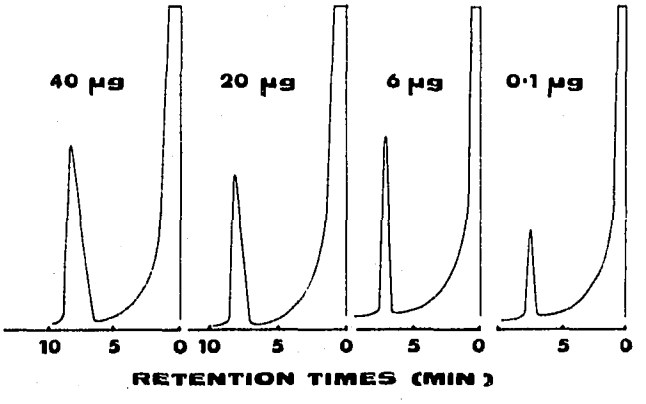

Fig. 2. Overloading of a column containing 0.5 % NGA on GCQ 60/80 mesh with pregnanediol. The sensitivity of the recorder was × 5 for 0.1 μg, × 200 for 6 μg, × 500 for 20 μg and 40 μg.

When 1–40 μg pregnanediol were injected and the effluents collected, recoveries for 15 injections were within the range 87–95 %, with a mean of 93 % $\pm$ 5.2 (S.D.). There was no relationship between amount injected and percentage collected.

Preliminary tests with other pure steroids indicate that the technique is useful for hydroxylated steroids and their polar derivatives (Table I).

TABLE I

COLLECTION OF STEROIDS IN GAS CHROMATOGRAPHY EFFLUENTS

Results are the mean of duplicate collections and triplicate injections of the steroids, eluted from the potassium bromide. All steroids were injected in 1 μl ethanol except pregnanetriol trimethylsilyl ether, which was injected in tetrahydrofuran.

The acetates were prepared with acetic anhydride in the presence of pyridine. The trimethylsilyl ether was prepared by heating the dry steroid at 60° in the absence of moisture with 0.3 ml of a freshly prepared mixture of trichloromethylsilane in hexamethyldisilazane (1:20, v/v).

Steroid	*Amount injected* (μg)	*Amount collected** (μg)	% *Collection*
Pregnanediol	40.0	38.1	95
Pregnanediol diacetate	25.1	21.6	86
Androsterone	20.3	10.0	49
Androsterone acetate	20.1	6.0	30
Pregnanetriol	20.4	19.7	98
Pregnanetriol trimethylsilyl ether	25.1	6.2	25
Pregnanedione	21.0	8.6	41
Pregnanolone	30.1	23.0	76

* Corrected for stream splitter ratio.

Discussion

Irreversible binding of steroids to the column or the potassium bromide may contribute to low recovery of some of the steroids tested. However, recoveries may be improved by using other solvents or mixtures of solvents for eluting adsorbed compounds from the potassium bromide. For example, improved recovery of androsterone and its acetate has been noted, when ethyl acetate–benzene (3:1, v/v) is used as eluent. By replacing or supplementing potassium bromide with other adsorbents such as silica gel[6], it should be possible to increase the versatility of the technique. Nevertheless, potassium bromide is particularly useful, as infrared spectrophotometry may be performed on a disc pressed directly from it.

Steroids have high boiling points and readily form fogs when cooled. The small particle size of these fogs prevents condensation and hence the low recoveries in procedures involving cooling of the gas stream. The fog may be dispersed electrostatically[7,8] or even prevented from forming by means of a temperature gradient trap[9], an evacuated receiver trap[10], or a differential temperature between the inner and outer wall of the collection tube[11]. This problem is overcome with the present technique. The heated stream splitter prevents fog formation and so the steroids in the effluent are adsorbed directly by the potassium bromide. Silanisation of the glass tubing prevents adsorption on its surface. This technique opens new possibilities for a study of the homogeneity of gas chromatography peaks.

Acknowledgements

Thanks are due to Dr. R. A. OSBORN and Mrs. M. POTTER. The study was financially supported by an anonymous benefactor and the Benevolent Society of N.S.W.

Royal Hospital for Women,
Paddington, New South Wales (Australia)

ROSALIND A. GREENSTREET*

1 H. H. WOTIZ, *Biochim. Biophys. Acta*, 74 (1963) 122.
2 S. C. BROOKS AND V. C. GODEFROI, *Anal. Biochem.*, 7 (1964) 135.
3 H. S. KROMAN AND S. R. BENDER, *J. Chromatog.*, 13 (1964) 242.
4 R. I. COX, *J. Chromatog.*, 12 (1963) 242.
5 E. C. HORNING, E. A. MOSCATELLI AND C. C. SWEELEY, *Chem. Ind. (London)*, (1959) 751.
6 K. WIDMARK AND G. WIDMARK, *Acta Chem. Scand.*, 16 (1962) 575.
7 P. KRATZ, M. JACOBS AND B. M. MITZNER, *Analyst*, 84 (1959) 671.
8 A. E. THOMPSON, *J. Chromatog.*, 6 (1961) 454.
9 D. H. VOLMAN, *J. Chem. Phys.*, 14 (1946) 707.
10 K. DORFNER, *Brennstoff-Chem.*, 44 (1963) 309.
11 R. K. STEVENS AND J. D. MOLD, *J. Chromatog.*, 10 (1963) 398.

Received October 15th, 1967

*Present address: Royal Postgraduate Medical School, London, W12 (Great Britain).