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Note

Comparative cannabis analysis

A comparison of high-pressure liquid chromatography with other chromatographic techniques

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The identification of cannabis for law enforcement purposes is a relatively simple procedure that is usually achieved by chromatography or microscopy. It is sometimes necessary, however, not only to identify samples but to compare them in order to establish a common origin or to trace distribution chains. In such cases, it is customary to compare the samples visually and then to use several different analytical methods, such as thin-layer chromatography (TLC)[1] and gas–liquid chromatography (GLC)[2], in order to provide a wide range of criteria on which to base the comparison. Despite this diversification of approach, existing techniques sometimes lack the discrimination required. We have therefore explored the potential of high-pressure liquid chromatography (HPLC) for comparative cannabis analysis and this paper compares the results obtained with those from TLC and GLC.

EXPERIMENTAL

HPLC

The column packing was prepared by chemical modification of a microparticulate porous silica (Partisil 5 of 7-μm average particle size; obtained from Reeve Angel Scientific, Maidstone, Great Britain). The silica was dried for 2 h at *ca.* 140° and 5 g were reacted at room temperature for 30 min with 20 ml of octadecyltrichlorosilane in 50 ml of sodium-dried light petroleum (boiling range 60–80°). The reaction was carried out in a stoppered tube agitated in an ultrasonic bath; the mixture was occasionally stirred with a glass rod to break up aggregates. The product was washed three times with 50 ml of light petroleum, Soxhlet extracted with the same solvent for 4 h, and dried at 80°. The material was sieved before use and a portion was ashed at *ca.* 600° to give some check on the level of organic coating (a weight loss of about 16% on ashing was normal).

A stainless-steel column 25 cm long, 6.35 mm (*i.e.* $^1/_4$ in.) O.D. and 4.9 mm I.D. was fitted with $^1/_4$-in. stainless-steel nuts (Swagelok) at each end. The bottom of the column was fitted with a $^1/_4$- to $^1/_{16}$-in. reducing union blocked with a plug of porous PTFE (4 mm thick and nominally 75-μm pore size). The column was packed at 5000–6000 p.s.i. with the modified silica using a balanced density slurry technique[3]

with 32% bromoform in chloroform as the suspending medium. A small portion of the modified silica was removed from the top of the column and a plug of porous PTFE was inserted. A modified ball valve, described elsewhere[4], was fitted to the top of the column to act as a stop-flow injection port.

Cannabis samples (resin or herbal) were prepared for HPLC by weighing 100-mg portions into glass tubes and adding 1-ml aliquots of the eluting solvent described below. The tubes were agitated in an ultrasonic bath for several minutes, and hard lumps of material were crushed with a glass rod. The resulting fine suspensions were allowed to settle and 0.5- to 2.0-μl aliquots of the supernatant solutions were injected on to the chromatographic column.

The conditions used for HPLC were as follows. Solvent, methanol–0.02 N sulphuric acid (80:20); pump, Waters Ass. Model No. 6000; flow-rate, 2 ml/min; pressure, *ca*. 2500 p.s.i.; detector, Varian UV detector operating at 254 nm; temperature, ambient.

GLC

Cannabis extracts for GLC were prepared in the same way as described above except that methanol was used as the solvent. The following chromatographic conditions were used: Column, 1.8 m of 2.1-mm-I.D. stainless-steel tubing packed with 3% OV-17 on Chromosorb W, 80–100 mesh, acid washed, DMCS treated; temperature, 240° isothermal or else programmed from 100° to 250° at a programme rate of 15°/min; carrier gas, nitrogen at 20 ml/min; detector, flame ionization detector.

TLC

TLC was carried out on methanol extracts of the cannabis samples using the method of Grlíc[5] with double development.

Samples examined

Thirty-four cannabis samples of known geographical origin have been examined. Twelve of these were cannabis resins obtained in Customs seizures and the remainder were samples of herbal material obtained from various countries via the United Nations. In addition, numerous samples of cannabis arising from the case work of this laboratory have been studied, including a number of cannabis extracts (*i.e.* the so-called hash oils).

RESULTS AND DISCUSSION

The many samples of resin and herbal material analysed by HPLC gave a variety of chromatograms differing in both qualitative and quantitative characteristics. Replicate analyses of different portions of the same sample gave virtually identical chromatograms, and with resin samples, no significant difference in the chromatograms was observed regardless of whether sampling occurred from the interior or exterior of slabs. Table I shows the degree of discrimination displayed by the various chromatographic techniques when applied to the samples of known geographical origin. It is apparent from the results that HPLC is superior to the other techniques for comparative cannabis analysis. It was found that those samples indistinguishable by

HPLC in this study were of similar geographical origin, thus two resin samples from Pakistan were indistinguishable, as were two resins from Morocco, two herbal samples from India and two herbal samples from Turkey. This may indicate that the HPLC method has some potential for indicating the origin of a sample, but further work on a much wider range of authenticated samples would be necessary before any conclusions could be drawn. In contrast, GLC, which has been suggested as a method capable of giving some indication of geographical origins[6], failed in three instances to distinguish between samples of widely different origins. None of the samples indistinguishable by HPLC could be distinguished by the other procedures.

TABLE I

A COMPARISON OF DIFFERENT METHODS OF CANNABIS DISCRIMINATION

Twelve cannabis resin samples and twenty-two herbal samples were examined, making a possible total of thirty-four distinct groups attainable if complete discrimination could be obtained.

	Method used for discrimination			
	Visual	TLC	GLC	HPLC
No. of resin groups	5	9	7	10
No. of herbal groups	6	3	18	20
Total number of groups	11	11*	25	30

* Two resins and two herbal samples comprised a single category.

The findings of this study have been supported by the results obtained with the much greater number of samples encountered in case work. A typical example of extracts displaying similar GLC characteristics which are readily distinguishable by HPLC is shown in Fig. 1. Fig. 2 gives some idea of the diversity of chromatograms obtained from different cannabis samples.

The HPLC conditions described above are the most suitable of those investigated for the comparison of cannabis samples. The resolution obtained using the microparticulate chemically bonded silica was markedly superior to that given by chemically similar, commercially available, pellicular materials of larger particle size.

The choice of a solvent system was dictated by the need to obtain chromatograms of maximum complexity within an analysis time of 10–15 min. Methanol–water combinations were found to give the best results and the resolution of the long-retained components was improved by incorporating traces of acid in the solvent system. Initially, acetic acid was used, but this appeared to attack the seals in the mechanical pump causing the pistons to seize. Low concentrations of sulphuric acid obviated this problem. The temperature at which the separation was carried out did have an influence on the chromatograms, with increasing temperature causing a decrease in retention time without any appreciable loss in resolution. For comparison purposes we normally lag the column to suppress any rapid temperature changes and operate at ambient temperature.

To achieve long-term, trouble-free operation of the HPLC system, it was found necessary to extract the cannabis samples with the eluting solvent. When methanol was used for extracting the samples, there was a noticable loss of resolution after fifteen to twenty analyses. This was probably due to a partial precipitation of the
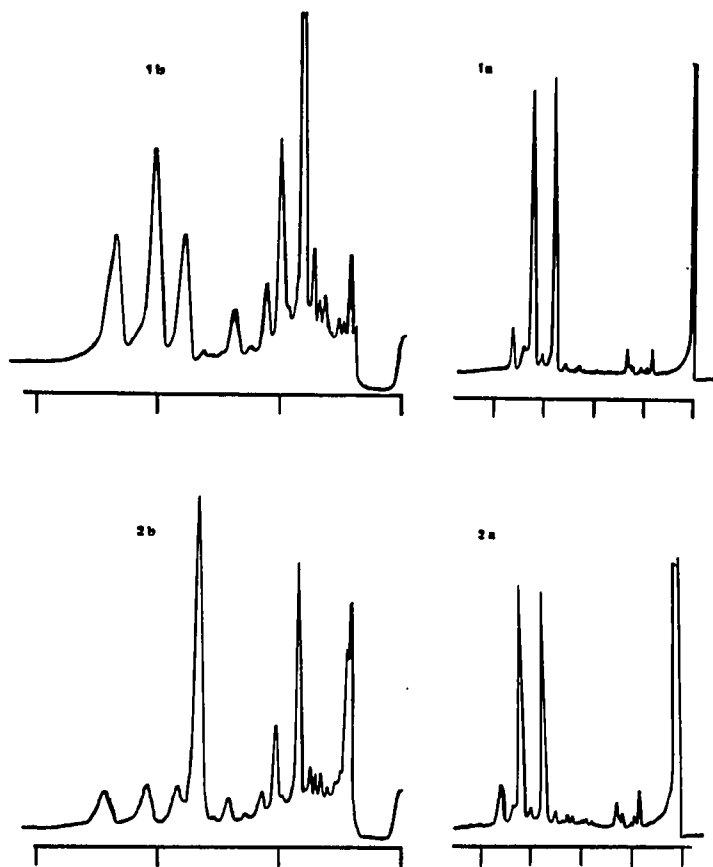
Fig. 1. Gas and liquid chromatograms of cannabis resin extracts. (1a) and (2a) are extracts examined by GLC; (1b) and (2b) are extracts examined by HPLC. For conditions, see text. The scale gradations represent 5-min intervals.
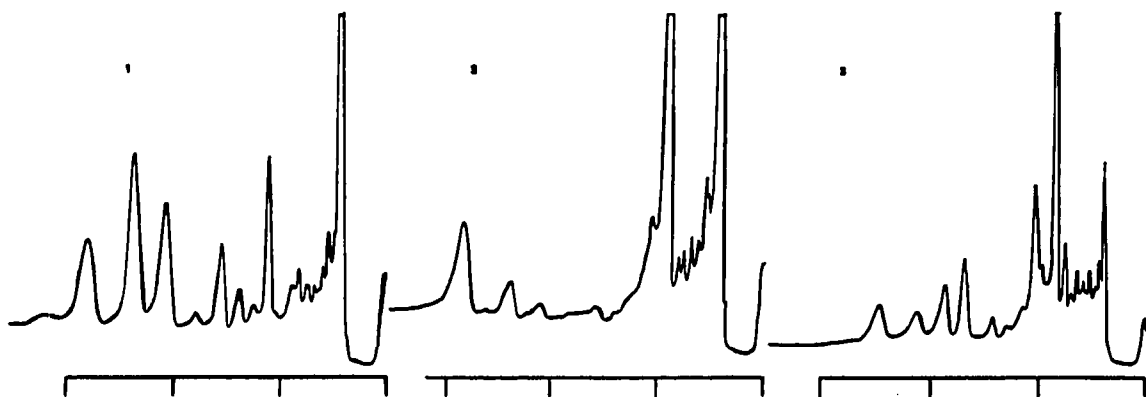

Fig. 2. Liquid chromatograms derived from cannabis samples of different geographical origin. (1) Herbal material from South Africa (UNC 335); (2) herbal material from Turkey (UNC 390); (3) resin from Morocco. For conditions, see text. The scale gradations represent 5-min intervals.

sample on the top of the column. The extraction time did not appear to be a critical factor in the analysis, but for comparison purposes we always extract samples under identical conditions. Ageing of the extracts over a period of several days did not affect the HPLC results.

The results obtained indicate that liquid chromatography provides a simple and reproducible method of cannabis discrimination. The technique also appears to provide information not readily obtainable by other chromatographic techniques. Further work is needed to elucidate the chemistry of the compounds being detected in the extracts, and to see whether any correlation exists between the geographical origin of the samples and these compounds.

REFERENCES

1 F. Korte and H. Sieper, *J. Chromatogr.*, 13 (1964) 90.
2 L. Strömberg, *J. Chromatogr.*, 68 (1972) 253.
3 R. E. Majors, *Anal. Chem.*, 44 (1972) 1722.
4 B. B. Wheals, C. G. Vaughan and M. J. Whitehouse, *J. Chromatogr.*, 106 (1975) 109.
5 L. Grlic, *J. Chromatogr.*, 48 (1970) 562.
6 T. W. M. Davis, C. G. Farmilo and M. Osadchuk, *Anal. Chem.*, 35 (1963) 751.