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A SIMPLE METHOD FOR CONCENTRATING DILUTE HIGH-BOILING SAMPLES FOR CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

Diluted samples of high-boiling compounds in organic solvents or derivatization reagents can be effectively concentrated prior to gas chromatography on a precolumn which contains a small amount of siliceous support provided and deactivated with a non-extractable (supposedly monomolecular) layer of stationary phase. It has been shown that this method is particularly suitable for concentration and sampling of biologic materials onto high-resolution capillary columns. This sampling procedure is superior to the low-temperature direct sample introduction via syringe. Excellent reproducibility with nanogram samples has been obtained.

INTRODUCTION

Several problems are encountered in high-sensitivity gas chromatographic analyses due to the unfavorably high ratios of solvent volume to that of trace constituents under determination. When introduced into chromatographic columns, large amounts of dilute solutions can cause numerous difficulties associated with the overlap of earlier fractions with the solvent zone, loss of sensitivity, and increase of electrical noise after the passage of a large quantity of solvent for a long time. Furthermore, repeated injections of large samples may frequently cause removal of the stationary phase from the top part of the column, and thus result in irreversible adsorption of subsequent samples on the bare solid support.

Therefore, the most effective concentration of samples prior to the separation process is desirable. However, simply removing excessive solvent does not always solve this problem. The formation of viscous solutions which are difficult to inject in a reproducible fashion impairs seriously the precision of analysis. Dry sampling[1–9] or removal of solvent by the precolumn technique[10–14] have been suggested to overcome some of the above-mentioned problems in the analysis of steroid hormones and other biological samples.

Recent introduction of high-resolution glass capillary columns for the chromatography of steroid metabolites[15–17] and other substances of biological interest[17–19] place even higher demands for improved sampling procedures. In particular, the inertness of the whole analytical system becomes mandatory in the analysis of nano-

gram and subnanogram quantities. Common experience indicates that the sample injection is the most critical point where sample decomposition and irreversible adsorption of extremely small samples occur. Considerable attention has been paid to improvements of injection techniques with high-resolution columns[20–24].

Direct injections of dilute samples onto capillary columns at lowered temperatures without splitting have been recommended and used in several studies[15,24,25], but a number of technical problems remain. Depending on the nature of the solvent, frequent passage of its large amounts may lead to a decreased life-time of thin-film capillary columns. Thus, an effective solvent removal appears extremely important for many improvements in trace analysis with capillary columns.

Another frequent problem in high-sensitivity determinations is that the final solution containing a sample dissolved in either an ordinary solvent or derivatization reagent may have a volume ranging from 50–500 μl, but no more than 0.5-μl aliquot can usually be utilized for the sample injection onto a capillary column. Thus, while it would be desirable to utilize the total sample because of sensitivity reasons, its major part remains often unused for the analysis.

In this communication we wish to report on a concentration method which can overcome most of the mentioned difficulties. The technique described below has been applied to the concentration of derivatized steroid hormones[26], marijuana constituents, and the trace analysis of air-pollutant polynuclear aromatic hydrocarbons[27]. However, its potential is obvious for many other trace analysis problems.

EXPERIMENTAL AND RESULTS

The technology of a precolumn and its assembly onto a gas chromatograph is particularly critical with high-resolution glass capillary columns and nanogram samples. In our procedure, a precolumn consists of a 1-mm-I.D. glass tube (from Hamilton, Reno, Nev., U.S.A.) which is filled at its end with about 2 mg of a packing material supported at both ends by small plugs of glass wool. This tube is ordinarily used as a part of the special injection port. The design of this injector with effective septum cooling is a modified version of that described by Van Rensburg *et al.*[28]. For the precolumn technique reported here, it is essential that the interruption of carrier gas and the precolumn removal be easily accomplished for each consecutive analysis. It is also important that all glass parts of the injector are well deactivated by either silanization or treatment with the phosphonium salt (benzyltriphenylphosphonium chloride). A 13-m × 0.25-mm-I.D. glass capillary column first deactivated with the identical substance[29], and then coated with SE-30 silicone gum has been mounted onto a Tracor Model 550 gas chromatograph with a Perkin-Elmer flame ionization detector.

For the sample introduction, an exact amount of a dilute solution is mechanically transferred onto the precolumn with the aid of a microsyringe or a small calibrated glass capillary. The latter alternative seems to present much less problems with contamination, which is so critical in the subnanogram analysis. The excessive solvent is flushed off on a gas line provided with the gas purifier and flow controller. In the case of hydrolyzable samples, the solvent removal is carried out on a vacuum line. Multiple applications of solutions containing common solvents or silylation agents present no problems primarily due to the nature of precolumn material as described below.

The thermal desorption of samples from the precolumn inserted into the injection port is then followed by their retrapping in the first part of a capillary column which is kept at sufficiently low temperature for a certain period of time. (The usual trapping time for nanogram quantities of trimethylsilyl steroids is between 20 and 30 min.) We have compared the suitability of direct microsyringe injection procedure followed by retrapping[24,25] and the precolumn technique for numerous samples, and always found the latter to be more effective. A typical example of such comparison carried out with an identical sample can be seen in Fig. 1. More intense peaks of model steroids and other compounds with the precolumn procedure were consistently observed, thus reflecting the negative influence of solvent in the first case. This could, perhaps, be explained by the expansion of a large total sample following evaporation and its diffusion outside the inert parts of the injection port, or a non-quantitative transfer of such samples from the syringe. When dealing with extremely small samples, the latter problem cannot be eliminated even through a "flush technique". Moreover, it is often observed that the later peaks of a chromatogram are often more intense when using the precolumn technique.

Now, it must be emphasized that the type of packing is extremely critical for the performance of such a concentration method with nanogram samples. While, for instance, 2,6-diphenyl-*p*-phenylene oxide porous polymer[30] is a very suitable concentration medium for more volatile samples[31,32], it proved unsuccessful for the concentration of derivatized steroids. Irreversible adsorption problems were also encountered with both bare silanized solid supports or glass wool. Naturally, any liquid-coated supports fail because of an easy wash-out of the stationary phase with large amounts of solutions and resultant uncovering of active sites, as well as the problems with the phase "bleeding" into the analytical column during the process of thermal stripping. Highly successful results were, however, obtained with the solid supports provided with thin films of bonded stationary phases according to the method reported by Aue *et al.*[33]. Highly deactivated packings are thus obtained which are essentially non-extractable with any organic solvents. The packings used for the preparation of precolumns used in this study were obtained by the treatment of ordinary diatomaceous earth supports (Chromosorb W and Gas-Chrom Q) with Carbowax 20M at 280° or the nonpolar fraction of Apiezon L grease (obtained through the silicic acid chromatography) at 320°. The packings were then exhaustively extracted with a series of solvents of different polarity, dried and used for the preparation of precolumns. It was found essential that the glass wool used to hold the precolumn material in place be treated in a similar fashion. For the work with methoxime-trimethylsilyl steroids, Apiezon-treated materials were found to be superior to Carbowax-bonded supports.

In addition to high-sensitivity steroid chromatography[26] and air-pollution studies[27], this concentration method is likely to find many other applications to other high-boiling samples. For example, Fig. 2 shows a complex chromatogram obtained after the concentration on the precolumn of a dilute solution of the methylene chloride extract of Mexican marijuana.

In summary, we feel that the following are the most important advantages of this concentration method: (1) an effective utilization of the total sample available from dilute solutions or derivatizations carried out in microscale, (2) improved chromatographic results due to overcoming usual problems associated with the solvent

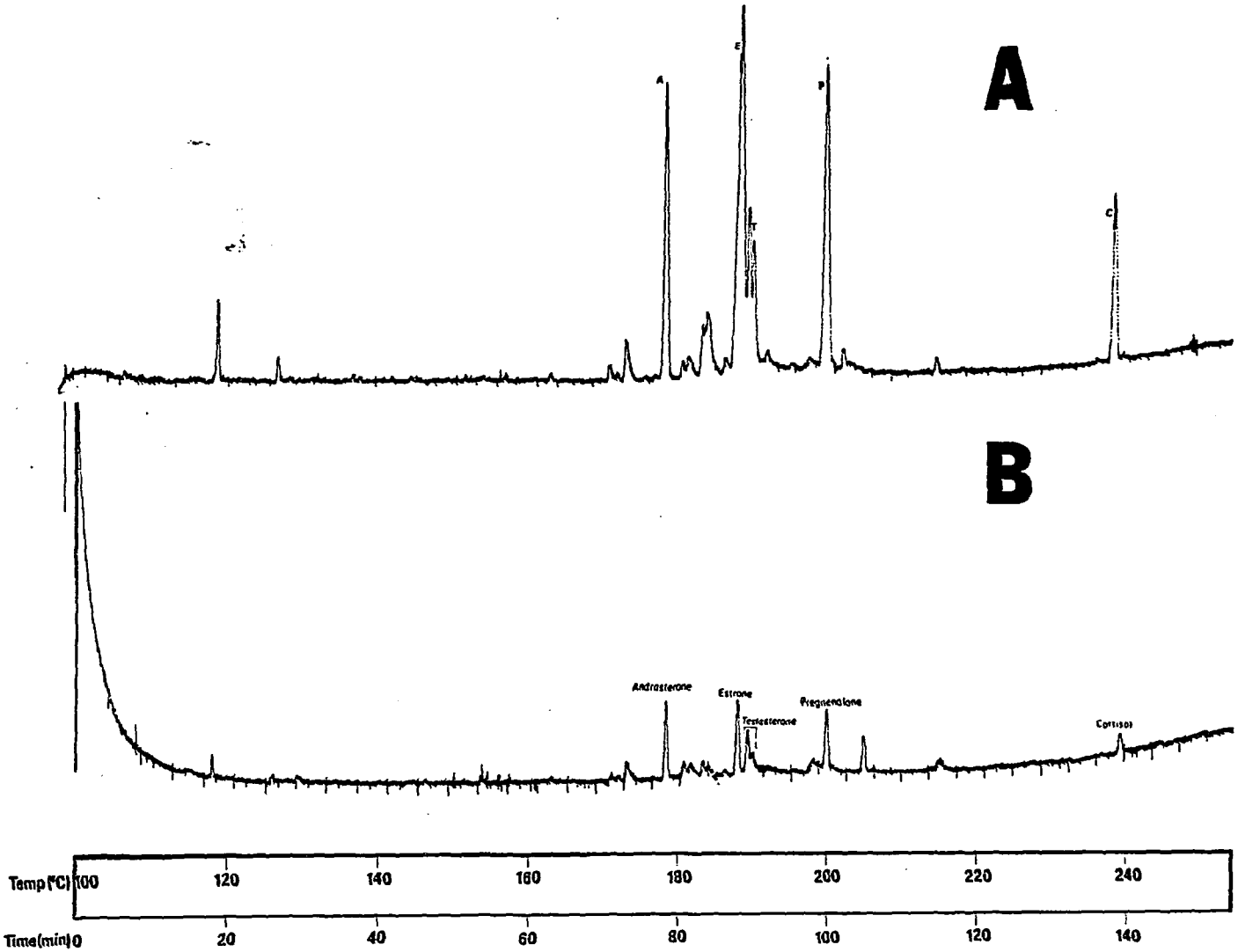

Fig. 1. Chromatograms of methoxime-trimethylsilyl derivatives of steroids. Conditions: 13-m × 0.25-mm-I.D. glass capillary column coated with SE-30 silicone gum; quantity injected, 10–15 ng each. A = Injection via the concentration precolumn; B = ordinary solvent injection (steroidal derivatives re-extracted into hexane); trapping period in both cases, 30 min.

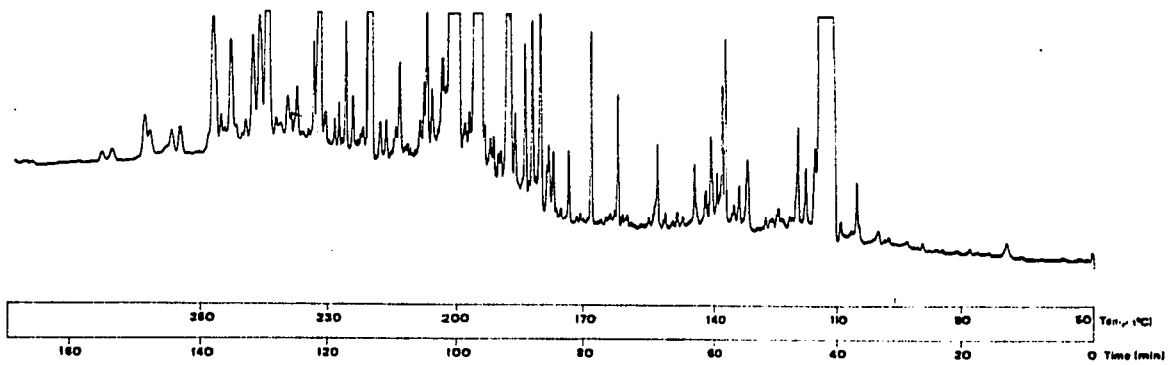

Fig. 2. Chromatogram of an aliquot of the methylene chloride extract of Mexican marijuana (a standard experimental cigarette obtained from the National Institute of Mental Health, Rockville, Md., U.S.A.). Conditions: 50-m × 0.23-mm-I.D. glass capillary column coated with SE-52 (methylphenyl silicone elastomer).

interference in both conventional gas chromatography and gas chromatography–mass spectrometry, contamination, and adsorption in the sampling port, (3) reduction of the problems resulting from the mechanical migration of heavy materials (often encountered with the extracts of biological samples) into the first portion of the analytical (capillary) columns, and (4) increased accuracy due to the fact that somewhat larger quantities of more accurately measured samples can now be introduced onto the precolumn.

An example of excellent sampling reproducibility can be seen in Table I, where four consecutive *n*-hydrocarbon samples (C_{24}, C_{26}, C_{28}, C_{30}) were chromatographed. Such a degree of repeatability could never be achieved when using a syringe injection method.

TABLE I

INJECTION REPRODUCIBILITY FOR 1- TO 5-ng QUANTITIES OF STANDARD HYDROCARBONS INJECTED ONTO AN SE-30 GLASS CAPILLARY COLUMN THROUGH THE PRECOLUMN TECHNIQUE

Sample run	*Peak area (mm²)*			
	C_{24}	C_{26}	C_{28}	C_{30}
1	42.00	36.25	22.50	17.50
2	39.60	28.00	22.50	18.75
3	32.00	33.75	23.75	15.00
4	43.00	43.75	27.50	17.50
Average	39.15	35.44	24.06	17.19
Standard deviation	4.98	6.53	2.37	1.57
$\frac{\text{Standard deviation}}{\text{Average}} \times 100\%$	12.7%	18.4%	9.8%	9.1%

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