On-Column Concentration of Trace High-Boiling Compounds for Quantitative Gas Chromatographic Analysis

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THE GAS CHROMATOGRAPHIC ANALYSIS of trace amounts of steroids, drugs, and other compounds of clinical or industrial interest has previously presented a problem because of the dilute form in which the samples are obtained for analysis. The usual procedure of concentrating the solutions to minute volumes is at best an awkward step which may lead to serious errors due to adsorption losses, decomposition, and sample volume changes resulting from evaporation of the solvent.

Several techniques have been proposed to resolve these difficulties but none has been totally satisfactory: the use of a solvent to which the detector is nearly insensitive; the placement of a four-port switching valve at the column exit for venting off the solvent; the evaporation of the solution on an inert powder for solid sampling; last, the use of an ingenious stratagem involving evaporation of the sample on metal boats or screens (*1*), their placement in an antechamber, and introduction into the injector compartment by means of an external magnet.

The frequent passage of large volumes of solvent through the column may cause, in time, serious contaminations resulting in high noise-to-signal ratio and loss of sensitivity. Solid sampling may lead to nonquantitative results. The evaporation of labile compounds on metal surfaces may cause decomposition and artifact formation. The introduction of powders or metal screens entails the periodic dismantling of the apparatus for cleaning or unloading the injector chamber.

A novel approach has been used to resolve these difficulties and greatly simplify the procedure. The dilute solution is injected as such and concentration takes place at the top of the column. The solvent and volatile impurities are vented out at the injection port. The labile, high-boiling compounds remain on the first 1 or 2 cm of packing and suffer no adverse conditions or loss. Furthermore, the column and detector remain free of the solvent and volatiles. An inexpensive, automatic solvent venting and carrier gas bleeding valve assembly connected to the injection system now permits the on-column concentration of trace, high-boiling, labile compounds for quantitative gas chromatographic analysis.

EXPERIMENTAL

A description of the solvent venting valve with injection port (Figure 1) and operating procedure are given below:

The side-arm (*A*) of a Barber-Colman glass U-shape column was equipped with a silicone rubber septum through which was inserted a $^1/_8$-inch tube made of Teflon (*B*) leading to an on-off valve actuated by a solenoid (*C*). The carrier gas line with solenoid and bleed valve (*D*) was rerouted through the injection septum (*E*) which was also equipped with the solenoid contact points (*F*). The column (*G*) was packed up to a distance of 6 cm from the side-arm and a 1 cm long glass-wool plug inserted. More packing was introduced to a length of 4 cm and a 1-cm glass-wool plug was inserted as a cap. Sample injections of 2.5 to 50 μl were made using a 100-μl Hamilton Syringe (*H*) with a 5-cm needle. The arrow at (*I*) shows the direction of flow of the carrier gas. On-off valves (*J*) and (*K*) remain shut for normal operation.

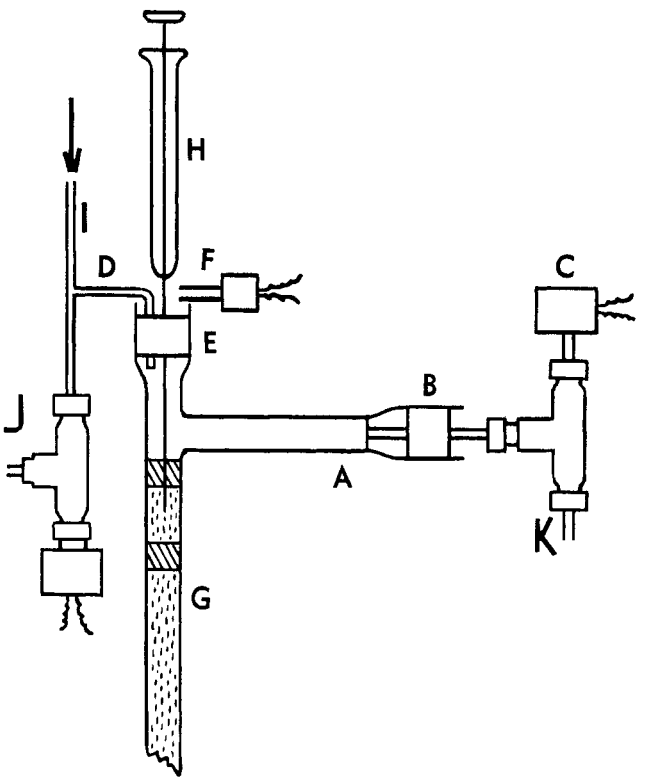

Figure 1. Solvent venting and carrier gas bleed valves with injection-port assembly

Gas chromatographic analysis was performed with a Barber-Colman Model 10 instrument equipped with tritium detector and 50-mv Brown-Honeywell recorder. The column was 6 ft × 4 mm i.d. packed with 1% SE-30 on Gas-Chrom Q (100–120 mesh). Injector, column, and detector temperatures were 310°, 215°, and 225°C, respectively. The carrier gas was argon at an inlet pressure of 30 psi. The detector voltage and electrometer settings were 1600 volts and 3×10^{-9} amp., respectively.

A standard solution containing 1.25×10^{-7} gram of cholestane and 2.17×10^{-7} gram of cholesterol per μl was prepared in hexane and then diluted 2, 4, and 20-fold with the solvent for increased injection volumes.

To operate, the solution was drawn in the syringe and measured, as usual, and the needle inserted through the septum to its full length, causing the solenoid contact points to meet and open the solvent venting valve (*K*) and the carrier gas bleed valve (*J*). The sample was injected into the 4 cm long packing at the top of the column. The syringe was

(1) E. Menini and J. K. Nerymberski, *Biochem. J.*, **95**, 1 (1965).

Table I. Comparative GLC Analyses of Cholestane–Cholesterol Mixtures at Different Dilutions and Injection Volumes Using the Solvent Venting Valve[a]

Solution	Dilution factor	Volume injected, μl	Solvent removed, %	Peak area in mm² Cholestane	Peak area in mm² Cholesterol	Area ratio[b]
I	1	2.5	0	728	870	1.20
II	2	5.0	50	780	956	1.22
III	4	10.0	75	1132	1267	1.11
IV	20	50.0	90	1188	1485	1.23

[a] Solution I was analyzed by GLC in the usual manner.
[b] Cholesterol/cholestane.

kept in place for 3 sec from the time of injection. Upon its withdrawal, the contacts opened thus closing the venting and bleeding valves and starting gas chromatography.

RESULTS AND DISCUSSION

When large volumes (5 to 50 μl) of hexane (bp 68° C) are injected into the packing at 310° C and the carrier gas sweeps through the injector chamber to the open valve, the instantly vaporized solvent takes the route of least resistance and is vented via the side-arm to the atmosphere. The high-boiling steroids (cholesterol bp 360° C) are dispersed in the packing and appear to be retained. Losses were found to be minimal or nil during valve openings of up to 5 sec. Table I lists the dilutions and injection volumes together with the peak areas and the corresponding cholesterol/cholestane ratios. Figure 2 is a scale replica of the actual GLC traces for solutions I and IV. Solution I was analyzed by GLC in the usual manner. The solvent venting valve was used in the analyses of solutions II to IV.

It is to be noted that, although no steroids were lost, the solvent was practically removed in toto. During valve openings of 3 sec, 50–90% of the solvent was vented off depending on the volume injected. The brief interruption of column flow caused but a slight pen deflection and return to the base line. Retention times of cholestane and cholesterol remained unchanged. For quantitative GLC analysis, it is imperative to use an internal standard whose behavior is closely related to the component(s) of interest. In this experiment, we purposely chose two compounds of different polarity: cholestane, a saturated hydrocarbon; and cholesterol, an unsaturated hydroxylated compound. The ratio of cholesterol/cholestane did not reveal a significant loss of cholestane during this experiment.

This technique also provides for the removal of some volatile impurities which are often found in biological extracts and which tend to obscure the trace components of interest. These impurities also tend to cause column and detector contamination, increasingly high noise levels, and shorter column life.

Preliminary experiments were also conducted with a precolumn in the following manner: The dilute sample was injected as previously but instead of venting off via the injector chamber, the solvent and volatiles were allowed to proceed downward, at a reduced flow rate, to a point one foot from the inlet where a venting port had been placed. Careful timing and adjustment of the parameters yielded equally good results.

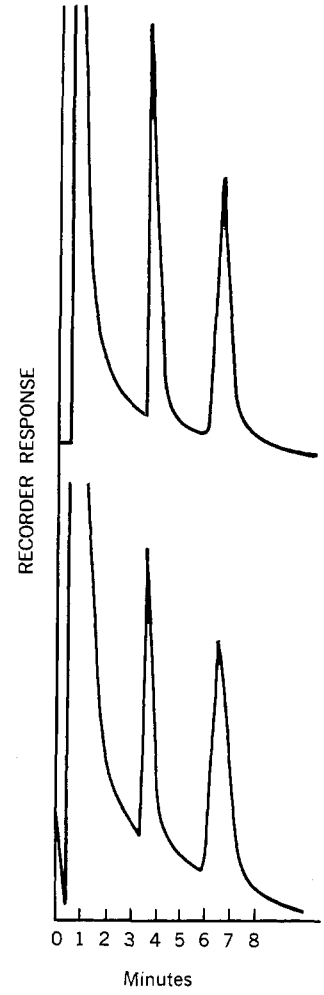

Figure 2. Gas chromatographic analysis of 2.5 μl of solution I in the usual manner (upper chart) and 50 μl of solution IV using the valve assembly (lower chart)

From left to right: hexane, cholestane, and cholesterol

It should also be noted that although the brief interruption of flow did not affect the argon ionization detector, it could cause difficulties with a flame detector. The conditions of analysis listed were found optimum for mixtures of cholesterol and cholestane and for like steroids. They may have to be changed to suit other situations.

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