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Splitless Injection on Capillary Columns, Part I. The Basic Technique; Steroid Analysis as an Example

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Abstract

A very simple procedure for splitless injection on capillary columns is discussed. In contrast to more sophisticated devices recently developed for the same purpose, the method described requires no additional equipment. The advantages of the method which broadens the use of capillary columns are discussed. Steroid analysis serves as an example of the applications.

Introduction

Injection on capillary columns without splitting has long been desired by practical chromatographers. Among the various reasons for this are the following:

Splitting may easily cause quantitative errors.

The refined equipment needed for proper splitting has large adsorptive surfaces and is difficult to keep clean.

Sample losses due to splitting are troublesome in the case of expensive or toxic samples.

Large volumes of dilute samples cannot be injected with splitting.

These and other reasons have led to considerable effort in developing injection techniques which would avoid splitting without affecting separation efficiency. Excellent results have been accomplished by workers at the Technical University of Eindhoven (1-3). The apparatus and methods described by these authors represent a further development of injection devices reported earlier (4,5). The latter consist in a trapping loop, i.e. a small capillary length, which is cooled during injection and, after introduction of the total sample, is quickly heated to start separation. While such devices work well, e.g., with dilute gaseous samples, difficulties are encountered with diluted solutions. Large amounts of solvent tend to plug the capillary when they are condensed in the chilled part.

We wish to demonstrate a much simpler method of splitless injection which provides maximum efficiency without additional equipment. We have found that a relatively moderately cooled column is itself a very efficient trapping system. If during injection the column temperature is held to at least 100° C below the boiling point of the most volatile sample component, and if after injection the column is slowly or rapidly heated to the normal analysis temperature, then full separation efficiency is observed even when the injection has been carried out very slowly or with interruption. This means that a substance boiling at 120° C may be ideally resolved after injection on a column at room temperature with subsequent heating to an analysis temperature of e.g., 80° C even when the well-known precautions of sample introduction on capillary columns have been neglected entirely.

The minimum difference between column temperature and boiling point of sample is strongly dependent upon the functionality of the sample as well as on the liquid phase.

The basic principle of this simple injection method was discussed by Rushneck (6) in 1965. Apparently, however, the author did not further study the broad applicability of the idea. A special application of the same principle was described by Lewins and Ikeda (7). Merrit, Walsh and coworkers (8), (with references to earlier work) gathered wide experience with injection on cold columns. Again, their purpose was not splitless injection but rather analysis of wide boiling mixtures.

In this paper we wish to present steroid analysis as an example for splitless injection on glass capillary columns. In a second part we shall discuss the detailed conditions and limits as well as more specialized applications of our injection method.

Experimental

We used a 20 m long and 0.26 mm diameter glass capillary pretreated for nonpolar coatings as described earlier (9). The liquid phase was silicone oil OV 101 with a film thickness of approximately .07 μ. Helium was the carrier gas flowing with an average velocity of

1. Cramers, C. A., and Van Kessel, M. M., J. Gas Chromatog. **6**, 577 (1968).
2. Groenendijk, H., and Van Kemenade, A. W. C., Chromatographia, **2**, 107 (1969).
3. Kuppens, P. S. H., Informal Symposium, GC-Discussion Group, London, March 1969.
4. Bartel, E. E., and Van der Walt, S. J., J. Gas Chromatog. **6**, 396 (1968).
5. Willis, D. E., Anal. Chem. **40**, 1597 (1968).
6. Rushneck, D. R., J. Gas Chromatog. **3**, 318 (1965).
7. Lewins, R. J., and Ikeda, R. M., J. Gas Chromatog. **6**, 331 (1968).
8. Merrit, C. Jr., Walsh, J. T., Forss, D. A., Angelini, P., and Swift, S. M., Anal. Chem. **36**, 1502 (1964).
9. Grob, K., Helvetica Chimica Acta **51**, 718 (1968).

60 cm per second, which corresponds to 1.6 ml per minute. The column was mounted in a Carlo Erba, (Milano) Model GI gas chromatograph with an all-glass system (Figure 1). The capillary starts inside a glass tube in which sample evaporation and splitting takes place. The tube is easily removed for inspection and cleaning. The outlet ends 1 mm below the 8 mm long quartz jet of the FID, which allows hydrogen to enter from the side, mix with the column stream, and flow to the flame. Thus the sample never contacts metal. Dead volume from column inlet to detector is practically zero. Vaporizer and detector can be heated above the temperature limit of the silicone rubber fittings since the latter are sufficiently distant from the heated parts as to be exposed to column temperature only.

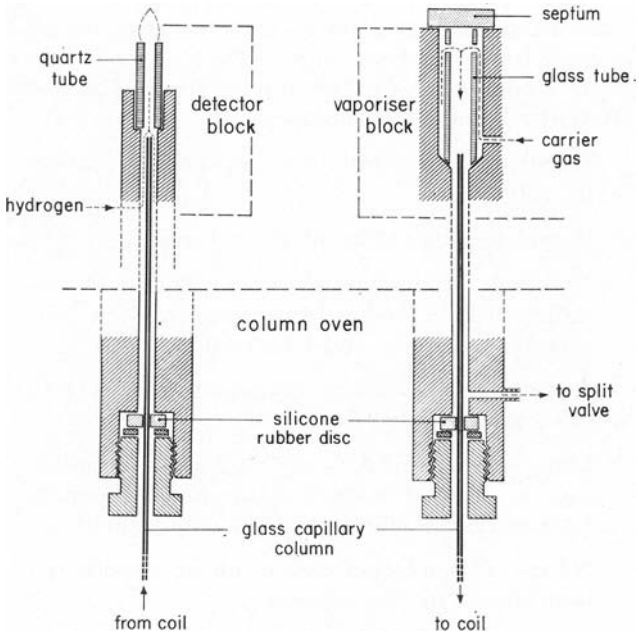

Figure 1. Diagram of column inlet and outlet system (not on scale).

The synthetic sample of 12 steroids-TMS was identical to that used in the work by Curtius and Müller (10). This sample as well as that extracted from urine was kindly supplied by the laboratory of Dr. Curtius. The individual steroids were present in a concentration of approximately .01%, the solvent being the silylating agent bis(trimethylsilyl)acetamide. For reproducible injection of small amounts of solution, we filled the syringe needle (Hamilton 701-N) with hexane, then introduced 0.3 μl of sample followed by 0.2 μl of air. During injection approximately 0.7 μl of liquid was evaporated whereby the known amount of sample was completely flushed out of the needle by the evaporated hexane. A similar injection procedure has recently been described by Kruppa (11).

The exact procedure leading to the chromatograms in Figure 2 was as follows:

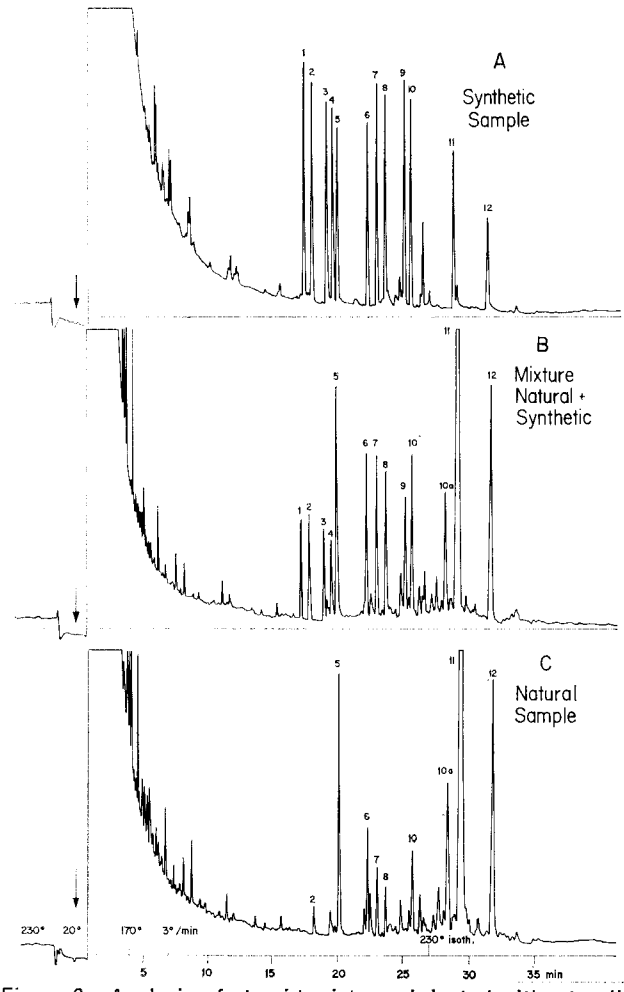

Figure 2. Analysis of steroid mixtures injected without splitting. Column: 20 m/.26 mm glass coated with OV 101. He flow 1.6 ml/min. FID. Samples and procedure see text.

1. Adjust base line at oven temperature 230° C, column flow 1.6 ml/min, split flow 20 ml/min.
2. Stop heating and open column oven to ambient air.
3. Close split valve.
4. When column has reached room temperature introduce sample as described in the foregoing paragraph.
5. 30 sec after injection open split valve to yield a side stream of 20 ml/min to clean up the injection port from back-diffused sample traces.
6. 60 sec after injection close column oven and heat rapidly to 170°C.
7. 7 min after injection start temperature program 3°/min from 170 - 230°C. (Isothermal period at 170°C depending on the need of separating solvent and its byproducts from sample peaks).

10. Curtius, H.-CH., and Müller, M., J. Chromatog, **32**, 222 (1968).
11. Kruppa, R. F., GC-Newsletters, Applied Science, Jan. 1969.

To obtain some tentative identification for the urine steroids we mixed the natural sample with the synthetic one (run B, Figure 2). Most of the urine peaks showed exact coincidence with peaks of the synthetic mixture.

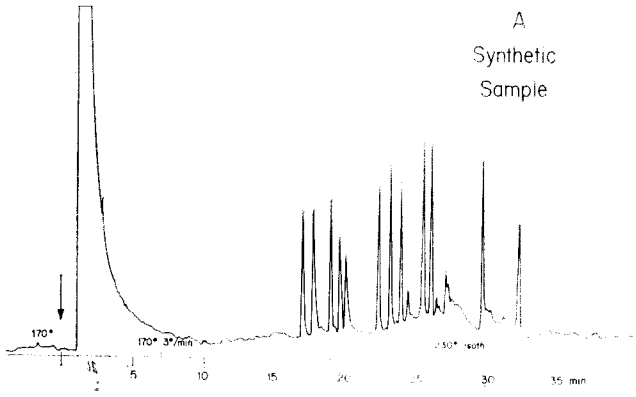

Figure 3. Sample and column as used for figure 2A. Ordinary injection with splitting at 170°.

The chromatogram shown in Figure 3 was obtained from the synthetic steroid mixture after ordinary injection with splitting at 170°C. Whereas for splitless injection the vaporizer temperature was 210°C, it was raised to 270°C for splitting to provide rapid evaporation. The somewhat poorer separation and the increased tailing (as compared with run A, Figure 2), is caused by minute impurities in the vaporizer tube. Traces of nonvolatile sample material or, more often, small particles of septum rubber strongly adsorb substances with low vapor pressure, such as steroids, and release them over a period sufficiently long to cause tailing. Cleaning the tube after every run reduces but normally does not eliminate the trouble. This is never observed after splitless injection since the sample is completely evaporated, even out of a dirty vaporizer tube, before separation starts. The poor baseline in Figure 3 is caused by septum bleed. It could not be overcome at our high sensitivity and high vaporizer temperature even after long continuous use. It can of course be made invisible by strictly using isothermal conditions.

Conclusions

The essential detail of our injection procedure is the temperature difference between the column and the boiling point of the most volatile sample component. During injection, the column temperature must be high enough to allow elution of the solvent and by-products or at least to prevent condensation. On the other hand, the temperature must be low enough to provide trapping of the most volatile sample component. Under well-adjusted conditions the procedure offers the following advantages:

Composition of the synthetic steroid mixture

Peak No.	1	Androsterone
	2	Etiocholanone
	3	Dehydroepiandorsterone
	4	11-Ketoandrosterone
	5	11-Ketoetiocholanone
	6	Pregnanolone
	7	11-Hydroxyandrosterone
	8	11-Hydroxyetiocholanone
	9	Allopregnanediol
	10	Pregnanediol
	11	Pregnanetriol
	12	Pregnanetriolone

Elimination of quantitative errors possible caused by splitting.

Complete elimination of sample losses.

No need for rapid evaporation. Thus, the vaporizer temperature is lower which leads to less sample breakdown and less septum bleed.

Analysis of very dilute samples without previous concentration.

Large amounts of air, e.g. head space samples, can be injected without harm to the column since oxygen is eluted from the cold column.

No loss of resolution due to adsorption-desorption effects in the vaporizer.

Very simple equipment with minimum adsorptive surface and easy cleaning instead of sophisticated devices, e.g. mixing chambers and linear splitters.

Whereas most of these advantages represent relative improvements of varying importance, the potential for analysis of very dilute gases or solutions opens a new use for capillary columns.

A drawback of our simple procedure resides in the impossibility of working isothermally. The effect is reduced by Groendijk's and Van Kemenade's (2) cold spot and heat sink, which does allow constant column temperature. According to our experience the corresponding injection device should, however, be much simplified since there is no need for a special design to include very high evaporation temperatures.

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