Direct sample introduction of high boiling compounds onto glass capillary columns. Comparison of manual and automatic sampling

Direct Sample Introduction of High Boiling Compounds Onto Glass Capillary Columns. Comparison of Manual and Automatic Sampling

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Summary

The performances of two systems for the direct introduction of high boiling compounds onto glass capillary columns are compared. Samples investigated consisted of hydrocarbons and steroids. The "Moving Needle" system (a manual version) developed in our laboratory is extensively described in the literature [1]. The standard deviation for quantitative measurements is approximately 2.5 % rel. Recently a Pye Auto Solids Injector has been modified so as to meet the requirements set by capillary gas chromatography. The very large dead volume of this sampling system, due to the use of sample holders prevents the direct coupling of this system to a capillary column. After introduction of a sample holder the solid sample evaporates slowly and the sample compounds are spread throughout a large volume of carrier gas, this badly affects the resolution obtainable. To overcome this problem the oven of the chromatograph contains a capillary pre-column (of some 50 cm length), which during a short period after sample introduction is cooled by a flow of air. During this cooling period the sample compounds are effectively trapped in the pre-column. After one minute the cooling medium is turned off, the pre-column rapidly heats in the column oven and a sharp reinjection of the sample occurs. A complete description of the system is given. The repeatability of quantitative measurements (σ_{rel}) is better than 1.5 %. There is no loss of resolution by using this sampling technique. Both systems described are compatible with isothermal and programmed operation. Also in both versions the solvent does not enter the capillary column to an appreciable extent.

Introduction

The high resolving power of capillary columns can only be exploited fully if quantitative sample introduction systems are developed. For many biochemical applications the use of stream splitters is prohibitive because of sample size requirements and nonlinearity of the splitting procedure for high boiling compounds. Several systems of direct sample introduction have been proposed [1–5]. *Van den Berg* of our laboratory [1] described an all glass "Moving Needle" system for the direct introduction of high boiling compounds onto capillary columns. With this system the standard deviation, σ_{rel}, for quantitative measurements was less than 2.7 % (for steroids and hydrocarbons).

Due to the very small dead volume of above-mentioned system the resolution of capillary columns is not affected.

Because of a demand for faster and more accurate analyses of an increasing number of samples automation of analytical methods is necessary. However, *Van den Berg*'s design does not lend itself to automation. Therefore, it was decided to adapt a Pye Auto Solids Injector to meet the requirements of capillary gas chromatography.

The principle of trapping and reinjection was used as already described by one of the authors [2] for volatile samples (manual method), *Rushneck* [3] and *Grob* [4, 5].

Description of the Adaptation of the Pye-Unicam Auto Solids Injector for Use with Glass Capillary Columns

The system was originally designed for the analysis of up to 35 solid samples on packed columns. It utilizes small, open ended, glass cylinders as sample holders into which solutions of the samples in a volatile solvent are intoduced after which the solvent is evaporated. After loading, the magazine with the sample holders is transferred to the injection system fitted to the chromatograph (See Fig. 1). Forward movement of the actuator(4) pushes the bottom sample holder in the magazine forward so that it drops into the position above the top of the column. Here the solid sample vaporizes and enters the column. The very large dead volume of this system prohibits its use with capillary columns. The sample components are spread throughout a large volume of carrier gas, this minimizes the resolution obtainable. To overcome this problem the oven of the chromatograph contains a capillary pre-column (length 50 cm, inside diameter 0.25 mm coated with SE-30), which during a selected period after sample introduction is cooled by a flow of air (from 240 °C to about 140 °C). During this cooling period the sample components are effectively trapped in the pre-column. Usually after one minute, the cooling is stopped, then the precolumn rapidly heats and a sharp reinjection of the sample occurs.

At the same time solenoid 6 is energized and ejects the empty sample holder into the reservoir mounted on the chromatograph.

Then the injection actuator (4) is forced backwards thus allowing the next sample holder to position for injection.

The sequence of events described above (including the actuating of a magnetic valve for the air cooling) is controlled from a cam timer fitted to the control unit of the Auto Solids Injector.

The connections (of very small dead volume) between the solid injector, pre-column, column and detector are made with teflon shrinking tubing.

In the version described (air as the cooling medium) and minimum temperature of the precolumn 140 °C, it should only be used for components less volatile than n-C20. In the Auto Solids Injection System the samples are kept under nitrogen (carrier gas) to avoid decomposition by contact with air. Also storage for longer periods prohibits the use of more volatile samples. The system with glass sample holders provides a convenient means of sample concentration when dealing with dilute solutions of involatiles in volatile solvents.

Chromatographic Conditions

The gas chromatograph used was a Pye Unicam Model 104 equipped with both the Auto Solids Injector and the all glass sampling system as described by *Van den Berg* [1]. Flame ionisation detection was used in all experiments. For the evaluation of both sampling devices a glass capillary column, 15 m X 0.25 mm coated with SE-30 was used. The column temperature was kept at 240 °C during all experiments. The injection region of the Auto Injector (near item 5 in Fig. 1) was maintained at 270 °C.

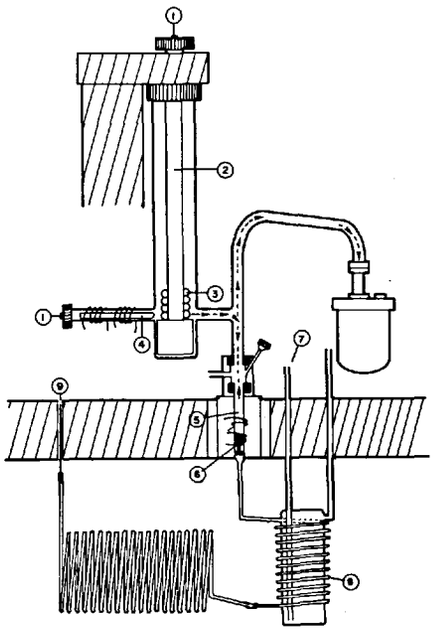

Fig. 1

- Adaptation of Pye Auto Solids Injector for direct sample introduction of high boiling compounds onto glass capillary columns.
  (1) sinter bleed, (2) magazine, (3) sample holders, (4) inject actuator, (5) injection heater, (6) eject actuator, (7) air flow, (8) capillary pre-column, (9) FID.

The standard mixtures of hydrocarbons and TMS-derivatives of steroids, as introduced onto the column, contain approximately 50 ng per component.

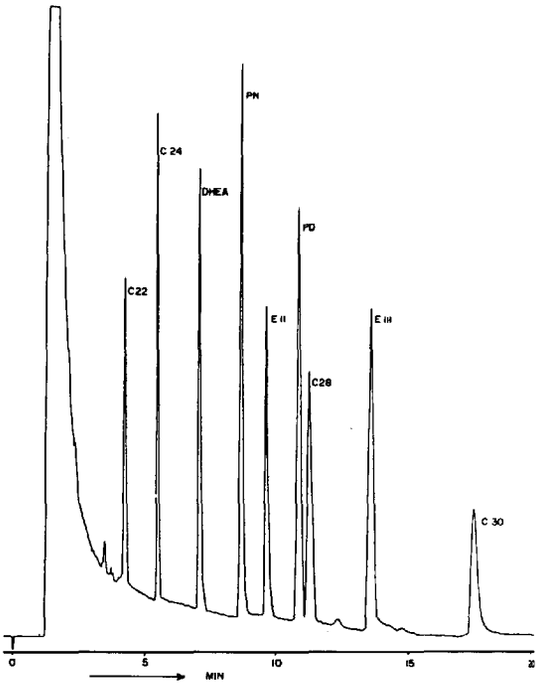

Fig. 2

- Chromatogram of some steroids and hydrocarbons. Using adapted Auto Injector (approximately 50 ng per component)
  Column: 15 m X 0.25 mm; SE-30.

  1 = n-C22 2 = n-C24 3 = DHEA = dehydroepiandrosterone
  4 = PN = pregnanolone 5 = EII = estradiol 7 = n-C28
  8 = EIII = estriol 9 = n-C30

Comparison of the Manual and Automatic Sampling Systems. ("Moving Needle" versus adapted Pye Solid-Sampler).

The same standard mixture of steroids (TMS-derivatives) and hydrocarbons was used in all experiments to avoid differences in composition due to, for example, derivative formation. The remaining solvent peak originates from the reagents used for derivative formation.

a. Qualitative Analysis

Retention indices for steroids obtained with both systems are in agreement within 1.5 index units. (Standard deviation on both systems 1.5 index units). No significant differences are observed.

b. Resolution

No significant differences in peak width at half height are found using both sample introduction systems. As pointed out already in reference [1] the loss in resolving power due to the sampling procedure calculated for n-C28 is smaller than 1 % for the manual version.

The peak shape is better using the automatic sample introduction (less tailing).

Table I.

Compound	Composition % w/w	"Moving Needle" Mean of 15 runs	σ_{rel} %	Automatic Injector Mean of 15 runs	σ_{rel} %
n-C20	16.6	17.8	2.7	16.3	1.4
n-C22	22.2	22.2	2.3	22.2	1.5
n-C24	28.0	28.0	2.4	28.6	1.1
n-C28	33.2	32.0	1.9	32.9	1.2

Table II.

Compound			"Moving Needle" Mean of 15 runs	σ_{rel} %	Automatic Injector Mean of 15 runs	σ_{rel} %
DHEA	=	Dehydroepi-androsterone	11.6	2.9	11.4	1.8
PN	=	Pregnanolone	22.8	2.3	22.1	1.3
EII	=	Estradiol	14.8	2.6	14.8	1.8
PD	=	Pregnanediol	25.7	2.8	25.4	1.5
EIII	=	Estriol	25.1	3.1	26.3	1.2

c. Quantitative Analysis

The repeatability and reproducibility of the Auto Injector and the manual system were tested on the basis of peak areas (Infotronics type CRS-208). Table I shows the results obtained from 15 runs of n-alkanes (about 50 ng per component).

The performance of both systems was also tested with TMS derivatives of steroids (see Table II).

Here also about 50 ng per component was introduced. The number of runs for steroids was also 15. Uncertainties in flame factors as well in completeness of derivative formation made the use of calibration samples of known quantitative composition impossible.

Conclusions

Apart from the obvious advantage of automatic analysis, the adapted version of the Pye Auto Solids Injector should be preferred because of better peak shape and better repeatability and reproducibility.

Both systems are compatible with isothermal and programmed analysis and the solvent does not enter the capillary column to an appreciable extent.

References

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