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TOBACCO CHEMISTRY*

II. ANALYSIS OF THE GAS PHASE OF TOBACCO SMOKE BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

The gas phase of tobacco smoke was injected, without prior condensation, through a glass injection system onto an efficient glass capillary column maintained at low temperature. Components eluted on temperature programming were studied by means of a mass spectrometer coupled to the column via a low-dead-volume molecule separator. By making use of the separation principle of the phase, thirty-four compounds were identified from their mass spectra.

INTRODUCTION

The gas phase of tobacco smoke is so complex[2–4] that currently the only effective method for the analysis of this material is gas chromatography using highly efficient columns[5–8]. GROB has developed a refined technique for the use of glass capillary columns in the separation of the components of the condensed gas phase[9–15] and in their identification by mass spectrometry[16,17], but a number of problems remain. For example, injection of fresh smoke is preferred[12], since changes may occur during the trapping process, requiring the introduction of large volumes of a diluted gas sample onto a capillary column without danger of serious loss of separating power. Moreover, it has been pointed out[12,13] that decomposition of some smoke constituents is likely if contact with a metal surface is allowed.

Interfacing of capillary columns with a mass spectrometer also presents some problems, particularly if a sample-enrichment device is used to meet sensitivity requirements. Difficulties caused by dead volume in the connection of capillary columns to a mass spectrometer via a molecule separator have also been experienced in the analysis of tobacco smoke[16,17].

The purpose of this work was to overcome the above-mentioned difficulties in analysing the gas phase of tobacco smoke. The problem of on-column concentration

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of a large smoke sample was solved by use of a low-temperature injection method[7,18]. In order to achieve a fairly inert analytical system, a glass injection device and efficient glass capillary columns were used. A mass spectrometer was coupled to the capillary column[19] through a low-dead-volume molecule separator[20], and the spectra of some of the separated components were recorded by means of a novel data-acquisition system[21].

EXPERIMENTAL

Capillary column gas chromatography

Glass capillaries were prepared on a commercial glass drawing machine (Dr. Hupe Apparatebau, Karlsruhe, G.F.R.). The original pyrex glass tubes were carefully washed with acetone and then dried. The inner surfaces of the glass capillaries were silanised with mixed vapours of hexamethyldisilazane and trimethylchlorosilane[22]. Their coating was carried out with a 10 % solution of Silicone Oil SF-96 in toluene by the dynamic method under controlled conditions[23]. Capillary columns, 48 and 120 m long with an I.D. of 0.2 mm, were used for the analysis of tobacco smoke; they had efficiencies, measured for toluene at room temperature, of 160,000 and 530,000 theoretical plates, respectively.

The all-glass injection splitter illustrated in Fig. 1 was used for mass spectrometry work. The glass-to-metal connections were made with the aid of Kovar alloy.

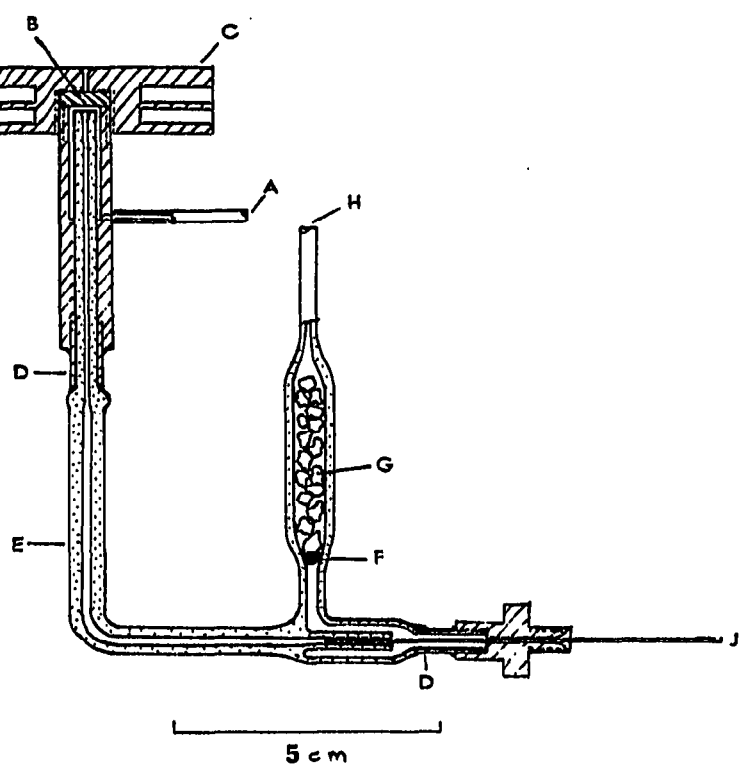


Fig. 1. Glass injection splitter. A = inlet for preheated carrier gas; B = septum; C = flanged nut for septum; D = Kovar alloy; E = glass tube; F = glasswool plug; G = charcoal; H = outlet to control valve; J = capillary column.

The inner glass tube was extended to the septum insert, and the end of the capillary was inserted directly into the mixing chamber so that contact of sample with metal was avoided. The buffer volume of the outlet tubing was filled with charcoal to prevent back diffusion and to protect the regulating valve.

A system which facilitates rapid heating was designed for efficient temperature programming of glass capillary columns. The small oven, shown in Fig. 2, consists of two complementary Marinite parts lined inside with a thin aluminium sheet. The glass capillary column is hooked onto the supports and connected to the splitter, situated in the same block, and the adapter of the flame ionisation detector. This adapter is interchangeable with another one for connection to the mass spectrometer. The column is heated with the aid of a concentrically arranged resistance spiral and an F and M Model 240 temperature programmer. Thermal gradients are minimised by the use of a small fan. The system is cooled by admitting an adjustable stream of liquid carbon dioxide through a jet[24]. The course of heating and cooling is followed with an extra thermocouple.

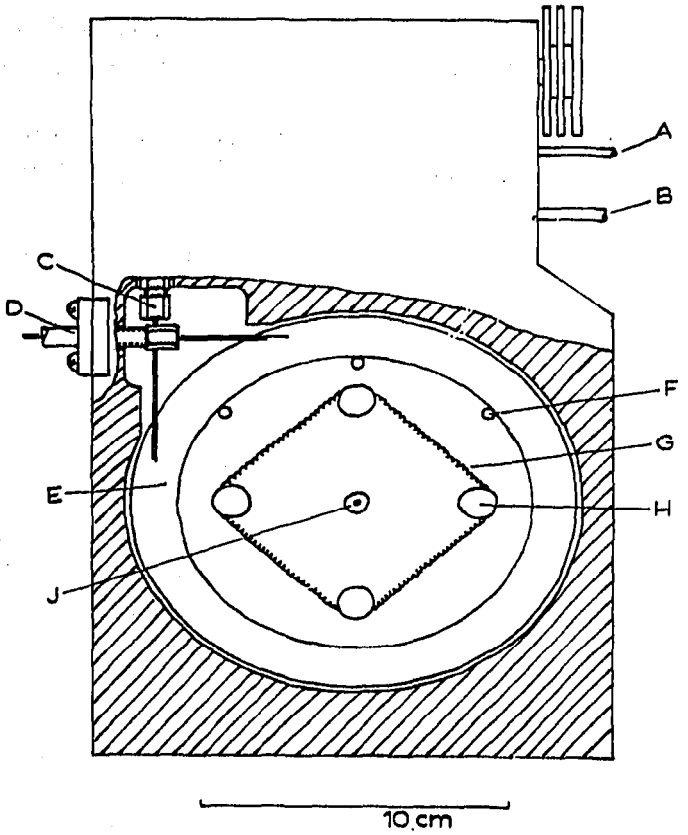


Fig. 2. Oven for capillary column chromatography. A = inlet for carrier gas; B = outlet to control valve; C = connection to injection splitter; D = adapter for FID-detector or connection to the mass spectrometer; E = glass capillary column bundle; F = support for capillary column; G = resistance spiral; H = support for resistance spiral; J = jet nozzle for liquid carbon dioxide.

Low-temperature injection

Commercial American blend cigarettes without filter tips were smoked in 30-ml puffs through a Cambridge filter by means of a glass syringe operated manually. Between 1 and 10-ml (usually 5-ml) samples of the gas phase were withdrawn from

this large syringe via a second glass syringe for injection into the column which had been cooled to −70°. The oven temperature was then programmed up to 130°. This enrichment method is essentially that proposed by RUSHNECK[7], but we found that a small splitting ratio was required to attain good resolution.

Problems concerning enrichment were experienced when the 120-m column was used, owing to the high inlet pressure necessary and the low ability of conventional silicone rubber seals to withhold gases at low temperature.

Coupling of the capillary column to the mass spectrometer

Capillary columns were connected to the LKB 9000 mass spectrometer through the Becker–Ryhage molecule separator. The performance of this separator in connection with capillary columns has already been studied by one of us[19], and it has been shown that only the second stage should be used, but with the jet nozzle having an I.D. of 0.24 mm replaced by the one from the first stage which has an I.D. of 0.10 mm.

Mass spectrometry

The mass spectrometric parameters were adjusted in order to fit the gas chromatographic conditions so that the slit widths were 0.20 and 0.30 mm, respectively, and the scan speed had a value corresponding to *m/e* 12–200 in 2 sec. Sensitivity and scan speed were consequently increased at the expense of resolution, which was 250 (10% valley definition).

The ion source temperature was 230°, the trap current 60 μA, and the electron energy 70 eV during all runs. The recording of mass spectra was made by means of the data acquisition system described recently by two of us[21]. After some reduction of data, the background was subtracted, and normalized mass spectra were plotted and printed out separately.

RESULTS AND DISCUSSION

It is generally recognised that the high efficiency of capillary columns, conveniently combined with temperature programming, is the first presupposition in the gas chromatographic separation of a complex mixture containing components with a wide range of boiling points. We have selected Silicone Oil SF-96 as a stationary phase owing to its ability to work over a wide temperature range[24] and to form very effective films on the inner wall of glass capillaries.

First attempts to obtain a good separation of the gas phase of tobacco smoke failed when injecting the sample onto an efficient capillary column at room temperature using a low splitting ratio (Fig. 3), and the need for a technique facilitating sample enrichment was realized. A simple and efficient method is apparently the low-temperature process proposed by RUSHNECK[7]. When the smoke sample was injected onto the same capillary column cooled to −70° and when the column was programmed from −70° to 130°, a considerable improvement was achieved, as can be seen from Fig. 4. The use of this technique is advantageous not only in increasing the possibility of separating the early peaks at low temperature but mainly in efficiently concentrating the sample components at the beginning of the column; no marked change was noted even when two smoke injections separated by a 15-sec interval were made.

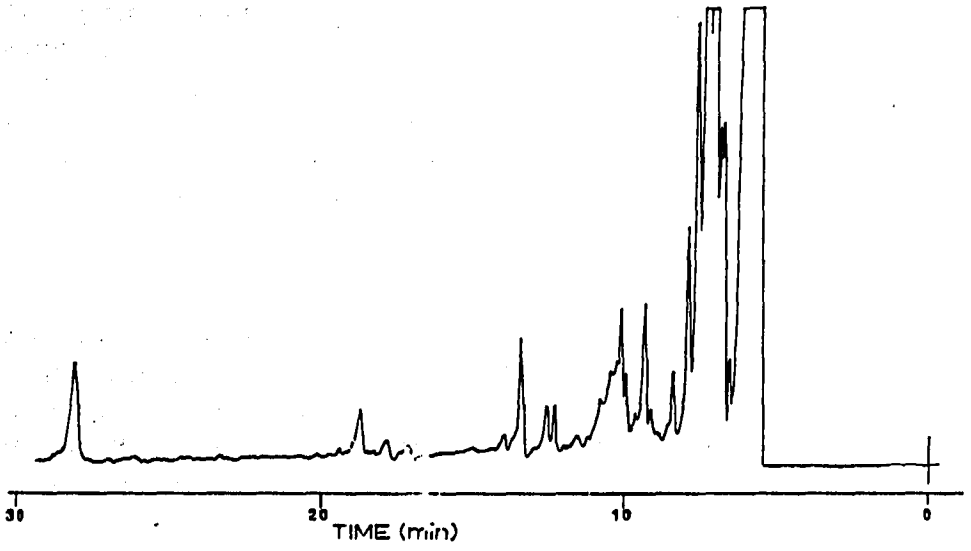


Fig. 3. FID chromatogram of 2 ml of tobacco smoke, split 200:1. Column: 48 m × 0.2 mm I.D. glass capillary, coated with SF-96, operated isothermally at 25°. N_2 flow rate, 0.3 ml/min.

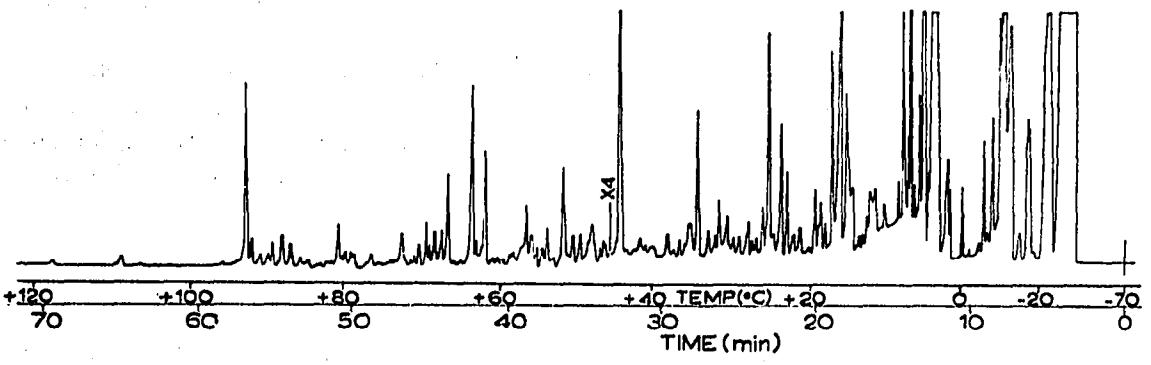


Fig. 4. FID chromatogram of 6 ml of tobacco smoke, split 10:1. Column as in Fig. 3. Initial column temperature, −70°. N_2 flow rate at 25°, 0.3 ml/min.

The broadness of the first peaks in the chromatograms with low-temperature injection may be attributable partly to the behaviour of the stationary phase at low temperature, as has been studied in detail by ALTENAU *et al.*[25] and CLAEYS AND FREUND[26], as well as to the overlap of components. The marked increase in the response of the flame ionisation detector for a column at low temperature, also observed in this work, has been discussed by SINGELTON *et al.*[27] and WALSH *et al.*[28].

For the separation and identification of some of the components of tobacco smoke, the 120-m capillary column was used. The chromatogram obtained with the aid of the total ion current (TIC) monitor of the mass spectrometer can be seen in Fig. 5, and some of the identified substances are listed in Table I. The mass spectra were of adequate analytical quality, but more enrichment is necessary for the trace components. The convenience of the method as a whole is exemplified by the identification of the four minor components 16–19 which are all eluted within a period of 40 sec. Fig. 6 shows the spectrum of a small peak (number 7), identified as isobutane,

TABLE I

COMPONENTS IDENTIFIED BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY IN CIGARETTE SMOKE

Peak No.[a]	*Substance*	*Peak No.*	*Substance*
1	CH_4, H_2S, NO	18	1,2-Dimethylcyclopropane (*trans*)
2	CO_2, CO, NO_2	19	*n*-Pentane
3	Ethane	20	2-Methyl-1,3-butadiene
4	Propene	21	CS_2 + 1,2-dimethylcyclopropane (*cis*)
5	Propane	22	2-Methyl-2-butene
6	Methyl chloride	23	Cyclopentadiene
7	Isobutane	24	2-Propanone
8	Ethanal	25	Acetonitrile + components not yet identified
9	1-Butene	26	Benzene
10	1,3-Butadiene	27	2,5-Dimethylfuran
11	*n*-Butane	28	Toluene
12	2-Butene (*trans*)	29	Ethylbenzene
13	2-Butene (*cis*)	30	Methyloctene
14	1,1-Dimethylcyclopropane	31	*m*-Xylene + *p*-xylene
15	2-Methylbutane	32	*o*-Xylene
16	1-Pentene	33	Heptyl chloride
17	Furan	34	1-Methyl-4-isopropenyl-1-cyclohexene

[a] See Fig. 5.

while Fig. 7 (peak 18) apparently corresponds to *trans*-1,2-dimethylcyclopropane, which to our knowledge has not been identified in tobacco smoke previously.

It has been pointed out[16,29] that distortion of mass spectra may occur in GC–MS work because of the change in concentration during scanning. In order to study any possible bias in our mass spectra, toluene was injected and its mass spectrum recorded at different points on the elution curve (see Fig. 8). The ratio of the ion intensities

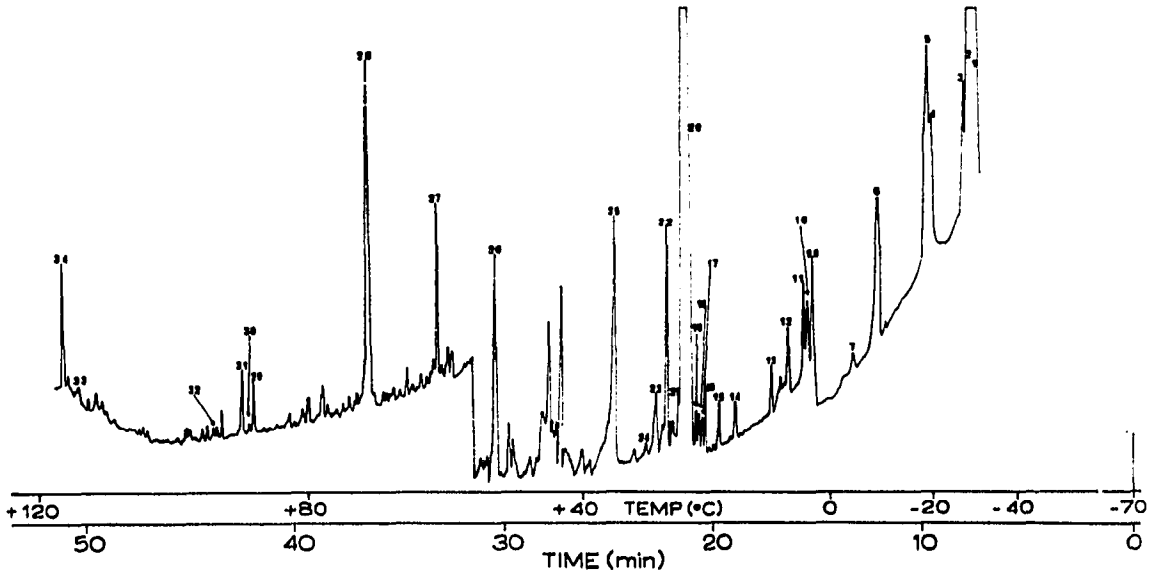
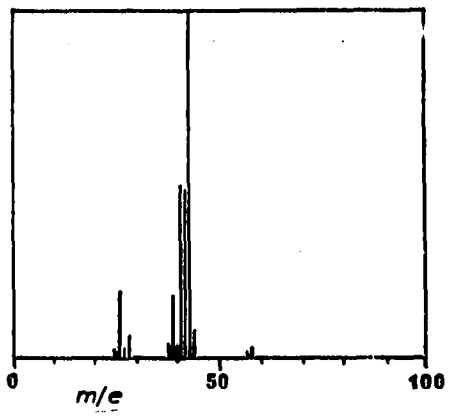


Fig. 5. TIC chromatogram of 5 ml of tobacco smoke split approximately 1:1. Column 120 m × 0.2 mm I.D. glass capillary coated with SF-96. Initial column temperature, −70°. He flow rate at 25°, 0.4 ml/min. Peak numbers refer to Table I.



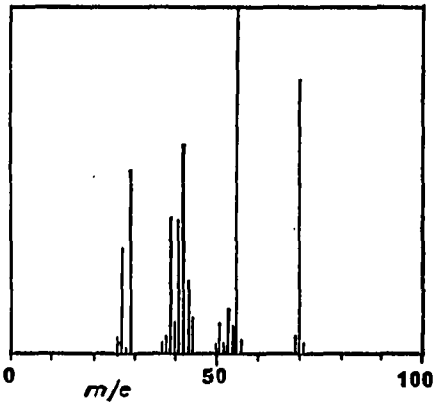


Fig. 6. Mass spectrum of peak 7 after background subtraction and normalisation.

Fig. 7. Mass spectrum of peak 18 after background subtraction and normalisation.

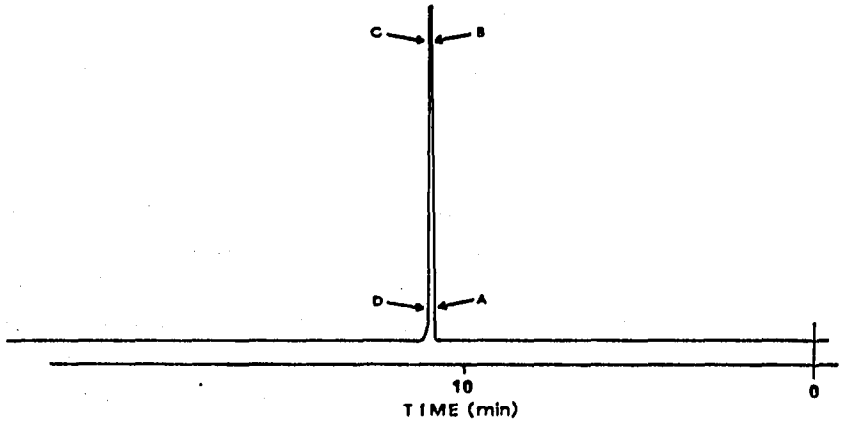


Fig. 8. TIC elution curve for toluene showing points at which mass spectra (Table II) were recorded.

at *m/e* 39 and 91 was taken as a measure of spectrum bias, and it was concluded that the scan speed used in our experiments was sufficient (Table II).

The use of a highly efficient column with a non-selective phase seems appropriate, since some closely related isomers which give similar mass spectra can be distinguished chromatographically and their structures confirmed when the separation principle

TABLE II

RATIO OF ION INTENSITIES IN MASS SPECTRUM OF TOLUENE AT DIFFERENT POINTS ON GC ELUTION CURVE

Points on the elution curve[a]	*Ratio of ion intensities at m/e 39 and 91*
A	0.08
B	0.15
C	0.12
D	0.10
Published spectrum	0.13–0.20

[a] See Fig. 8.

is known. For example, the *cis*- and *trans*-isomers of 2-butene, which give almost identical mass spectra, were differentiated in this way, while *o*-xylene was similarly distinguished from the *meta*- and *para*-isomers. On the other hand, some fractions overlap even when a capillary column with efficiency as high as 530,000 theoretical plates is used. For a more complete analysis, complementary use of an efficient polar capillary column is clearly necessary.

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