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SEPARATION OF FRESH TOBACCO SMOKE ON A PACKED POLAR GAS CHROMATOGRAPHIC COLUMN PRIOR TO ON-LINE ANALYSIS BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY USING A NON-POLAR CAPILLARY COLUMN

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SUMMARY

Gaseous samples of fresh tobacco smoke were injected on to a packed polar column (2,2-oxydipropionitrile) in an all-glass chromatographic system. With the aid of a warm syringe, selected fractions were withdrawn from the bottom of the electron capture detector of the packed column for further injection into an efficient non-polar glass capillary column (SF-96), connectable on-line to a mass spectrometer.

The method has permitted the separation and identification of some polar and non-polar components of tobacco smoke, which gave previously mixed, broad, tailing peaks when the smoke was injected directly into a non-polar capillary column.

INTRODUCTION

A number of investigators[1-4] have demonstrated that the gas phase of tobacco smoke is a complex mixture containing volatile components with a wide range of polarities and volatilities. Gas chromatography is assumed to have the resolving capacity for such a complex mixture, provided that highly efficient capillary columns are used. At present, mass spectrometry is considered to be the only on-line identification technique that has a separating capacity similar to that of capillary column gas chromatography. However, without taking specific measures, less volatile, highly adsorbent or reactive compounds, in forms such as physiological active ions and radicals, are not likely to be detected by this combined analytical technique. Moreover, the possible increase in the number of organic components at lower concentrations forms a general limitation for any tracer study, a matter which, as a "tracer cosmos" concept, has been discussed by one of the present authors[5].

In this laboratory, the non-polar stationary phase SF-96 was chosen for the analysis of tobacco volatiles on long glass capillary columns and a routine procedure was developed for loading silanized glass capillaries with it. Using toluene as a typical test substance, theoretical plate numbers up to 10^6 were recorded for 150-m columns. Most of the columns acceptable for tobacco smoke analysis were found to be stable and to last for more than a 100 analyses, even when the columns were sprayed with

liquid nitrogen during each injection cycle and then temperature programmed up to 130°.

An obvious drawback to the use of non-polar capillary columns is that polar components tend to appear as broad, tailing peaks, sometimes only seen as an elevation of the baseline, on which non-polar components of the tobacco smoke appear as needle-sharp peaks. This reduction in the chromatographic intensity results in insufficient utilization of the material in the mass spectrometer. Thus, polar components on non-polar capillary columns are usually suitable for identification studies only at high concentrations. Also, mass spectrometric investigations of efficiently separated non-polar compounds may be considerably hindered by a few intense fragments of tailing compounds.

In this investigation, we have attempted to solve the typical polarity problems mentioned by pre-separation of the fresh tobacco smoke on a long, packed polar column, withdrawing selected gaseous fractions with a heated syringe, and then injecting each of these fractions into the non-polar capillary column for on-line mass spectrometric investigations. This transfer technique was found to give yields better than 70%, with a typical tobacco smoke sample such as that represented by the gas chromatogram in Fig. 1. Only fresh, uncondensed samples of the smoke, and subsequently very mild conditions for handling the sample during separation, were used, thus hopefully avoiding possible secondary reactions[6,7].

EXPERIMENTAL

Injection of tobacco smoke

Smoking through a Cambridge filter at room temperature of a standard cigarette (research cigarettes, Code 1R1, University of Kentucky) was accomplished with an all-glass 50-ml Hamilton syringe in accordance with the method described earlier[3]. The first two puffs were discarded, and the 100 ml of smoke used for analysis consisted of two 50-ml puffs each lasting 2 sec and taken with a 1-min interval. As each 50-ml sample was injected into the long packed column within less than 1 min, with the risk of back-flush into the carrier-gas line, a PTFE stop-cock, fitted on the carrier gas connection to the injection part, was closed during the whole period of the two injections including that of evaporation of the lowest boiling part of the sample.

Before injection, the carrier gas pressure was temporarily reduced to 0.5 kg/cm² in order to facilitate the injection and also the condensation of the volatile organics of the sample in the outer U-shaped first loop of the column, which was then kept at liquid nitrogen temperature. Three minutes after injection of the sample, the liquid nitrogen bath was replaced with an ethanol–dry-ice bath, which gave rise to a violent and sudden increase of pressure in the column that only carefully packed columns could withstand. Two minutes later, the stop-cock to the carrier gas was opened, and the pressure regulated to 2.0 kg/cm², corresponding to a flow-rate of 10 ml/min. After a further 5 min, the most volatile compounds had eluted, e.g., ethane, hydrogen sulphide and chloroethane, and the ethanol–dry-ice bath was replaced with an oven (80°).

Gas chromatograph

The gas chromatograph was constructed in this laboratory with the intention

that the smoke should not come into contact with any metal other than that of the needles of the syringes, the splitter of the detector and the Kovar tubing of the electron capture detector. The column (length 3 m, I.D. 1.5 mm, O.D. 3.0 mm; spiralled in 100-mm O.D. coils), the U-shaped loop for condensation, the injection port and the tubes leading into the detector and the carrier gas regulator were all made in one piece; gaskets were made of PTFE. The column and the loop for condensation were rinsed and silanized before filling with 3% 2,2-oxydipropionitrile on Chromosorb W, 80–100 mesh, acid washed and silanized in the laboratory. Throughout the investigation, the column was maintained at 55° as it was placed tightly over a heavy walled aluminium tube, forming the fan-operated thermostat of the chromatograph.

Two detectors were used simultaneously, a flame ionization detector (FID) and an electron capture detector (ECD), both obtained from Varian (Palo Alto, Calif., U.S.A.) but modified in this laboratory to accept the column tubings within the detectors and sealed with PTFE to the quartz tip of the FID and the Kovar tubing of the ECD. By means of a two-way split, 20% of the carriergas flow was usually led to the FID and the remainder to the ECD. When attempting to analyze low-concentration samples, all of the exit gas was led through the ECD, which was found to give acceptable guidance for the withdrawal of selected fractions of tobacco smoke.

To the top of the Kovar tubing of the ECD was attached a PTFE tube (length 30 mm, I.D. 2 mm). The needle of the warm (60°) gas-tight 10-ml (in some instances 5-ml) syringe fitted loosely into this tubing, which thus served as a needle guide for the manually operated syringe. When, as indicated by the chromatograms (ECD and/or FID), a fraction was to be collected, usually of 2–5 ml, a syringe with attached needle was removed from a clean oven and the needle was introduced a distance of 40 ± 5 mm into the ECD. Aided by a stop-watch, a trained operator could draw the plunger at an even rate, corresponding to the flow-rate of the carrier gas, and thus not disturb the chromatograms.

The withdrawn fraction was immediately injected into the capillary column gas chromatograph, the oven of which was then cooled to −70° by spraying liquid nitrogen over the column. After injection, the temperature was increased at the rate of 2°/min to 130°. In some instances, the splitter was closed during the injection, and then the splitting facilities were utilized according to Grob and Grob[8] to vent the injection compartment, after which the sample was condensed in the first part of the column.

The capillary column gas chromatograph has been described earlier[2,3], but a few modifications were made. In the injection splitter, a mixing chamber for sample and carrier gas was added. The column oven was fitted with a more effective fan that was found to give better linearity on temperature programming. The glass capillary column (length 150 m, I.D. 0.22 mm) used throughout this investigation was coated with SF-96, and was found to have an efficiency of 950,000 theoretical plates under isothermal conditions (room temperature) with injection of a liquid sample of toluene and using an FID.

For identification studies, the column was connected to an LKB mass spectrometer through a one-step molecule separator[9]. The end of the capillary column was then placed carefully concentrically with and close to the second jet of the LKB separator.

TABLE I

COMPONENTS FOUND AND IDENTIFIED IN FRACTIONS OF CIGARETTE SMOKE

Prior to the injection into the capillary column of the GC–MS instrument, pre-separation was performed on a packed polar column gas chromatograph.

Fraction No. (cf., Fig. 2)	Sampling interval (sec from start of analysis)	Component	Peak No. in Fig. 1	Reference
1	144–156	1-Butene-3-yne	2	10
		1,2-Butadiene	3	10
		2-Methylbutane	4	3, 4, 10
		1-Pentene	5	3, 4, 10
		n-Pentane	6	3, 4, 10
		2-Methyl-1,3-butadiene	7	3, 4, 10
2	420–460	Chloromethane	1	3, 4, 10
		1,3-Butadiene	2	3, 4, 10
		Ethanal	3	3, 4, 10
		1-Hexene	11	3, 4, 10
		n-Hexane	13	3, 4, 10
		3-Methyl-2-pentene	16	10
		3,3-Dimethyl-1-pentene	19	
		1-Heptene	24	
		n-Heptane	25	10
		trans-2-Heptene	26	
		Dimethyl disulphide	27	10
		2-Methylheptane	29	
		1-Octene	31	
		n-Octane	32	10
3	456–486	Propanal	8	10
		2-Propanone	9	3, 4, 10
		2-Methylfuran	12	3, 4, 10
		3,5-Hexadien-1-yne	15	3, 4
		Benzene	17	3, 4, 10
4	480–492	Propenal	10	3, 10
		Ethyl acetate	10	10
		2-Methylpropanal	10	10
		2-Methylfuran	12	3, 4, 10
		Benzene	17	3, 4, 10
5	512–524	2-Butanone	14	3, 4, 10
		Benzene	17	3, 4, 10
		Thiophene	18	3, 10
		3-Methylbutanal	19	10
		2-Methylbutanal	20	10
		Toluene	28	3, 4, 10
6	516–558	2-Butanone	14	3, 4, 10
		3-Methylbutanal	19	10
		2-Methylbutanal	20	10
		3-Methyl-2-butanone	21	10
		3-Methyl-3-buten-2-one	22	4, 10
		3-Pentanone	23	10
		Toluene	28	3, 4, 10

TABLE I *(continued)*

Fraction No. *(cf., Fig. 2)*	Sampling interval *(sec from start of analysis)*	Component	Peak No. in Fig. 1	Reference
7	558–588	Acetonitrile	14	10
		2,3-Butanedione	17	3, 10
		Toluene	28	3, 10
8	550–580	2,3-Butanedione	17	3, 10
		3-Methyl-3-buten-2-one	22	4, 10
		2-Pentanone	23	10
9	578–608	Acetonitrile	14	10
		Propionitrile	22	10
		2-Butenal	25	3, 10
		n-Propylbenzene	34	
10	641–671	1-Butyronitrile	24	10
		Styrene	33	3, 4, 10
		Allylbenzene	35	
11	720–732	1-Methylpyrrole	30	3, 4, 10
		tert.-Butylbenzene	36	3, 4
12	1128–1140	5-Hexen-2-one	33	

RESULTS AND DISCUSSION

Components identified in tobacco smoke are listed in Table I and gas chromatograms are shown in Figs. 1–6. Although having been produced by a glass capillary column with an efficiency of almost 10^6 theoretical plates, the gas chromatogram in Fig. 1 bears some obvious signs of poor separation of the fresh sample of tobacco smoke injected directly into that column. Among and mixed with the many needlesharp peaks there are a few apparently deformed peaks, numbered I–V. In the same gas chromatogram, the peaks denoted by Arabic numerals (1–36) are those for which acceptable mass spectra were obtained on a re-injected sample which corresponded satisfactorily to available literature data[11,12] or to those of standard samples. A few compounds listed in Table I have not been reported previously in the literature on tobacco. Quantitative aspects are not dealt with in this paper.

Owing to the polar components in the smoke, peaks I–V are so broad and tailing that even doubling the number of plates of this non-polar column (SF-96) would not be likely to improve the analytical usefulness of the chromatograms. Only further deactivation of the capillary column would do so, but applying the manual method of using a polar column for pre-separation described here, it was demonstrated that typical mixed peaks can be resolved. Thus, fraction 1 (Table I) of the pre-column is freed from the polar components chloromethane and ethanal (see Fig. 4) compared with the directly injected sample (see Fig. 1). The two polar compounds are then found in fraction 2, where they cause far less interference in the mass spectrometric identification studies. In the capillary gas chromatogram of fraction 3 (Fig. 3), the broad peaks II and III of the polar compounds propanal and 2-propanone are separated fully from peaks 3, 4 and 5. In the original chromatogram (Fig. 1), these
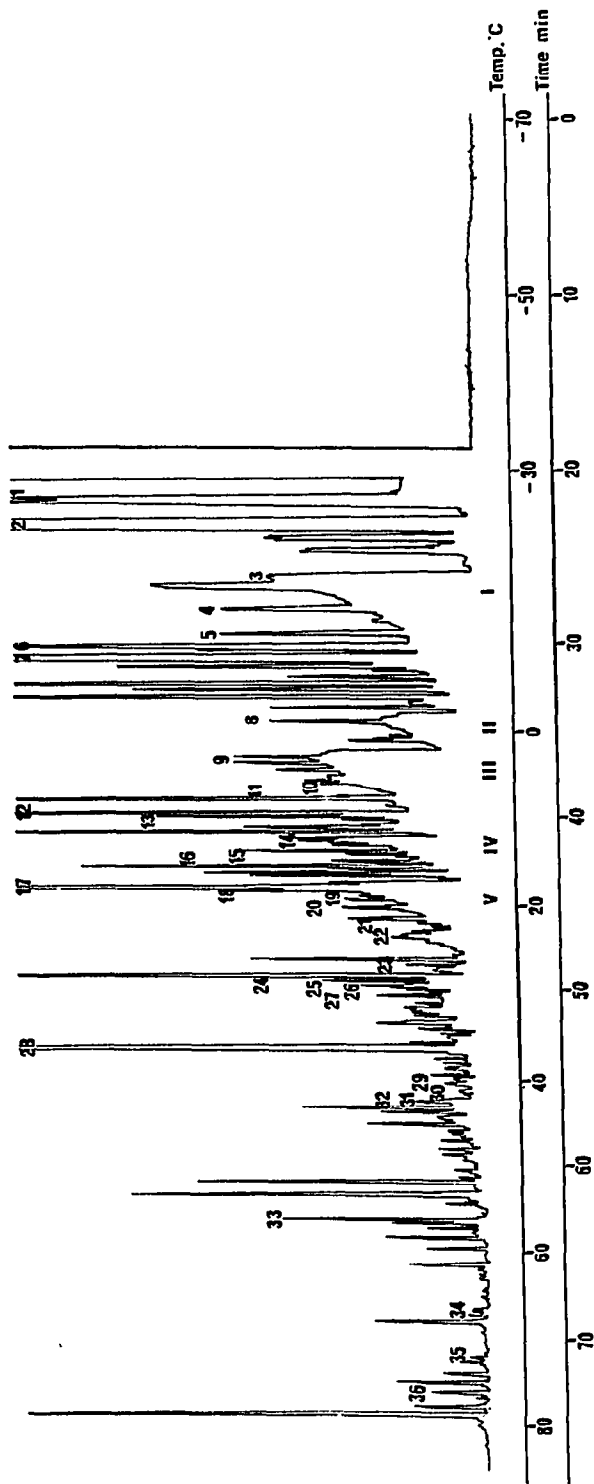
Fig. 1. Gas chromatogram (FID) of 10 ml of tobacco smoke; splitting ratio, 1:40. Glass capillary column, length 150 m, I.D. 0.22 mm, coated with SF-96. Initial temperature on injection, −70°. Carrier gas (nitrogen) flow-rate at 25°, 0.25 ml/min. Roman numerals indicate tailing fractions: I = ethanal; II = 2-propanone + propenal; III = 2-propanone + propenal; IV = acetonitrile + 2-butanone + 3-butene-2-one; V = 2,3-butanedione + 3-methylbutanal + 2-methyl-butanal. Peak numbers with arabic numerals refer to Table I.
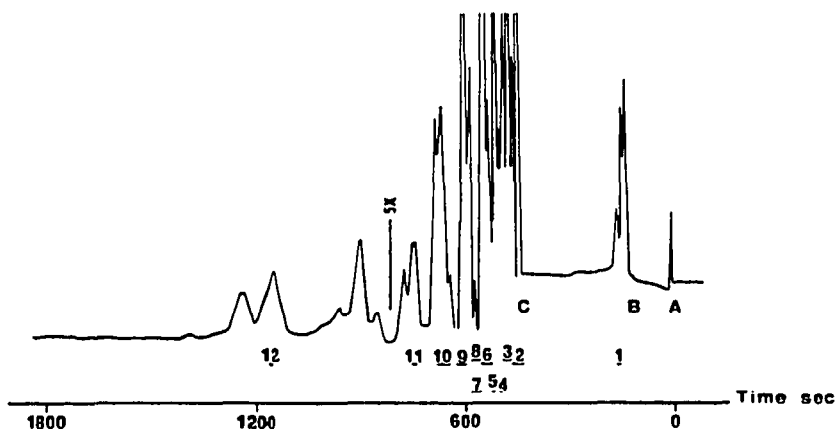
Fig. 2. Gas chromatogram (FID) from the pre-separation of 100 ml of tobacco smoke. Glass column, length 3 m, I.D. 1.5 mm, packed with 3% 2,2-oxydipropionitrile, operated isothermally at 55°. Carrier gas (nitrogen) flow-rate, 10 ml/min. A = coolant of trapping loop changed to dry-ice; B = carrier gas switched on; C = temperature of trapping loop increased to 80°. The fraction sampling intervals are given in Table I.

latter peaks are mixed with the broad peaks IV and V, resulting in less favourable conditions for identification.

On injection into the SF-96 capillary column of a polar column pre-separated fraction containing only a few polar components, but freed from the previously accompanying ones, there will not necessarily be an improvement in the shapes, as is clearly demonstrated by the first two broad peaks in Fig. 3. This also means that highly polar components will still be detected less sensitively than non-polar components in both gas chromatography and mass spectrometry. Comparing the peak heights of peak 8 (propanal) and 17 (benzene) in Fig. 1, that of the less polar benzene is considerably higher, although quantitative determinations[10] have shown the opposite relationship (40 and 30 μg per cigarette).

When polar compounds are present in sufficient amounts to respond on mass spectrometric detection, the absence of non-polar components gives better conditions for identification studies. Upon re-injection, the possible homogeneity of a broad, tailing fraction can be investigated by recording several mass spectra, as indicated in the chromatograms by short vertical lines on the broad peaks. On examination of fractions corresponding to the deformed peaks I–V (Fig. 1), fractions I and II were each found to contain only one major polar component, namely ethanal and propanal. In comparison, peaks III, IV and V were found to be mixtures; III was 2-propanone and propenal, IV was 2-butanone, acetonitrile and 3-butene-2-one, and V was 2,3-butanedione, 3-methylbutanal and 2-methylbutanal (see Table II). By selecting carefully the fractions withdrawn from the packed polar column, it has been found possible to separate all of the above polar compounds as single-component peaks after re-injection into the SF-96 capillary column. In Figs. 5 and 6, this is demonstrated for 2-butanone and acetonitrile, respectively.

Removal of polar compounds also improves the conditions for identification of the non-polar compounds, e.g., 1-heptene and n-heptane in fraction 2 (Table I).
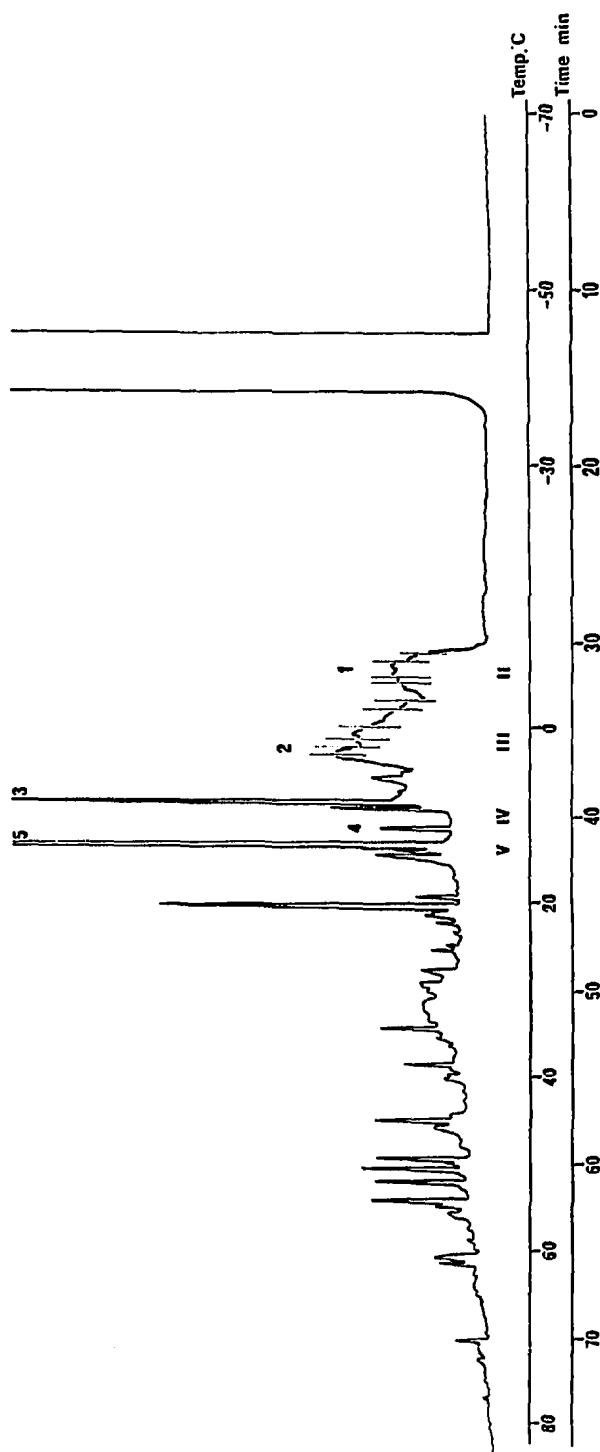
Fig. 3. Gas chromatogram (total ionization current (TIC)–MS detection) of fraction 3 in Fig. 2; splitting ratio, 1:10. Glass capillary column, length 150 m, I.D. 0.22 mm, coated with SF-96. Initial temperature on injection, −70°. Carrier gas (helium) flow-rate at 25°, 0.40 ml/min; electron ionizing voltage, 70 eV. 1 = Propanal; 2 = 2-propanone: 3 = 2-methylfuran; 4 = 3,5-hexadien-1-yne; 5 = benzene. Roman numerals indicate the positions of the mass spectra shown in Table II. Vertical lines indicate the positions of the recordings of broad tailing peaks as in Fig. 1.
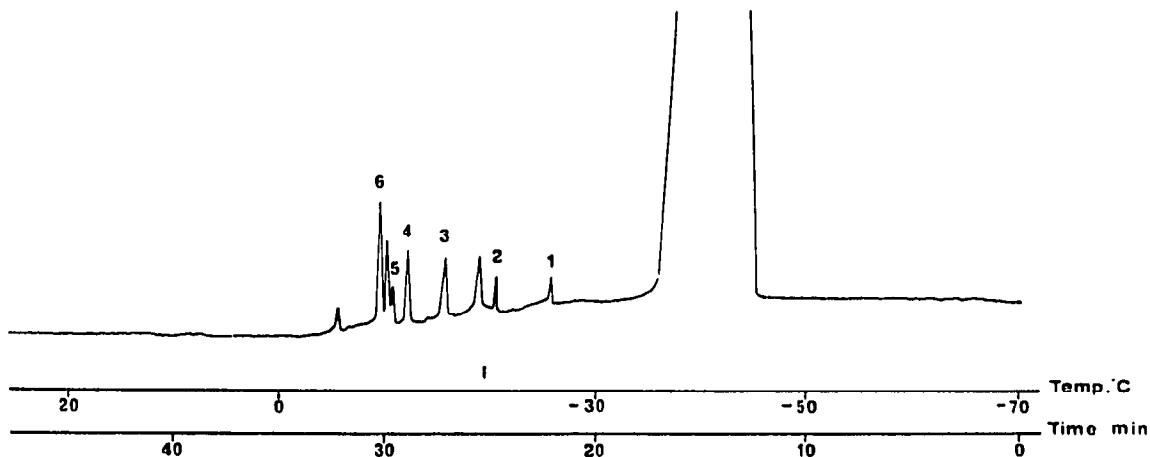
Fig. 4. Gas chromatogram (TIC) of fraction 1 in Fig. 2; splitting ratio, 1:10. Column and conditions as in Fig. 3. 1 = 1-Buten-3-yne; 2 = 1,2-butadiene; 3 = 2-methylbutane; 4 = 1-pentene; 5 = *n*-pentane; 6 = 2-methyl-1,3-butadiene. Roman numerals indicate the positions of broad tailing peaks as in Fig. 1.

The mass spectrometric identification of these two compounds was hampered greatly by the simultaneous elution (see Fig. 1) of 2-butenal, which, after pre-separation on this long polar column, appears as late as in fraction 9 (Table I). Another example is 1,2-butadiene (Fig. 4), which before pre-separation was mixed with ethanal, causing interference. When dealing with compounds that have relatively small differences in polarity, more care is necessary in the selection of the limits of the fractions withdrawn. However, the isomers 3- and 2-pentanone are easily separated by the 2,2'-oxydipropionitrile column so that they elute with fractions 6 and 8, respectively. These examples might be taken as an indication that the polar column used in this investigation was too long. In general, the separation efficiency of the pre-column should not be too high, as this would lead to a large number of fractions to be handled with subsequent time-consuming analysis on the capillary column. Thus, in order to obtain the optimal pre-separation suitable for the specific task of a particular investigation, all of the gas chromatographic parameters have to be chosen with great care.

The concentration achieved with this technique is noteworthy. From a 100-ml smoke sample injected into the packed column, the whole of a withdrawn fraction of interest, say 2–3 ml, can be injected without splitting into the capillary column. In this way it has been found possible to achieve interpretable mass spectra of 5-hexene-2-one, a polar trace component of tobacco not previously reported (*cf.*, fraction 12, Table I).

The separation of trace components eluted directly before a major component might be disturbed owing to the retaining effect of the major component[13]. When increasing the amount of sample, a minor component eluted directly after a major component might be included in the latter, *cf.*, benzene (17) and thiophene (18) in Fig. 1. Fraction 5 (Table I) is carefully collected under conditions such that the benzene content is substantially reduced. Splitless injection then makes the identification of thiophene possible.
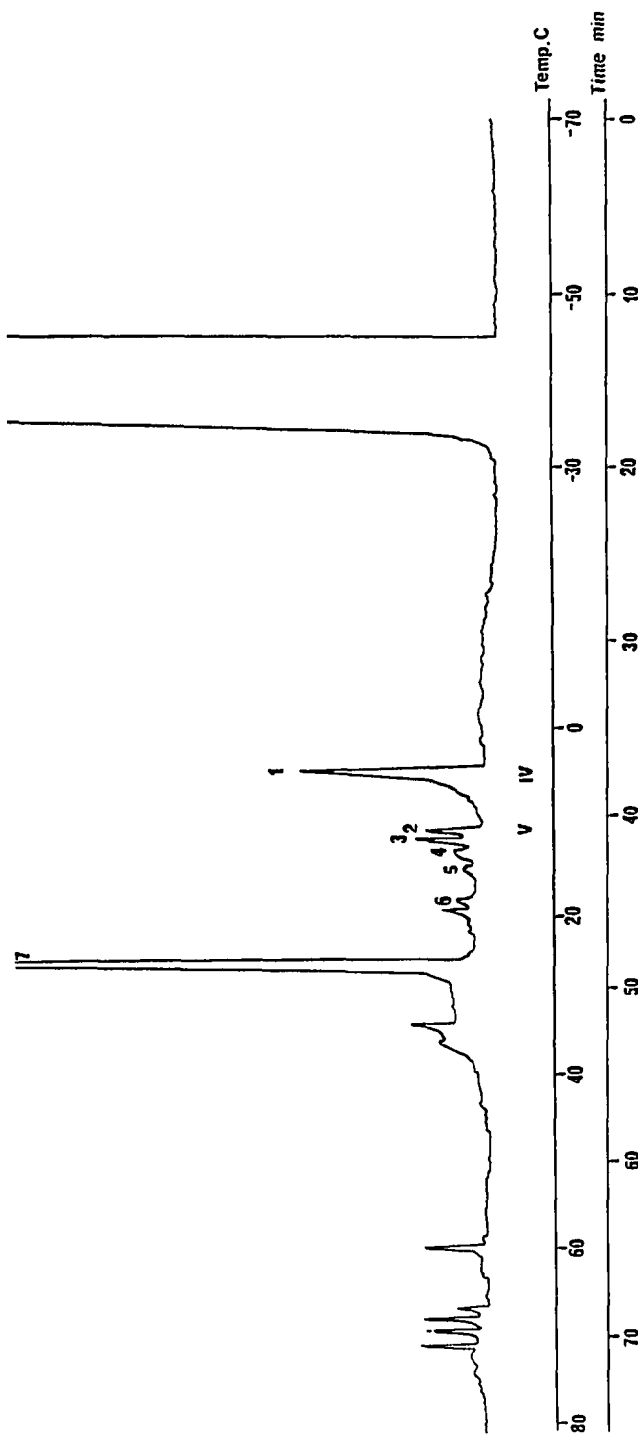
Fig. 5. Gas chromatogram (TIC) of fraction 6 in Fig. 2; splitting ratio, 1:10. Column and conditions as in Fig. 3. 1 = 2-Butanone; 2 = 3-methylbutanal; 3 = 2-methylbutanal; 4 = 3-methyl-2-butanone 5 = 3-methyl-3-buten-2-one; 6 = 3-pentanone; 7 = toluene. Roman numerals indicate the positions of broad tailing peaks as in Fig. 1.
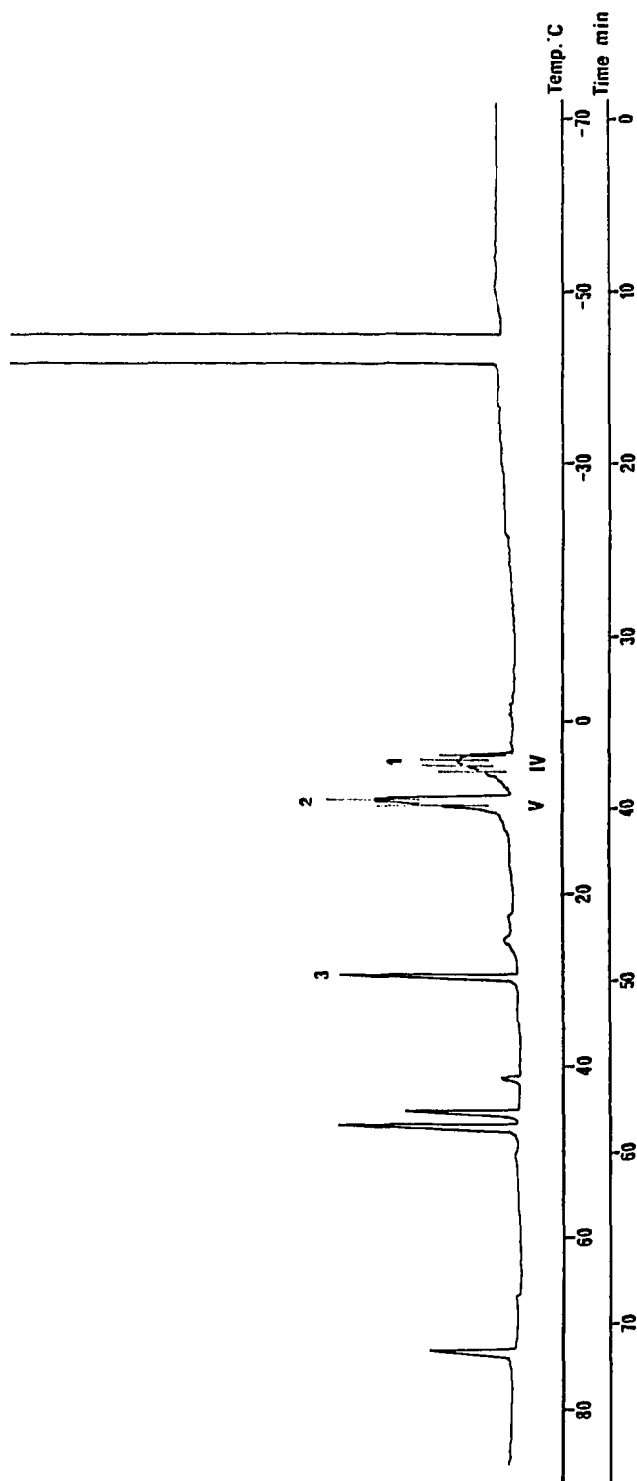
Fig. 6. Gas chromatogram (TIC) of fraction 7 in Fig. 2; splitting ratio, 1:15. Column and conditions as in Fig. 3. 1 = Acetonitrile; 2 = 2,3-butanedione; 3 = toluene. Roman numerals indicate the positions of broad tailing peaks as in Fig. 1. Vertical lines indicate the positions of the recordings of the mass spectra shown in Table II.

TABLE II

MASS SPECTRA RECORDED AT INTERVALS DURING THE ELUTION OF TAILING
FRACTIONS FROM THE SF-96 CAPILLARY COLUMN

These mass spectra, demonstrating homogeneity of the fractions, were recorded after pre-separation
on the packed polar column.

Compound	m/e	MS intensities at the positions indicated in Figs. 3 and 6					
Propanal	29	1000	935	1000	626		
(cf., Fig. 3)	27	935	1000	870	1000		
	28	550	780	666	474		
	58	420	770	815	356		
	55	240					
	57		274	390			
	56				300		
2-Propanone	43	1000	1000	1000	1000	1000	1000
(cf., Fig. 3)	58	428	426	331	338	205	250
	27	80	69	100	78	53	53
	42	48	41	63	63	61	53
Acetonitrile	41	1000	1000	1000	1000		
(cf., Fig. 6)	40	670	510	480	520		
	39	360	170	170	150		
	38	220	100	110	120		
2,3-Butanedione	43	1000	1000				
(cf., Fig. 6)	86	170	140				
	15	160	230				
	41	140	48				
	14	70	62				

It is usually considered that manual re-injection techniques give transfer losses that are too high to be applied in practical analytical work. In this investigation, as in previous work in this laboratory[14], it has been shown that after some training and with the use of warm, tight glass syringes, yields of about 90% can be regularly obtained. On decreasing the amount of sample to the picogram level, the relative losses due to transfer increase drastically. This was demonstrated with a series of injections of carbon tetrachloride, known to be most sensitively detected by the ECD. At the 20-ng level, the mean recovery from ten injections was 90%, but when the same number of analyses were made with a sample of 30–40 pg, a recovery of only 10% was obtained.

With reference to the above examples, we claim that this manual technique is a versatile and powerful tool for more efficient identifications. We are aware that this technique is experimentally demanding, and that careful training is essential in order to utilize fully the potentialities of the technique, and also that there is a further need for advanced technical transfer aids. We realize that there would be certain advantages in using a reversed system, i.e., a packed non-polar column followed by a highly efficient polar capillary column. As yet, we have been unable to prepare such polar capillary columns, but the present system prevents water, high-boiling compounds and, not least, easily decomposed aerosols from entering the delicate capillary column. We presume that this chromatographic imperfection was a major reason why earlier workers[4], using only direct injection techniques, were unable to report the presence of some oxygen-containing constituents of tobacco smoke.

REFERENCES

1 K. Grob, *Beitr. Tabakforsch.*, 3 (1966) 403.
2 K. D. Bartle, L. Bergstedt, M. Novotny and G. Widmark, *J. Chromatogr.*, 45 (1969) 256.
3 C. R. Enzell, E. Bergstedt, T. Dalhamn and W. H. Johnson, *Beitr. Tabakforsch.*, 6 (1971) 41.
4 C. R. Enzell, E. Bergstedt, T. Dalhamn and W. H. Johnson, *Beitr. Tabakforsch.*, 6 (1972) 96.
5 G. Widmark, *International Symposium on Identification and Measurement of Environmental Pollutants, June 1971*, National Research Council of Canada, Ottawa, 1971, p. 396.
6 K. Grob, *J. Gas Chromatogr.*, 3 (1965) 52.
7 K. Grob, *Beitr. Tabakforsch.*, 3 (1965) 243.
8 K. Grob and G. Grob, *J. Chromatogr. Sci.*, 7 (1969) 584.
9 M. Novotny, *Chromatographia*, 2 (1969) 350.
10 H. Elmenhorst and Ch. Schultz, *Beitr. Tabakforsch.*, 4 (1968) 90.
11 A. Cornu and R. Massot, *Compilation of Mass Spectral Data*, Heyden, London, 1966; 1st Suppl., 1967; 2nd Suppl., 1971.
12 E. Stenhagen, S. Abrahamsson and S. W. McLafferty, *Atlas of Mass Spectral Data*, Wiley, New York, 1969.
13 W. E. Harris, *J. Chromatogr. Sci.*, 11 (1973) 184.
14 G. Widmark and K. Widmark, *Acta Chem. Scand.*, 16 (1962) 575.