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THE USE OF HIGH-EFFICIENCY PACKED COLUMNS FOR GAS–SOLID CHROMATOGRAPHY

IV. THE GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC ANALYSIS OF COMPLEX ORGANIC MIXTURES

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SUMMARY

Complex organic mixtures, such as essential oils and food extracts, have been analysed by means of gas adsorption on packed columns and mass spectrometry.

An original double-detection coupling system that permits the chromatographic resolution to be completely preserved is also described.

Minor peaks have been identified.

INTRODUCTION

In previous papers of this series[1–3], and in a paper published elsewhere[4], we described the application of gas–liquid–solid chromatography (GLSC) to the separation of isotopic molecules. Such difficult analyses can be performed only with very efficient columns, up to 100 m in length and with about 10^5 theoretical plates.

GLSC columns were also tested and their performances evaluated in the analysis of polar[5] and reactive[6] compounds.

In this paper, the first results of the application of such columns to gas chromatographic–mass spectrometric analyses are reported. The main purpose is to obtain packed columns with a resolving power similar to that of capillary columns. This, if an efficient coupling system is available, would allow the mass spectrometric identification not only of the major but also of the minor constituents of complex mixtures. Their identification may be of great help in food and flavour chemistry.

GAS–LIQUID–SOLID CHROMATOGRAPHY (GLSC)

GLSC was chosen for the purposes of the present work because of the particular features of this technique, which can be summarized as follows:

(1) GLSC exploits the separation power of gas–solid chromatography (GSC)

because its operating mechanism is based mainly on an adsorption process. The use of carbon black as an adsorbent results in the best separation factors among compounds of analogous structure[7] and with very close boiling points. In many instances the column length necessary for a certain separation can be shortened by a factor of ten with respect to gas–liquid chromatography (GLC).

(2) The presence, in controlled amounts, of an appropriate liquid phase permits the use of linear gas chromatography with polar and hydrogen-bonding compounds. However, owing to the influence of the support, hydrocarbon-type compounds can also be eluted with satisfactory separation factors. The column is thus of general use.

(3) Bleeding of GLSC columns is largely reduced for a particular liquid phase at a particular temperature compared with the same phase used in GLC. This occurs because the liquid phase is strongly adsorbed by the support, and as it is present in a range between less than one and two or three molecular layers, its vapour pressure is lower than in the bulk liquid. This improves the results obtained by coupling gas chromatography with mass spectrometry. Cleaner spectra are obtained and the ion source and gas lines remain uncontaminated for a much longer period.

EXPERIMENTAL

Gas chromatography

The gas chromatograph used was a Carlo Erba Model GI 450 equipped with a double-trace recorder (Leeds and Northrup Speedomax, Model XL682) when required or a single-trace recorder (Model XL681).

Columns were made of stainless steel (2 mm I.D.) packed with Sterling FT, which is a graphitized carbon black with a surface area of about 15 m^2/g, obtained from Elettrocarbonium, Research and Development Division, Milan, Italy. This carbon black, acting as an adsorptive support, is coated with the desired liquid phase according to the usual GLC procedure. After coating, the column is kept overnight at 200° under a stream of helium.

The elution time of methane is used to measure the linear gas velocity, provided that it is found to be equal to the retention time of helium as measured with a thermal conductivity detector.

Mass spectrometry

An AEI MS12 mass spectrometer was used. The coupling line, developed in our laboratory as a modification of the design given by the manufacturer, is made entirely of glass. The coupling line is shown schematically in Fig. 1, and the details have been published elsewhere[8]. The equipment was originally equipped with the double-detection system comprising a flame ionization detector (FID) and a total ion monitor (TIM). The numbers of theoretical plates measured from the two signals were the same, showing that coupling of the gas chromatograph with the mass spectrometer does not affect the chromatographic efficiency.

The double-detection system is not used in routine work and only the FID is used in recording the chromatograms. The output of the mass spectrometer is connected to an oscillograph that continuously monitors the spectra of the compounds as they reach the ion source. The repetitive scanning (8 sec/dec.) is carried

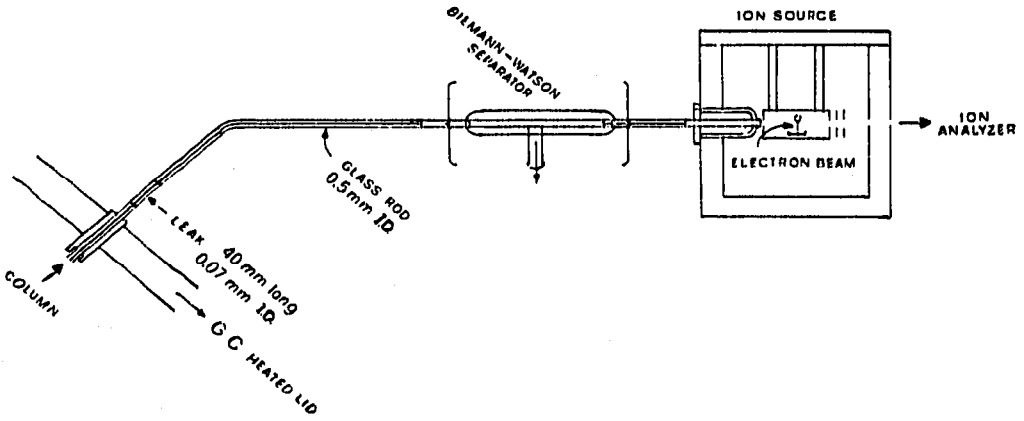


Fig. 1. Scheme of the coupling line.

out continuously during the recording of the chromatogram. Spectra were taken for every significant peak when the intensity of the spectrum on the oscillograph was sufficient to ensure a clear recording.

RESULTS AND DISCUSSION

Three 5-m columns were prepared and tested in the present work. The first was coated with 2.5% SE-52, the second with 1.5% FFAP and the third with 1% Carbowax 20M. In Fig. 2, the Van Deemter plots for these three columns are reported. An important property of these curves is their shape, which is characteristic of GLSC, as reported earlier[4]. The characteristic hollowness of the curves was forecast by MYERS AND GIDDINGS[9] for very homogeneous adsorbents. The linear part of the curve has a low slope, which enables a high linear gas velocity to be

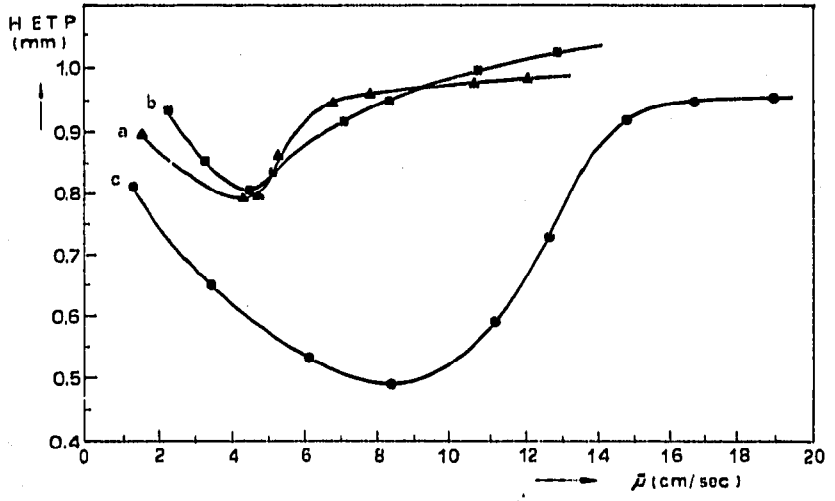


Fig. 2. Van Deemter plots for some GLSC columns. Column packing: 5 m Sterling FT + (a), 2.5% SE-52, (b), 1.5% FFAP, and (c), 1% Carbowax 20M.

used while maintaining an HETP value of less than 1 mm. If the high separation factors that are common in GLSC and the above factor are taken into consideration, the advantages of this technique as far as rapid analysis is concerned can be easily understood. The disadvantage of having high retention volumes compared with GLC is overcome by the possibility of using high linear gas velocities.

A remarkable difference is observed between the Carbowax 20M column and the other two. The curve is flatter and the minimum much lower. This is not attributed to the nature of the liquid phase, but rather to its concentration on the adsorbent. The concentration of Carbowax 20M is in fact 1%, which represents

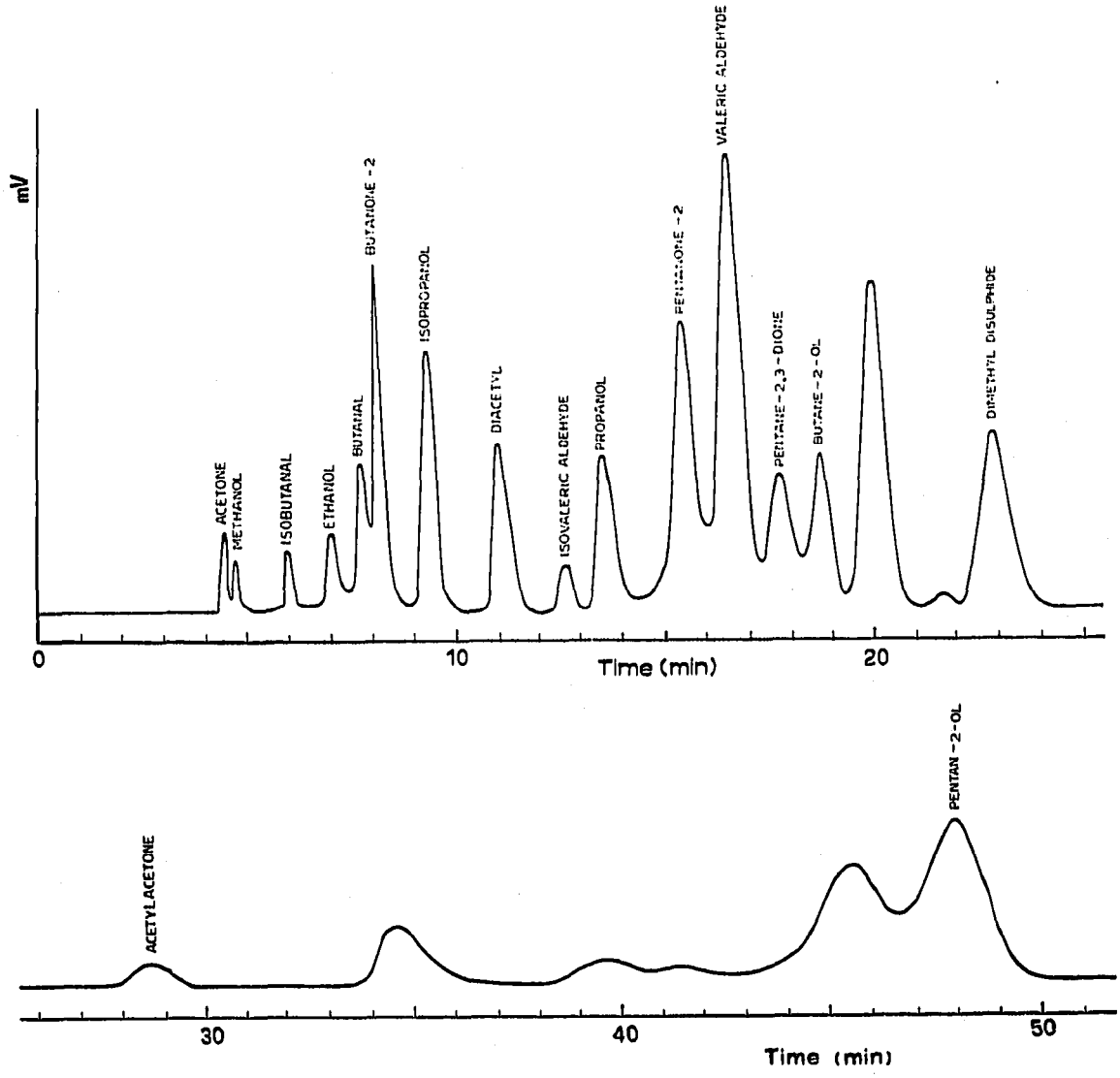


Fig. 3. Gas chromatogram of an artificial mixture representing a milk extract. Temperature, 66 °; inlet pressure, 3.5 kg/cm².

a little less than a monolayer, while in the other two instances the concentration corresponds to two or three molecular layers. As found in our laboratory (*e.g.*, ref. 17), the best kinetic conditions for GLSC occur when the amount of liquid phase present is insufficient to form a monolayer on the adsorbent. A compromise between column efficiency and reasonable retention times is reached when the extent of coverage is about a monolayer.

For the analyses of the mixtures reported here, only the Carbowax 20M and FFAP columns were used. With the SE-52 column, tailed peaks for polar compounds were obtained and the use of this column for this work was discontinued.

Fig. 3 shows a chromatogram obtained from an artificial mixture representing the approximate composition of a milk extract (volatile constituents). The identification is made with the aid of the mass spectra obtained.

This separation shows the high resolving power of the column used, especially for isomers. Sharp peaks are obtained for polar compounds and the number of theoretical plates is between 6000 and 7000 under the operating conditions used.

Fig. 4 shows a double-trace chromatogram obtained by using both the TIM and the FID. The mixture, eluted on the 5-m FFAP column, is artificial and analogous to that of a tea aroma. It was assumed that this mixture consisted of 23 constituents, but in our chromatogram 88 peaks were found. Of these compounds, 23 were identified by means of their mass spectra only.

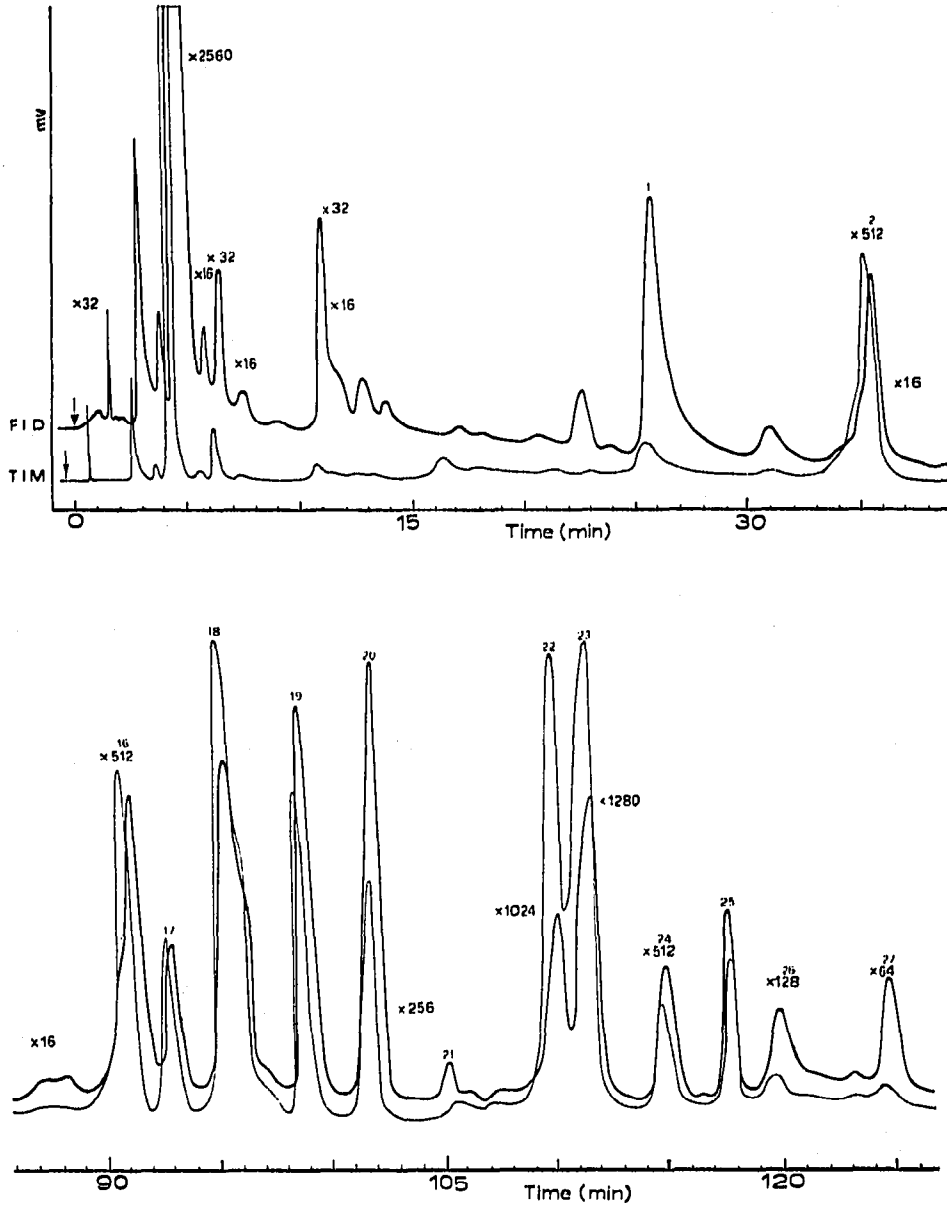
Few of the compounds that should have been present in the sample have not been identified, while six of the compounds identified in the mixture were not thought to be constituents. Of these substances, two were present in very small amounts (less than 0.2% of the mixture), namely α-ionone and benzyl alcohol. The good separation of α-ionone and β-ionone (peaks Nos. 27 and 28) shows the very high resolving power of the column for these isomers.

In Table I the results of the analysis of the mixture shown in Fig. 4 are reported. The structure has been assigned only when the relative spectrum has been found univocally in the literature.

In Fig. 5 a chromatogram of a Spanish lemon oil is reported. Essential oils of this type contain more than 80% of limonene, while up to several hundred minor constituents are present. Among these, some oxygenated compounds are responsible for the flavour and odour, and their identification is very important for characterizing the organoleptic properties of the oil from a chemical point of view. High-resolution gas chromatography was used for several years to characterize roughly the composition of essential oils by using capillary columns[10,17].

With packed columns, a satisfactory chromatographic spectrum cannot usually be obtained, while the mass spectrographic identification of the constituents of very complex mixtures is still a difficult problem to solve. Of course, apart from mass spectrometric and gas chromatographic information, the complete study of such mixtures requires a long time, synthetic work and UV, IR and NMR studies in order to characterize them with a high degree of certainty. The mass spectrum can be useful for definite identification only if the spectrum of the pure compound is available.

In Table II the results of the information obtained from the mass spectra of lemon oil are collected. Few of the compounds have been identified because of the lack of mass spectra of terpenes in the literature and because these compounds



have very similar mass spectra, the relative intensities of the peaks often being the only way they can be distinguished. In Table II, the seven most intense peaks are reported together with the molecular ions and some information about the possible structures of the unidentified compounds. The analytical results obtained from Table II are of little importance for the identification of the constituents of lemon oil. On the other hand, the scope of this paper is to show the potentiality

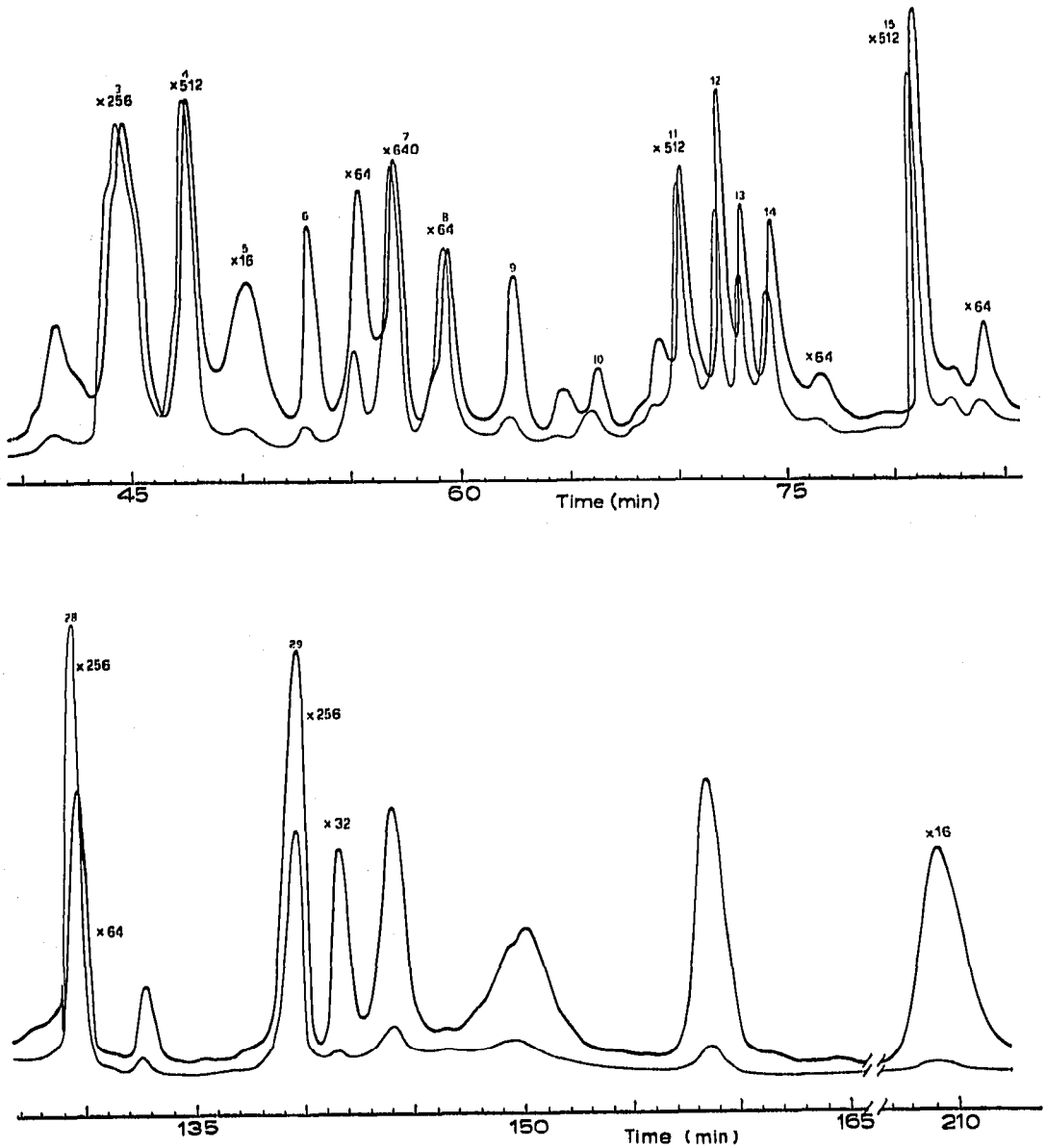


Fig. 4. Gas chromatogram of an artificial mixture representing a tea aroma. Inlet pressure, 7 kg/cm²; temperature, isothermal at 50° for 5 min, programmed at 1°/min to 215°, then isothermal at 215° to the end; sample size, 16 μl.

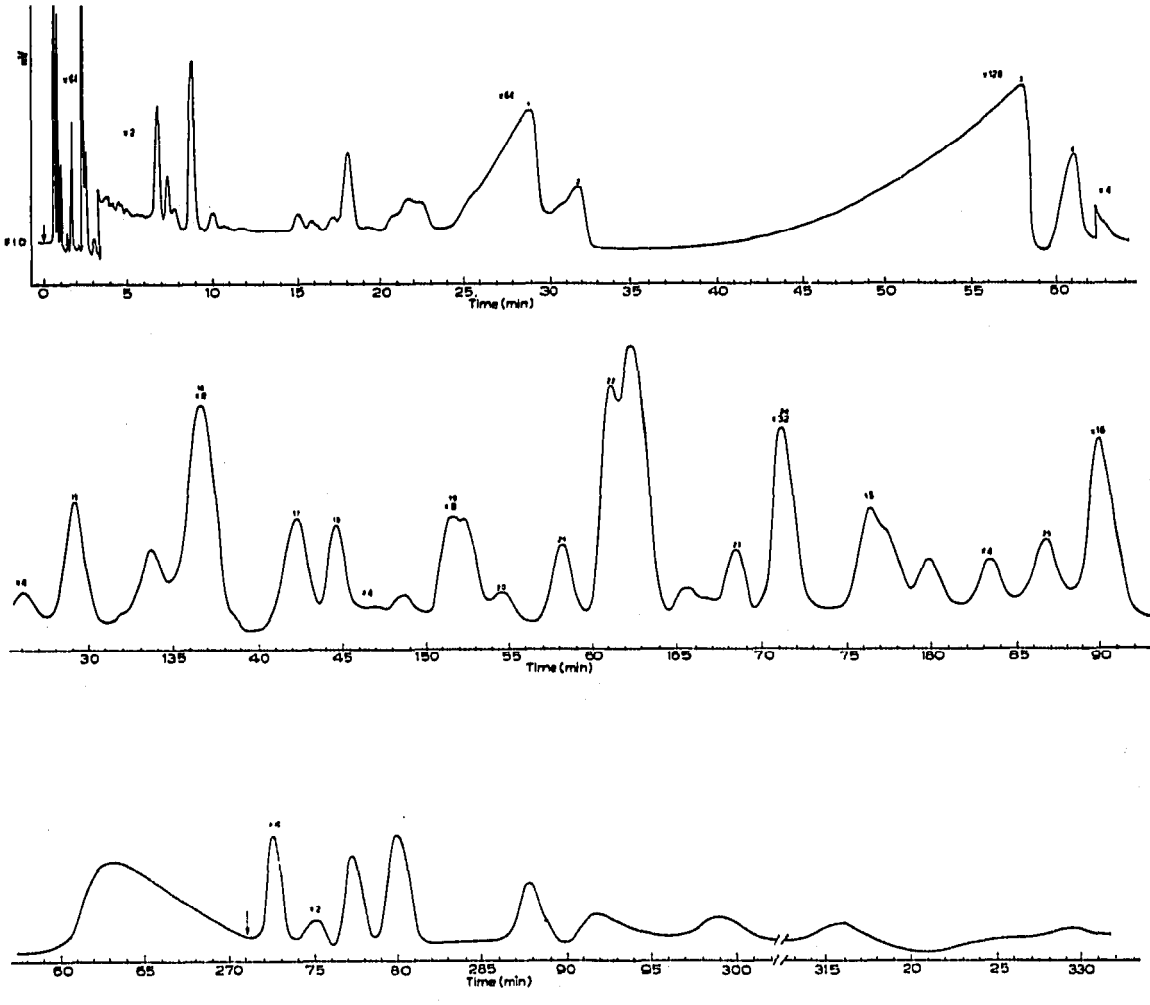


TABLE I

COMPOUNDS CORRESPONDING TO CHROMATOGRAM IN FIG. 4

Peak No.	*Structure assigned*[11–18]	*Peak No.*	*Structure assigned*	*Peak No.*	*Structure assigned*
1	3-Hexene	12	4-Hexen-1-al	22	β-Phenylethanol
2	1-Penten-3-ol	13	Mixture	23	Phenyl acetonitrile + methyl salicylate
3	Mixture	14	2,4-Hexadienal		
4	Hexanal	15	Benzaldehyde	24	Mixture
5	Mixture	16	Phenyl acetaldehyde	25	Geraniol
6	3-Hexen-1-ol	17	Linalool-ox	26	Mixture
7	4-Hexadien-3-ol	18	Acetophenone + methyl benzoate	27	α-Ionone
8	Mixture			28	β-Ionone
9	Mixture	19	Linalool	29	5-*n*-Propyl-γ-butyrolactone
10	Mixture	20	Benzyl acetate		
11	1-Penten-3-methyl-3-ol	21	Benzyl alcohol		

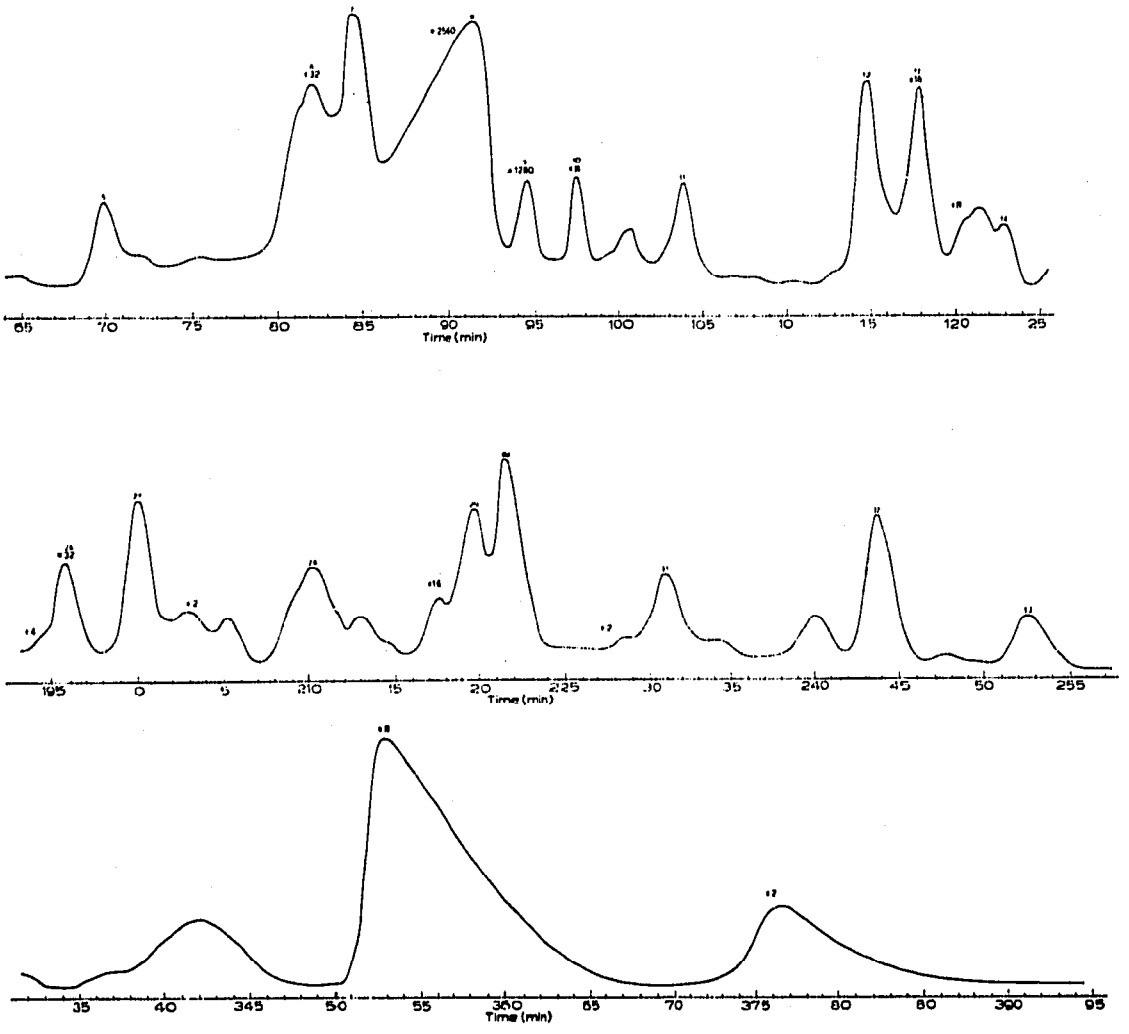


Fig. 5. Chromatogram of a natural lemon oil. Column: 5 m Sterling FT (60–80 mesh) + 1.0% Carbowax 20M. Inlet pressure, 7 kg/cm² increased to 8.5 kg/cm² 15 min after starting the final isothermal; temperature, 5 min isothermal at 50°, programmed at 0.5°/min up to 215°, then isothermal at 215° to the end; Sample size, 13 μl.

TABLE II

COMPOUNDS CORRESPONDING TO CHROMATOGRAM IN FIG. 5

Peak No.	*Structure assigned*	*Molecular ion*	*Base peak*	*Most intense peaks*	*Structural information*	*Reference*
1	—	136	93	91–92–77 79–41–121	Terpene	
2	—	136	93	91–77–92 41–79–105	Terpene	
3	β-Pinene					14
4	Sabinene					14
5	—	154	43	41–93–67 68–81–55	Terpene, oxygen	
6	Myrcene					14
7	α-Phellandrene					14
8	Limonene					14
9	—	136	93	91–77–136– 121–41–79	Terpene	
10	—	136	93	91–41–79 77–105–121	Terpene	
11	—	136	93	41–91–79 77–105–121	Terpene	
12	—	152	41	91–67–81 79–119–55	Terpene, oxygen	
13	—	152	91	41–67–93 81–79–119	Terpene, oxygen	
14	—	152	41	55–119–91 67–81–79	Terpene, oxygen	
15	Carveol					15
16	—	152	91	41–79–119 77–55–134		
17	—	152	41	91–79–67 55–93–94		
18	—	136	41	93–91–69 79–77–121	Terpene	
19	—	134	91	41–119–77 79–65–105	Aromatic	
20	—	150	41	91–79–67 69–107–135		
21	Carvone					16
22	—	152	91	41–119–77 79–69–134	Terpene	
23	—	150	107	91–132–105 65–77–79	Aromatic, oxygen	
24	—	152	41	69–91–119 77–79–84	Terpene, oxygen	
25	—	150	43	41–91–79 107–77–135		
26	—	204	41	93–69–91 79–77–105	Sesquiterpene	
27	—	204	93	41–69–119 55–91–79	Sesquiterpene	
28	—	204	41	91–79–55 105–119–77	Sesquiterpene	

TABLE II (*continued*)

Peak No.	*Structure assigned*	*Molecular ion*	*Base peak*	*Most intense peaks*	*Structural information*
29	—	204	41	67–81–91 79–77–93	Sesquiterpene
30	—	204	41	69–93–79 91–55–109	Sesquiterpene
31	—	204	41	55–67–93 91–81–79	Sesquiterpene
32	—	202	41	91–79–55 67–93–105	Sesquiterpene
33	—	202	41	121–77–159	Sesquiterpene

of GLSC rather than to carry out analytical work on the mixtures eluted as examples.

Our recent studies[18] have shown that columns can be made much longer and the number of the compounds identified can be increased, while still maintaining the analysis time within reasonable limits, by choosing the appropriate temperature programming and slow flow-rates.

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