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복합 유기혼합물체로부터 휘발성이 서로 다른 세 유기 화합물 그룹의 분리 농축방법의 연구

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Isolation and Concentration of Organic Components from a Complex Matrix into Three Fractions of Different Volatilities

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요 약. 복합 유기혼합체로부터 휘발성이 다른 세 그룹을 분리 농축할 수 있는 간단한 소형장치가 고안되었다.

이 장치를 사용하여 휘발성이 높은 유기성분들은 테넥스지씨에 헤드스페이스 트랩핑하고 남은 시료를 유기용매로 용출하여 얻어진 용출물을 휘발성 및 비휘발성 그룹으로 분리 농축할 수 있었다.

얻어진 휘발성 성분그룹은 고성능 캐피러리 가스크로마토그래피로 분석하였고, 비휘발성 성분그룹은 고성능 액체크로마토그래피에 의해서 분석하였다. 상세한 실험방법 및 유기 혼합물질인 잎담배를 시료로 사용하여 얻어진 분석 결과가 언급되어 있다.

ABSTRACT. A simple micro-sampling system is described which facilitates isolation and concentration of complex organic constituents into three fractions of different volatilities.

The method involves the headspace trapping of very volatile components from a complex matrix onto a porous polymer, Tenax GC, followed by the solvent elution of the matrix and the subsequent fractionation of the eluate into volatile and less-volatile fractions. The headspace and the volatile fractions are then analyzed by high-resolution capillary gas chromatography.

The less-volatile fraction is analyzed by high-performance liquid chromatography. Experimental details and the results obtained using tobacco leaves as a complex organic matrix are presented.

INTRODUCTION

The success of any chemical analysis depends largely on the methods employed in isolation and concentration of the compounds from a complex matrix. Commonly employed techniques are solvent extraction, steam distillation, vacuum distillation, headspace enrichment, sublimation, precipitation and chromatographic fractionation.

An ideal sampling method will permit maximum yield of compounds of interest with no contamination, artifacts or decomposition, with minimal manipulation, with rapidity and high reliability. To minimize the chance for contamination, the procedure should be as simple with fewer manipulation as possible, and applicable on a microscale. However, no single sampling procedure is uniformly satisfactory, as Jennings *et al.*,[1] mentioned. Depending on the sample composition and the compounds of interest, one or some combination of those te-

chniques may improve the overall performance of the sampling system.

Very innovative sampling systems have been reported in recent publications, namely; a bomb apparatus for multiple extraction[2]; the well-known apparatus for simultaneous steam distillation and extraction[3,4]; vacuum steam distillation and headspace condensation systems[5]; adsorption and partition chromatographic systems[6]; a transevaporator used for combined headspace and extraction[7]; and a simple apparatus for pigment enrichment[8].

It is highly desirable to prepare samples which are amenable to gas chromatographic and high-performance liquid chromatographic analyses from very complex organic matrix containing multicomponents of a wide range of volatilities, polarities and functional groups. Presently, we are engaged in composition studies on aromatic tobaccos of different varieties. Attempts have been made to design an efficient sampling scheme for obtaining suitable samples. A simple sampling apparatus thus developed is introduced here which allows the headspace trapping of very volatile components from a complex matrix and the elution of the matrix with organic solvent, followed by the separation of the eluate into two fractions of different volatilities in a continuous manner.

EXPERIMEMTAL

Tobacco Sample. Sun-cured aromatic tobacco, Sohyang (*Nicotiana tabacuum* L.) was dried (60 °C) and pulverized to pass 16 mesh. The ground tobacco was stored in a colored container.

Reagents. Dichloromethane, methanol, and *n*-hexane (Lichrosolv®, chromatographic grade, E. Merck, Darmstadt, Germany). Dichloromethane (GR grade, E. Merck, Darmstadt, Germany). Tenax GC, 60/80 mesh (Alltech Associates, Arlington Heights, Ill, USA).

Apparatus. The sampling apparatus consists basically of six custom-built parts, all made of Pyrex glass, as shown in *Fig.* 1, : an isolator, a connector for holding a column packed with a sample in question, a condenser for removing water vapor, a concentrator for collection of the solvent eluate, fitted with a transfer tube, and a graduated concentrator for condensing the evaporate.

Headspace Sampling. Very volatile components were enriched using a sampling system as shown in *Fig.* 2.

The tobacco column (glass tubing, 12.5×0.6 cm I.D.) was tightly packed with a 1 g portion of ground tobacco, between glass wool plugs. The concentration column is a glass tubing (10.5×0.2 cm I.D.; 0.37 cm O.D.) containing 50 mg of Tenax GC secured with glass wool at both ends. It was conditioned at 280° by purging with nitrogen at 10 m*l*/min for 6 hr before use. Glass wool was prewashed sequentially with acid (6 *N* HCl), base (6*N* NaOH), distilled water, methanol, acetone, and dichloromethane, followed by baking at 300 °C for 3 hr *in vacuo*. A stream of nitrogen was allowed to pass upward through the tobacco in the column (A) at 30 m*l*/min for 30 min, carrying the volatile components in the headspace of the tobacco into the condenser (B), finally onto the concentration column (C) to be adsorbed on Tenax GC. The condenser was chilled with ice-water. At the end of the sampling period, the concentration column was disconnected and stored in a Teflon-lined screw-capped culture tube in a freezer before analysis.

Column Elution. After the headspace sampling was finished, the volatile and the less-volatile components were then fractionated from the same tobacco matrix. *Fig.* 3 depicts the sampling system, where the condenser is repla-

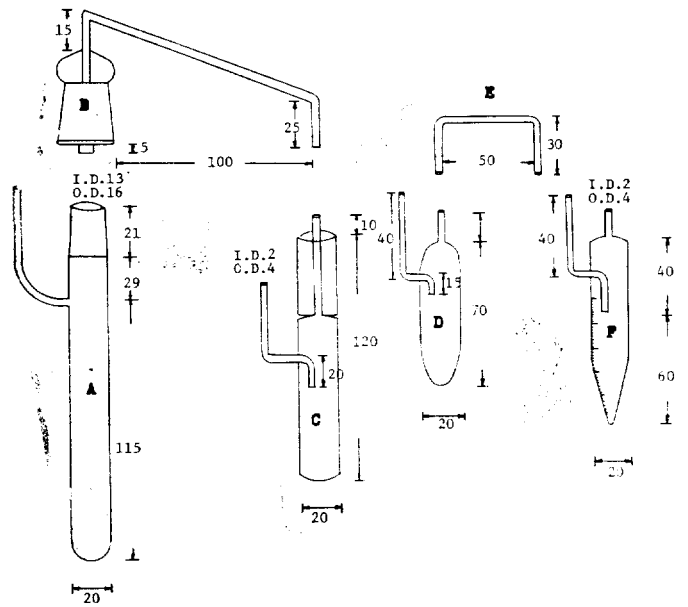

Fig. 1. Diagrams of basic parts of apparatus. (A) Isolator; (B) connector; (C) condenser; (D) concentrator; (E) transfer tube; (F) graduated concentrator. Dimension in mm.

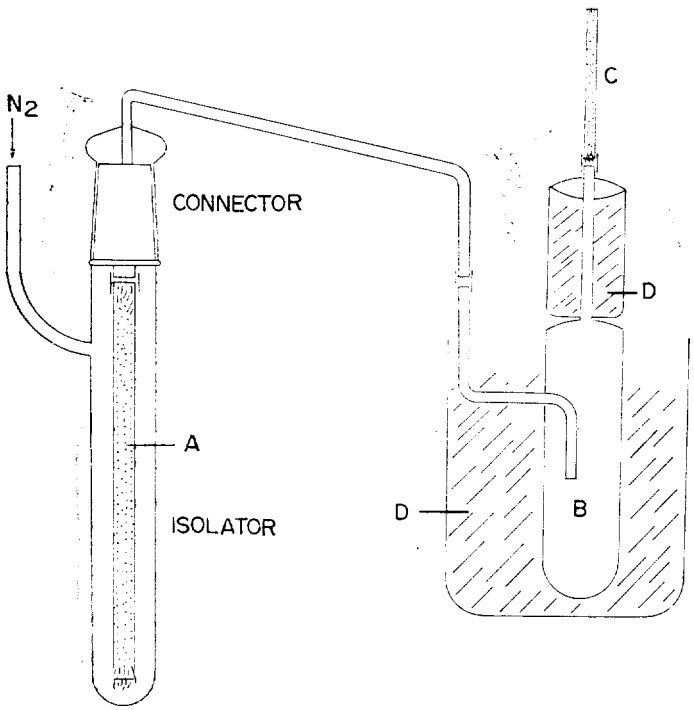

Fig. 2. Headspace sampling system. (A) Tobacco column; (B) condenser; (C) concentration column; (D) ice-water.

ced by a concentrator and a graduated concentrator. A 5 *ml* portion of dichloromethane was placed into the isolator (A) and forced to pass upward through the tobacco (B) held at ambient temperature under a slow stream of nitrogen for a period of 50 min. The eluate was then collected into the concentrator (C). The eluate was evaporated to dryness at an elevated temperature (water bath of 60 °C) and under increased flow of nitrogen stream (0.7 kg/cm^2), yielding a less-volatile fraction in the concentrator, while the evaporate was condensed in the graduated concentrator (D) cooled at ice temperature. After the completion of condensation, the coolant was removed and purging with nitrogen at the further increased flow-rate (2.8 kg/cm^2) was continued until the condensate was reduced to a volume of 200 μl, suitable for direct injection to a gas chromatograph. The less-volatile residue was dissolved in 1 m*l* of dichloromethane containing 1 % methanol for high-performance liquid chromatographic analysis.

Gas Chromatography. Analyses of both the headspace fraction and the volatile fraction were performed on a Hewlett-Packard Model 5840 gas chromatograph connected

to a Model 5840 GC terminal, equipped with a flame ionization detector and a Model 18835 B capillary inlet system (Hewlett-Packard, Avondale, Pa, USA).

Headspace Fraction. A flexible fused silica capillary column (12 m×0.20 mm I.D.; coated with methyl silicone fluid; Hewlett-Packard) was connected to a standard injector port maintained at 280 °C through which nitrogen was passed at 2 m*l*/min. The first 3 m portion of the column was reformed into small coils and was used as a precolumn cold trap. It was chilled with dry ice-ethanol. The concentration column was inserted into the injector and the volatile components desorbed from the Tenax GC were then transferred to the chilled part of the column for a period of 15 min. After the transference, the column inlet was switched to a capillary inlet system, through which nitrogen carrier gas was passed at 0.55 m*l*/min and 30 m*l*/min nitrogen makeup gas was supplied through the detector maintained at 280°. The cold bath was removed and the chromatographic analysis was started isothermally at 35 °C for 10 min, then programmed at 4 °C/min to 160 °C, and held at 160 °C for 10 min.

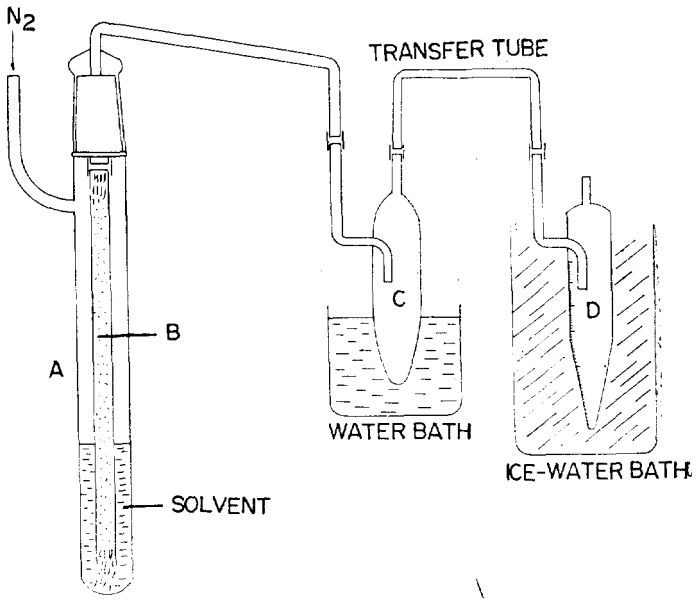

Fig. 3. Column elution sampling system. (A) Isolator; (B) tobacco column; (C) concentrator; (D) graduated concentrator.

Volatile Fraction. A glass capillary column (40 m×0.25 mm I.D.; coated with methyl silicone gum SE-30; Supelco, Inc., Bellefonte, Pa, USA) was used for this study. Injector port was maintained at 200 °C and capillary inlet system was operated at splitless injection mode: a splitless glass liner was installed; inlet purge flow was adjusted to 50 m*l*/min; purge delay time was set to 0.5 min; septum/seal purge flow was adjusted to 0.5 m*l*/min. Column inlet pressure was adjusted to 0.7 kg/cm^2 so that the flow of nitrogen through the column was 0.75 m*l*/min, and makeup gas flow at 30 m*l*/min was furnished through the detector kept at 280°C. An aliquot of 1.5 μl was injected and oven was kept at 30°C for 10min, then programmed at 5°C/min to 200°C, and maintained at this temperature for 10 min.

High-Performance Liquid Chromatography (HPLC). The less-volatile fraction was analyzed using a Waters Associates Model ALC/GPC 200 liquid chromatograph equipped with dual Model 6000 A solvent delivery systems, a Model U6K universal injector, a Model 660 solvent programmer, and a Model 440 UV absorbance detector. A 5 μl aliquot was injected into a μ-Porasil column (30×0.39 cm I.D.; Waters Associates) which was eluted at a flow-rate of 2 m*l*/min, with solvent systems, A=*n*-hexane:

and B=1 % methanol in dichloromethane, linearly programmed at 5 % B/min, from 0 % to 100 % B, and held at 100 % B for 20 min. UV absorbance was monitored at 254 nm.

RESULTS AND DISCUSSION

The present sampling device allows consecutive enrichments of the headspace volatile, the less-volatile and the volatile fractions from a given tobacco sample. Solvent elution, evaporation and concentration steps after the headspace trapping were continuous procedures with no other manipulations than the elevation of the temperature of the water bath, the increase of the flow-rate of nitrogen and the removal of ice-water bath. The sampling procedure was performed on a microscale under the continuous stream of inert gas. Therefore, the chance of introducing contaminants or creating artifacts were minimized. The sampling for all fractions could be completed within 2 hr.

Headspace sampling[9] is a well-established procedure to collect highly volatile compounds either in major or trace amounts. In the course of the collection of headspace volatile components, Tenax GC was used as the trapping sorbent. Because of its high adsorptivity, desorptivity, thermal stability and hydrophobicity Tenax GC[10] has become very popular for the concentration of trace amounts of volatile compounds.

The evaporation of the solvent eluate effects

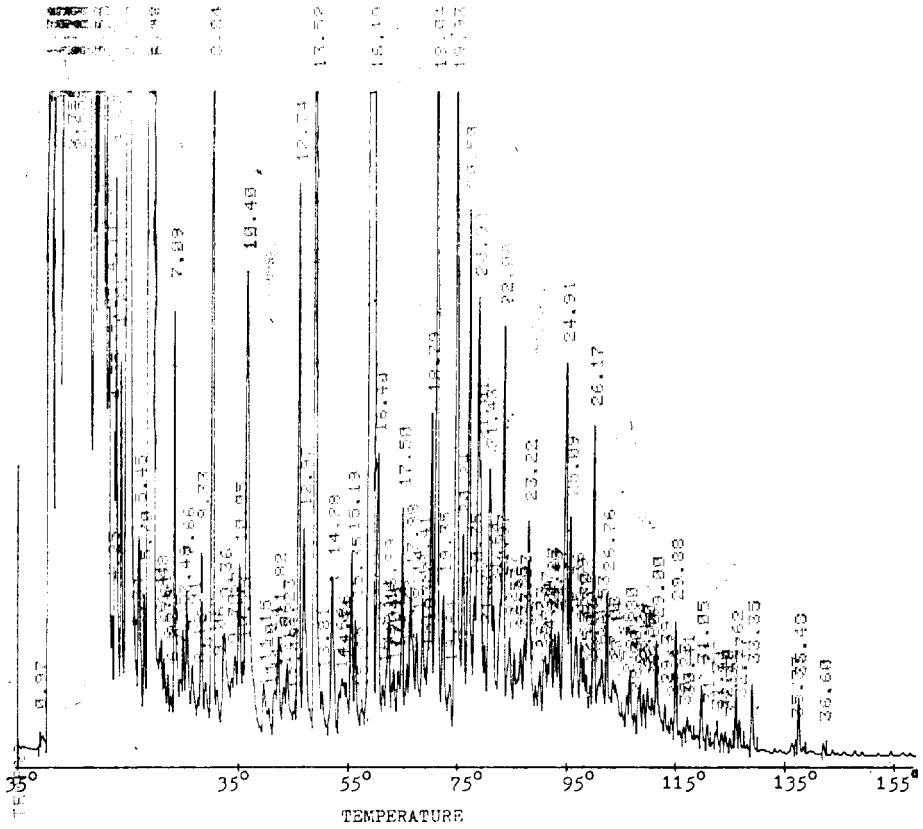

Fig. 4. GC profile of tobacco headspace fraction. Column, fused silica capillary column (12 m×0.2 mm I.D.) coated with methyl silicone fluid; cold trapping, 2 ml/min for 10 min using dry ice-ethanol coolant; N_2 carrier, 0.55 ml/min; oven temperature, isothermally at 35 °C for 10 min and programmed at 4° C/min to 160 °C; injector, 280 °C; detector, 280 °C.

a separation of the volatile components from the less-volatile substances which interfere with GC analysis.

In the analyses of the headspace and the volatile fractions, a fused silica and a Pyrex glass capillary columns were employed. Because of the high separation power and inertness, the use of glass capillary columns in GC has been rapidly increased[11]. Fused silica capillary[12,13], an advanced version of glass capillary, in terms of the durability and inertness, was recently developed. Its inherent flexibility enables to reform the first couple of meters of the column into a precolumn cold trap. Headspace volatile components thermally desorbed from the Tenax GC trap were swept and introduced into the chilled precolumn and then condensed in the form of a plug. This insures a sharp plug injection of the components, which is a necessary requirement for obtaining high resolution of the sample.

The headspace fraction thereby collected and desorbed into a GC are representative of the composition of the volatile flavors emitted from tobacco leaves. The complexity of the tobacco headspace volatiles is demonstrated in *Fig.* 4. As can be seen from the profile, low boiling components at the early part were in high concentration and the run could be finished before the column reached the temperature of 150 °C, which proves that the headspace enrichment is efficient for concentrating low boiling components.

The volatile fraction, even after concentration, was still too diluted to be directly injected to a capillary column without overloading it.

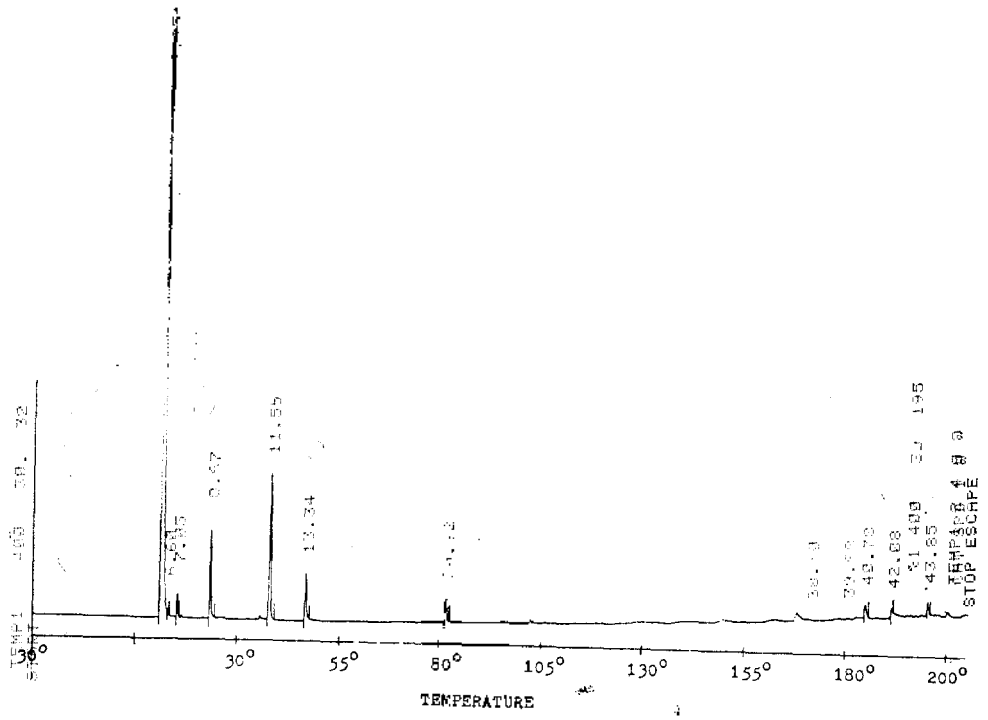

Fig. 5. GC profile of tobacco volatile fraction (split mode). Column, glass capillary (40 m×0.25 mm I.D.) coated with SE-30; N_2 carrier flow-rate, 0.75 ml/min; 0.6 μl injection in split mode operation; split ratio, 100:1; column temperature, 30 °C for 10 min then programmed to 200 °C at 5 °C/min and held at 200 °C for 10 min; injector, 200 °C; detector, 280 °C.

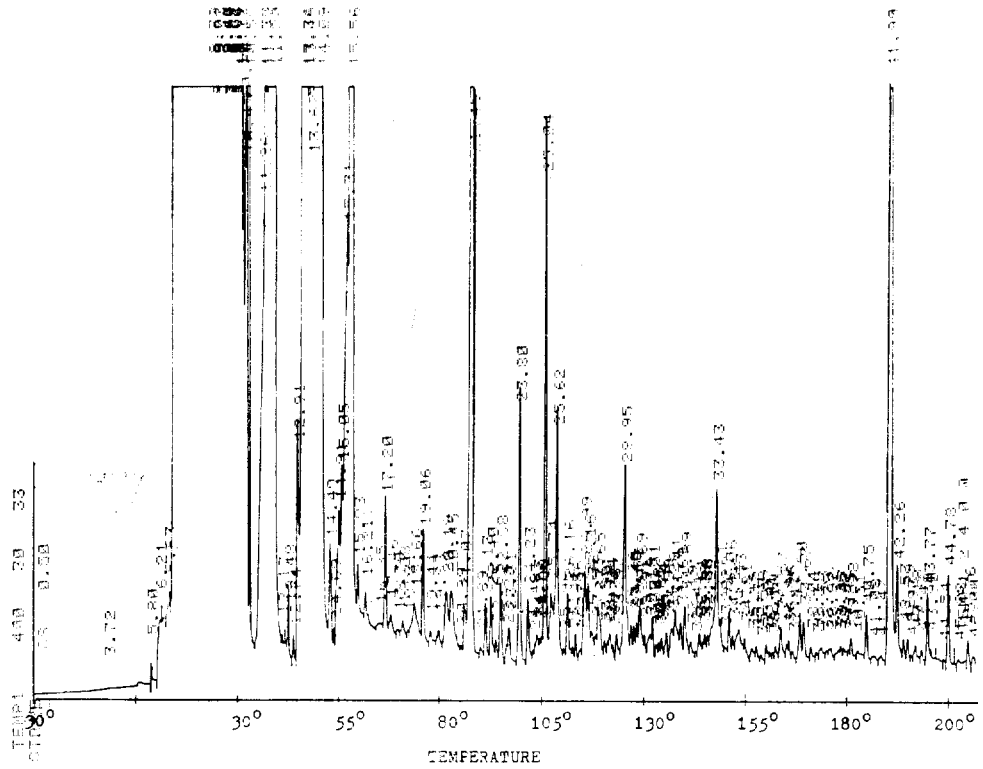

Fig. 6. GC profile of tobacco volatile fraction (splitless mode). 1.5 μl injection in splitless mode operation; purge flow, 50 ml/min; purge delay time, 0.5 min; septum/seal flow, 0.5 ml/min; other conditions are the same as in *Fig.* 5.

"Grob splitless" injection technique[14~16] was thus employed to inject the volatile fraction onto the capillary column since a relatively large amount of dilute sample (0.5~6 μl) can be injected without affecting resolution. This splitless technique has been found to be best suited for trace analysis[16]. *Fig.* 5 and 6 show the GC profile of the volatile fraction in the split injection mode and splitless injection mode, respectively. Components in trace amounts were not detected as seen in *Fig.* 5. The early portion of the chromatogram in *Fig.* 6 is dominated by solvent peaks. The levels of components are low except for several major peaks. Some volatile compounds seem to be lost during the concentration step which was done under the high stream of nitrogen. Further refinements are needed to minimize the loss of the volatiles.

The less-volatile fraction was analyzed by normal phase HPLC. To improve the overall resolution of the complex mixture, gradient elution mode was applied. *Fig.* 7 illustrates the high-resolution HPLC profile. Ultraviolet absorbing components were only detected.

CONCLUSION

The designed separation scheme provided the fingerprinting profiles of the three fractions, from which analytical information could be traced. The technique has already been used for the characterization of Korean aromatic tobacco varieties as compared with Oriental-type in our laboratory.

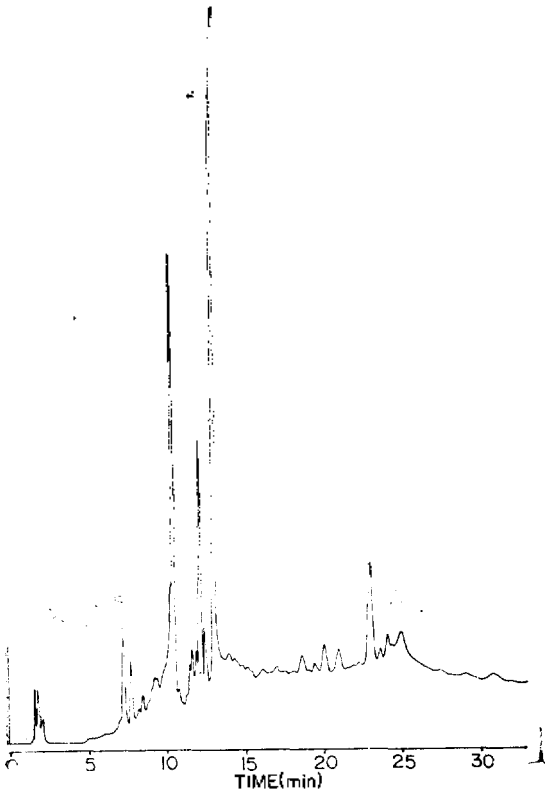

Fig. 7. HPLC profile of tobacco less-volatile fraction. Column, μ-Porasil (30 cm×0.39 cm I.D.); flow-rate, 2 ml/min; elution, gradient (linear) in 20 min; mobile phase, *n*-hexane to dichloromethane containing 1 % methanol and held at final solvent for 20 min; detector, UV at 254 nm; sensitivity, 0.1 aufs.

This simple but efficient sampling procedure should be applicable to other complex matrices such as foods, natural flavor products and plant or animal tissues.

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