Some Aspects of High-Resolution Gas Chromatographic Analysis of Complex Volatile Samples

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Volatile samples from food aroma, odors, air pollution, tobacco smoke, and physiological fluids are of such complexity that only high-efficiency capillary columns are capable of an adequate degree of resolution. The use of high-resolution chromatography for both research and routine analytical purposes requires extended use of computer techniques. The application of computers is considered in view of the problems associated with gas chromatography–mass spectrometry, technology of capillary columns, and reproducibility of retention data and quantitation. The value of a new efficient sampling method for routine headspace analysis is suggested. Examples of complex mixtures from aroma and biomedical research are illustrated and the application of computer-based pattern recognition to such samples is discussed.

A steadily increasing amount of analytical information has been obtained from complex volatile mixtures since high performance chromatographic columns have provided greater resolution and more sensitive and selective detectors have become available. Up to several hundred constituents in complex volatile mixtures, such as those encountered in aromas, tobacco smoke, air pollution, and biological samples, can now be resolved with high-efficiency capillary columns. Moreover, additional compounds are often revealed in complex chromatograms with the combination of gas chromatography and mass spectrometry as well as by the use of selective detectors.

Different approaches to the analysis are usually chosen for research and routine purposes. The primary task of researchers in all mentioned fields is usually to separate (isolate) biologically active compounds and elucidate their structures. In doing so, they often have to determine all mixture constituents. At present, combined gas chromatography–mass spectrometry has gained considerable prominence in such problems, but other ancillary techniques may sometimes be useful. Once the sample composition is more or less known, high-resolution capillary gas chromatography can be routinely used to observe qualitative and quantitative changes in samples under different circumstances. High-resolution gas chromatography is an ideal method for screening purposes, since many compounds can be simultaneously recorded. For instance, any changes in fruit aroma due to ripening, storage conditions, etc., can be followed. In a quite similar fashion, the differences in volatile metabolites of urine or blood due to pathological conditions may be traced from high-resolution chromatograms. The source of water or air pollution can be similarly identified from a complex chromatographic "fingerprint." Precise chromatographic information is usually sufficient in such cases.

Both types of analyses share a common feature: the amount of data from both gas chromatography–mass spectrometry or high-resolution profiling methods alone is so excessive that the use of computer techniques for evaluation becomes mandatory. While the future of routine coupling of data acquisition systems and computers with the concerned analytical units is undisputable, many technological problems remain yet to be solved. The number of problems generally increases with a decrease in the volatility of studied samples. In this article, we will limit our discussion to the mixtures of volatiles which can be eluted from capillary columns at temperatures not appreciably higher than 200°.

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HIGH-RESOLUTION GAS CHROMATOGRAPHY–MASS SPECTROMETRY AS A RESEARCH TOOL

Even the most impressive chromatograms obtained with high-efficiency capillary columns are of little use without the positive identification of separated peaks. Mass spectrometry is the only choice for identification purposes due to sample size requirements for capillary columns. Although capillary columns had been used in early developments in the gc–ms field, only a few laboratories have accomplished a successful coupling of the two methods at this time. Molecule separators with low dead volumes[18,24] are generally applicable, but the current trend seems to go toward the "no separator" approach, where powerful pumping systems must be employed.[12,34] Efficient couplings of high-resolution glass capillary columns with mass spectrometers allow identifications to be carried out on nanogram quantities as, for instance, demonstrated in tobacco[3,13,34] and air pollution[11] analysis. Due to sensitivity requirements and fast scanning speeds used in recording sharp capillary peaks, mass spectrometric resolution and measurement precision must often be sacrificed.

With complex chromatograms containing up to several hundred constituents it is obvious that the use of computers becomes inevitable. Computer methods applied to high-resolution gc–ms may be important in several directions. An appropriate system can quite easily handle enormous amounts of data and convert them precisely to useful analytical information. Both off- and on-line processing may be used. Even with complex capillary column chromatograms, mass spectra can be taken virtually throughout the whole analysis on an automatic basis at either preset intervals or as the chromatographic peaks emerge from the column. For instance, Kaiser and Schulze[34] were able to store up to 1800 recorded spectra with a low-cost computer system.

Further, computers with larger capacities can simultaneously perform a number of tasks which are essentially impossible through manual operation. These are, for instance, continuous checking of the baseline position or peak shapes, corrections for spectra distortions due to pressure changes during measurement, automatic adjustment of sensitivity, rapid information on overlapped peaks, etc. Computer-aided interpretation of spectra through comparison with a collection of stored spectra is another well known aspect of computer usage. General and specific roles of computers in gc–ms have been summarized in an excellent review by Henneberg *et al.*[17]

CHROMATOGRAPHIC PROFILE METHODS

When reproducible chromatograms are obtained from complex samples, both qualitative and quantitative dif-

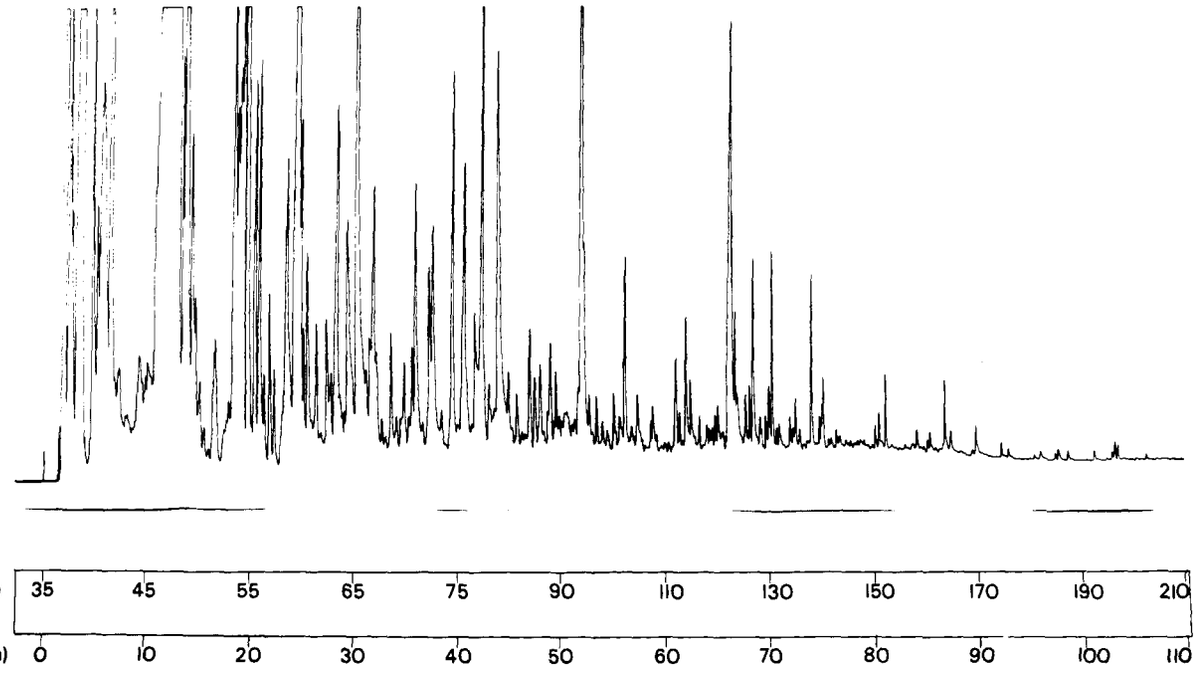

Figure 1. Chromatogram of volatiles from the urine of a normal male. The sample was obtained by a headspace concentration method at 100°. Conditions: 60 m × 0.25 mm i.d., glass capillary column coated with SF-96 silicone oil.

ferences due to various circumstances may be observed. This approach is becoming increasingly important in the biomedical field, where such sample fingerprints have been referred to as "metabolic profiles."[8,19] In such cases, multicomponent determinations are carried out with physiological fluids in order to diagnose pathological conditions, to study drug effects, to observe the environmental influence on the organism, etc. Due to extreme complexity and limited stability of many biological samples, it often becomes mandatory to use high-efficiency glass capillary columns.[29]

Only recently have the techniques used by flavor research chemists been applied to study volatile constituents of body fluids.[28,38,41,43] Many low molecular weight compounds which constitute such volatile profiles have already been identified.[41,43] A typical urinary profile of a normal male, obtained by a headspace sampling technique[28,42] and chromatography on a glass capillary column coated with a nonpolar silicone fluid, is shown in Figure 1.

Based on similar chromatographic procedures, the identification of oil spill[1] and air pollution sources[4] has been attempted. The potential of food quality fingerprinting (so called "aromagrams") has clearly been indicated.[37] An extended use of these methods, particularly for routine purposes, in the near future can be expected.

SAMPLING PROCEDURES

It is not always realized that a reproducible multicomponent analysis is impaired more often by sample preparation and treatment than the following chromatographic step. Both extraction and headspace sampling techniques may be used to prepare samples of volatiles for analysis. The determination of trace organics present in the headspace is of primary importance in the cases where extractions fail to provide desired information (*e.g.*, in aroma-related problems). A concentration of headspace samples becomes necessary with high-resolution capillary columns because of the requirements for sample size. Direct injections are sometimes satisfactory with the use of the so-called "cryogenic procedure."[3,16,32] Significant advances in headspace sampling methods have been recently achieved[27,42] through the application of an unusually stable porous polymer[39] known under the commercial name TENAX-GC (2,6-diphenyl-*p*-phenylene oxide, available from Applied Science Laboratories, College Station, Pa.). This organic porous adsorbent proved to be a suitable medium for the efficient trapping and transfer to a gas chromatograph of the headspace constituents of human urine,[28,41,42] breath,[42] polluted air,[42] and marijuana smoke in a room atmosphere.[26] Good reproducibility is an important advantage of this method. All chromatograms shown in this publication have been obtained in this way. Figure 2 shows a headspace chromatogram of dry sage leaves sampled at 70° for 10 min (upper chromatogram). The lower chromatogram shows the blank of our sampling system at high sensitivity. Quantitative aspects of the sampling method will be reported elsewhere.[27]

Due to its rapidity and simplicity, this sampling technique may prove useful in the rapid evaluation of the headspace "fingerprints" for routine purposes, quality control, etc. One possible application of this method for evaluation of tobacco flavor is suggested by Figure 3, where "aromagrams" of three different cigarette products are compared.

HIGH-PRECISION CAPILLARY GAS CHROMATOGRAPHY

Although gas chromatography–mass spectrometry usually yields the ultimate answer in the identification of chromatographic peaks, routine analyses can hardly depend on this method. Since each compound in a complex chromatogram is, to a certain extent, characterized by its retention and response value, this type of information is likely to find more extensive use in routine work once the mixture composition is generally established. Furthermore, retention data can often times yield additional structural information which is difficult to achieve by any other means. As indicated by Schomburg and Dielmann,[35] reproducibility of retention data with high-efficiency glass capillary columns up to 0.05 Kováts index units with nonpolar and 0.1 units with polar stationary phases is now available through computer evaluation. High separating power of a column is mandatory to resolve two solutes which differ by 1 index unit or less.

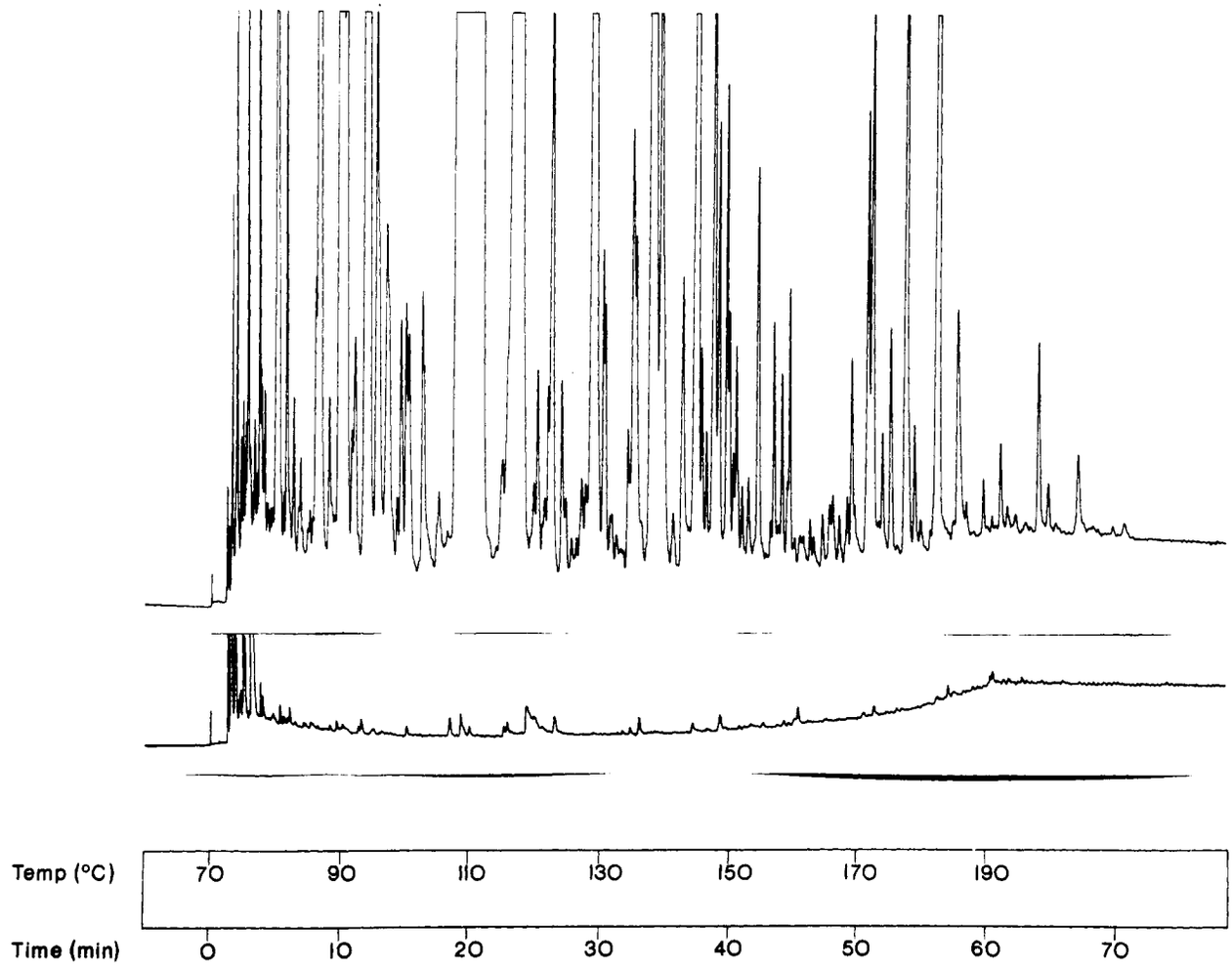

Figure 2. Chromatogram of the headspace of sage leaves at 70° with the blank chromatogram of the sampling system. Conditions: 40 m × 0.3 mm i.d., glass capillary column coated with Emulphor.

Since fully resolved peaks in a chromatographic profile may be separated by only seconds or less, an unambiguous peak identification places high requirement on reproducibility. Consequently, some technological advances are still needed for fine control of temperature and carrier gas flow rate. In addition, technology of capillary columns with reproducible sorption properties is of serious concern. This situation is likely to improve with advancing knowledge of the surface chemistry of high-resolution columns.[2,5,25]

Distinction among individually analyzed samples is generally complicated by the natural range of variation in concentration of constituents. Such a case is the range of values considered normal[2,36] in metabolic patterns. If meaningful routine evaluation is attempted by computer techniques (*e.g.*, pattern recognition), it is essential that such a range not be extended by an imprecise quantitative method. Both column technology and detection parameters must, therefore, be brought under strict control. Hopefully, more extensive use of currently available autosamplers in the future will significantly improve the precision of quantitative measurements.

SIMPLIFICATION OF CHROMATOGRAPHIC PROFILES

Certain natural mixtures are of such complexity that a great number of peaks still overlap, even with columns possessing several hundred thousand theoretical plates. Moreover, small chromatographic fractions (not necessarily unimportant ones) may often be obscured by the presence of major constituents and therefore remain unnoticed. Trace analysis is sometimes further complicated by the maximum total sample size used without column overloading. Thus, selective enrichment is often necessary.

Fractionation and group separation schemes based on different forms of chromatography or various chemical principles are most commonly sought; however, formation of artifacts and destruction of labile constituents is often too high a price to pay for enrichment, and more straightforward methods are indeed desirable. While no satisfactory general solution of these problems can be offered at present, at least three promising methods appear to have some future.

(1) Column-Switching Techniques. Multiple column systems have primarily been used in work with packed columns based on the method as described by Deans,[9] but similar applications with capillary columns are rare.[10,33] This is undoubtedly due to the problems with insufficient inertness and excessive volumes in connections between two high-resolution columns. By switching a portion of the effluent from one column to another, certain portions of a complex chromatogram can be investigated in greater detail. Better resolution can then be accomplished through either different operating conditions of the second column or a stationary phase of different selectivity.

(2) Subtractive Precolumns. Although subtraction techniques are quite common in petroleum analysis (*e.g.*, when dealing with mixtures of saturated and unsaturated or normal and branched hydrocarbons), similar approaches should be investigated with mixtures of polar compounds. Such methods would not only simplify the task of

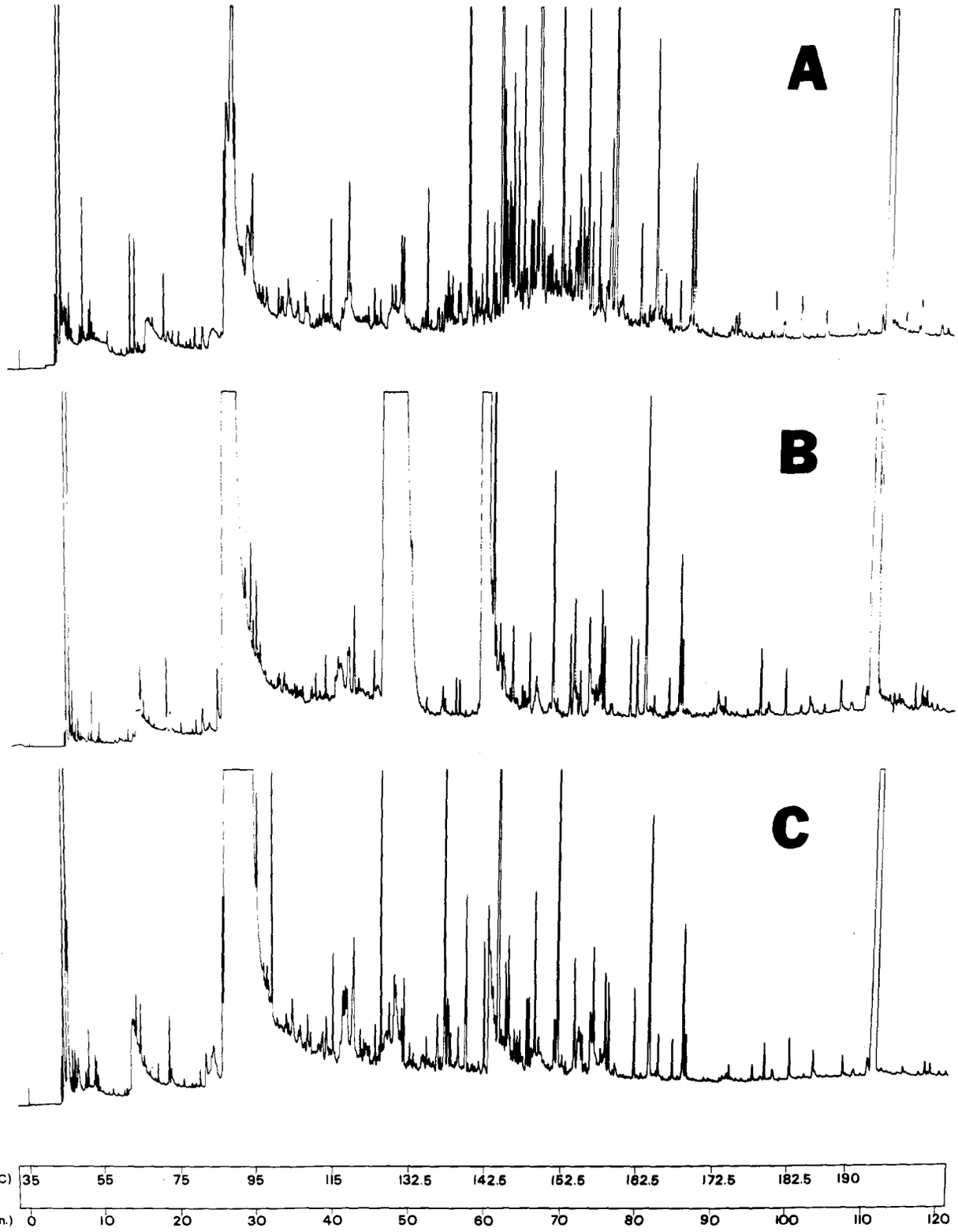

Figure 3. Headspace chromatograms of three different cigarette tobacco samples at 70°. Conditions as in Figure 1. A = standard tobacco cigarette obtained from Tobacco Health Research Institute, University of Kentucky, Lexington, Ky. B = commercial menthol cigarette. C = commercial cigarette.

resolving complex mixtures but also provide valuable qualitative information.

(3) Selective Detection. Selective detectors based on different principles have already found wide application to different biomedical and environmental problems. However, only a few of them possess suitable parameters for efficient coupling with high-resolution columns. The three most obvious and useful selective detectors applicable to the monitoring of capillary column effluents are the combined flame ionization-flame photometric, thermionic, and electron capture detectors.

The use of a flame photometric detector is particularly attractive for flavor studies, where trace sulfur compounds are known to be significant. A high degree of selectivity and sensitivity in the picogram range can be obtained with this detector. The flame photometric (sulfur-sensi-

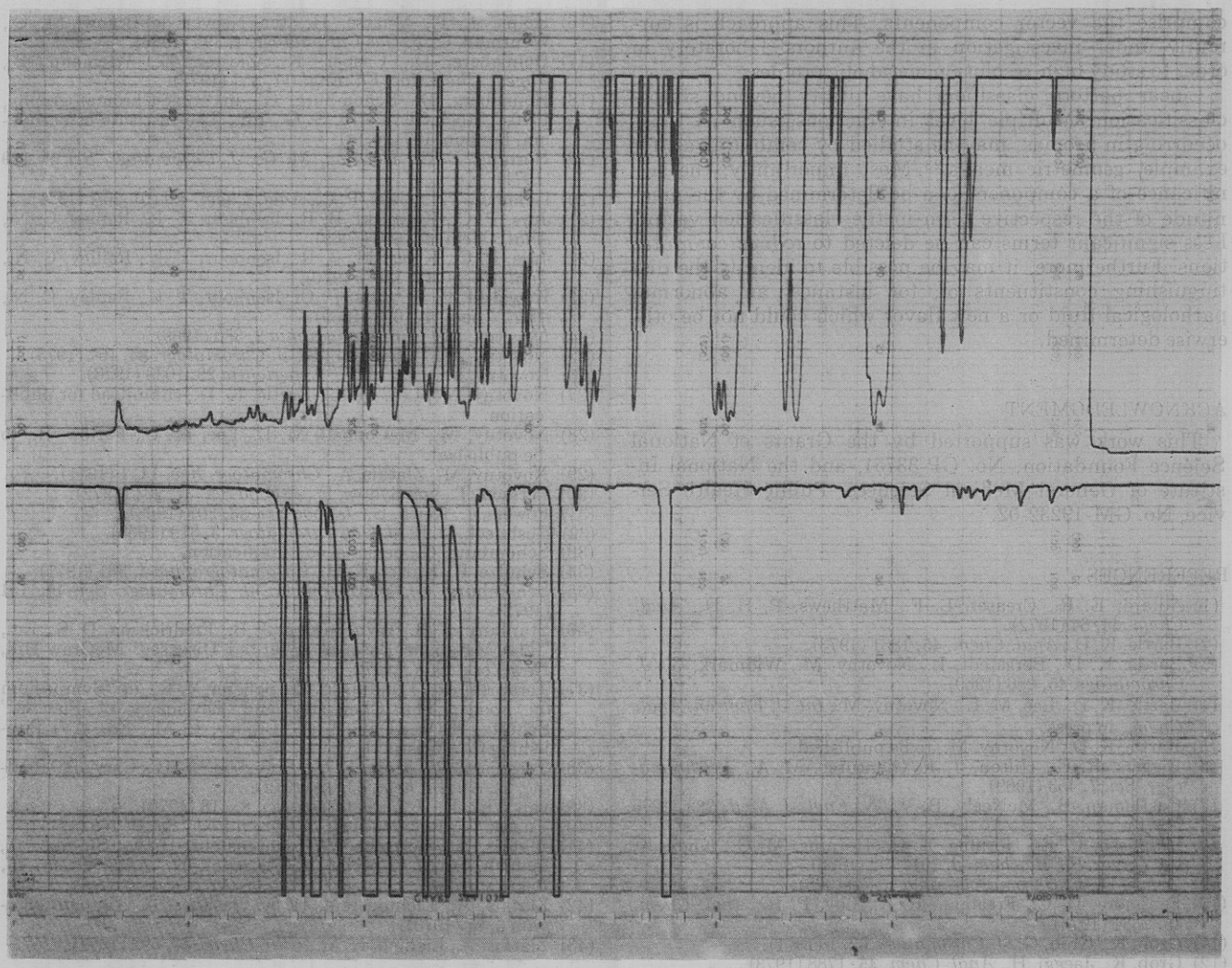

Figure 4. Dual chromatogram of an 800 cm^3 sample of natural gas trapped on a porous polymer[42] and detected by a combined flame ionization–flame photometric detector. The lower chart corresponds to the flame photometric detection signal. Chromatographic conditions: 100 m × 0.5 mm i.d., nickel capillary column coated with Emulphor. This chromatogram was obtained through the courtesy of Drs. W. Bertsch and A. Zlatkis.

tive) signal can be simultaneously recorded opposite the flame ionization trace, thus quite safely indicating which peaks correspond to sulfur compounds. Figure 4, which shows the resolution of organic constituents concentrated on a porous polymer precolumn[42] from 800 cm^3 of natural gas, is a typical example of such selective recording.

COMPUTER-AIDED DATA EVALUATION

Automated data processing in the routine analysis of multicomponent mixtures offers distinct advantages over manual handling. The accuracy, precision, and speed of measurements of retention parameters and peak areas are significantly enhanced by the use of digital integrators and computers. Commercial software for fused peak processing, baseline correction, etc., is now readily available. The evaluation of chromatograms in digital form can further be improved and extended by computer techniques.

Various techniques have been described to correlate chromatographic and flavor data by statistical computation.[6,14,15,30,31,40] Hawkes and Wheaton,[15] for instance, classified peppermint oils statistically on the basis of geographic origin. More recently, this classification was repeated[14] utilizing a form of discriminant analysis. The organoleptic flavors of coffee[6,31] and potato chips[31] have been correlated with gas chromatographic data by another form of discriminant analysis.

Biggers *et al.*[6] have analyzed 32 of about 70 gas chromatographic peaks obtained from steam distillates of coffee, yielding satisfactory evaluation of one type of coffee independent of the degree of roast. Since a particular flavor may result from component interactions of several compounds, peak height ratios rather than simple peak heights were examined. This approach does not allow for multiple interactions. The range of each peak ratio was then determined for different qualitites of coffee, and those ratios which were nonoverlapping were used as criteria for evaluation of other coffees. Such techniques are satisfactory for the analysis of up to about 40 peaks. However, their utilization for the evaluation of all data from a high-resolution chromatogram would be computationally unwieldy.

A potential solution for high-resolution chromatography may be the use of linear pattern classifiers as applied by Jurs *et al.*[20,21,22,23] to the analysis of low-resolution mass spectra. The peak intensity at each mass unit is represented here as the component of a vector. A classification vector is computed by an iterative training or learning machine procedure using known spectra that may overlap in any or all components. A new spectrum may be classified by simply computing its dot product with the classification vector. This method can be quite easily applied to patterns of high dimensionality and appears suitable for classifying chromatograms with a high degree of complexity. Peak areas in uniform retention time intervals should

comprise the vector components. This approach is currently under investigation in the authors' laboratory in order to study high-resolution metabolic profiles.

Linear pattern classifiers have utility beyond simple classification. Multiple level interactions, such as those occurring in aromas, may be studied by combining as, for example, geometric means.[23] Most importantly, the significance of a component can be determined by the magnitude of the respective term in the classification vector. Less significant terms can be deleted to reduce computations. Furthermore, it may be possible to identify the distinguishing constituents of, for instance, an abnormal pathological fluid or a new flavor which could not be otherwise determined.

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