

## Milestones in Chromatography

This "Milestones in Chromatography" column discusses four major application fields in which gas chromatography changed the way chemists carry out analyses.

# The Evolution of the Application of Gas Chromatography

New analytical techniques usually create some excitement. However, they become part of our standard methodology only if they provide some distinct advantages over existing and well-established methods and permit analysts to make measurements that were hitherto impossible. This situation was the case with gas chromatography (GC) when it was introduced 50 years ago. Its rapid acceptance was due mainly to the fact that it provided the immediate solution to the analysis of a number of important sample types. This situation also continued in the following years, and GC provided the solution to a number of analytical problems.

Looking back upon the early evolution of GC, I would like to point particularly to four fields in which its use revolutionized analytical chemists' methodology. These fields were the analysis of complex hydrocarbon mixtures, the determination of individual fatty acids present in lipids, the investigation of naturally occurring flavor compounds, and the detection of trace toxic impurities in the environment and food. Without the use of GC, scientists probably would still be, figuratively speaking, in the Dark Ages of analytical chemistry.

This "Milestones in Chromatography" column will describe how, within one decade after its introduction, GC changed scientists' perceptions of these very complex, naturally occurring sample types. GC not only permitted the fast and relatively easy determination of their already known constituents but also revealed the presence of important trace compounds that previously were unknown. In this way, GC significantly contributed to the understanding of the environment and broadened scientists' knowledge of the human body and the food humans consume.

demanding the accurate analysis of different sample types. In chemical synthesis, the so-called light petroleum gases (LPG), which consist of hydrocarbons with carbon numbers as large as approximately  $C_5$ – $C_6$ , represent a very important raw material. Early techniques developed for LPG analysis were complicated and tedious; they required large gas samples (a few hundred milliliters) and 8–12 h analysis times. Despite this effort, analysts could obtain only partial results (1–3).

The introduction of GC changed the situation; in fact, the very first advertisement of a commercial gas chromatograph (Perkin-Elmer's model 154) used a chromatogram of the  $C_1$ – $C_5$  hydrocarbons, including all the  $C_4$  saturated and unsaturated isomers (Figure 1) (4). Instead of a large gas sample, this analysis needed only 1–5 mL gas samples. And instead of many hours, the analysis was finished in 23 min. Thus, it is not surprising that GC replaced the existing, complicated, and time-consuming techniques almost immediately. Present-day chromatographers do not realize the importance of the breakthrough represented by GC in the petrochemical field. This also was how I started in GC 46 years ago, and I had to analyze a number of such samples each day. In fact, establishing the peak area (at that time no integrators were available) and the calculation of the sample composition took longer than the analysis itself.

Many other analytical problems in the petroleum and petrochemical fields also found solutions with the introduction of GC, among them the complex problem of the elucidation of the composition of petroleum. Petroleum contains hundreds of various hydrocarbons in a wide boiling range, and scientists had desired to learn as much as possible about its composition. In 1927, the American Petroleum Institute initiated Research Project Number 6, which sought to separate and identify the constituents of crude petroleum. The substance

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Editor

### Hydrocarbon Analysis

During and after World War II, the petrochemical industry evolved very rapidly and



problems (dimerization of the acids in the stationary phase) the technique worked, and they soon succeeded in separating the lower fatty acids ( $C_1$ – $C_{12}$ ). Their paper

not only reported on this analysis but also presented the theory of GC (12). Soon, others demonstrated that it was preferable to analyze the fatty acids as their methyl

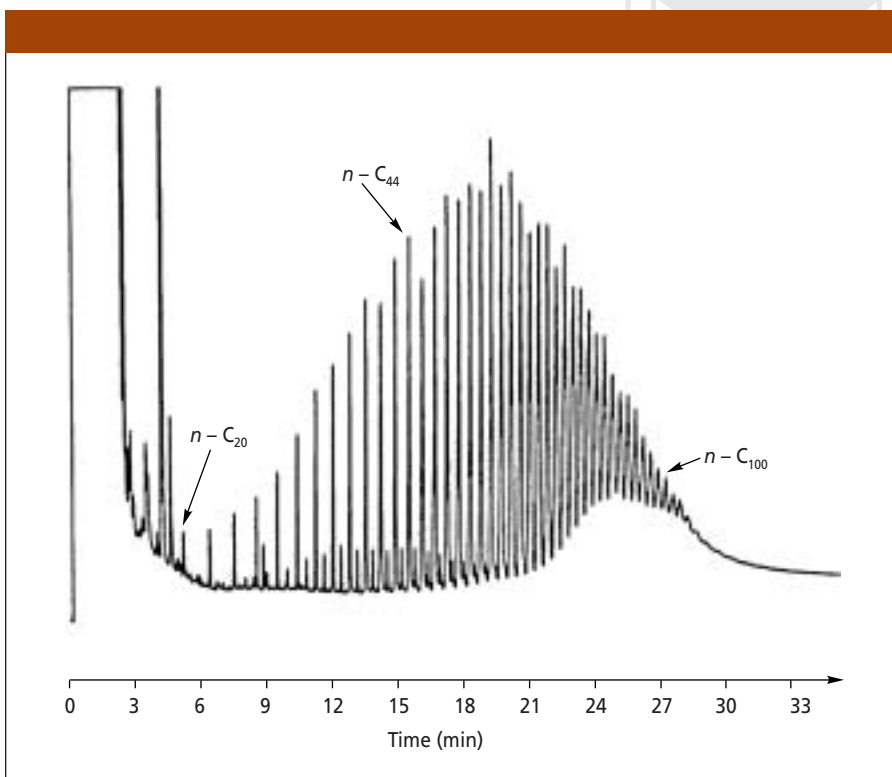
esters, which extended the range to  $C_{22}$  acids (13).

In the beginning, scientists had few choices for usable stationary phases: only a couple nonpolar phases were stable at the necessary high temperatures (200–230 °C), and these phases had insufficient selectivity to separate saturated and unsaturated acids that contained the same number of carbon atoms. A breakthrough occurred in 1958 with the introduction of Reoplex 400, which was a polyester-type plasticizer used as the stationary phase (14,15). Soon small companies (most notably Applied Science Laboratories [State College, Pennsylvania]) started to produce and market various polyesters synthesized specially for use as GC stationary phases. Figure 4 shows a typical chromatogram of a fairly complex fatty acid methyl ester (FAME) mixture (16).

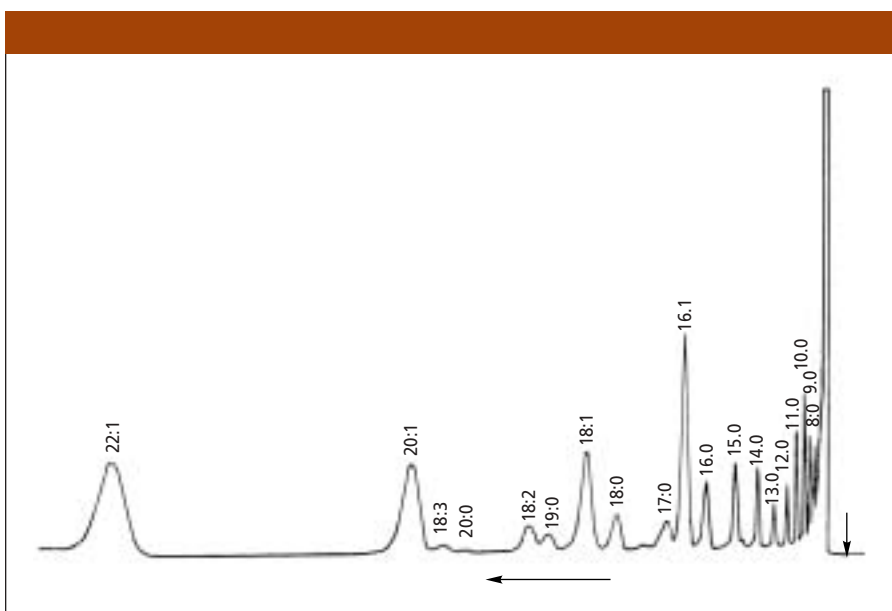
The possibility of GC analysis of FAMES was very important. Until then, fats and oils essentially were characterized by some highly inadequate methods that relied mainly upon physical characteristics and by a few primitive chemical tests such as the bromine number to indicate the degree of unsaturation. With GC, the individual constituents could be determined fairly easily, which completely changed the situation. I just want to mention one example from my own experience.

Circa 1960–1962, Perkin-Elmer's representative in Austria contacted me for help. A legal process had been initiated against a vendor of sunflower oil, in which he was accused of adulterating the commercial product by using carefully blended cheaper oils. The vendor evidently was clever, and his product fulfilled the then-existing general specifications for sunflower oil; thus officially, his product looked genuine. However, a young chemist in a government laboratory who had served as the expert witness to the court wanted to investigate the adulterated product by GC. Comparing it with pure sunflower oil samples, the difference in composition was clear. As I was told, this instance was the first time that GC analysis was used as evidence in a court of law.

After the introduction of capillary columns, ionization detectors, and even more stable selective stationary phases, FAME analysis could be further refined to also permitting the separation of *cis*–*trans* isomers of the unsaturated fatty acids (17). Soon such analyses became part of our standard methodology and an indispens-



**Figure 3:** Chromatogram of a Polywax 1000 sample containing hydrocarbons with carbon numbers as large as  $C_{100+}$ , from 1987 (9). 8 m  $\times$  0.25 mm aluminum-clad fused-silica capillary column coated with bonded methylsilicone stationary phase ( $d_f = 0.1\text{-}\mu\text{m}$ ). Column temperature programmed from 50 °C to 430 °C at 15 °C/min. Programmed-temperature vaporizer, heated from 50 °C to 440 °C. Sample: 0.5  $\mu\text{L}$  of a 1-mg/mL solution in 1:1  $\text{CS}_2$ –*n*-decane, split 1:20. Carrier gas: hydrogen. Flame ionization detector.



**Figure 4:** Chromatogram of fatty acid methyl esters, from 1964 (16). 10 ft  $\times$   $\frac{1}{8}$  in. o.d. packed column containing 10% ethylene glycol cyanoethyl succinate on silanized support. Temperature: 194 °C; flame ionization detector.

able tool in agriculture and food chemistry and biochemistry to assess the fat content and the relative amount of the individual acids in lipids or triglycerides. In fact, the fingerprint of the fatty acid composition often can be used to identify the organisms.

The newest breakthrough in this field is represented by the possibility of identifying bacteria by the analysis of FAMES that have been extracted from the bacteria cell walls. As reported recently, MIDI Inc. (Newark, Delaware) established a library of FAME profiles of more than 1500 species of bacteria. The most exciting use of this library was in the fall of 2001, when it was used to confirm anthrax infection as the cause of the death of a 94-year-old woman living in Oxford, Connecticut (10).

For fatty acid analysis, the triglycerides must be hydrolyzed and then the methyl esters formed. This process usually is per-

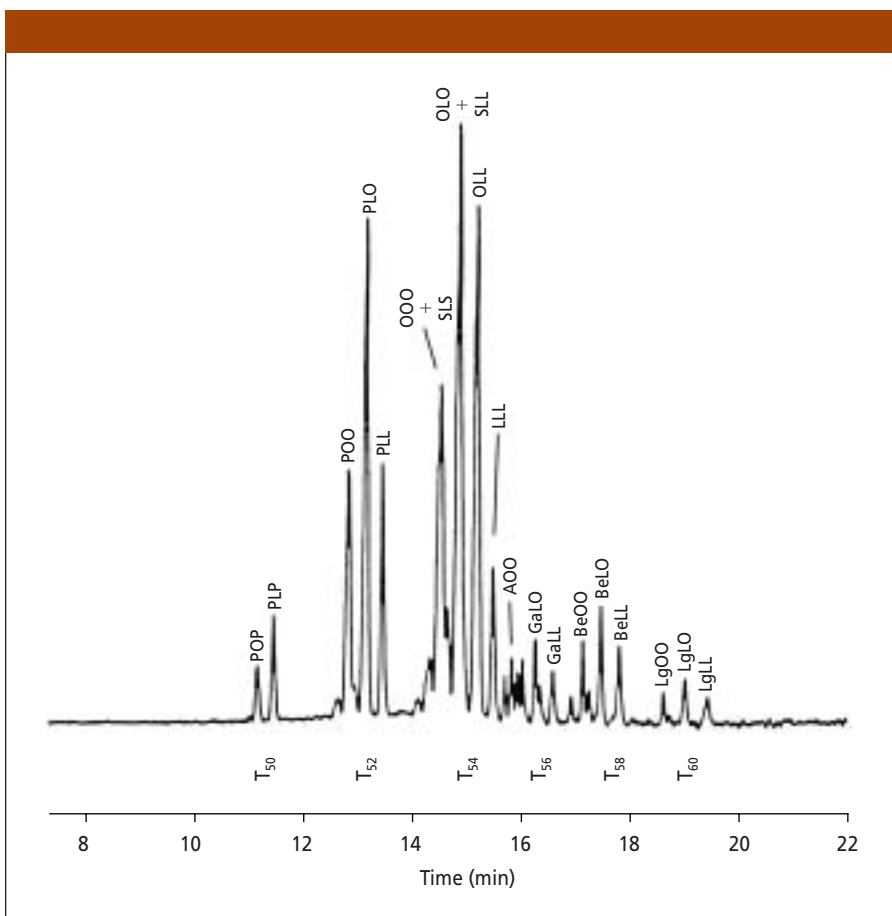
formed in one step and generally is a routine procedure. However, the recent advent of capillary columns that permit operation at high temperatures now allows even direct analysis of the triglycerides without hydrolysis. In particular, the group of P. Sandra in Belgium (18,19) pioneered this application. The chromatograms showed clusters of peaks, each cluster representing isomers with the same carbon number. Within each cluster, the individual peaks corresponded to the individual triglycerides. Figure 5 shows a typical chromatogram, which illustrated the analysis of the triglycerides of peanut oil (20). For example, the cluster marked as T<sub>54</sub> contains the peaks of triglycerides in which the number of carbon atoms of the three fatty acids totals 54. This again is an example of the recent extension of the range of compounds amenable to GC.

## Flavor Compounds and Essential Oils

One field in which the superiority of GC is obvious is the analysis of flavor compounds, fragrances, and essential oils. In the first years of its evolution, GC often was compared with the human nose: everything that a human can smell can be determined by GC. In fact, some of the first demonstrations of the superiority of GC were in this field.

The potential of the technique is best illustrated by the story of Keene P. Dimick, originally an agricultural chemist working at the Western Regional Research Laboratory of the U.S. Department of Agriculture in Albany, California (21). Starting in 1946, his research project dealt with the investigation of the natural aroma compounds of strawberries. It took him six years of hard work to reduce 30 tons of strawberries to approximately 35 mL of an oil that represented the essence of the fruit; however, it was still an open question as to how to further investigate this oil. Because of the lack of appropriate analytical methods available to separate and identify its important components, he was going to terminate the project until he first heard about GC in 1953. GC completely changed the situation. With his associates he built a gas chromatograph using a Gow-Mac thermal-conductivity detector and used it to investigate the strawberry essence. Their first report was presented at the famous symposium on GC held during the 129th National American Chemical Society Meeting in April 1956 in Dallas, Texas, coinciding with two publications in *Food Technology* (22,23). This work and subsequent studies became so famous that the title page of the February 1958 issue of *Analytical Chemistry* used one of the strawberry essence chromatograms as an illustration of the potential of GC (24). Another consequence of this work was the incorporation of Wilkens Instrument and Research Co. in December 1956 by Dimick, his wife, and his brother-in-law. Wilkens Instrument and Research was a highly successful company that developed and marketed gas chromatographs under the name Aerograph. The company later merged with Varian and today represents its chromatography division.

Another example of the potential of GC in flavor analysis is the classical work of E.sz. Kováts at the Federal Technical University (Zurich, Switzerland). In 1955, Kováts started the investigation of essential oils in the laboratory of Leopold Ruzicka



**Figure 5:** Chromatogram of the triglycerides of peanut oil from 1986 (20). 15 m × 0.25 mm fused-silica capillary column coated with bonded phenyl (65%) methyl silicone stationary phase ( $d_f = 0.1\text{-}\mu\text{m}$ ). Column temperature programmed from 300 °C to 360 °C at 4 °C/min. Programmed-temperature vaporizer, heated from 50 °C to 400 °C. Sample: 0.5  $\mu\text{L}$  of a 20- $\mu\text{g}/\text{mL}$  solution in isooctane, split 1:50. Carrier gas: hydrogen. Flame ionization detector. The T numbers give the total carbon number of the three fatty acids in the triglyceride. The individual fatty acids are indicated by the following code: P = palmitic acid (16:0); S = stearic acid (18:0); O = oleic acid (18:1, *cis*); L = linoleic acid (18:2, *cis-cis*); A = arachidic acid (20:0); Ga = gadoleic acid (20:1, *cis*); Be = behenic acid (22:0); Lg = lignoceric acid (24:0).



(recipient of the 1939 Nobel Prize in Chemistry). He built gas chromatographs, both for analytical and preparative use, and through long, painstaking work finally succeeded in elucidating the composition of mandarin peel oil (25), lime oil (26), and rose oil. (Due to confidentiality requirements by the sponsor, the results of the analysis of rose oil could be published only 25 years later; see reference 27.) To illustrate the complexity of the investigations, it is worthwhile to mention that Kováts succeeded in separating 145 compounds in rose oil, of which 127 could be identified; in addition, he showed the presence of 87

additional but not fully separated compounds. Remember that this work was performed before the availability of mass spectrometry for compound identification.

Early investigations of essential oils were carried out using packed columns. By today's standards, these separations were fairly inadequate, although still better than possible with other methodologies. As in other fields, the introduction of capillary columns represented a breakthrough in these investigations, which can be seen by comparing Figures 6 and 7. Figure 6 was obtained in 1958 using a packed column (28), and Figure 7 is from an early work

that demonstrates the superiority of capillary columns (29).

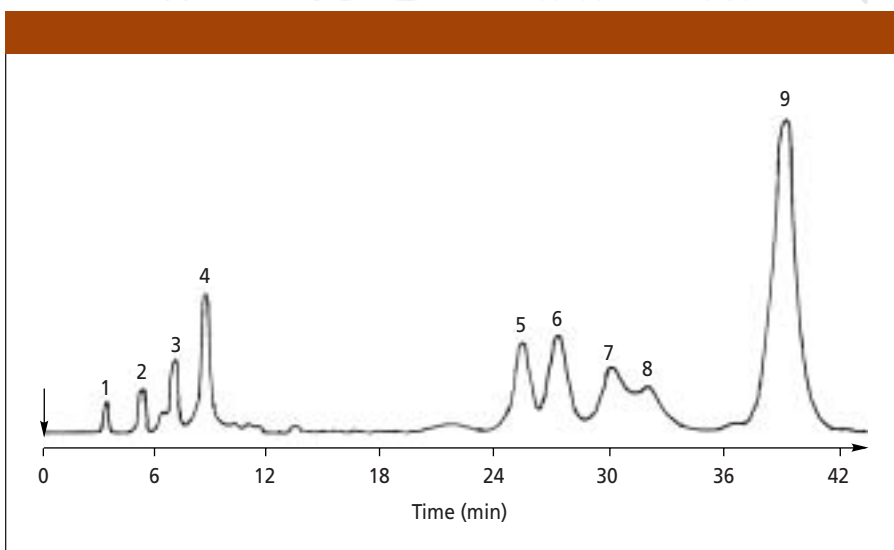
### Environmental Analysis

In 1962 the publication of the book *Silent Spring* created a sensation (30). In it, Rachel Carson documented without any doubt the detrimental influence of pesticides to the environment. The *silent spring* was the result of their indiscriminate use: these chemicals pass from one organism to another through the links of the food chain, eventually poisoning birds and silencing the forests and meadows.

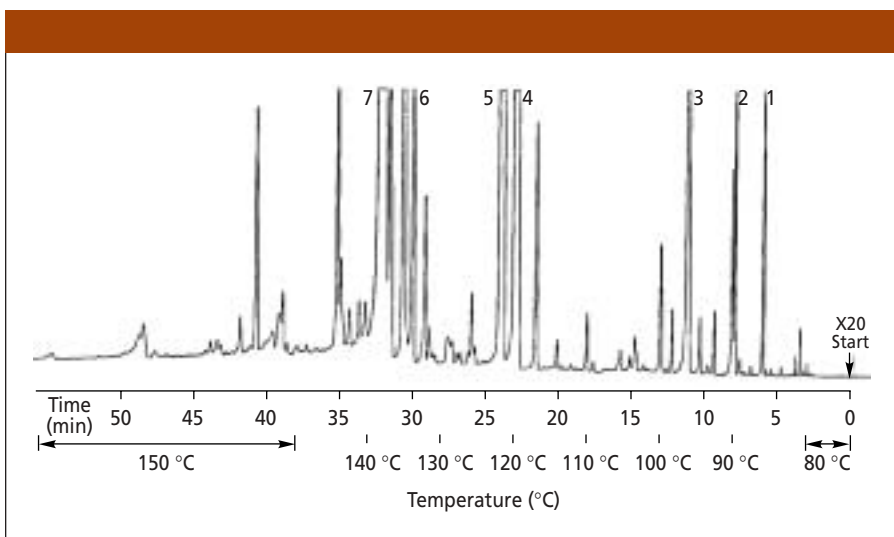
Actually, when Carson's book was published, the detrimental effect of chlorinated pesticides on fauna and even humans already was known. Still, the impact of her book was tremendous and in the introduction of the book's 1994 edition, Al Gore, then Vice President of the United States, compared the book's effect with that of *Uncle Tom's Cabin*. As he said, "both rank among the rare books that have transformed our society" (30). This book created the environmental protection movement, initiated government regulations of the manufacture and use of pesticides, and ultimately resulted in the formation of the U.S. Environmental Protection Agency (EPA) and similar agencies in most developed countries.

By the end of the 1950s, GC already had been used to analyze pesticide residues in combination with other instrumental techniques such as infrared spectroscopy for compound identification (31). A major step forward was Coulson and Cavanagh's invention of the microcoulometric detector, which provided higher sensitivity and selectivity than those of then-existing detectors (32,33). However, the real breakthrough was the 1960 introduction by J.E. Lovelock of the electron-capture detector, which provided unsurpassed sensitivity — orders of magnitude better than that of any other detector (34,35). Soon this detector was produced by Ionics Research, a small company founded by A. Zlatkis (University of Houston) and Lovelock (Figure 8), followed by the individual instrument companies producing gas chromatographs.

I remember the title page of one of the 1962 issues of *Aerograph Research Notes*, the quarterly magazine of Wilkens Instruments and Research: it showed a chromatogram with a peak corresponding to  $10^{-12}$  g of a pesticide. The headline, printed in large, boldface characters, asked the question: "Have you ever seen a picogram?" (36). Figure 9 shows another souvenir from this



**Figure 6:** Packed column chromatogram of peppermint oil of Yakima Valley from 1958 (28). Isothermal conditions, thermal conductivity detector. Peaks: 1 =  $\alpha$ -pinene; 2 =  $\beta$ -pinene; 3 = limonene; 4 = eucalyptol; 5 = menthone; 6 = menthofuran, 7 = menthyl acetate; 8 = unknown, 9 = menthol.



**Figure 7:** Capillary chromatogram of peppermint oil of Yakima Valley from 1962 (29). 150 ft  $\times$  0.25 mm stainless steel capillary column coated with Ucon Oil 50-HP-2000 poly(propylene glycol). Sample volume: 1  $\mu$ L (liquid), split. Flame ionization detector. Major peaks: 1 =  $\alpha$ -pinene; 2 =  $\beta$ -pinene; 3 = eucalyptol; 4 = menthone; 5 = menthofuran; 6 = menthyl acetate; 7 = menthol.

early period — a chromatogram used in an advertisement from the Barber-Colman Co., a major supplier of GC instrumentation in the first part of the 1960s (37).

So far, I have dealt only with the analysis of pesticide residues, but with respect to the environment, the determination of impurities in air was just as important. A special organization — the Air Pollution Control Association — had been formed in the United States circa 1950, and by the end of the 1950s the potential of GC in air pollution studies was a regular feature of the journals (38,39). A breakthrough in this field — almost equal to *Silent Spring* — occurred in 1971–1972 when Lovelock participated in the voyage of the ship *Shackleton* from the United Kingdom to Montevideo, Uruguay. During the voyage, Lovelock took air samples over the Atlantic Ocean using GC with electron-capture detection (ECD). He demonstrated that the presence of trace concentrations of chlorofluorocarbons (CFCs) originated from human pollution (40).

Soon after, Rowland and Molina postulated that the CFCs decompose in the stratosphere due to the influence of UV radiation from the sun and release their halogen atoms, which in turn contribute to the depletion of stratospheric ozone (41). For their work, Rowland and Molina (together with Paul Crutzen of The Netherlands) received the 1995 Nobel Prize in Chemistry. The recollections of Rowland (42) and Lovelock (43) provide a good summary of this work, the evolution of our knowledge of the presence of CFCs in the stratosphere, and their detrimental effect on the balance of our atmosphere.

Analytical chemistry related to environmental pollution investigations is very complex: scientists must determine trace concentrations of a wide variety of compounds in very complex matrices; therefore, their instruments must have sufficient separation power, selectivity, and sensitivity. Present-day EPA regulations list a large number of volatile organic compounds (VOCs), the possible presence of which must be regularly evaluated. This tracking would be impossible without modern GC, high-resolution capillary columns, and ultrasensitive detectors. Today, capillary columns specially tailored for such applications can provide the separation and analysis of as many as 100 compounds in less than 20 min (representative chromatograms can be found in the catalogs of the supply houses; for example, see reference 44).

Environmental protection — the analysis of air, soil, and water for trace and ultratrace contaminants — is the fourth major field in which the application of GC changed scientists' understanding of the world around them.

### Evolution of Chromatography and the Scientific Instrument Industry

I tried to point out the four most important applications fields in which GC permitted analyses that previously had been considered impossible. But GC also helped the evolution in two additional fields.

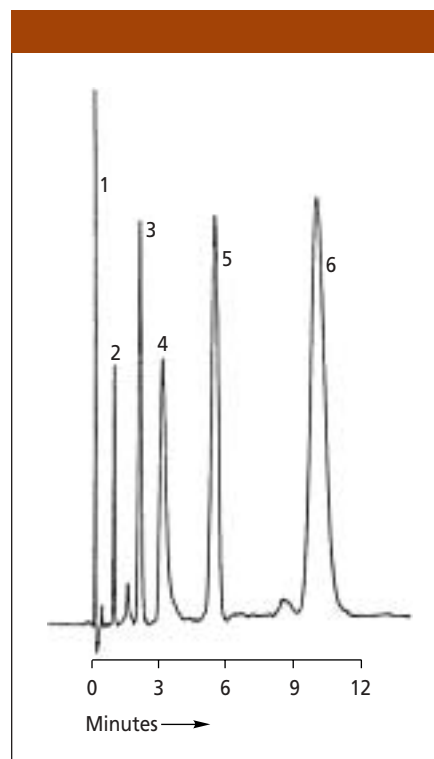
The first is *liquid chromatography*. Classic LC is 100 years old; however, it changed very little for many decades. In the 1940s, Martin developed the theory of partition chromatography and introduced paper chromatography. However, the practice of column LC changed very little: chemists continued to use small, short glass columns filled with an adsorbent and to have the gravimetric pressure control the slow flow of the liquid mobile phase through the column. Finally during the mid-1960s, the theory of GC and the experiences gained in its use induced a few researchers — Calvin Giddings, Csaba Horváth, and others — to consider how to modernize liquid column chromatography. HPLC was the result of these considerations. It is safe to say that without the advances in GC, the breakthrough in LC — the development of HPLC — could not have happened either.

The introduction of GC 50 years ago had also another effect: it helped to create a new industrial branch, the *scientific instrument industry*. Gas chromatography is par excellence an instrumental technique, and a symbiosis between it and the scientific instrument industry existed — the rapid evolution of the former could not happen without the involvement of the lat-



**Figure 8:** J.E. Lovelock (left) and A. Zlatkis, in 1961. Zlatkis holds the new electron-capture detector manufactured by Ionics Research (28).

ter and vice versa. The scientific instrument industry had a slow start after World War II, but the introduction of GC in the 1950s rapidly increased the number of companies involved in this industrial branch. Although seven companies produced gas chromatographs in 1956, the number increased to 23 by 1962. Naturally, as the companies strengthened their operations, they added other instrument types to their product lines. By 1975, the sales volume of the scientific instrument industry grew to approximately \$1 billion. Today, 28 years later, the worldwide instrument market is estimated to be worth \$22 billion, with GC representing roughly 7% and LC approximately twice as much. These figures mean that today, even in the highly diversified instrumental analysis field, chromatography represents approximately 20% of the scientific instrument industry (45,46). These data are the best illustration of the importance of GC in the scientific evolution of the past 50 years.



**Figure 9:** Chromatogram of a pesticide mixture from a 1963 advertisement of a gas chromatograph with an electron-capture detector (37). 2 ft × 3 mm glass packed column containing 2.5% SF 96 methylsilicone on silanized support. Column temperature: 180 °C. Sample volume: 0.5 μL (liquid). Peaks: 1 = solvent (*n*-heptane); 2 = lindane; 3 = parathion; 4 = sulphenone; 5 = ethion; 6 = EPN. Sample concentration was in the nanogram-per-microliter (ppm) range.

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