Rapid Analysis of Natural Essences by Combined Flow and Temperature-Programmed Capillary Gas Chromatography

Pressure programming in conjunction with temperature-programmed gas chromatography significantly shortens analysis times of lime, peppermint, and grapefruit essences on capillary columns without adversely affecting resolution.

Natural essences used as seasonings, flavorings, and scents may vary widely in origin and composition and yet share common problems in their analysis. Generally, they are complex mixtures of moderately volatile and labile organic compounds which require the ultimate in high resolution gas chromatography under mild vaporizing conditions. Cieplinski and Averill (1962) have demonstrated the utility of capillaries, especially when the partitioning phase has a wide effective temperature range—e.g., Apiezon L, OV-101, OV-17, OS-138, or Carbowax 20M. However, in routine applications such as quality control, the resolution of lesser constituents frequently must be sacrificed on the short columns necessary for fast chromatograms. A way to ease this problem was first demonstrated by Scott (1965), who programmed both temperature and carrier gas flow on a packed column. Subsequently, Zlatkis *et al.* (1965) and Teranishi *et al.* (1966) extended simultaneous flow and temperature programming to capillaries. The results reported herein show that by a suitable choice of flow and temperature programs, complex essences may be analyzed in 10 to 15 minutes with good resolution of minor constituents.

EXPERIMENTAL

Analyses were carried out on a Perkin-Elmer Model 226 hydrogen-flame-ionization gas chromatograph equipped with a 100-foot × 0.010-inch i.d. stainless steel capillary coated with decolorized Apiezon L containing 10% w./w. Igepal CO-880. A Perco Flo-trol programmer was inserted in the He carrier gas line just upstream from the injector block inlet. Microliter samples of natural essences were injected with a Hamilton 701 microsyringe into the inlet-splitter at 120° C. Upon vaporization, the sample was split 100 to 1 and the smaller portion was swept into the capillary for analysis. Simultaneously, the column temperature was linearly programmed from 70° to 180° C. at a rate of 10° C. per minute, and the carrier gas flow was programmed according to an exponential inlet pressure program from 15 to 82 p.s.i.g. during 12 minutes. Essence constituents identified in Table I were determined by mass spectral comparisons with known standards and were obtained on a Hitachi RMU-6E mass spectrometer connected to the gas chromatograph.

Table I. Essence Constituents Identified by Retention Time and Mass Spectral Analysis

Peak No.	Compound	Peak No.	Compound	Peak No.	Compound
1	α-Thujene	10	γ-Terpinene	19	Geranial
2	α-Pinene	11	Terpinolene	20	Piperitone
3	Camphene	12	Citronellal	21	Menthyl acetate
4	Sabinene	13	Menthone	22	Neryl acetate
5	Myrcene	14	Menthofuran	23	Geranyl acetate
6	β-Pinene	15	Menthol	24	α-Bergamotene
7	1,8-Cineole	16	Terpinene-4-ol	25	Caryophyllene
8	*p*-Cymene	17	Neral	26	β-Bisabolene
9	Limonene	18	α-Terpineol	27	Nootkatone

RESULTS AND DISCUSSION

The utility of a policy of programming carrier gas flow and column temperature simultaneously to obtain faster chromatograms at a minimal cost in resolution can be seen in the chromatograms of expressed lime oil. Figure 1 shows a conventional temperature-programmed chromatogram on a 300-foot × 0.010-inch i.d. capillary. With this research-type chromatogram almost 2 hours were required to elute the sesquiterpenes (peaks 24 to 26), whereas in Figure 2 the dual programmed chromatogram provides essentially the same information in one tenth the time. The disparity in analysis times suggests that the operation of the column in Figure 1 may not be as efficient as possible, perhaps because of the negative flow program which occurs during temperature programming at constant inlet pressure as a result of increased flow resistance with increasing temperature.

The same procedure also is suitable for natural peppermint distillate (Figure 3). The principal flavor components, 1,8-cineole, menthone, menthofuran, menthol, and menthyl acetate (peaks 7, 13, 14, 15, and 21, respectively), were well resolved and readily identified by their mass spectra. Excellent base line stability was obtained with Apiezon L, even in single column mode.

In the analysis of an extract of grapefruit peel (Figure 4), an important C_{15} ketone, nootkatone (peak 27), was eluted without peak distortion in 15 minutes despite vaporization at 120° C., where according to MacLeod and Buigues (1964) its vapor pressure is only a few millimeters

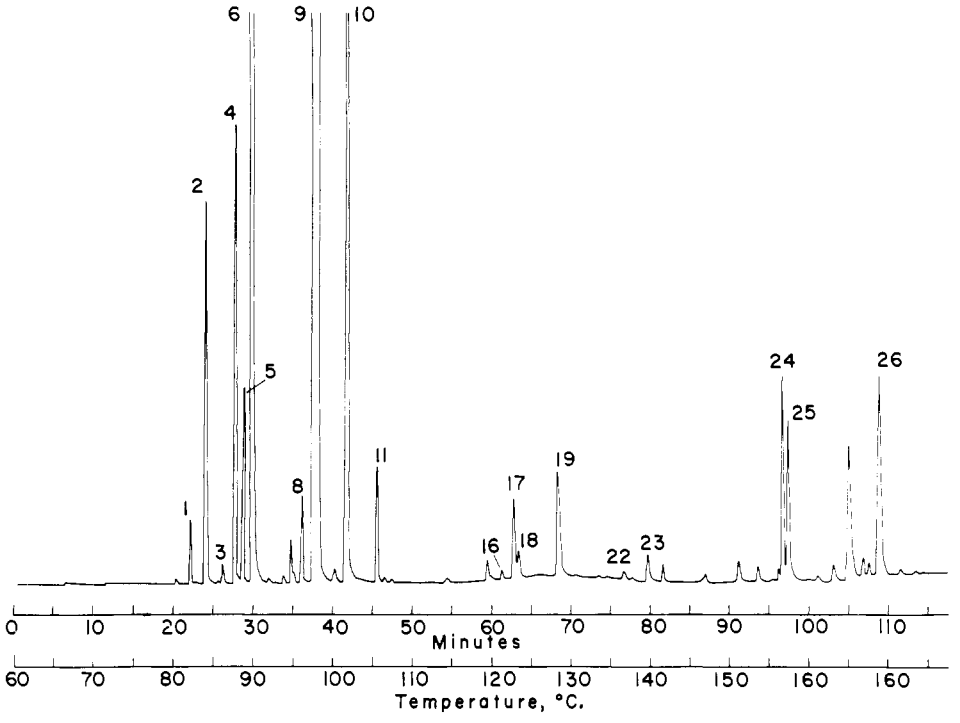

Figure 1. Temperature-programmed gas chromatogram of expressed lime oil

Column, 300-foot × 0.010-inch i.d. capillary, coated with decolorized Apiezon L containing 5% w./w. CO-880. Sample, 3μl., split 100 to 1; carrier gas inlet pressure, 40 p.s.i.g.; peak identities, Table I

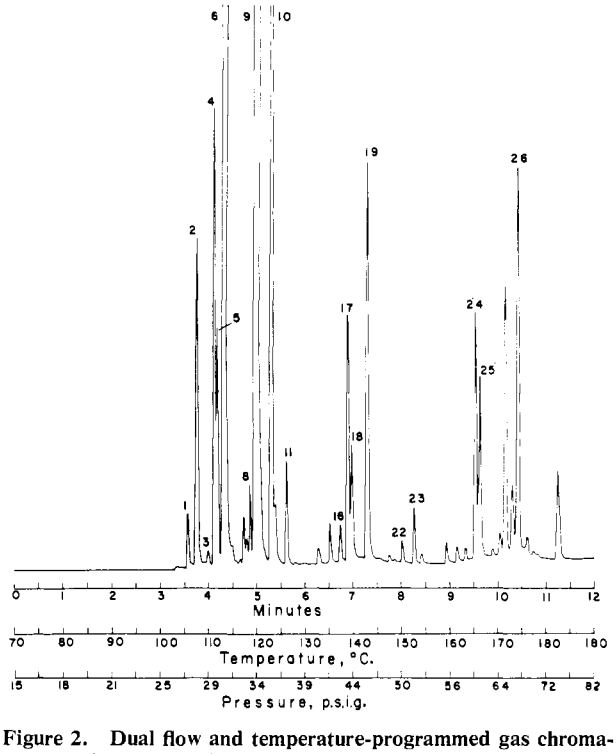

Figure 2. Dual flow and temperature-programmed gas chromatogram of expressed lime oil

Column, 100-foot × 0.010-inch i.d. capillary coated with decolorized Apiezon L containing 10% w./w. CO-880. Sample, 1 μl., split 100 to 1; peak identities, Table I

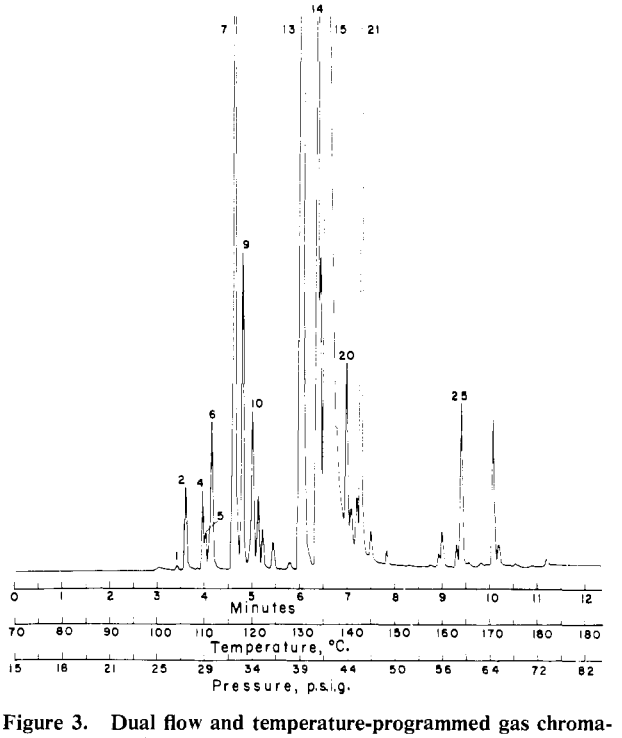

Figure 3. Dual flow and temperature-programmed gas chromatograms of distilled peppermint oil

Chromatogram conditions same as in Figure 2

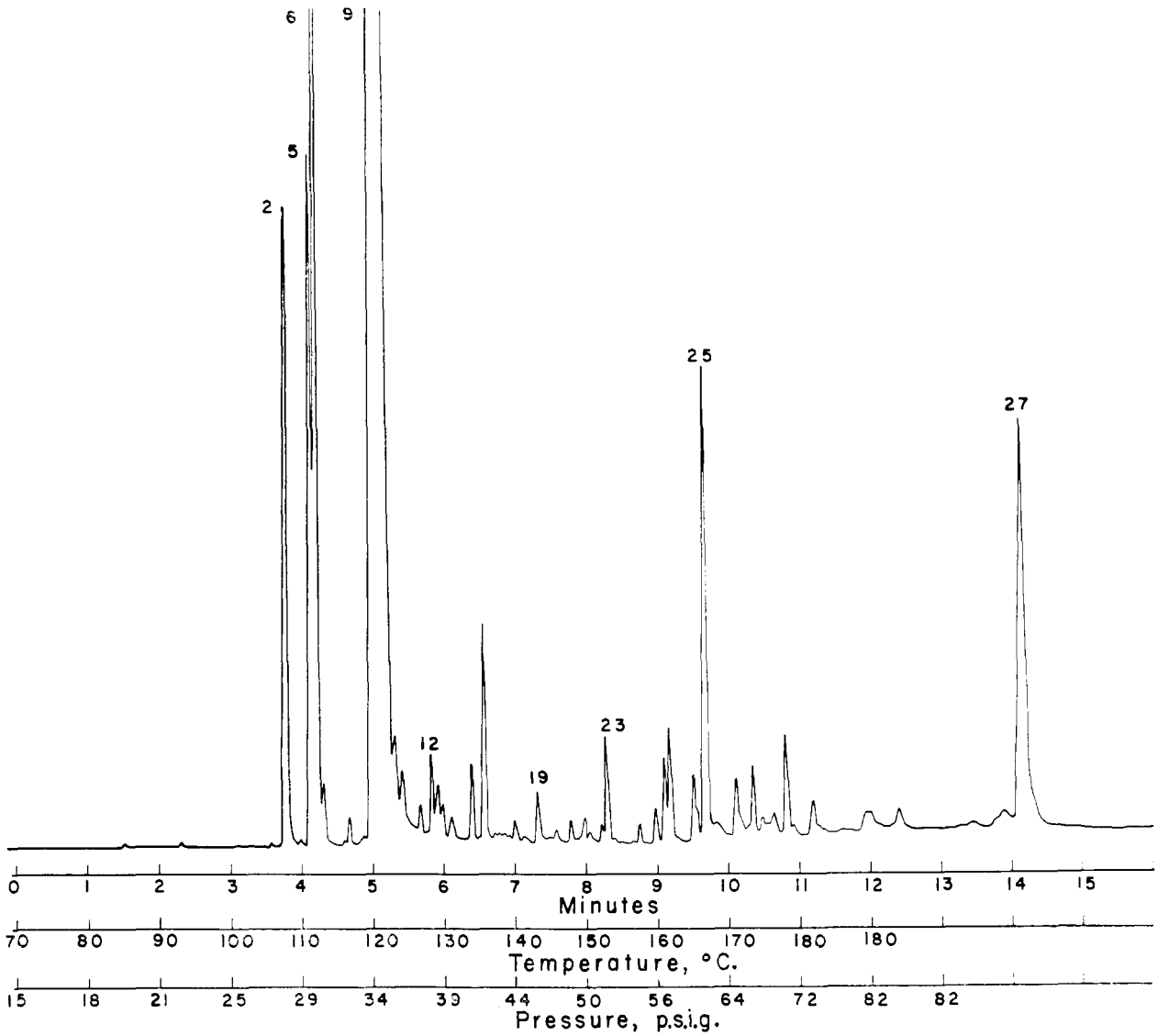

Figure 4. Dual flow and temperature-programmed gas chromatograms of expressed grapefruit oil

Chromatogram conditions same as in Figure 2

of Hg. Their isothermal chromatogram of the same sample at 215° C. on a 10-foot × 0.25-inch o.d. packed column not only required a longer time for nootkatone to emerge, but also resolved few of the other constituents evident in Figure 4. These examples indicate that combined flow and temperature-programmed gas chromatography can substantially reduce the analysis times of complex essences, while retaining good chromatographic resolution of minor constituents.

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