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Fractional Factorial Design for the Identification of Important Instrumental Factors Affecting the Quality of Gas Chromatographic Separation of Whiskey Volatiles

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Abstract: A fractional-factorial screening experiment that helps students identify important factors in gas chromatographic method development is described. The experiment gives practical experience in relating chromatographic theory to practice. The results of sixteen gas chromatography runs of a single whiskey sample enable students to deduce that mobile-phase flow rate has the major effect on the quality of the separation of this complex mixture. Three other individual factors and an interaction of two factors exert a minor influence on the quality of the separation.

Introduction

There is a gap between the topics discussed in an instrumental methods course and the practical details of method development in gas chromatography (GC). Topics such as band broadening, differential migration, stationary-phase polarity, the need for temperature programming, and the choice of detector are well-covered in modern instrumental-methods texts [1, 2]. Knowledge of these topics and practical information available in many catalogs [3] can guide the chromatographer to choose the correct column configuration and detector to solve a particular separation problem; however, the details of the method, including settings of temperature, flow, and time, must be established by iterative experimental investigation. These latter elements of method development are often puzzling to students, especially for temperature-programmed methods, due to a paucity of examples available in text and lecture examples. With the exception of one article describing the investigation of variables associated with the split/splitless injector [4] and a recently published GC simulator [5], I am unaware of any published experiments in the chemical education literature directed at systematic GC method development. The experiment described here is an attempt to fill the gap. It is a screening experiment that gives students an opportunity to systematically identify the relative importance of variables that can be adjusted to improve a GC separation of a complex mixture.

The goal of this experiment is to establish chromatographic conditions that maximize the number of integratable bands eluting after ethanol when a sample of whiskey is injected into the column. In particular, mobile-phase flow rate, injector purge time, initial oven temperature, final oven temperature, and temperature ramp rate are investigated. The GC method developed here is then used later in the semester to determine if fusel alcohol content and chromatographic complexity correlate with whiskey quality [6]. Because whiskey is a complex mixture of many dozens of volatile compounds, maximizing the number of chromatographic bands observed has the effect of maximizing the probability of observing correlations.

While it is possible to study these five factors individually, the one-factor-at-a-time approach is inefficient. A more efficient approach is to do a screening experiment, in which the effects of all five factors are studied simultaneously using a two-level fractional factorial design [7–9] using only sixteen experiments. Students observe that adjusting one factor, mobile-phase flow rate, has a major effect on the number of bands in the chromatogram. Three other factors (injector purge time, final oven temperature, and temperature-programming ramp rate) have a minor effect on the number of integratable bands. There is also an interaction effect involving flow rate and ramp rate. The initial temperature has no effect, at least for the values chosen in this experiment.

Experimental

A capillary gas chromatograph with autosampler (Hewlett Packard 6890) and associated software (HP GC ChemStation version 5.01) were used with the following conditions:

column:	30 m in length, 0.45 mm I.D.
stationary phase:	Carbowax (polyethylene glycol), 1.0 µm film thickness
mobile phase:	helium, flow rate set to either 1.0 mL/min or 2.0 mL/min
injection volume:	1.0 µL.
injector:	splitless, purge time set to either 0.2 min or 0.5 min
oven:	temperature programmed, temperatures and rates are given in Table 1.
detector:	flame ionization
chromatographic run time:	30 min

Integration was done with the enhanced integrator and the following settings:

slope sensitivity = 20
minimum peak width = 0.05 min
minimum peak area = 5 pA s
minimum peak height = 2 pA

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Table 1. Design of Sixteen Experiments

		Factor A	Factor B	Factor C	Factor D	Factor E
Standard order	Random run order	Init. Temp, °C	Ramp rate, °C	Final temp, °C	Helium flow rate, mL/min	purge time, min
1	3	40	8	220	1	0.5
2	8	60	8	220	1	0.2
3	4	40	16	220	1	0.2
4	16	60	16	220	1	0.5
5	2	40	8	250	1	0.2
6	9	60	8	250	1	0.5
7	6	40	16	250	1	0.5
8	14	60	16	250	1	0.2
9	7	40	8	220	2	0.2
10	12	60	8	220	2	0.5
11	10	40	16	220	2	0.5
12	15	60	16	220	2	0.2
13	5	40	8	250	2	0.5
14	1	60	8	250	2	0.2
15	13	40	16	250	2	0.2
16	11	60	16	250	2	0.5

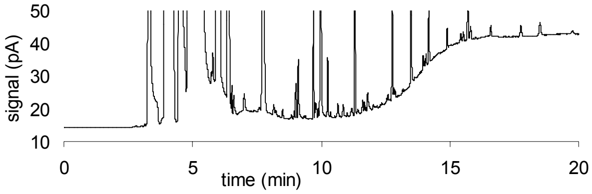

Figure 1. Chromatogram of standard order run 16 with 40 integrated bands observed beyond ethanol. The broad, off-scale peak near 5 min is ethanol.

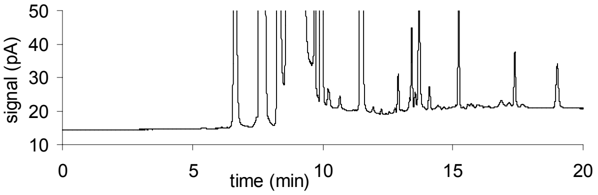

Figure 2. Chromatogram of standard order run 3 with 13 integrated bands observed beyond ethanol. The broad, off-scale peak near 9 minutes is ethanol.

These settings give integrated values for part-per-million level components when the bands are moderately sharp. When less sensitive settings were used (minimum peak area = 20 pA s, minimum peak height = 5 pA), fewer bands were integrated, and fewer significant factors were observed.

The sixteen experiments were generated using Design Expert software (version 5.0, Stat-Ease, Inc., Minneapolis, MN), using a 2^{5-1} fractional factorial design. The sixteen GC methods (Table 1) were stored on the computer operating the GC. A GC sequence was programmed to inject a single sample of Dewar's White Label whiskey using each of the methods in random order. The sequence was run overnight under computer control.

The response measured was the number of chromatographic bands integrated with retention time greater than that for ethanol. The effect of the five variables on the response was determined using Design Expert 5 software. The data can also be interpreted graphically in the absence of specialized software.

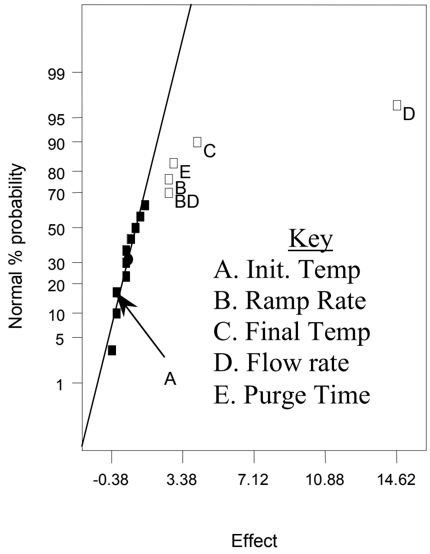

Figure 3. Normal probability plot of effects. The open symbols represent factors whose effect sizes are statistically significant.

Results and Discussion

The effect of adjusting the five variables shown in Table 1 to two levels is substantial. There is a factor-of-three difference in the number of integrated bands observed in the best and the worst of the sixteen chromatograms (Figures 1, 2).

The number of observed bands for all sixteen experiments are given in Table 2. (All sixteen chromatograms are included in the supplementary material.)

The data in Table 2 may be analyzed and interpreted using either of two approaches: evaluation of effect size or pairwise comparison of the number of bands. The evaluation of effect size with normal probability plots (similar to normal quantile plots in reference 9) gives a direct visual interpretation of the data set, but the procedure requires specialized software and instruction of students. The interpretation of normal probability plots and the mechanics of constructing them are described elsewhere [9, 10]. (A student handout describing the rationale of factorial-design experiments and the use of normal

Table 2. Response (Number of Integrated Bands) for the Sixteen Experiments in Standard Order

Run	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
# Bands	18	14	13	16	17	19	21	18	27	27	32	31	33	29	34	40

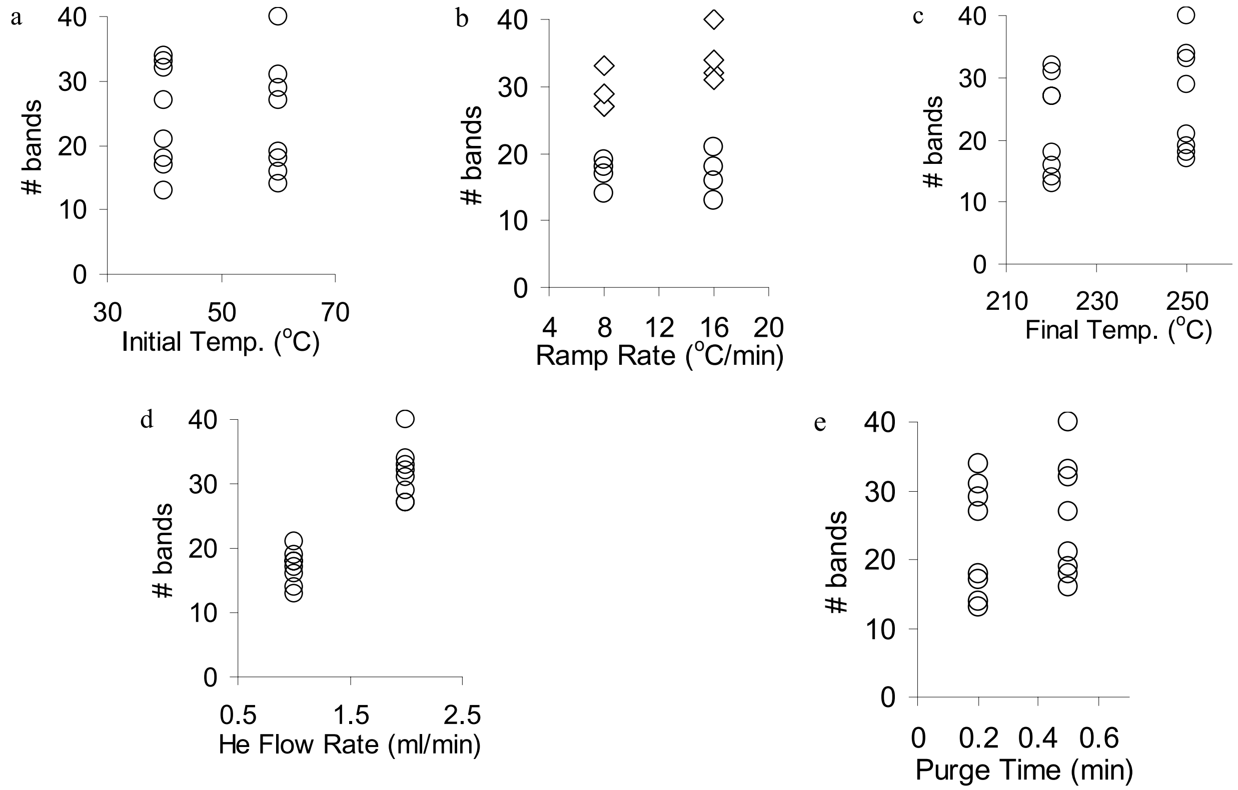

Figure 4. Number of bands observed at each (a) initial temperature, (b) ramp rate, (c) final temperature, (d) flow rate, and (e) purge time. In (b), circles are results at flow = 1 mL/min and diamonds are at flow = 2 mL/min.

probability plots has been included in the supplementary material.) Pairwise comparison is done easily, but construction of the plots is labor-intensive and interpretation of the plots can be subjective.

In the absence of specialized software, effect size may be calculated for each factor and the interaction of factors by reference to the experimental design in Table 1 and the responses in Table 2. For example, the effect size of 14.6 for factor D, helium flow rate, is calculated as the difference between the average response of 31.6 at high flow rate (standard order 9–16) and the average response of 17.0 at low flow rate (standard order 1–8). The interaction effects are calculated in an analogous fashion, considering the product of the coded (–1 for low value, +1 for high value of a variable) levels of each factor in an experiment. The "high-level" experiments for the BD interaction are standard order 1, 2, 5, 6 (B and D both –1), 11, 12, 15, 16 (B and D both +1), and the average response is 25.6. The eight other "low-level" experiments have an average response of 23.0. The effect size for the BD interaction is the difference of 2.6. The fifteen rank-ordered effects are plotted on normal probability paper. Effects for nonsignificant factors are random deviates from zero, and they plot on a straight line near zero. Effects for statistically significant factors deviate from that straight line, either to the far right and below the line or to the far left and above the line. Rigorously objective criteria may be used to establish the significance of factors. The Design Expert software uses the method described by Lenth [11].

The normal probability plot of effects (Figure 3) indicates that there are four statistically significant factors and one significant interaction. The major factor affecting the number of integratable bands is mobile-phase flow rate (factor D). Approximately twice the number of bands are integrated when a near-optimum flow rate of 2 mL/min is used, rather than a far-from-optimum flow rate of 1 mL/min (Figure 4d). The very low flow rate produces a larger plate height and consequently broader, less intense bands and consequently fewer integrated features than the higher flow rate.

Other, subtler effects are observable in this screening experiment. They have only a small practical effect on the chromatography. On average, a final temperature of 250 °C results in one or two more bands than a final temperature of 220 °C (Figure 4c). A 0.5-min purge time gives about one more band than a purge time of 0.2 min (Figure 4e). The effect of ramp rate on the number of bands is also small, and there is an interaction with the mobile-phase flow rate (Figure 4b). For a carrier-gas flow rate of 1 mL/min, the average number of bands observed is 17 for both ramp rates. For a carrier-gas flow rate of 2 mL/min, the average number of bands observed is 29 for a ramp rate of 8 °C/min and 34 for a ramp rate of 16 °C/min. The initial temperature, factor A, has no observable effect on the number of chromatographic bands, at least over the range of temperatures studied (Figures 3, 4a).

Pairwise comparison of the results of two-level factorial-design experiments can give immediate insight into the presence of active factors that are of large magnitude [7]. The plots in Figure 4, which are constructed using data from Table 2, make it clear that factor D, mobile-phase flow rate, is the major source of variation in the number of integrated bands.

The importance of the other four factors may be ambiguous to the neophyte, but this is an opportunity for further learning of the strategy of experimental design. Because the goal of a screening experiment is to identify potentially significant factors that will be studied in more detail in subsequent experiments, we should suggest that a liberal interpretation of the data is desirable. The consequences of a false positive are minor at the early stages of experimentation, but the consequences of a false negative are more severe. In the context of this experiment, a second experimental iteration could be done where the major factor(s) identified could be adjusted over a range of values to achieve the desired goals of the separation. Our students have not done this, as the chromatograms developed using the conditions of standard order 16 were adequate for characterizing and classifying whiskeys of different type and price [6].

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Supporting Material. The sixteen chromatograms, a rationale of factorial-design experiments, and a student laboratory handout are available as an Adobe Acrobat PDF file (http://dx.doi.org/10.1007/s00897010504b).

References and Notes

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