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Laboratories and Demonstrations

Extraction and Gas Chromatographic Determination of Ethanol in Beverages

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…the experiment can be used in an organic or an analytical laboratory…[or it] also can be modified for use in general chemistry.

We describe an experiment that teaches students liquid-liquid extraction and gas-liquid chromatography, two basic techniques in which students need to obtain background and experience. In addition to fulfilling our pedagogical objectives, the experiment presents an analytical problem that students consider meaningful and interesting.

The students who perform the experiment are enrolled in the first semester of both the introductory organic and analytical chemistry lecture courses or have completed them previously. Because of the large number of students enrolled and the relatively small number of instruments we have available, only a portion of the total number of students perform this experiment

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in any week (ca. 25%); thus, the experiment does not necessarily match the lecture content in time. Because of the timing difficulties, the theory for both techniques is examined in the lecture course, the introduction to the experiment in the laboratory needs to describe some of that theory, in addition to the experimental details. Students perform this experiment over two three-hour laboratory periods in consecutive weeks. The first day is primarily used to acquaint them with gas chromatography concepts and to develop some experience in the procedures (e.g., liquid-liquid extraction, injection technique, and instrument operations). The second laboratory period applies these techniques to the analysis of ethanol in four beverage samples.

The focus of the course that contains this experiment is the separation and identification of organic and bioorganic materials; thus, this experiment does involve a "real" analytical problem, albeit one about which most of us are not terribly concerned. From the organic perspective, it includes a very propitious example of extraction, done on a scale and in a manner not normally used in organic chemistry (i.e., nothing is isolated). With appropriate changes in focus, the experiment can be used in an organic or an analytical laboratory (or a combined one as appears in our curriculum). Furthermore, with a suitably simplified introduction, this experiment also can be modified for use in general chemistry. Because we offer only one organic lecture and laboratory sequence and require the analytical course as a corequisite, the students who perform the experiment come from the myriad of majors which require chemistry through the organic sequence.

Extraction of Ethanol from Aqueous Solutions

The literature for the analysis of ethanol in aqueous solutions, including beverages, reports the use of pycnometric techniques [1, 2] in addition to a variety of instrumental methods that include the use of: high performance liquid chromatography [3–6], diffusion through membranes [7], electrodes modified with surface microbes or enzymes [8–9], NMR [10], conductivity [11], fuel cell sensors [12], and others that were not amenable to the undergraduate laboratory or did not fulfill our goals. It seemed reasonable that we could contrive a liquid-liquid extraction procedure to partition ethanol (but not water or salts, proteins, or carbohydrates) into a reasonably volatile organic solvent that would be suitable for GC analysis (*vide infra*). Ethanol and related molecules have been previously extracted from complex aqueous solutions such as orange juice [13] and fermentation mixtures [14, 15].

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Table 1 contains data from the variety of organic solvents that we initially tried. It lists the partition coefficients *K* for the equilibrium:

$$(\text{ethanol})_{\text{water}} \overset{K}{\rightleftharpoons} (\text{ethanol})_{\text{solvent}} \quad (1)$$

The criteria for the extraction solvent include moderate to low volatility, limited solubility in water, and an appropriate range of gas chromatographic retention times. Using these standards and the candidates in Table 1, the best three solvents were judged to be 1-butanol, 1-pentanol, and ethyl acetate. Because we are not trying to isolate all of the ethanol by extraction, we are not limited to solvents with the highest partition coefficients. It is sufficient that the solvent extract enough ethanol to make the procedure reproducible and capable of measuring relatively small ethanol contents in the aqueous sample (i.e., 0.1% ethanol as the minimum). The dynamic range of the student gas chromatographs and the conditions under which we used them would have allowed the experiment to detect the ethanol at even lower levels (i.e., 10 ppm).

Although we chose 1-pentanol, it has several minor disadvantages. Because it has the longest retention time, it determines the time per injection. The students do not like to wait for the elution of a peak which is not the actual “data” that they need to collect. In spite of the fact that 1-pentanol is not very volatile and sample vials are supposed to be kept sealed, some students comment about its odor.

Gas Chromatographic Analysis of Ethanol in Organic Solvents

Gas chromatography was chosen as the analysis method because of the ease of performance and sensitivity of the technique. Better separations were generally observed using polar liquid phases (e.g., Carbowax 20M or Carbowax 1520) rather than nonpolar ones (e.g., SF96[1], SE30[1], 2, 4-dinitrophenylhydrazine or dinonyl phthalate), although many satisfactory separations could be accomplished using members of the latter group. Our concern was that the minor differences (e.g., packing of the columns, flow rate, or oven temperature) among the 24 instruments in use necessitated truly “foolproof” conditions. The selected parameters are described below, and retention time data at two

[1]Alltech Associates, 2051 Waukegan Road, Deerfield, IL 60015; and Supelco Inc., Supleco Park, Belefonte, PA 16823.

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TABLE 1. Partition Coefficients for Ethanol and GC Retention Times.

solvent	K[a]	retention time, min (25°C)[b]	retention time, min (95°C)[c]
hexanes	0.017	1	1
carbon tetrachloride	0.064	4.5	--
toluene	0.090	17	--
anisole	0.20	>30	--
methylene chloride	0.55	6	1
ethyl acetate	0.91	6	--
chloroform	0.92	12.5	--
diethyl ether	1.3	1	1
methyl *t*-butyl ether	1.3	1.5	1
1-pentanol	1.6	>40	10
1-butanol	2.4	36	6.5
ethanol	--	8.5	2.5
water	--	>14	4

[a]Partition coefficient for ethanol equilibrated between equal volumes (2 mL) of water saturated with NaCl and the indicated organic solvents (see eqn 1, text);
[b]Retention time from gas chromatographic analysis on a 5' × 0.125" OD 20% Carbowax 20M column at 25°C (see experimental);
[c]Retention time on another GC with a 5' × 0.125" OD 20% Carbowax 20M column at 95°C . The manifestation of the effect of temperature on retention time can be seen by comparing the chromatograms in Figures 1 and 2.

temperatures for the solvents (using the final GC conditions) are given in Table 1. Figure 1 is a representative chromatogram of the GC separation of various homologous alcohols and water. That chromatogram includes alcohols that were possible extraction solvents(i.e., 1- or 2-propanol, 1-butanol, and 1-pentanol), the extraction target ethanol, and two potential contaminants (i.e., water and methanol).

Experimental

1-Pentanol (Fisher or Aldrich) and absolute ethanol (Gold Seal) were used as obtained. NaCl (reagent grade or table salt) was used to saturate aqueous solutions. Beer (from freshly opened cans or bottles) was poured into bottle-top dispensers (Brinkmann or Wheaton, 1-5 mL, 0.1 mL gradations) as needed. The beer dispensers were stored in a refrigerator between uses to prevent spoilage. The carbonation from beer poured directly

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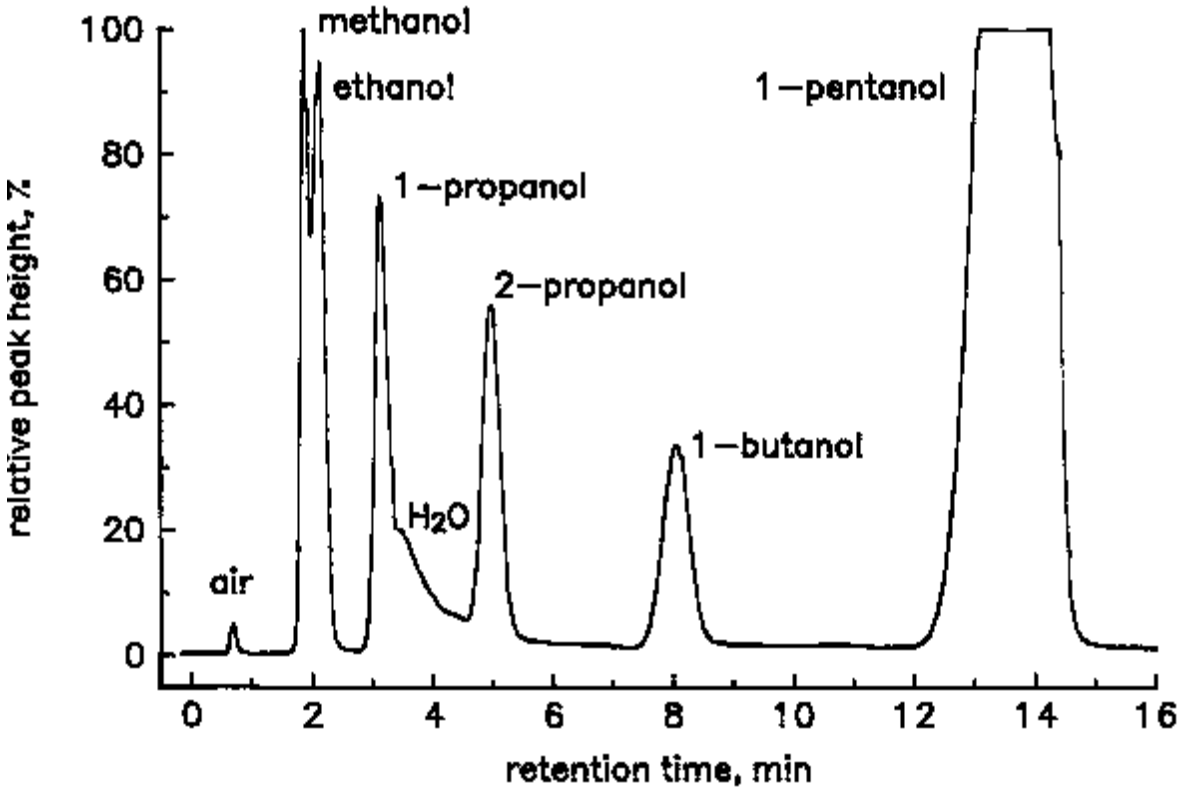

FIGURE 1. CHROMATOGRAM SHOWING SEPARATION OF HOMOLOGOUS ALCOHOLS AND WATER. THE COLUMN OVEN IS AT CA. 95°C, AND THE REMAINING CONDITIONS ARE GIVEN IN THE EXPERIMENTAL. THE SOLUTION CONTAINS CA. 2% (V:V) OF EACH ALCOHOL AND WATER IN 1-PENTANOL.

from the can or bottle into the dispenser does not appear to reduce the accuracy or reproducibility of the measurement. As a result, no special effort was made to remove the carbonation. There does not appear to be any loss of ethanol from solutions stored in this way.

The 24 gas chromatographs (Gow Mac Model 69–350 connected to a Recorder Company strip chart recorder) are in a separate instrumentation room dedicated to their use. The experiment schedule rotates so that in each two-week period only a fraction of the total course enrollment is performing this experiment. In other words, two of eight sections perform the GC experiment at a given time while pairs of the remaining six sections conduct other experiments using other equipment. In this way, 24 GCs serve ca. 700 students per semester. One can devise variations that require even fewer instruments. The students work in pairs with each person sharing the activities; however, two complete sets of data are collected from a single set of samples in the allotted time.

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The students record all actual conditions, but in practice are encouraged only to adjust the flow rate, attenuation, current on the thermal conductivity detector, or any of the recorder settings. (Instrument temperatures are preset because changes requiring reequilibration take 30–90 min.) Helium-carrier-gas flow rates are measured using rotameters (Matheson Gas Products Model 600) with glass and stainless steel floats, a method much more efficient than the soap bubble flowmeters. The chromatographs are each outfitted with a 5 ft × 0.125 in OD stainless steel column, which were packed here or purchased from Gow-Mac and contain 20% Carbowax 20M on 80/100 mesh Chromosorb W. The conditions for the gas chromatographs are as follows: helium pressure, 30 psi; helium flow rate, 12–18 mL/min; injector temperature, 160–180°C; detector temperature, 160–180°C; column (oven) temperature, 95–110°C; attenuation, 1–8, as needed; filament current, 100 mA. The settings for the recorders are: range (maximum), 2 mv; chart speed, 4 cm/min (to measure ethanol). The typical student activities are summarized by day below.

Day 1.

The students begin the day with a quiz and watch a short videotape (produced in-house) that demonstrates how to control all parameters with which they have to become involved (i.e., measuring and adjusting the flow rate, turning on the thermal conductivity bridge and adjusting the current, setting the scale and chart speed on the recorder). We also warn them about potential burn hazards.

A Hamilton student syringe (no longer commercially available) or a Hamilton 10 μL syringe (with a flexible plunger shaft or Cheney adapter) and a set of samples are provided at each instrument station. The five samples contained in 5-mL glass vials are: (a) solutions of 2, 4, and 6% ethanol (v:v) in 1-pentanol; (b) a liquid-liquid extraction sample, prepared from a mixture of 2 mL of water saturated with NaCl and 2 mL of 5% ethanol (v:v) in 1-pentanol; and (c) a solution of ca. 2–4% undecane in hexanes. The students are instructed to record two “acceptable” chromatograms of each sample, that is, with the ethanol peak at least half the height of the chart paper and > 5 mm wide. It is pointed out that the ethanol peak heights from the replicate injections are the most discriminating indicator of good injection technique. The injection size is always nominally 2.5 μL, the capacity of the student syringe. Students are reminded several times to inject the pentanol or upper layer of the extraction mixture. The appropriate peaks are obvious since a typical chromatogram is included in the experiment

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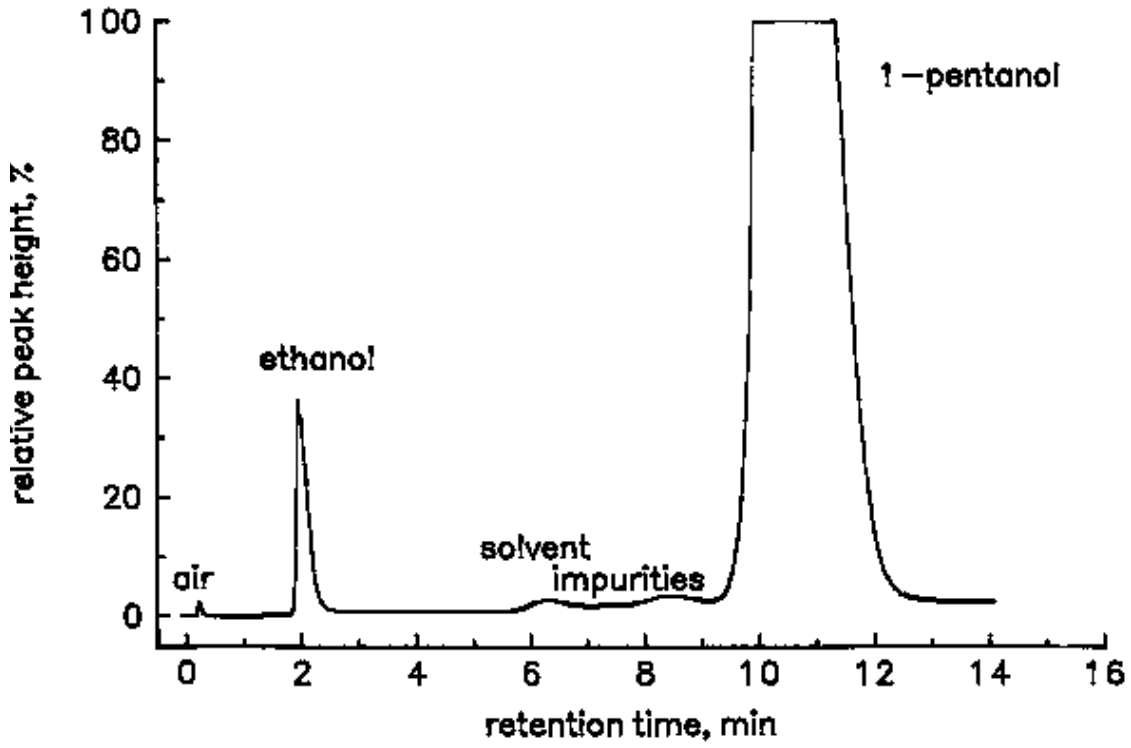

FIGURE 2. CHROMATOGRAM SHOWING SEPARATION OF A TYPICAL EXTRACTION SAMPLE OF BEER. A 1-mL PORTION OF THE BEER SATURATED WITH NACL WAS SHAKEN WITH 1 mL OF 1-PENTANOL. THE ORGANIC LAYER IS INJECTED INTO A GC USING THE CONDITIONS GIVEN IN THE EXPERIMENTAL. THE COLUMN OVEN IS AT CA. 105°C. THE PEAKS HAVE BEEN IDENTIFIED BY COMPARISON WITH AUTHENTIC SAMPLES. THE SMALL PEAKS AT CA. 6–9 min ARE IMPURITIES CONTAINED IN THE BATCH OF 1-PENTANOL USED TO MAKE THE SAMPLES.

description and is included here as Figure 2. The duration of the stay in the instrument room is ca. 1.75–2.25 hours (maximum of 12 samples; ca. 10 min/sample).

The after-lab assignments include measuring the peak areas (calculated from the height times the width at half height and separately by cut-and-weigh), plotting a standard curve (peak area vs. % alcohol for 0, 2, 4 and 6 % ethanol), determining the *K* value (from the actual percentage of alcohol in the 1-pentanol layer of the 5% ethanol sample that was equilibrated between 1-pentanol and NaCl-saturated water and from the plot above), and the height equivalent per theoretical plate (HETP, calculated using the undecane peak width and retention times). This HETP and the measured flow rate are compared with a van Deemter plot given in the experiment description and taken from data collected on one of instruments (see Figure 3).

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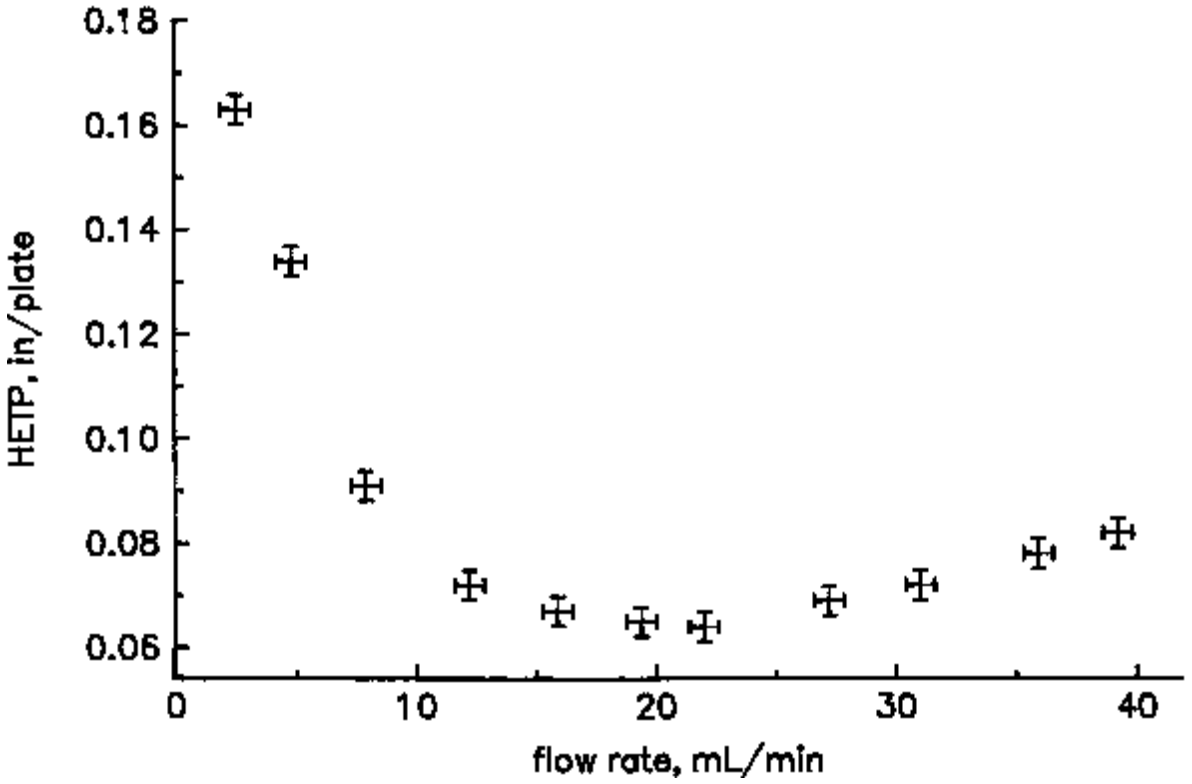

FIGURE 3. A VAN DEEMTER PLOT OBTAINED USING DATA COLLECTED ON A SINGLE REPRESENTATIVE CHROMATOGRAPH. ALL GC CONDITIONS ARE THE SAME EXCEPT FOR THE FLOW RATE, WHICH WAS ADJUSTED USING THE INSTRUMENT NEEDLE VALVE AND MEASURED WITH A SOAP BUBBLE FLOWMETER OR ROTAMER. HETP MEASUREMENTS WERE MADE USING INJECTIONS OF A HEXANES SOLUTION CONTAINING 2–4% UNDECANE.

Day 2.

Four beers are provided in bottles equipped with Brinkmann or Wheaton bottle-top dispensers. The students add 1.0 mL of 1-pentanol and 1.0 mL of beer into a 5 mL glass vial, followed by ca. 200 mg of NaCl. For one beer, three additional samples are made that make use of the technique of additions to determine the alcohol content: 2% ethanol in 1-pentanol, 4% ethanol in 1-pentanol and 6% ethanol in 1-pentanol. These three samples are assembled from 1 mL of the ethanol/pentanol solution, 1 mL of beer, and the salt. The seven samples are taken to the instrument room and two acceptable chromatograms of each are recorded.

The after-lab assignments include measuring the peak areas (calculated using the height times the width at half-height and cut-and-weigh), a plot of peak area versus % ethanol

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added (for 0, 2, 4, and 6% ethanol), and a determination of the percentage alcohol in each beer using the standard addition plot above (i.e., the slope of the line above is peak area/% ethanol).

Results and Discussion

The experiment has been performed by about 3500 students in the last four calendar years (four summer sections, ca. 50 each; four fall groups, 700 each; four spring groups, 100 each) with only minor revisions along the way. It has been very successful from our point of view and very popular from the students' perspective. The students have generally all used the same set of four beers (although not necessarily the same brand). A typical chromatogram is shown in Figure 2. Baseline separation of ethanol, water, impurities in 1-pentanol, and the solvent itself is obvious for the critical components. The beers were selected to cover a range of values and include a malt liquor, brand X, the "light" version of brand X, and the alcohol "free" version of brand X. Typical results are as follows:

sample	% ethanol
malt liquor	5.4
Brand X	4.5
"light" version of Brand X	3.8
alcohol "free" version of Brand X	0.3

We have examined wines, cough medicines, and fruit juices during method development and found that the samples gave satisfactory, reproducible results. Wines and cough syrups can be used neat or after dilution. The method of standard additions and our analyses appear to be linear over the entire range that was tested (0.1–15% ethanol).

Figures 1 and 2 show data collected on two different gas chromatographs under the conditions given above. Note that one is equilibrated at ca. 95°C and the other at ca. 105°C, and as a result, the retention times are somewhat different. Nonetheless, the separations are quite satisfactory except perhaps if a methanol impurity was present (see Figure 1). Figure 3 shows a van Deemter plot (height equivalent per theoretical plate versus carrier flow rate) using data obtained on a single chromatograph. The instrument conditions were constant except that the flow rate was changed using the needle valve knob on the front of the GC. Although the students here obtained only one point each,

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they could, in principle, collect enough data to prepare their own plot. The additional time required (6 injections @ 10 minutes), however, is currently not available in our schedule. The HETP sample uses a nonpolar solvent (hexanes) and eluent (undecane) to guarantee a symmetrical peak shape. The intention of this part of the experiment is to experimentally calculate the number of theoretical plates and use the calculated HETP to verify that the GC is within the optimal flow range. The experimental data in Figure 3 suggest that virtually any rate between 10 and 30 mL/min will be satisfactory since the van Deemter plot is so flat in this range of flow rates.

In earlier versions of the experiment, we required that the students make the standard solutions of ethanol in 1-pentanol (2, 4, and 6%) by adding μL quantities of ethanol directly into the 5 mL vials. This effort ranged from moderately unsuccessful to disastrous. We ascribe that to the potential for confusion among the many vials (in spite of repeated warning to label them clearly), contamination, and/or poor technique using syringes (a shortcoming that is overcome by the middle of the first day). Some of these problems could be eliminated by preparing larger samples of the solutions, an option that was immediately forsaken as wasteful. The current experiment could be classified as microscale and the volumes reduced even further. During development, we used as little as 0.3 mL of 1-pentanol and beer each. In practice, these small volumes led to a higher incidence of inadvertent injection of the water layer, since the interface between solvents is less obvious with the smaller volumes. However, the data from 1- or 0.3-mL samples seems to be equally reliable. The standard solutions of ethanol in 1-pentanol (2, 4, and 6%) are replaced periodically since the students have the tendency to leave the caps off and store the vials on top of the hot chromatographs.

The introduction to the laboratory includes a discussion on the importance of knowing the partition coefficient *K*, especially if one is intending to use multiple extractions to isolate the solute of interest. Since the students are not provided with the data in Table 1, they make a single determination of the value of *K*. We emphasize that it is not necessary to isolate all of the ethanol in this experiment; however, in another experiment in this course they do a more traditional extraction of an organic molecule in which the intention is to isolate all of it.

Two methods of integration have been used: (1) cut-and-weigh and (2) measuring the areas from the height times the width at half-height. During development, this was supplemented by the use of a planimeter and by electronic integration (Hewlett Packard

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Model 3390A Integrator) as a means to check the accuracy and reproducibility of the previous two techniques. In the students' hands, the cut-and-weigh method has not been very reproducible, because the peaks are generally not large enough and the available balances only have 1-mg resolution. Both of these shortcomings could be readily remedied but have not been because the other method works so well. Nonetheless, cut-and-weigh remains in the experiment as a demonstration of an alternative integration method. We chose not to use electronic integration for the students because the strip chart recorder visually demonstrates the relationship among the peaks from 2, 4, and 6% ethanol in 1-pentanol and provides the students with experience in how to use attenuation. The extra injection or two that may be necessary to get the first peak on-scale is warranted, considering the experience gained with peak-size adjustment. Chemistry majors get experience with integrators in a subsequent instrumental analysis course.

The students are introduced to two methods that enable them to convert the chromatographic peak area to an actual alcohol percentage: (1) a calibration or standard plot and (2) the method of standard addition. They seem to find the former more intuitive but gather most of their data from the method of standard additions. Almost all students get straight lines based on the four points they collect for one of the beers. In cases where they don't, most often the problem is with errors in area measurement based on incorrect use (or no use) of changes in attenuation. We chose not to use an internal standard based on early trials in which students became confused about which peaks are significant. Furthermore, picking the internal standard complicates the selection of GC parameters. Although the 16–22 students in a section compare their results, no formal error analysis is conducted. That exercise would take little additional class time but is already covered extensively in our laboratory curriculum in the quantitative analysis of unknowns in general chemistry.

In summary, we have found the combination of liquid-liquid extraction and gas chromatographic analysis of the resulting solutions quite fruitful. The experiment described is practical and very simple conceptually, and it has proven to be popular with our clientele. This method for the determination of ethanol in complex aqueous solutions is a simpler and more pragmatic one than many of those reported in the literature. The potential to vary most, if not all, of the conditions (e.g., column liquid phase, extraction solvent, samples) makes this experiment quite versatile and robust.

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