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Quantitative resolution of severely overlapping gas chromatographic peaks

Isothermal and temperature-programmed operation

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ABSTRACT

Severely overlapping gas chromatographic peaks ($R \leq 0.35$) are quantitatively resolved by a mathematical method. Accurate analyses may be obtained under the worst of overlapping conditions even with simple chromatographic instrumentation. For the method to be successful either the peak shapes of each component must differ if the retention times are identical, or their retention times must be slightly different if the peak shapes are identical. The method has been tested on both isothermal and temperature-programmed data. The computer method proposed requires correction for fluctuations in carrier gas flow-rate and programmed column temperature, both of which cause irreproducibility in peak position. A procedure for accomplishing this correction is presented.

INTRODUCTION

Quantitative analysis of severely overlapping gas chromatographic (GC) peaks has been a serious problem since the introduction of the technique. Whenever possible, resolution has historically been increased by varying the experimental conditions of separation, *i.e.*, column packing, temperature, flow-rate, choice of liquid stationary phase, etc. As samples become more complex, however, the problem of overlapping peaks becomes more acute, and changing the conditions to resolve one set of components generally causes another set to become unresolved. The selection of proper conditions for satisfactory separation can be extremely time consuming, and for complex systems sufficient resolution for accurate analysis by conventional methods is often impossible. Rather than physically resolving the peaks, another approach is to resolve them mathematically.

In an earlier paper on the theory of this approach, Lundeen and Juvet [1] reviewed previous mathematical methods and proposed a new method which gave promising results with computer-simulated data. D'Allura and Juvet [2] applied this method successfully to liquid chromatography. In this work, the method is applied

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to both isothermal and temperature-programmed gas chromatography. The detector response is measured at numerous locations throughout the width of the peak. Since peak shapes change with sample size, linear calibration graphs are not obtained except near the peak maxima. An example of experimentally measured calibration graphs obtained from various regions of a chromatographic peak is given in Fig. 1. Pure standards are used to fit the detector response at a given time to a second-degree polynomial of each component's concentration. With higher order polynomials, calibration points can be fitted exactly; random fluctuations, however, are better smoothed by using lower order polynomials [3]. The response from the overlapping mixture is taken as the sum of the responses from each of the components present in the mixture. A series of simultaneous equations are solved to give the concentration of each component of the mixture. Although no instance has been found in any of our studies for the non-additivity of component detector responses, if a possibility were to exist for interference in the flame ionization detection (FID) of two substances eluted simultaneously, the suitability of the analysis would best be tested using a test mixture of the pair at known concentrations. The method is successful if either the peak shape of each overlapping component is slightly different or if there is a slight difference in retention. The analysis of isotopic mixtures in which peak shapes are identical but retentions slightly different has been reported elsewhere [4].

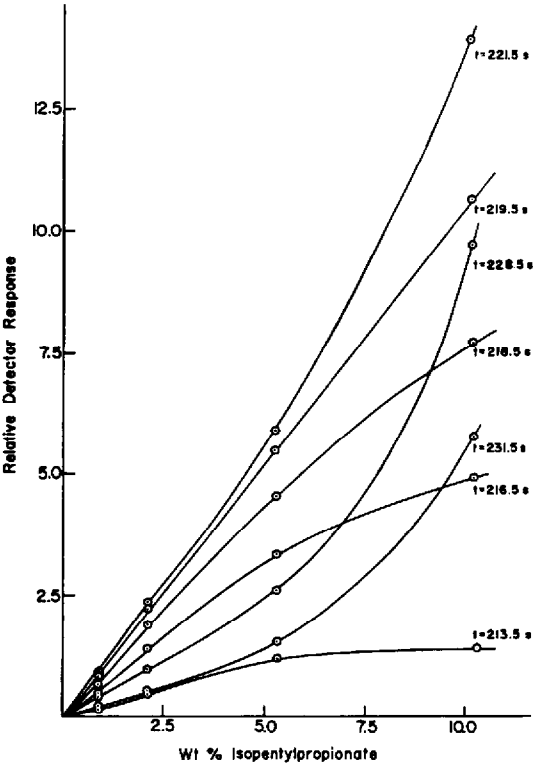

Fig. 1. Calibration graphs for isopentyl propionate. Variation in detector response with sample size at various positions throughout the width of the chromatographic peak. Times given are from moment of injection.

The peak positions must be accurately reproduced between the chromatograms of the standards and that of the unknown mixture. Changes in anything that affects the peak position, such as unexpected variations in flow-rate or in column temperature, affect the accuracy of the proposed method if proper corrections are not made. Therefore, either the flow-rate and column temperature must be held constant so that the peak position doet not vary or else these parameters must be accurately measured and the peak position adjusted to the position it would have had these parameters not varied. The latter approach was chosen.

THEORY

A well established equation in gas chromatography [5,6] is

$$1 = \int_0^{t_R} (\bar{F}/V_R^0)\,dt \tag{1}$$

where dt is the time element, t_R is the observed retention time, V_R^0 is the corrected retention volume and $\bar{F}$ is the average flow-rate in the column. This equation applies to both isothermal and to temperature-programmed gas chromatography as long as the flow-rate and corrected retention volume are both expressed at the same temperature. Both $\bar{F}$ and V_R^0 have the gas compressibility factor applied so that eqn. 1 may be rewritten using the directly observed quantities rather than the pressure-corrected quantities:

$$1 = \int_0^{t_R} (F_c/V_R)\,dt \tag{2}$$

where F_c is the corrected flow-rate expressed at the column outlet and V_R is the observed retention volume. If the flow-rates and volumes are expressed at some standard temperature, T_S, such as 25°C, rather than being expressed at the column temperature, T_c, then

$$F_S = (T_S/T_c)F_c \tag{3}$$

$$V_S = (T_S/T_c)V_R \tag{4}$$

where F_S and V_S correspond to F_c and V_R, respectively, corrected to the standard temperature. Eqn. 1 may therefore be written in terms of these new quantities as

$$1 = \int_0^{t_R} (F_S/V_S)\,dt \tag{5}$$

The adjusted retention volume, V_R', of a compound is the observed retention volume of the compound, V_R, minus the volume of the mobile phase, V_M, generally measured from the retention volume of some unretained compound such as air, V_a:

$$V_R' = V_R - V_M \tag{6}$$

The adjusted retention volume varies with the absolute temperature of the column [5] according to

$$V'_R = A \exp(-\Delta H_S/RT_c) \tag{7}$$

where ΔH_S is the heat of solution of the solute in the stationary phase, R is the gas constant and A is a constant for the particular solute. Both ΔH_S and A vary slightly with temperature, but over a temperature range of 40–60°C can be assumed to be constant. The observed retention volume is then

$$V_R = V_M + A \exp(-\Delta H_S/RT_c) \tag{8}$$

Habgood and Harris [5] have shown that this equation is more accurate if the volume are expressed at a standard temperature rather than at the column temperature. The following approximate equation will be substituted:

$$V_S = B \exp(C/RT_c) \tag{9}$$

Fig. 2 shows a plot of eqn. 9 for *o*-xylene and dodecane. It can be seen that the relationship is sufficiently linear over the temperature range studied to justify the use of eqn. 9. Substituting eqn. 9 into eqn. 5 yields

$$1 = \int_0^{t_R} F_S/[B \exp(C/RT_c)]\,dt \tag{10}$$

The integration could be carried to some time t_i rather than t_R. If $t_i < t_R$ then the integral would be < 1, and if $t_i > t_R$, then the integral would be > 1.

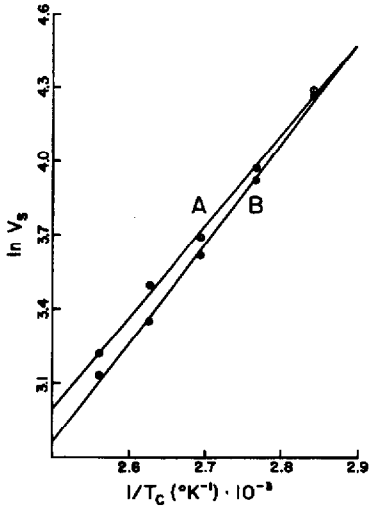

Fig. 2. Retention volume *vs.* $1/T$. Observed retention volumes corrected to 25.00°C. (A) *o*-Xylene; (B) dodecane.

Assume that the same compound is injected two different times under slightly different conditions of temperature and flow-rate. If one of the runs is chosen as standard and the second run is compared with it, the ratio of the two integrals is

$$K = \frac{\int_0^{t_1} (F_S)_2 \exp[-C/R(T_c)_2]\,dt}{\int_0^{t_1} (F_S)_1 \exp[-C/R(T_c)_1]\,dt} \tag{11}$$

As two runs with the same compound are being compared, the constant B will be the same in both integrals and will cancel. If $K > 1$, then under the second set of conditions the compound eluted earlier, and its retention time is

$$(t_R)_2 = (t_R)_1/K \tag{12}$$

This equation is not limited to the retention times of peak maxima but can be used to relate any time within a peak from one set of conditions to the other. If $K < 1$, then the compound eluted later under the second set of conditions but its retention time and other corresponding points on the peak are still given by eqn. 12. By using this equation, the position of a compound injected several times may be corrected for variation in the column temperature and flow-rate of the carrier gas. This equation is applicable for both isothermal and temperature-programmed gas chromatography.

Determination of the exact value of C for both compounds to be resolved would make the above corrections time consuming and impractical for day-to-day use. In order to simplify the procedure, an average value of C may be assumed for all compounds. This assumption implies that the B value for each overlapping component will also be identical since the components elute together.

Therefore, by monitoring the flow-rate and column temperature, the position of peaks eluting under different sets of conditions may be corrected to a set of standard conditions.

EXPERIMENTAL

Gas chromatograph

A Perkin-Elmer (Norwalk, CT, USA) Model 3920 gas chromatograph was used, equipped with a dual flame ionization detector and a 5 ft. × 1/8 in. I.D. column containing 12% Carbowax 20M (Supelco, Bellefonte, PA, USA) on 60 80-mesh Chromosorb W AW. A Model CXSS-18E-12 Chromel–Constantan thermocouple (Omega Engineering, Stamford, CT, USA) was installed through a hole drilled in the side of the column so that the temperature-measuring junction was near the center of the column and in contact with the column packing. The thermocouple was referenced against an ice-bath and calibrated against a precision thermometer. The thermocouple could read to 0.01°C over the temperature range 80–120°C. The injection port was replaced with a Seiscor (Seismograph Service, Tulsa, OK, USA) Model VIII

liquid sampling valve. Tubing from the sample valve output was run through a heated aluminum block maintained at 160°C for rapid and reproducible vaporization of the sample. The sample was dissolved in a solvent and ca. 0.5 μl was injected reproducibly with a standard deviation of 1.6% and a maximum deviation of 4.1%. The volume injected was maintained constant to improve reproducibility in sample vaporization. Nitrogen was used as the carrier gas, and no flow controller was required as corrections were made for variations in flow and temperature. Hence accurate analyses may be obtained using this technique with simple instrumentation.

Flow monitor

The flow monitor used was similar to that reported by Juvet *et al.* [7]. The flow monitor was positioned in the carrier gas line before the liquid sample injection valve. Under computer control, a Seiscor Model VIII gas sampling valve was used to inject a small sample of helium periodically into the carrier gas. The helium introduced passed through the first pair of cells of a Gow-Mac micro-thermal conductivity cell (Model 10-952; Gow-Mac Instrument, Bridgewater, NJ, USA), through an empty capillary delay loop to the second pair of cells. Both thermal conductivity cell pairs were wired to form the four arms of a Wheatstone bridge. The capillary delay loop was made of a 6-ft. length of 1/16-in. O.D. stainless-steel tubing. The time required for the helium to travel through the delay loop was a function of the flow-rate of the carrier gas. A computer automatically injected a 30-μl sample of helium every 30 s and then sampled the output from the Wheatstone bridge at a rate of 200 Hz. The time required for the helium to travel through the delay loop was calibrated over the flow-range 16–22 ml/min against a soap-bubble flow meter. Flow-rates were then converted to a standard temperature of 25°C. Using the flow monitor, the flow-rate of the carrier gas could be measured to ± 0.02 ml/min. Results from the flow monitor are not affected by changes in the temperature of the GC oven. The presence of helium from time to time in the carrier gas had no effect on the FID of the organic compounds injected owing to the insensitivity of the detector to helium.

Computer

A Digital Equipment (Maynard, MA, USA) PDP-8/E computer was used for taking the data reported here, although a Commodore (Santa Clara, CA, USA) 32K microcomputer has been programmed for similar studies involving GC analysis of isotopic mixtures reported elsewhere [4]. The PDP-8/E was equipped with 16K of memory, a KL8-E asynchronous data control board, a DK8-EP real-time programmable clock, an RX02 dual density floppy disk system and a DECwriter II. Programs written used the OS8 V3D operating system. An interface was built to control a Phoenix Data (Phoenix, AZ, USA) Model ADC-312 12-bit analog-to-digital converter (ADC), an eight-channel multiplexer, four relay lines and four switch-sensing lines. Three Analog Devices Model 610K instrumental amplifiers were used to adjust the signals from the thermocouple, flame ionization detector and flow monitor to the 0–5 V range required for the ADC. A Rikadenki-Kogyo (Tokyo, Japan) Model B-161 recorder was sometimes attached to the output of the amplifiers for visual monitoring of the signals.

Procedure

An assembly language program was used to collect data during each injection from the three signal sources. The program sampled the temperature and FID signals at a selectable rate (0.25–100 points/s) and calculated the flow-rate once every 30 s. With 16K of memory both the temperature and FID channel buffers have a maximum data capacity of 3938 points. The buffer on the flow channel will not fill before the other buffers. The order of sampling was four standards of the first component, four standards of the second component, and as many sample mixtures as desired. The data were then analyzed by three BASIC programs. The first program corrected the data for fluctuations in flow and temperature, the second was used to find the peaks of interest and subtract off the baseline and the third did the resolution calculations.

Chemicals

Samples were dissolved in a mixture of hexane isomers (Certified ACS grade, Fisher Scientific, Fair Lawn, NJ, USA). Other reagents were analytical-reagent grade isopentyl propionate, dodecane (99% pure), *o*-xylene (97% pure), tridecane (99+% pure) and 2-octanone (95% pure) (Aldrich, Milwaukee, WI, USA).

RESULTS AND DISCUSSION

Fig. 3 shows typical chromatograms of mixtures of two components with almost identical retentions that have been resolved by this resolution method. Note that no shoulder is apparent on the two peaks eluting at 100 and 160 s in Fig. 3A and B, respectively. No other mathematical resolution method can handle the analysis of such mixtures. The output signals from the flame ionization detector and the thermocouple together with the calculated flow-rates are shown along a common time axis. The off-scale peak and the small peak on its tail in both chromatograms are from the hexane solvent. The temperature of the column packing was measured rather than that of the oven, as partitioning of the sample takes place in the packing. Under isothermal conditions, the packing temperature was constant with a standard deviation of only 0.028°C. With temperature programming, the temperature inside the column shows a significant lag behind the oven temperature owing to the insulating properties of the solid support. The flow-rate is greatest at the time of injection because of vaporization of the sample. It then returns over a period of several seconds to a more constant value. Since the flow monitor averages the flow over the *ca.* 10 s of measurement made each 30 s, any rapid oscillations in flow-rate that might occur at injection are not observed. Flow-rates varied by 0.06 ml/min or *ca.* 0.3% over the period of a chromatographic run if the momentary initial flow increase is disregarded. If it is not, the flow varied by 0.30 ml/min or about 1.3%. Measured flow-rates are used in making retention time corrections rather than an average of these values.

In Fig. 3A the single peak at *ca.* 100 s actually consists of two severely overlapping peaks of *o*-xylene and dodecane with a resolution of only 0.35. Quantitative results for this chromatogram and other mixtures of the same two compounds are given in Table I. The average error before corrections for variations in flow-rate and temperature was *ca.* 13%, and this was reduced to *ca.* 7% after making retention time corrections, an error not much larger than the 2–5% error generally quoted for completely resolved peaks in conventional GC analyses. The corrections were made

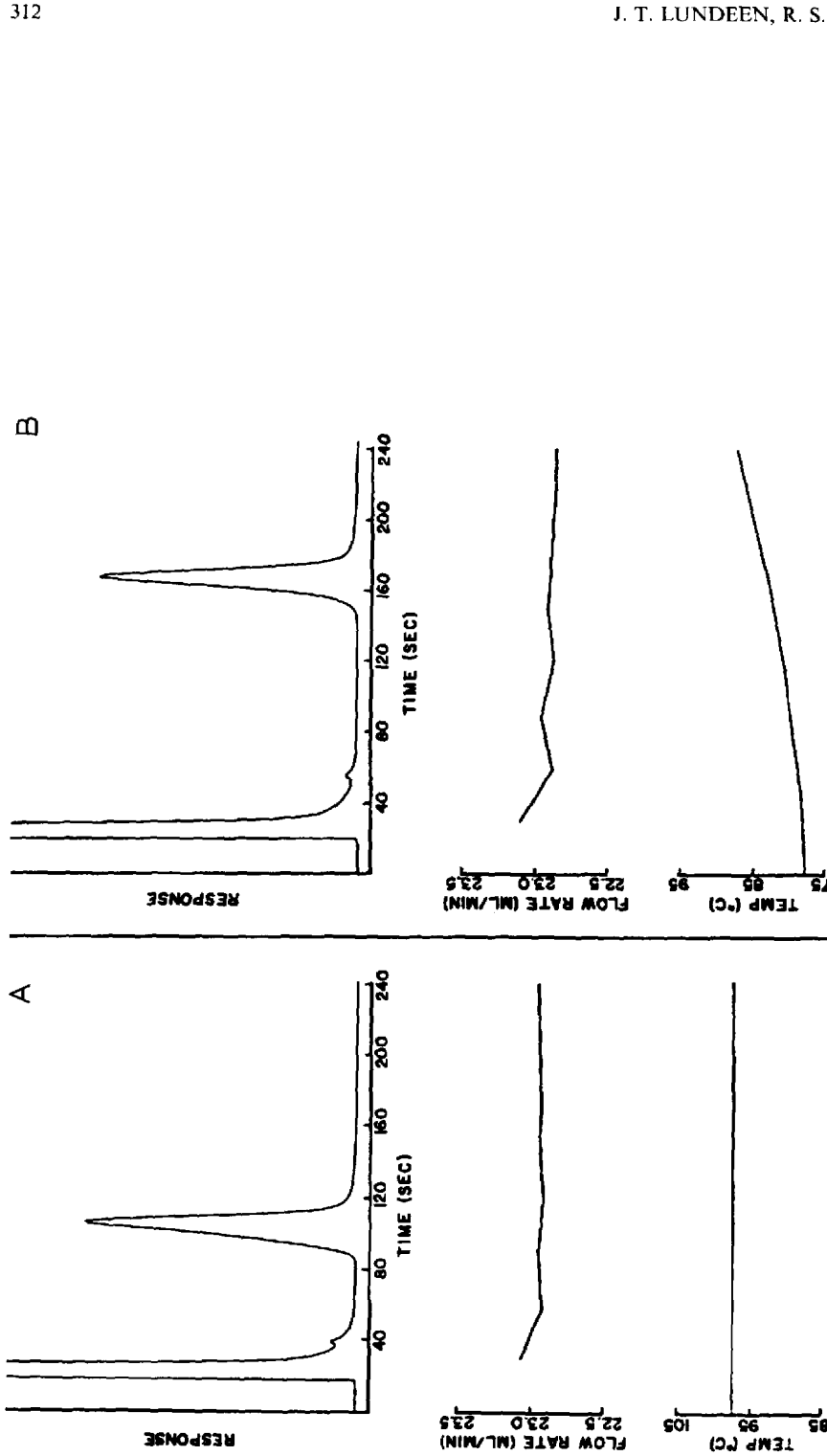

Fig. 3. (A) Isothermal analysis of dodecane and *o*-xylene. Column temperature: 97.4°C. Peak resolution: 0.35. Mixture: 3.00% (v/v) *o*-xylene and 3.00% (v/v) dodecane in hexane (first two peaks from hexane solvent). Measured flow-rate and column temperature shown at the time indicated. Column: 12% Carbowax 20M on 60–80-mesh Chromosorb W AW. (B) Temperature-programmed analysis of dodecane and *o*-xylene. Oven temperature: initially 77.9°C, then programmed at *ca.* 3.5°C/min for 4 min. Peak resolution: 0.24. Mixture, measured flow-rate and column temperature and column as in (A).

using a C value of 8.0 kcal/mol. The corrections were made using a range of C values of $\pm 20\%$ from this value, and the quantitative results varied by less than 1%. Hence correction is not strongly dependent on the choice of C when variations in temperature are small.

Table II gives the results for the resolution of tridecane and 2-octanone analyzed isothermally. The peaks had a resolution of only 0.20, a value so small that no indication of a shoulder is evident on the peak. However, the average error in composition after corrections for fluctuations in flow-rate and temperature is only *ca.* 9%.

Fig. 3B is a typical temperature-programmed chromatogram. The peak eluting at *ca.* 160 s is composed of a mixture of dodecane and *o*-xylene, the resolution of which is only 0.24 under the experimental conditions used. Table III gives the analytical results for various mixtures of these two components. The average error before corrections for variations in temperature and flow-rate was *ca.* 28% and after corrections was *ca.* 24%. As expected, these errors are considerably larger than those found under isothermal conditions. Not only is the flow changing during the analysis, but also the temperature is being deliberately changed. Changes in temperature have a dramatic effect on retention times, hence any small irregularities in the rate of heating between different samples will affect the results. Equally important, peak shapes are more symmetrical and similar in temperature-programmed GC than in isothermal determinations. The correction method was designed to correct for these changes in temperature and flow-rate, and it did improve the results. Considering that the two components involved had almost identical retention times ($R = 0.24$), any result at all under such conditions must be considered remarkable. No other method of resolution can approach the accuracy obtained for these severely overlapping peaks. The small difference in retention and the similarity in peak shapes contribute significantly to the errors obtained in temperature-programmed operation.

CONCLUSIONS

The resolution method developed earlier by Lundeen and Juvet [1] using computer-simulated chromatographic peaks has been shown to work well in practice under isothermal conditions and reasonably well in temperature-programmed determinations under conditions of resolution that no other mathematical resolution method can handle. As this method provides relatively good accuracy with isothermal determinations of severely overlapping peaks, some might choose to analyze the sample isothermally at higher temperatures, accepting overlapping of early eluting peaks, rather than to use temperature-programmed elution. A large part of the error is undoubtedly caused by the $\pm 1.6\%$ sampling error. The flow monitor measures the average flow-rate for periods of 10 s once every 30 s. If this period could be reduced or a continous monitor designed, as was done for our work in liquid chromatography [2], a more accurate profile of the flow-rate could be made. This should further improve retention corrections. Moreover, in this work all peaks were measured using the same amplifier gain from the gas chromatograph. This meant that on the smaller peaks only about 15% of the range of the ADC was used. If the gain of the amplifier were accurately calibrated at different gain settings, the height of each peak could be adjusted to take full advantage of the ADC, probably leading to further improvements in accuracy.

TABLE I

ISOTHERMAL ANALYSIS OF DODECANE AND *o*-XYLENE

Improvement in quantitative results through correction for flow and temperature variations. Conditions as in Fig. 3A. Peak resolution: 0.35. Component 1 is *o*-xylene and 2 is dodecane.

Actual		Uncorrected				Corrected			
Amount of 1 (%, v/v)	Amount of 2 (%, v/v)	Amount of 1 (%, v/v)	Error (%)	Amount of 2 (%, v/v)	Error (%)	Amount of 1 (%, v/v)	Error (%)	Amount of 2 (%, v/v)	Error (%)
3.00	3.00	3.44	+14.7	2.48	−17.3	3.33	+11.0	2.74	−8.7
2.00	4.00	2.39	+19.5	3.73	− 6.8	2.30	+15.0	3.90	−2.5
4.00	2.00	4.38	+ 9.5	1.84	− 8.0	4.29	+ 7.2	2.00	0.0

TABLE II

ISOTHERMAL ANALYSIS OF TRIDECANE AND 2-OCTANONE

Improvement in quantitative results through correction for flow and temperature variations. Column temperature: 87.7°C. Peak resolution: 0.20. Component 1 is tridecane and 2 is 2-octanone.

Actual		Uncorrected				Corrected			
Amount of 1 (%, v/v)	Amount of 2 (%, v/v)	Amount of 1 (%, v/v)	Error (%)	Amount of 2 (%, v/v)	Error (%)	Amount of 1 (%, v/v)	Error (%)	Amount of 2 (%, v/v)	Error (%)
2.00	4.00	1.92	−4.0	4.37	+ 9.2	2.03	+ 1.5	4.31	+ 7.8
2.00	4.00	1.93	−3.5	4.39	+ 9.8	2.04	+ 2.0	4.34	+ 8.5
3.00	3.00	3.22	+7.3	3.59	+19.7	3.32	+10.7	3.47	+15.7
3.00	3.00	3.23	+7.7	3.58	+19.3	3.34	+11.3	3.47	+15.7
2.00	4.00	1.93	−3.5	4.43	+10.8	1.91	− 4.5	4.45	+11.2
2.00	4.00	2.03	+1.5	4.41	+10.2	1.95	− 2.5	4.45	+11.2
3.00	3.00	3.18	+6.0	3.57	+19.0	3.27	+ 9.0	3.46	+15.3
3.00	3.00	3.14	+4.7	3.60	+20.0	3.26	+ 8.7	3.47	+15.7

TABLE III

PROGRAMMED TEMPERATURE ANALYSIS OF DODECANE AND *o*-XYLENE

Improvement in quantitative results through correction for flow and temperature variations. Conditions as in Fig. 3B. Peak resolution: 0.24. Component 1 is *o*-xylene and 2 is dodecane.

Actual		Uncorrected				Corrected			
Amount of 1 (%, v/v)	Amount of 2 (%, v/v)	Amount of 1 (%, v/v)	Error (%)	Amount of 2 (%, v/v)	Error (%)	Amount of 1 (%, v/v)	Error (%)	Amount of 2 (%, v/v)	Error (%)
3.00	3.00	3.10	+ 3.3	4.29	+43.0	2.93	− 2.3	4.51	+50.3
3.00	3.00	3.16	+ 5.3	4.44	+48.0	3.01	+ 0.3	4.53	+51.0
2.00	4.00	2.76	+38.0	3.82	− 4.5	2.49	+24.5	4.20	+ 5.0
2.00	4.00	2.74	+37.0	3.83	− 4.2	2.46	+23.0	4.27	+ 6.8
4.00	2.00	5.28	+32.0	0.72	−64.0	4.33	+ 8.3	3.27	+63.5

Although only packed columns have been employed in the study reported here, peak widths of 20–30 s were handled with ease. Since temperature and FID signals can be sampled as rapidly as 100 points/s, open-tubular columns with a good quality, chemically bonded liquid stationary phase could possibly be used.

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