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SPLITLESS SAMPLING FOR CAPILLARY-COLUMN GAS CHROMATOGRAPHY

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SUMMARY

The experimental aspects of the "Grob splitless" sampling technique are discussed in terms of the sampling time, purge activation time, solvent type, sample size, column temperature, injector temperature and column flow-rate. Guidelines are developed for using and simplifying splitless sampling. Optimized experimental parameters are given from the results of optimization by simplex. Precision data for retention times and peak areas are shown to be 0.05 and 1%, respectively, for an automated splitless capillary system.

INTRODUCTION

High-resolution gas chromatography (GC) is developing rapidly as the GC technique of choice for complex separations and for trace organic analysis. This technology is due to recent developments in capillary-column GC instrumentation, high-sensitivity and low-volume detectors, glass capillary column technology and new sampling and injection techniques. These developments have recently been reviewed elsewhere[1,2].

Good sampling and injection techniques are particularly crucial for obtaining high-efficiency separations and highly reproducible, accurate and representative quantitative results on very small sample sizes. High-performance capillary sampling systems should meet the following criteria: (1) the solvent peak should not interfere with the quantitation of solute peaks; (2) there should be no discrimination of sample components in terms of their boiling points, polarity, molecular weight, *etc.*; (3) retention times should be reproducible to $<0.1\%$ relative standard deviation; (4) normalized peak areas should be reproducible to $<1\%$ relative standard deviation; (5) thermal degradation, adsorption, rearrangements and other solute reactions should be negligible at the picogram level; (6) the sampling system should introduce a negligible loss in column efficiency; (7) the sample recovery should be quantitative and representative for both trace and major sample components; and (8) changes in the column operating conditions should not influence the sampling processes. Several different types of sampling and injection techniques have been developed for capillary-column GC separations such that it is now important to choose the proper sampling technique according to the type of sample to be analyzed.

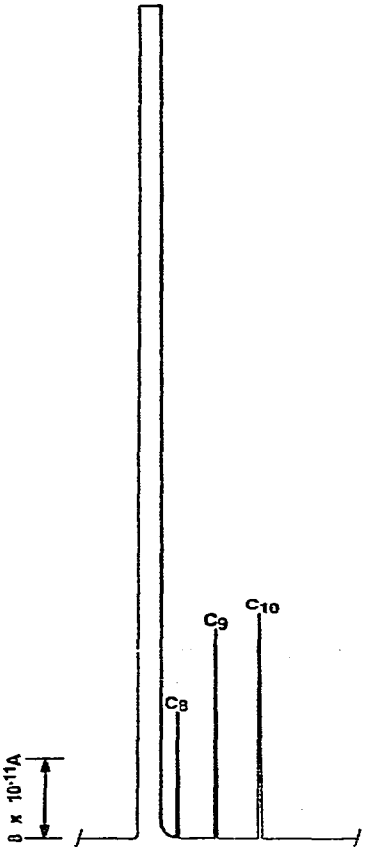
The "direct" or "on-column" and "selective" sampling techniques developed by Schomburg and co-workers[3–5] have demonstrated excellent precision and accuracy for mixtures of alkanes up to n-C_{34}. These techniques have the advantages of minimizing thermal effects of sensitive or unstable compounds because a heated injector is not required to vaporize the sample. The "moving needle" sampling technique is reported to have a reproducibility of better than 1.5% for high-boiling compounds and is amenable to automation[6,7]. Techniques such as "heart cutting"[8] and "pre-column sampling"[9] are also useful in the quantitative analysis of complex mixtures and trace components because they make use of enhanced chemical selectivity and decrease the amount of sample clean-up required. Splitters are well known in capillary-column GC sampling[10–12].

The "splitless" sampling technique of Grob and co-workers[13–15] has been found to be particularly useful for the analysis of (a) very dilute solutions, (b) samples with components eluting near the tail of the solvent peak, (c) thermally labile compounds and (d) samples with very polar components such as natural products and environmental extracts. The advantages of the technique include the following: (1) dynamic splitting of the samples is not required; (2) the volume of the samples injected can be measured directly with a syringe (in the microliter range); (3) rapid vaporization of the sample is not required and, consequently, relatively low injection port temperatures can be used to minimize sample degradation; (4) septum bleed is minimized by the use of a septum purge; (5) analysis of very dilute samples without pre-concentration is possible; (6) sorption losses in injectors are minimized because the internal surface area is low; (7) injector inserts are easily replaced and cleaned; and (8) the technique is easily automated. However, the successful application and performance of the splitless sampling technique depend on a large number of experimental variables, including the type and boiling point of the solvent, sample size, sampling time, injector purge time, initial column temperature, injector temperature, column flow-rate and type of column. The effects of these variables on the separation efficiency and quantitative sample recovery and their optimization have not been previously reported. Systematic studies, the effect of critical instrument parameters on chromatographic performance and the optimization by simplex techniques of the splitless sampling technique are described in this paper.

Splitless sampling is a particularly attractive technique for trace analysis because a large dynamic range of sample concentrations can be injected. By optimizing the length to volume ratio of the splitless glass insert for the Varian 3700 capillary inlet system, excellent separations, quantitative sample recovery and good solvent peak shapes were obtained on samples as small as 0.1 μl injected, as is shown in Fig. 1. Normally, at least 2 μl are required in order to obtain the solvent effect. The reasons why a good solvent effect is obtained with small sample sizes on 0.25 mm I.D. columns will be discussed elsewhere[16]. The upper limit of sample capacity of this splitless system is shown to be $\geqslant$10 μl elsewhere in this paper.

EXPERIMENTAL

All experimental data were measured on a Varian Model 3700 capillary gas chromatograph equipped with an FID, a Varian Model 8000 AutoSampler, a CDS-111 chromatography data system, a Model 9176 recorder and an External Events



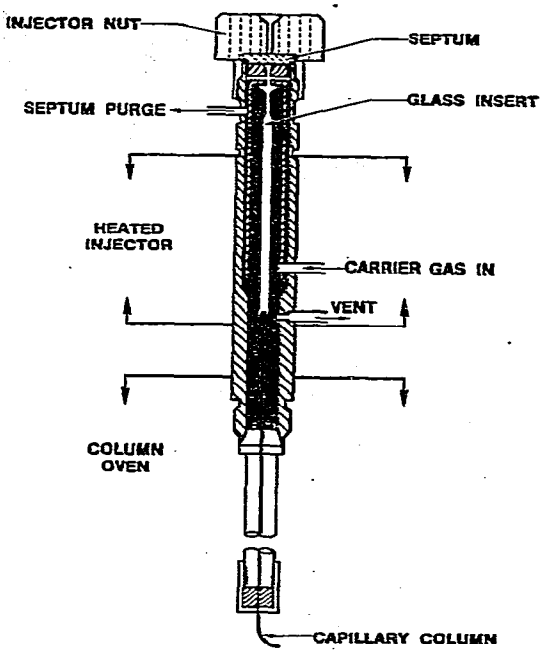


Fig. 1. Splitless capillary sampling for a 0.1-μl sample, diluted 10^3:1. Solute concentration, 3 ng/μl in isooctane. Purge activation time, 40 sec; column, 20 m × 0.25 mm I.D. OV-101; temperature program, 60° to 140° at 7°/min with an initial hold of 2 min.

Fig. 2. Cross-sectional view of the Varian Model 3700 splitless capillary injector.

Module. The column efficiencies, retention times, peak areas, peak widths and other peak parameter measurements were made in real time and calculated on a Varian CDS-220 data system using EBASIC. Helium was used as both the carrier and make-up gas to the FID.

Splitless injector

A cross-sectional view of the Varian Model 3700 capillary injector with the splitless insert installed is shown in Fig. 2. The carrier gas enters the injector body through pre-heating coils. This increases the temperature of the carrier gas to that of the injector before reaching the sample vaporization chamber, eliminates condensation if back-flash occurs during sample injection and sweeps the outside of the glass insert. At the top of the glass insert, the stream of carrier gas flows in two directions, as shown in Fig. 3: part flows into the injector insert and on to the column at the normal column flow-rate. A second flow stream is used for septum purge, which is vented to the atmosphere.

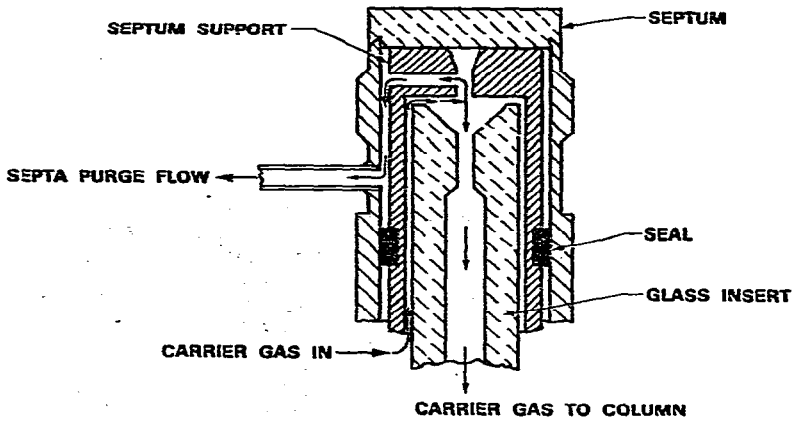


Fig. 3. Cross-sectional view of the Varian Model 3700 capillary injector septum purge.

The septum purge flow prevents septum contamination from reaching the glass insert and the column. Unlike split sampling, where the split flow vents 80–99.9% of the incoming contaminants to the atmosphere (for splitting ratios of 5:1 to 1000:1), splitless sampling would transfer all contaminants (principally from the septum) directly to the glass insert and column when operating in the standby or pre-injection modes if it were not for the septum purge flow. The septum purge flow is prevented from recirculating through the injector by a seal just below the septum purge outlet (see Fig. 3). Because of the septum purge feature, the split flow can be turned off when not in use without allowing septum bleed to contaminate the chromatographic system and, at the same time, save large amounts of carrier gas.

The splitless glass insert, shown in Fig. 2, is easily installed and removed from the top of the injector. This is particularly important for splitless sampling where large volumes of "dirty samples" are injected. For optimal quantitative results with trace and polar components, it is recommended that the glass insert be changed regularly, if not after every sample injection. The small volume of the insert (*ca.* 150 μl) minimizes mixing of the sample vapor and carrier gas before transfer to the column. It should be noted that the split tip fills most of the lower portions of the 2-mm I.D. glass insert to minimize diffusion chamber effects and sample losses.

Other important design considerations of a splitless insert require a minimum internal surface area and a restrictor section on the insert to minimize sample back-flash. During the sampling time, the syringe needle (nominally 0.45 mm O.D.) in the capillary section (nominally 0.55 mm I.D.) of the glass insert acts as a restrictor to minimize sample back-flash because the linear carrier gas velocity in this region is very high. Finally, it is imperative that the sampling point or split tip be located in the heated injector region to eliminate sample discrimination and/or condensation.

The effect of the design of the glass insert on the chromatographic performance is shown in Fig. 4. Keeping the sampling and analysis conditions fixed, two glass inserts of different lengths and inside diameters were tested. The insert with the larger inside diameter created a larger diffusion volume below the split tip, which contributed to the excessive solvent peak tailing and loss of solute peak area. The larger insert

also allowed mixing of the sample and carrier gas which interfered with the "solvent effect". This is reflected in the retention time shifts, wider peaks and reduced column efficiency measured for the n-C_8 and n-C_9 peaks in comparison with the results from the 2-mm I.D. insert. These effects are most pronounced for the peaks near the solvent peak. An ideal solvent effect is demonstrated by the 2-mm I.D. insert in that peak sharpening occurs on peaks that elute close to the solvent peak and a baseline separation is obtained between the n-C_8 and isooctane peaks. This is remarkable when one considers that peaks 1–10 sec wide are produced with a slow injection technique that initially spreads peaks out over the time period from sample introduction until the injector is purged (nominally 30–60 sec). The solvent effect negates the effects of the slow vaporization and mass transfer and allows the high efficiency of the capillary column to be realized.

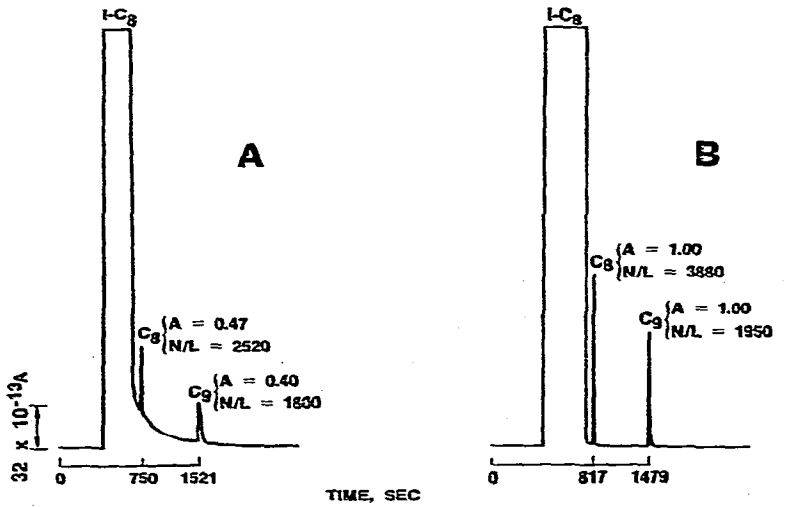


Fig. 4. Effect of the length-to-volume ratio and diffusion chambers in a splitless capillary injector. Inserts: (A) 4 and (B) 2 mm I.D. Column, 25 m × 0.25 mm I.D. OV-101. A = relative peak area normalized to the 2-mm I.D. insert; N/L = number of theoretical plates/m.

RESULTS AND DISCUSSION

Solvent effect

The solvent effect is a function of virtually all of the GC, capillary column and sample variables. Fig. 5A shows the experimental operating procedure for splitless sampling by plotting the carrier gas flow-rate through the injector as a function of time, where the injector purge delay time is denoted as Δt_2. A greatly attenuated solvent peak profile is shown in Fig. 5B. From the shape of this curve the solvent effect in the column can be understood[16]. The sharp back side of the solvent peak will be obtained only if a step function in the injector carrier gas flow-rate is obtained at the time of the injector purge. For the system described here, the injector purge time is of the order of 200 msec at a purge flow-rate of 100 ml/min. This rapid flushing carries all detectable sample residuals out of the sampling region, prevents solute

peak tailing and removes the classical "diffusion tail" from the solvent peak. This step function in the injector volumetric flow-rate does not change the column flow-rate because pressure-regulated pneumatics are used.

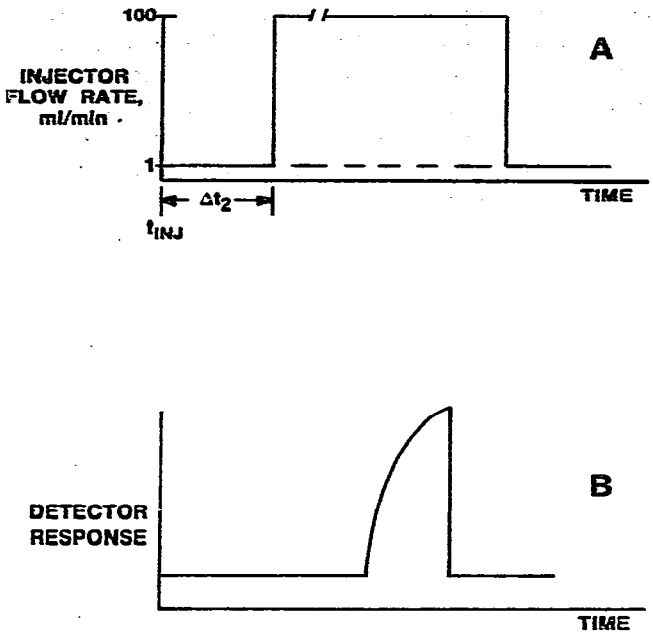


Fig. 5. (A) Carrier gas flow-rate profile as a function of time for the splitless sampling mode. Δt_2 denotes the injector purge delay time. Nominal column (1 ml/min) and injector purge (100 ml/min) flow-rates are shown for narrow-bore WCOT columns. (B) True solvent peak shape in the splitless mode when attenuated to 10^{-8} A full-scale.

The solvent peak shape shows why solute peaks eluting just ahead of the solvent front cannot be separated quantitatively, and why the splitless mode may not be satisfactory for applications such as solvent impurity analyses. Therefore, low-boiling solvents are required for the analysis of very volatile sample constituents. In general, the boiling point of the solvent should be at least 25° below that of the first peak of interest, otherwise they will co-elute with the solvent peak. This is illustrated by the isooctane–*n*-C_8 separation shown in Fig. 4, where the boiling point difference is 26.5°. Another example is the loss of the light ends in the separation of a volatile coal hydrogenation product run in the splitless mode (Fig. 6). Fig. 6 should be compared with the results for the same sample run "neat" in the split mode, shown in Fig. 7. Because dichloromethane (boiling point 40.2°) was used for the solvent effect in Fig. 6, it can be predicted that sample components with boiling points less than 65–70° will not be resolved from the solvent peak.

Sampling technique

The effects of various sampling techniques were investigated because it was found that the quantitative results may be dependent on the operator and the technique. The rate of sample injection or delivery to the injector is important in order

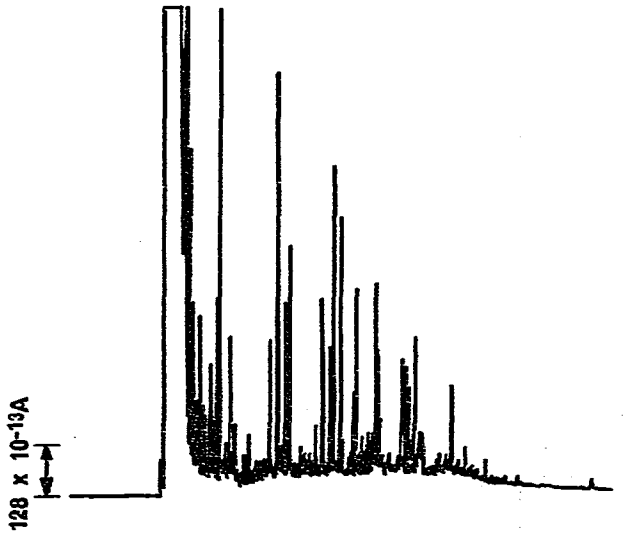


Fig. 6. Coal hydrogen product capillary separation using splitless sampling. Dilution, 10^5:1 in dichloromethane; 6.0 μl injected. Note the loss of resolution for the light hydrocarbons due to the solvent effect. Temperature program, from 27° to 200° at 2°/min with an initial hold of 15 min.

to insure uniform rate of sample vaporization without molecular weight discrimination and to avoid sample back-flash out of the injector insert. A maximum rate of sample injection of 1 μl/sec was found to give the best chromatographic results when using the splitless mode. This relatively slow rate of sample introduction is feasible and advantageous because the solvent effect resharpens the broad injection profile and overloading of the injector is avoided. Because the sampling and vaporization processes are relatively slow, it is possible to operate at lower injector temperatures, even with samples that contain high-boiling components. We have rarely experienced the need for injector temperatures above 230° when operating in the splitless mode. These low injection port temperatures are a distinct advantage in analyzing thermally labile compounds, reducing septum bleed and increasing septa lifetimes.

The sampling time, Δt_1, must also be considered in order to minimize sample discrimination according to vapor pressure differences, sample back-flash and to insure quantitative sample recovery. The sampling time is defined here as the period of time in which the syringe needle is resident in the heated region of the splitless injector. Fig. 8 shows the relative peak height as a function of the sampling time for peaks with both large and small k'. The relative peak heights are seen to converge after sampling times longer than 20 sec. For $\Delta t_1 < 20$ sec it can be seen that the more volatile components ($k' = 0.4$) are preferentially lost, probably due to sample diffusion into diffusion chambers, back-flash and venting out through the septum purge. Therefore, all peak heights in the chromatogram will be a function of Δt_1 for $\Delta t_1 < 20$ sec. Conditions for this study were chosen to represent a worst-case analysis, *i.e.*, a low-boiling solvent (b.p. 46°) with a high injection port temperature (250°). Although higher boiling solvents and/or lower injection port temperatures will reduce the required sampling time, a sampling time of $\Delta t_1 > 20$ sec is recommended for most applications.

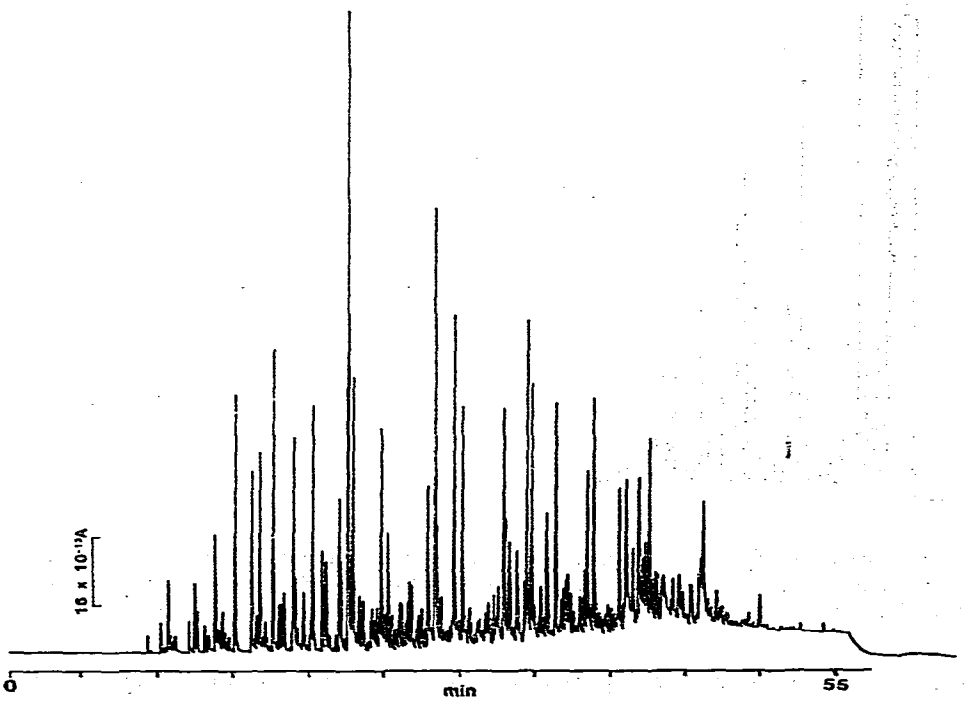


Fig. 7. Coal hydrogenation product capillary separation using split sampling on the neat sample. Column, 112 m × 0.25 mm I.D. OV-101; temperature program, from 40° to 200° at 5°/min with initial and final hold times of 10 min.

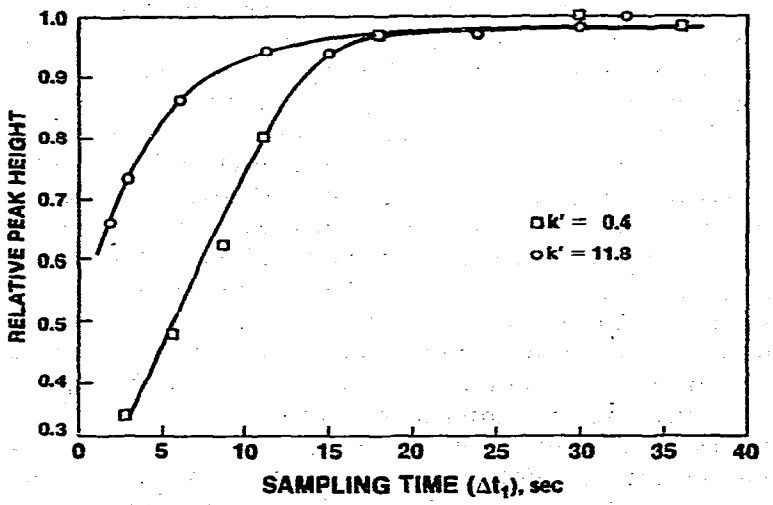


Fig. 8. Effect of splitless sampling time (Δt_1) on the sample recovery and sample discrimination on the basis of k'. Sample, 2 μl of n-C_8 ($k' = 0.4$) and n-C_{10} ($k' = 11.8$) diluted 10^5:1 in carbon disulfide. Column, 25 m × 0.25 mm I.D. OV-101, isothermal. □, $k' = 0.4$; ○, $k' = 11.8$.

The delay time in activating the injector purge flow, Δt_2, is also an important experimental variable. The purge activation delay time is the time between the start of sample injection and the time at which the sample/solvent residual vapors are purged out of the injector. Purging the injector too soon (*e.g.*, $\Delta t_2 < 5$ sec) vents a very large fraction of the total sample out of the injector such that sample recovery is low and the least volatile components are preferentially sampled on to the column At $\Delta t_2 > 2$–3 min, back-diffusion of the sample in the injector on to the column begins to occur. The result is that the column efficiency is degraded and the solvent peak profile begins to tail appreciably.

Fig. 9 shows that (a) for optimal sample recovery, $\Delta t_2 > 40$ sec is recommended, (b) the sample recovery is independent of k' and (c) the column and system efficiency (N/L plates per meter) is independent of Δt_2. In this injector design, a value of $\Delta t_2 = 40$ sec corresponds to more than six injector purge volumes at a column flow-rate of 1 ml/min. This appears to be the minimum number required for this length to volume ratio, which implies that the mixing mechanism is not one of linear dilution. Fig. 9 illustrates why some workers have reported obtaining different chro-

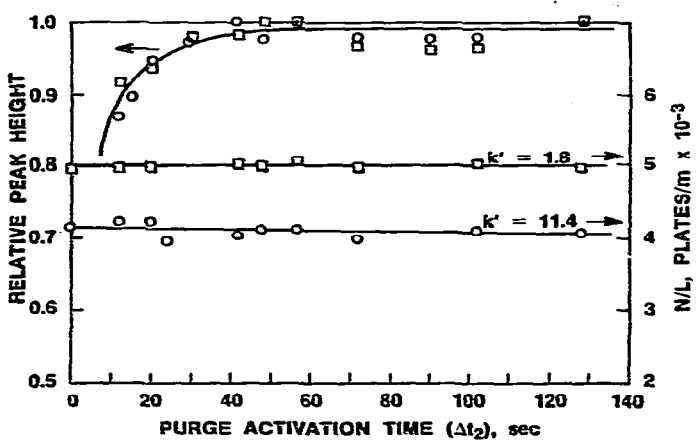


Fig. 9. Effect of purge activation time (Δt_2) on the sample recovery (relative peak height) and separation efficiency (N/L, plates/m). Sample, 2 μl of n-C_8 ($k' = 1.8$) and n-C_{10} ($k' = 11.4$) diluted 10^5:1 in carbon disulfide. Column, 25 m × 0.25 mm I.D. OV-101, isothermal. □, $k' = 1.8$; ○, $k' = 11.4$.

matograms when Δt_2 was varied by only 1–2 sec and one of the reasons why their quantitative reproducibility was poor. Contrary to popular belief, peaks eluting close to the solvent peak are not broader than peaks with a large k' and the column efficiency is not a function of Δt_2. In fact, our real-time computer measurements have shown that the column efficiency is higher for early than late peaks, and that peaks eluting immediately after the solvent show more theoretical plates than would be predicted from theory.

Sample effects

The effects of the volume of sample injected are shown in Fig. 10. Both samples are the same and the GC conditions are identical. The most obvious effect is the

dependence of retention time on the sample volume injected. The retention time shifts, the differences in separation efficiency and the separation number for homologous pairs are summarized in Table I.

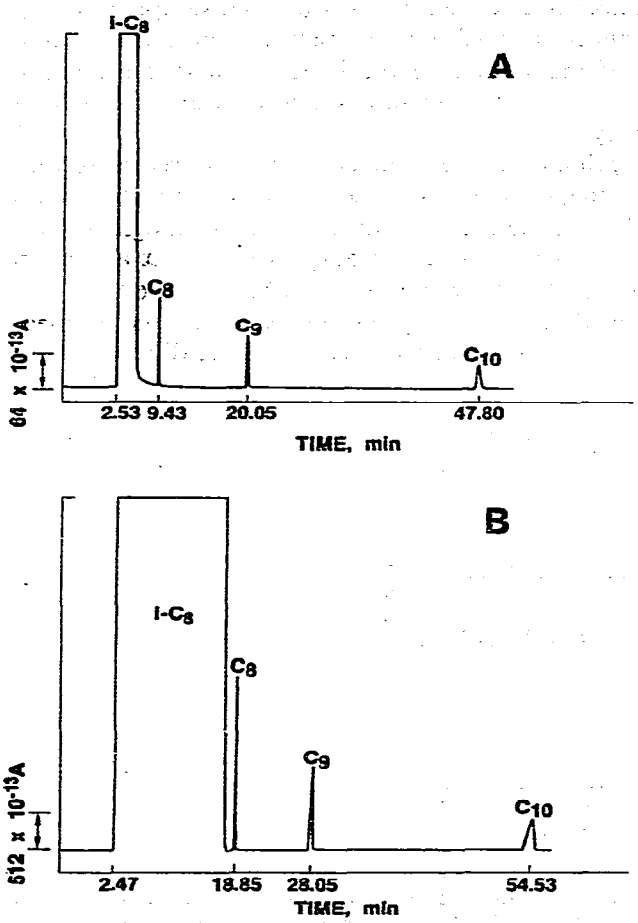


Fig. 10. Effect of the sample size in the splitless mode on the retention time, separation efficiency and resolution. (A) 1.0 μl injection of n-C_8, n-C_9 and n-C_{10} diluted 10^4:1 in isooctane. (B) 9.5-μl injection of the same sample. Column, 25 m × 0.25 mm I.D. OV-101, isothermal at 34°.

Table I shows that: (a) precise reproducibility of sample size is required from run to run if reproducibility of retention time is required, especially for peaks eluting immediately after the solvent peak (change in retention time decreases with k'); (b) because the separation number and the column efficiency depend on the retention time, they will vary as the retention times are shifted; (c) the column efficiency for n-C_8 is abnormally high for the given k' and increases considerably with sample size because the only effect is a retention time shift. The mechanism and causes of these effects are discussed in detail elsewhere[16].

TABLE I

EFFECTS OF SAMPLE VOLUME ON CAPILLARY-COLUMN GC PERFORMANCE WITH SPLITLESS SAMPLING

Sample volume (μl)	*Property*	*Isooctane**	*n-C₈*	*n-C₉*	*n-C₁₀*
1	Retention time (t_R) (min)	2.5	9.43	20.05	47.80
9.5		2.5	18.85	28.05	54.53
	Difference** (%)	—	100	40	15
1	Theoretical plates/m (N/L)	—	4390	2930	2750
9.5		—	14.400	2360	935
	Difference** (%)	—	230	20	—***
1	Separation number (SN)	—	43	45	
9.5		—	25	23	
	Difference** (%)	—	40	50	

* Retention time of the solvent peak represents the elution time for the leading edge of the solvent front.

** Differences calculated relative to the 1.0-μl sample size.

*** Calculation is not meaningful because the large sample injection resulted in a column overloading for n-C_{10}.

The solvent effect is seen to be equally effective for both injections because the n-C_8 peak widths at half-height for the 1.0- and 9.5-μl injections are 4.02 and 4.45 sec, respectively. One of the column efficiencies reported in Table I (*e.g.*, 14,400 plates/m) clearly exceeds the theoretical limits calculated from the Van Deemter equation. Measured values in Tables I and II exceeding 5000 plates/meter should be considered as "anomalous values" of the total number of theoretical plates because it is seen that the peak width is approximately independent of the sample size because of the peak sharpening by the solvent effect. The shift in retention time of 100% for the n-C_8 peak therefore gives abnormally high values for N/L owing to the fundamental behavior of the solvent effect. All of the measurements and calculations reported here were made on the real-time minicomputer-based data system. Therefore, peak widths reported to 0.01 sec and column efficiencies calculated to three significant figures are consistent with the accuracy of the computer system and the algorithms used.

Although it is seen that excellent chromatographic results and solvent peak profiles are obtained with sample injections of up to 10 μl, the limitation of such large injections may be overloading of the column. This is indicated by the triangular n-C_{10} peak for the 9.5-μl injection in Fig. 10. Obviously, smaller sample sizes than 9.5 μl could have been injected, or more dilute solutions could have been used because the signal-to-noise ratio for n-C_{10} in the latter chromatogram was >2000:1. Smaller sample sizes for splitless injections have the advantage of minimizing liquid phase stripping in the column and, therefore, increasing the column lifetime.

Fig. 11 illustrates the effect of the type of solvent used and the relationship of its boiling point to the initial column oven temperature. The results are summarized in Table II.

Although the difference in the number of carbon atoms in the two solvents is only two, the difference in boiling points is 62.3°. Therefore, the n-C_5 separation

was run only 4° below its boiling point. It is evident that a good solvent effect was not obtained from the fact that the solvent peak shape is poor and shows some tailing and the column efficiency is not comparable to the n-C_7 separation even though the same column, flow-rates, temperatures and sample sizes were used for both chromatograms. The n-C_7 separation was run 66° below the boiling point of the solvent and the improvement is clearly evident.

It should be noted that the higher boiling solvent increased the retention times because of the large difference between the boiling point of the solvent and the column oven temperature. Again, the difference in column efficiencies can be accounted for by the fact that the peak widths of the n-C_8 peak for the n-C_5 and n-C_7 solvents were about the same (4.84 and 4.08 sec, respectively), whereas the retention times changed by a factor of 1.7, resulting in an anomalous number of theoretical plates.

In order to minimize the analysis time and to obtain good solvent effects, it is recommended that the initial column temperature be at least 15°, 30–40° if possible,

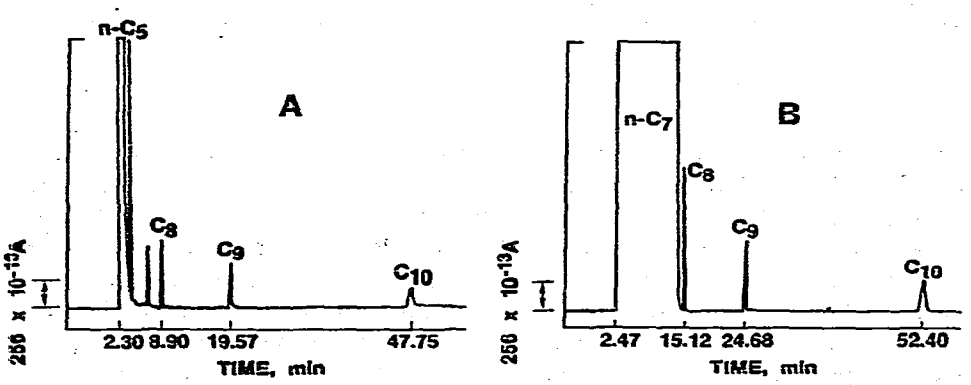


Fig. 11. Effect of the solvent type and column temperature in splitless sampling on the retention time, separation efficiency and resolution. (A) 6.0-μl injection of n-C_8, n-C_9 and n-C_{10} diluted 10^5:1 in n-C_5; (B) 6.0-μl injection of the same sample on the same column (Fig. 10) also at 32°.

TABLE II

EFFECTS OF SOLVENT TYPE ON CAPILLARY-COLUMN GC PERFORMANCE WITH SPLITLESS SAMPLING

Solvent	*Property*	*Isooctane**	*n-C₈*	*n-C₉*	*n-C₁₀*
n-C_5	Retention time (t_R) (min)	2.3	8.9	19.57	47.75
n-C_7		2.5	15.12	24.68	52.40
	Difference** (%)	10	70	25	10
n-C_5	Theoretical plates/m (N/L)	—	2700	1900	1450
n-C_7		—	10,900	2600	1350
	Difference* (%)	—	300	35	5
n-C_5	Separation number (SN)	—	36	34	
n-C_7		—	31	30	
	Difference** (%)	—	15	15	

* Retention time of the solvent peak represents the elution time for the leading edge of the solvent front.

** Differences calculated relative to the n-C_5 solvent.

below the boiling point of the solvent. Thus, if the initial column temperature for the n-C_7 separation had been 70–80°, the solvent peak width would have been decreased, the solute retention times would have been decreased and a good solvent effect and column efficiency would have been achieved. Therefore, control and resettability of the initial column oven temperature is essential, especially for low-boiling solvents.

The shift in the retention time of n-C_{10} is shown to correlate well with the boiling point of the solvent in Fig. 12. A number of different solvent types on a

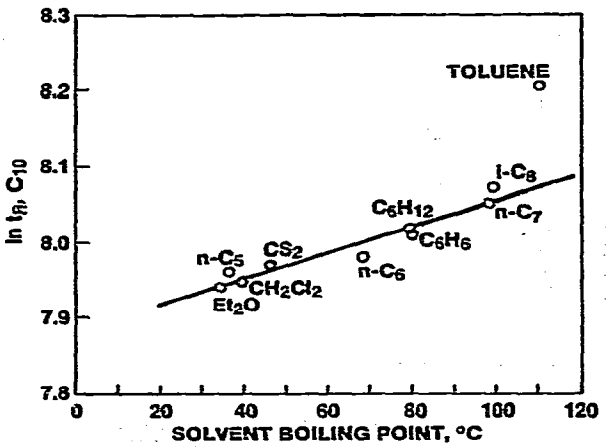


Fig. 12. Relationship between the retention time shift for splitless sampling and the boiling point of the solvent. Test solute, n-C_{10}; column, OV-101 at 30°.

non-polar column (OV-101) at 30° covering a boiling point range of 75° were found to follow the expression

$$\ln t_R = 1.67 \cdot 10^{-3} T_b + 7.89 \quad (1)$$

where T_b is the boiling point of the solvent. Eqn. 1 will give the same slope for all low-boiling solvents on non-polar columns. However, the intercept will be a function of the initial or isothermal column oven temperature. It is interesting that all of the solvents show an excellent fit to eqn. 1 regardless of the types of functional groups, aromatic character, etc., with the exception of toluene. Toluene does not follow eqn. 1 because it has a large dipole moment *and* a high boiling point relative to the column oven temperature, *i.e.*, the dipole moment is effective in inducing very large retention time shifts, whereas the low-boiling solvents are so volatile that there is no dipole moment effect*. The solvent boiling point effect shown in Fig. 12 shifts the retention time of n-C_{10} by >6 min by changing only from diethyl ether (boiling point 34.6°) to isooctane (boiling point 99.3°) when all other conditions are held constant. The

* A detailed explanation of the dipole moment effect will be given in a future publication[16].

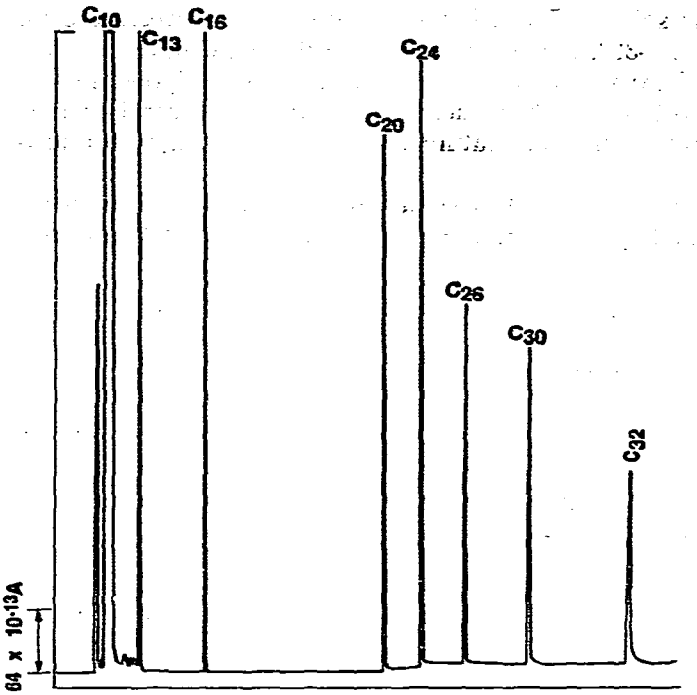


Fig. 13. Splitless capillary separation of n-C_{13}–n-C_{32} hydrocarbons in n-C_{10}. Sample size, 2 μl; Δt_1 = 3 sec; Δt_2 = 42 sec; column, 30 m × 0.25 mm I.D.; temperature program, from 120° to 280° at 3°/min with a final hold of 43 min.

relative retention time shift of solutes with a smaller k' than that of n-C_{10} will be even larger.

Precision and automation

The data presented here show the necessity of having high-precision instrumentation for capillary-column GC for control, regulation and resettability. Using the commercially available instrumentation previously described, a sample of n-C_{13}–n-C_{32} was used to test the reproducibility of the system in the temperature-programmed mode. A typical chromatogram is shown in Fig. 13 and the results of eleven consecutive determinations are given in Table III.

From Table III it can be seen that by complete automation of a splitless capillary system, that reproducibility of the retention time is nominally of the order of 0.05% (or 900 msec in 30 min) and peak areas are reproducible to about 1%. The precision of the peak area is therefore basically limited by the reproducibility of the AutoSampler for injections of this sample size.

The dramatic effects of the sample size and solvent type on retention time can introduce errors in the most sophisticated computerized peak identification and reporting techniques. The CDS-111 uses two time reference peaks to compensate for the non-linear time shifts that occur with variations in sample size in splitless sampling.

TABLE III

REPRODUCIBILITY OF SPLITLESS SAMPLING ELEVEN CONSECUTIVE DETERMINATIONS

Peak	t_R *(min)*	*Relative standard deviation of* t_R *(%)*	*Normalized peak area, A (%)*	*Relative standard deviation of A (%)*
n-C_{13}	9.7	0.10	15.35	0.99
n-C_{16}	19.95	0.03	18.26	0.57
n-C_{20}	47.98	0.02	10.65	1.20
n-C_{24}	53.89	0.02	12.38	0.89
n-C_{26}	60.66	0.03	10.52	1.05
n-C_{30}	70.64	0.05	15.97	0.76
n-C_{32}	86.19	0.06	16.88	1.23
Mean		0.04		0.95

However, the computer can also handle the large changes in retention times that may occur when a different sample size or solvent is used in other sample preparation or analysis procedures. A separate computer method is called up from memory (manually or automatically) when the analysis changes. Each method is tailored for specific retention times, which allows the computer to identify peaks reliably.

Simplex optimization

Because of the number of experimental variables involved in splitless sampling for capillary-column GC and the general confusion regarding the effects or optimization of these variables, the results of a simplex optimization study are reported here for the purposes of simplifying the procedure and making general recommendations for the application of the technique. Simplex is a sequential experimental technique for optimizing the performance of a system, such as in GC. It produces a new list of parameter settings that will shift the operation of the system towards a better performance, based upon the previous experimental results. As it is an empirical technique, the system under study does not have to be mathematically modeled in order to be optimized.

Simplex can simultaneously adjust all parameter settings after each experiment, which reduces the total number of experiments necessary for achieving optimal performance. There is also a reduction in the number of experiments required in comparison with classical experiment design (evaluation of five parameters at four points requires 1024 experiments). Simplex and its application have been described elsewhere[17–20]. The variable-sized simplex of Nelder and Mead[21] was used because of its ability to cover the entire range of each parameter in the initial series of experiments, thereby improving the chance of settling on the "global" or true optimum and not a local maximum in the response.

The range of values studied for the five most important splitless parameters is shown in Table IV, together with the optimized values.

Four criteria were used to evaluate the quality of the separation from the splitless injection for purposes of optimization: (a) the ability to separate a peak from the back side of the solvent peak (percentage valley height); (b) the fraction of the sample injected that was actually transferred to the column (sample recovery);

TABLE IV

SIMPLEX OPTIMIZATION OF SPLITLESS SAMPLING

Conditions: Column temperature, 32°; solvent, isooctane (boiling point 99.3°).

Parameter	*Initial range of values*	*Optimized values*
T_{inj} (°C)	180–300	220
Δt_1 (sec)	5–10	7
Δt_2 (sec)	20–50	30
F_C (cm³/min)	0.5–1.0	0.6
Sample size (μl)	2–10	6

(c) the discrimination effects of the sampling procedure on sample composition (linearity); and (d) the relative efficiency of the column $(N/L)_{Rel}$. These four parameters are shown in the chromatogram in Fig. 14, and the equations are as follows:

$$\text{Valley height (\%)} = 500 \exp\left(\frac{100a}{-7.2b}\right) \tag{2}$$

where a is the valley height above the baseline and b is the height of the solute peak. The value of −7.2 produces 50% of the maximum valley height score at a 5% valley height.

$$\text{Sample recovery} = 100\left[\frac{(A_{C_{10}})_{exp}/\mu l}{(A_{C_{10}})_{max.}/\mu l}\right] \tag{3}$$

where A is the normalized peak area. The maximum n-C_{10} area/μl normalizes the results to the largest n-C_{10} area/μl value observed.

$$\text{Linearity} = 300\left(1 - \left|\frac{R_T - R_E}{R_T}\right|\right) \tag{4}$$

where R_T is the true C_{10}/C_9 area ratio and R_E is the experimentally measured area ratio.

$$N/L_{Rel} = 100\left[\frac{(N/L)_{C_{10},\,exp}}{(N/L)_{C_{10},\,max.}}\right] \tag{5}$$

where the $(N/L)_{C_{10},max}$ normalizes the results to the largest $(N/L)_{C_{10}}$ observed in the experimental set.

Note that the four criteria were weighted in the ratio of 5:3:1:1. It seemed reasonable in this instance to assume that one instrumental parameter may affect more than one of the analytical criteria and that two or more instrumental parameters could interact with each other. For example, the injector temperature can affect the sample recovery (back-flash at high temperatures), linearity (loss of higher molecular weight compounds at low temperatures) and sample size (back-flash at high sample

sizes and injector temperatures). In these instances, only multi-dimensional optimization techniques are feasible as one-parameter variant studies often result in the location of pseudo-optima.

It was desirable to optimize the instrumental parameters for a specific sample according to the four criteria mentioned. This would allow the conclusions from the individual parameter studies to be tested in a real application. The sample (n-C_8, n-C_9 and n-C_{10} in isooctane at a 10^5:1 dilution) and column temperature (32°) were held constant. Six initial experiments were required, after which simplex computed the next experimental conditions based on the previous results. Each experimental chromatogram was evaluated by computer to determine N/L, peak areas, peak heights and valley heights. The four criteria were calculated and the overall rating of each experiment was the sum of the four weighted criteria.

The optimization was stopped after 44 experiments, when the numerical ratings of the experiments became virtually constant and the simplex had undergone repeated contractions, indicating the optimum was probably within the area bounded by the simplex. The resulting chromatogram is shown in Fig. 14. The optimized values agree with the general guidelines developed earlier. The injector temperature was moderate (22°). The sampling and purge activation times were smaller than those determined with carbon disulfide as solvent, but are reasonable and would probably be in the vicinity of the "knee" in the peak height *versus* Δt_1 and Δt_2 curves for the solvent used. The column flow-rate settled near the optimal linear velocity of the carrier gas (helium, 20 cm/sec). The sample size was found to be larger (6 μl) than normally used. This is thought to be due primarily to a faster increase in the n-C_8 peak height than valley height with sample sizes up to 6 μl. Selection of different criteria (*e.g.*, column resolution or length of analysis) would result in different optimal values. particularly the column flow-rate, sample size and column temperature.

CONCLUSIONS

This study has shown the effects of the operating parameters on splitless sampling in capillary-column GC and that high-precision results with automated systems can be routinely obtained. The following general guidelines result from these studies and should be implemented for the successful practice of splitless sampling:

(1) Sample sizes between 0.1 and 10 μl may be injected.

(2) Rate of sample injection $\approx$ 1 μl/sec.

(3) Sampling time, $\Delta t_1 \geqslant 20$ sec.

(4) Injector purge delay time, $\Delta t_2 \geqslant 40$ sec.

(5) Effective dynamic range of sample dilution is 10^5:1.

(6) $T_{INJ} \leqslant 230°$, nominally for solvents with boiling points $<100°$.

(7) Use low-boiling solvents such that the solvent boiling point is 25° below that of the first peak of interest. Solutes with a smaller boiling-point difference will co-elute with the solvent.

(8) Initial column oven temperature should be 15–30° below the boiling point of the solvent.

(9) Solutes that elute ahead of the solvent will not be separated efficiently or quantitatively unless there is a large $\Delta k'$ between the solutes of interest and the solvent peak.

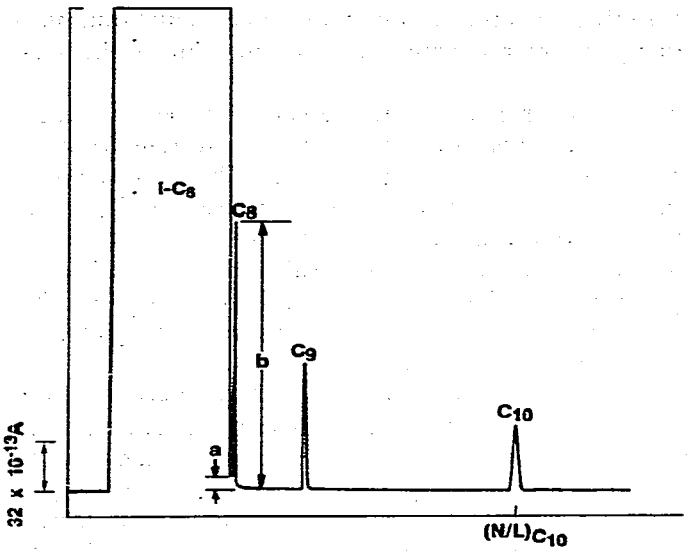


Fig. 14. Optimized splitless sampling capillary chromatogram, showing the criteria used for the simplex optimization. The valley height was calculated from the peak height (*b*) and valley height (*a*). Linearity was calculated for the *n*-C_{10}–*n*-C_9 pair, and separation efficiency from the *n*-C_{10} peak. Dilution, 10^6:1; $\Delta t_1 = 7$ sec; $\Delta t_2 = 30$ sec; sample size, 6 μl; carrier gas flow-rate, 0.6 cm³/min; column, 18 m × 0.25 mm I.D. OV-101.

It has been shown in this work that the limitations of splitless sampling for capillary-column GC cited elsewhere[5] are not of general concern. Specifically, (a) the mass transfer of the vaporized sample from the injector to the column is a slow process, but does not cause peak broadening when proper experimental procedures are followed. In fact, the peak sharpening of the solvent effect results in very high separation efficiencies for peaks with low k' and the same efficiencies for peaks with large k', as in other capillary-column GC sampling modes. (b) The solvent effect does shift the retention times significantly but not the retention order. Therefore, the polarity of the stationary phase is not changed for peaks that are well resolved from the solvent peak. (c) There is no high-boiling sample discrimination in the splitless mode if Δt_2 is roughly optimized for the solvent used, and all of the "ground rules" for splitless sampling are followed (see above). (d) Long residence times in the injector are not necessarily detrimental for most samples analyzed by capillary-column GC because the volume-to-surface area ratio is large, lower injector temperatures are used than for splitters and the solvent-to-solute concentration ratio is very large so that surface effects are blocked by the solvent molecules. Therefore, we believe that this work has shown that splitless sampling is an effective technique for use in capillary-column GC.

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