Application of High Speed Gas Chromatographic Techniques to Flavor Systems

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Introduction

Flavor systems, in general, are complex mixtures of organic compounds. Since many of the components are volatile, they often can be analyzed using gas chromatography (GC). Flavor systems are evaluated by GC for a number of reasons. Flavor extracts might be tested for quality control purposes, or to determine if a new material matches a desired flavor profile; or, flavor systems can be "reverse engineered" to estimate the volatile composition. With a wide range of compound types, boiling points and concentrations, the analysis times can be quite long. Additionally, complete resolution of all components might require two different stationary phases. For routine analyses, an ideal separation would involve a single, rapid run with complete resolution of all target components. Two approaches for achieving high speed GC separations will be discussed.

One approach to reduced analysis times utilizes fast oven temperature programs, particularly useful if a wide range of boiling points are represented. To ensure fast, reproducible temperature gradients an auxiliary heating unit can be introduced into the GC oven. For complex systems that require a dual column analysis for complete characterization, two-dimensional GC techniques can significantly reduce analysis times. Flow-modified selectivity tuning using two dissimilar stationary phases in series will be discussed. In this technique, the columns are joined at a junction point connected to a source of carrier gas. The flow through the first column can be stopped for short periods of time, enhancing the separation of target compounds.

Fast GC Using Rapid Temperature Programs

Fast temperature programs can be used in GC to speed up the elution of high boiling point compounds and late eluters. This is especially useful if compounds with widely varying boiling points are present in the sample. While many GCs can be temperature programmed at rates 70°C/min. or higher, at higher temperatures these programs can be difficult to maintain. The temperature profile also tends to become less reproducible as the temperature and the programmed ramp rate increases.

Auxiliary heaters, such as the GC Racer temperature programmer, can be used to maintain the desired temperature gradient. The GC Racer consists of a resistive heating element that is placed on the floor of the GC oven, and a controller that connects with the main PC board of the GC. In Figures 1 and 2, the actual temperature in the oven of an Agilent 6890 GC, with and without the GC Racer, is shown at two different temperature programs. In Figure 3, a high-speed GC separation of volatile compounds is shown. This was done with and without the GC Racer, using the instrument conditions given in Table I.

Figure 1. Actual temperature vs. time for an Agilent 6890 GC, using a temperature program of 20°C/min.

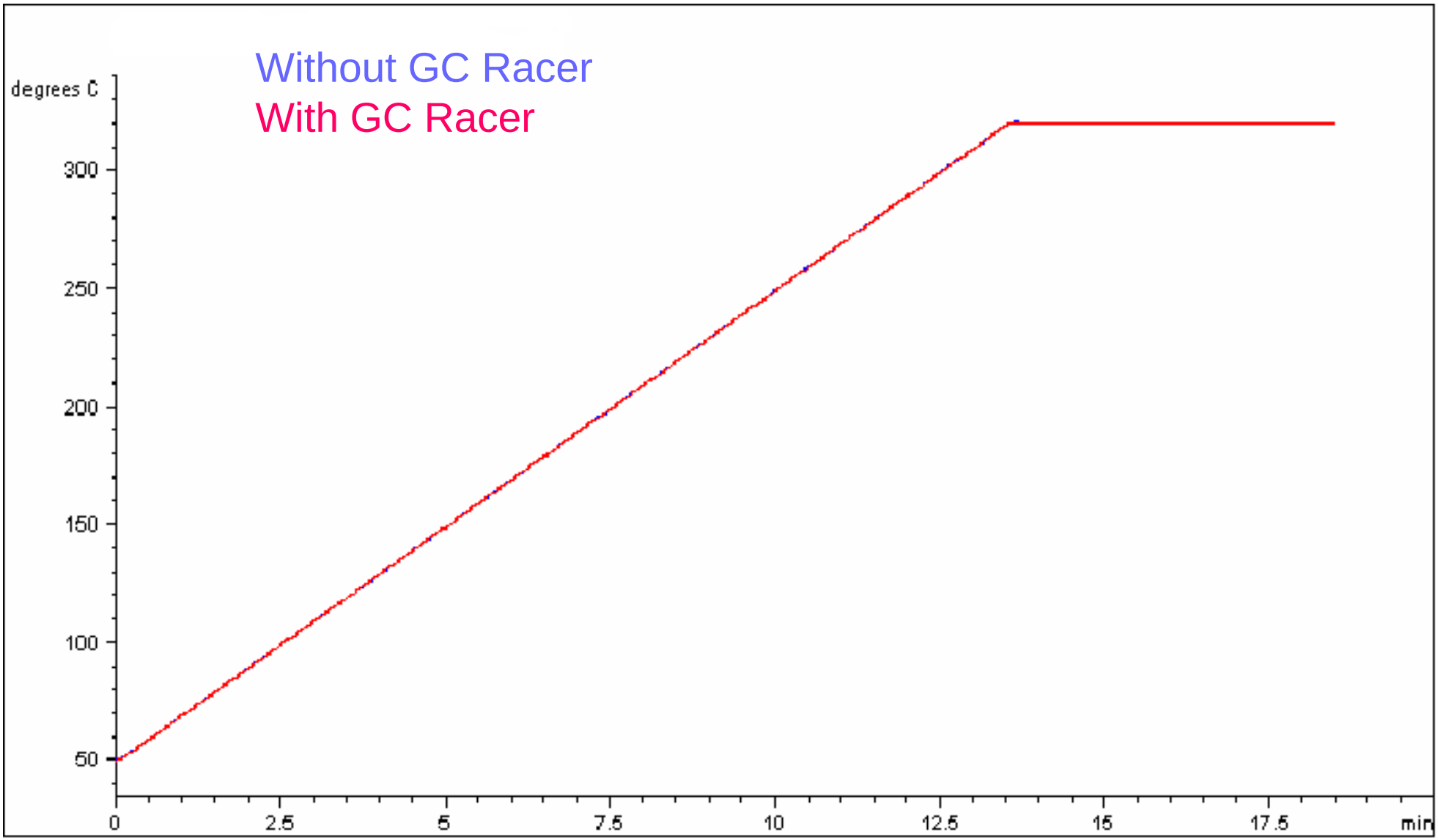


Figure 2. Actual temperature vs. time for an Agilent 6890 GC, using a temperature program of 60°C/min.

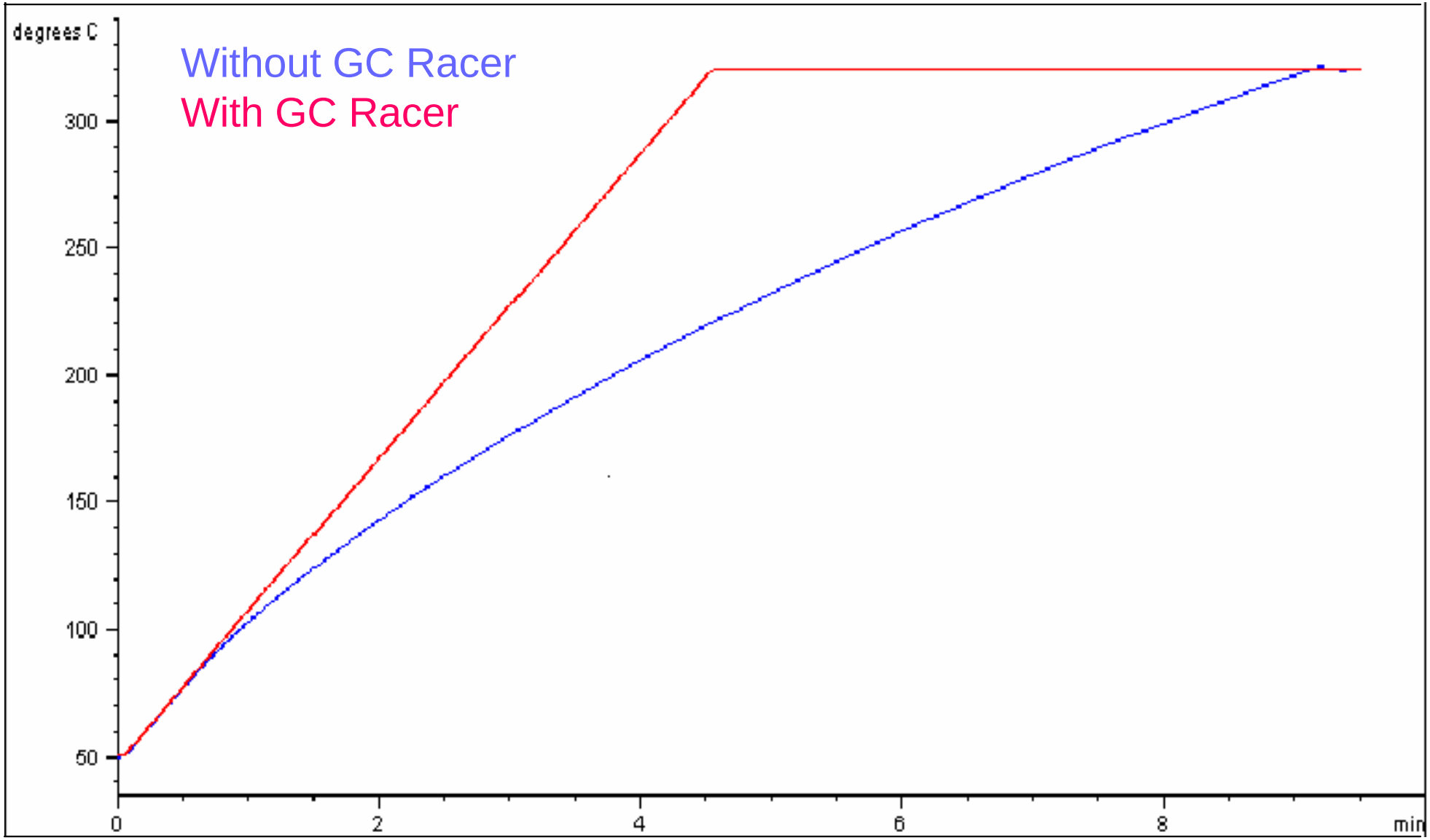


Figure 3. Separation of volatile compounds, with and without the GC Racer system.

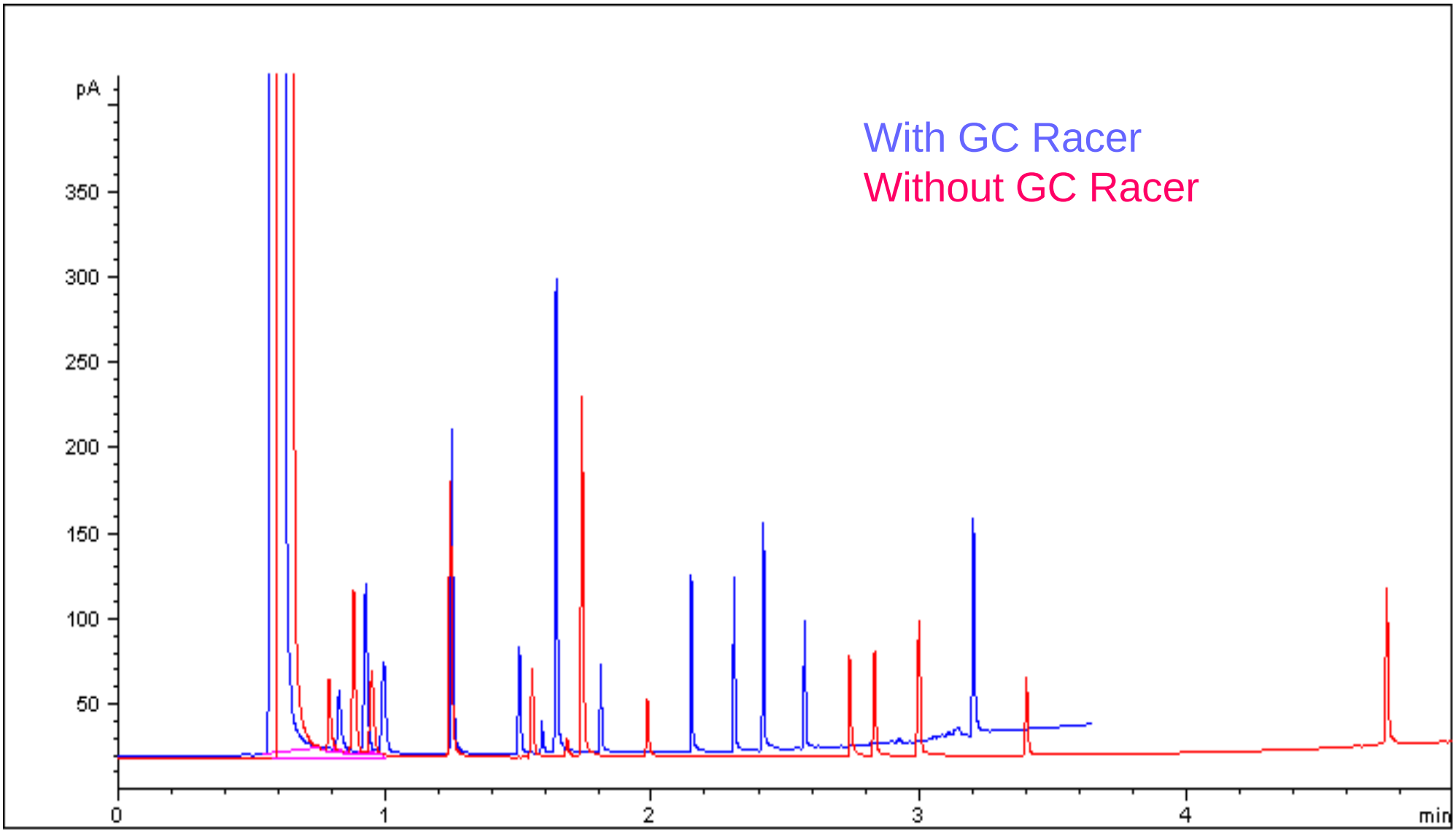


Table I. GC program for the volatile compounds analyzed in Figure 3.

Columns:	Rtx®-VMS, 10m x 0.18mm x 1.0µm
	Stabilwax®-DA, 10m x 0.18mm x 0.18µm
Injector:	250°C, 1 µL split injection (150:1)
Carrier Gas:	hydrogen at 25 psi, constant pressure
Oven:	35°C (0.5 min. hold)
	to 45°C at 20°C/min.
	to 210°C at 100°C/min. (1-4 min. hold)
Detector:	FID @ 250°C
	Air @ 400cc/min.
	H2 @ 40cc/min.
	N2 @ 40cc/min.

Fast GC Using Pressure Tunable Selectivity

A powerful technique for separating complex mixtures, developed by Sacks, et. al.[1,2] at the University of Michigan, uses pressure-tunable selectivity and a series-coupled column ensemble. In this technique (Stop-Flow GC), two standard-dimension capillary columns with dissimilar stationary phases are connected in series, using a 4-way junction. At this junction point, a source of carrier gas also is connected, with the pressure controlled by an external Electronic Pressure Control (EPC) unit. An external valve controls the flow of the carrier gas to the junction point. A detector is connected to the fourth port of the junction, and monitors the components as they elute from the first column.

When an injection is made, the sample components move through the first and second columns. There are 4 possible scenarios for closely-eluting compounds A and B:

(1) A and B are resolved after passing through both columns
(2) A and B are not resolved after the first column, but are resolved after the second column
(3) A and B are resolved after the first column, but not after the second column
(4) A and B are not resolved after either column

In the first two scenarios, the separation can be allowed to proceed normally. In the 4th scenario, different column chemistries should be selected. Stop-Flow GC can be used to significantly improve the resolution described in the 3rd scenario.

For compounds that resolve on the first column, but coelute at the end of the second column, a stop-flow pulse can be introduced between the two compounds as they elute from the first column. This is done by opening the valve to the external gas source after the first compound has passed the junction point. The first compound continues on through the second column, while the second compound is stopped (or moved slightly backward) on the first column. In this way, the separation between the two components can be increased, as demonstrated in Figure 5. In Figure 5a, no stop-flow pulses have been applied, resulting in the coelution of A and B, as well as C and D. In Figure 5b, one pulse at 28 seconds has been applied, effectively resolving A and B at the outlet of the second column. In Figure 5c, both sets of coeluting compounds have been resolved by using stop-flow pulses at 28 and 43 seconds.

In Figures 6 and 7, the stop-flow technique was applied to a mixture of volatile organic compounds. In particular, pyridine, p-xylene, and m-xylene were difficult to resolve on the selected stationary phases. Using a series of 3 stop-flow pulses, the resolution of these compounds can be greatly improved. The timing and duration of the stop-flow pulses, as well as the resulting chromatogram, are shown in Figure 7.

Figure 4. Diagram of the Stop-Flow GC system, originally described by Sacks, et. al.[1,2]

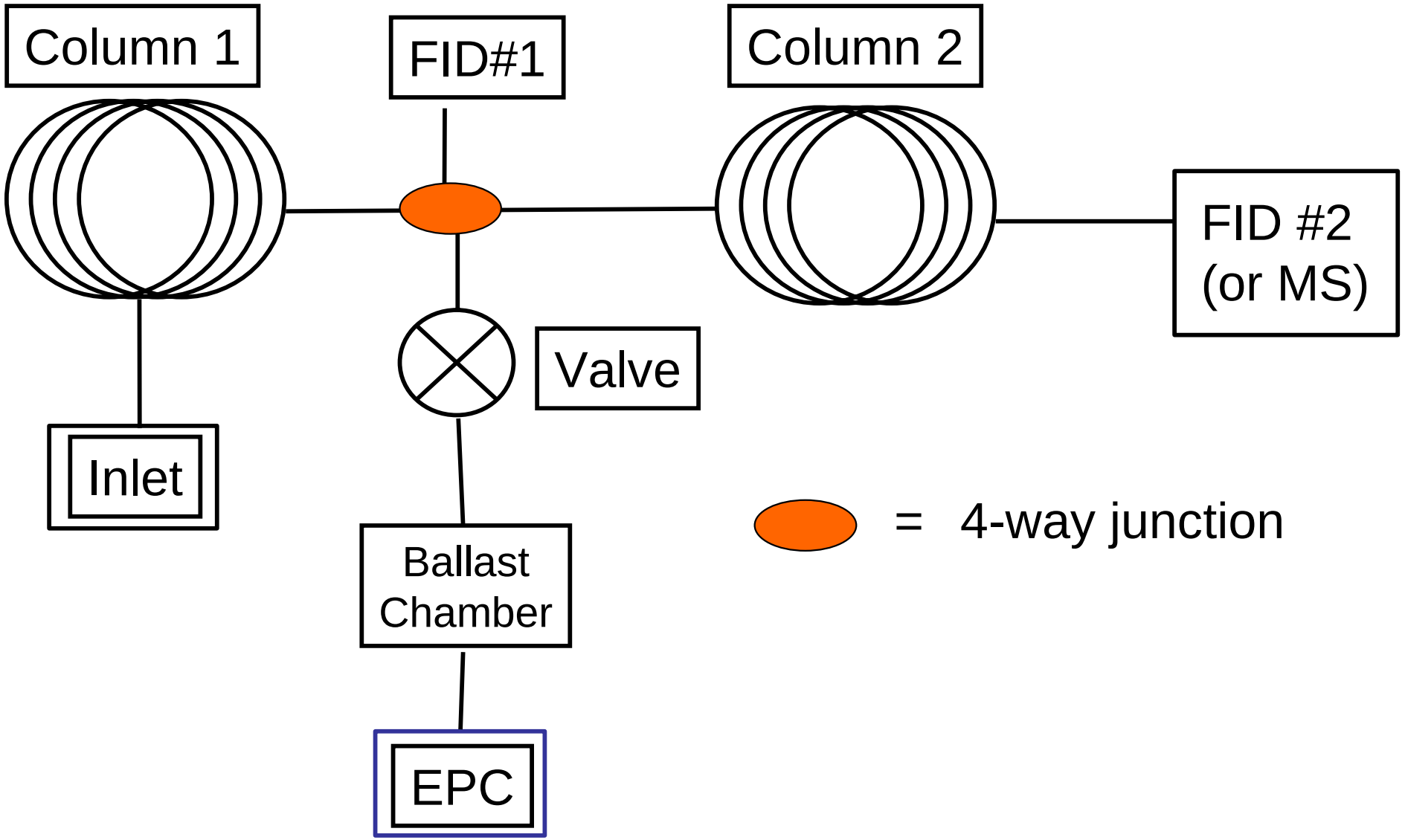


Figure 5. Stop-Flow GC for optimizing the separation of coeluting compounds: (a) no stop-flow pulses; (b) pulse at 28s; (c) pulses at 28s & 43s.

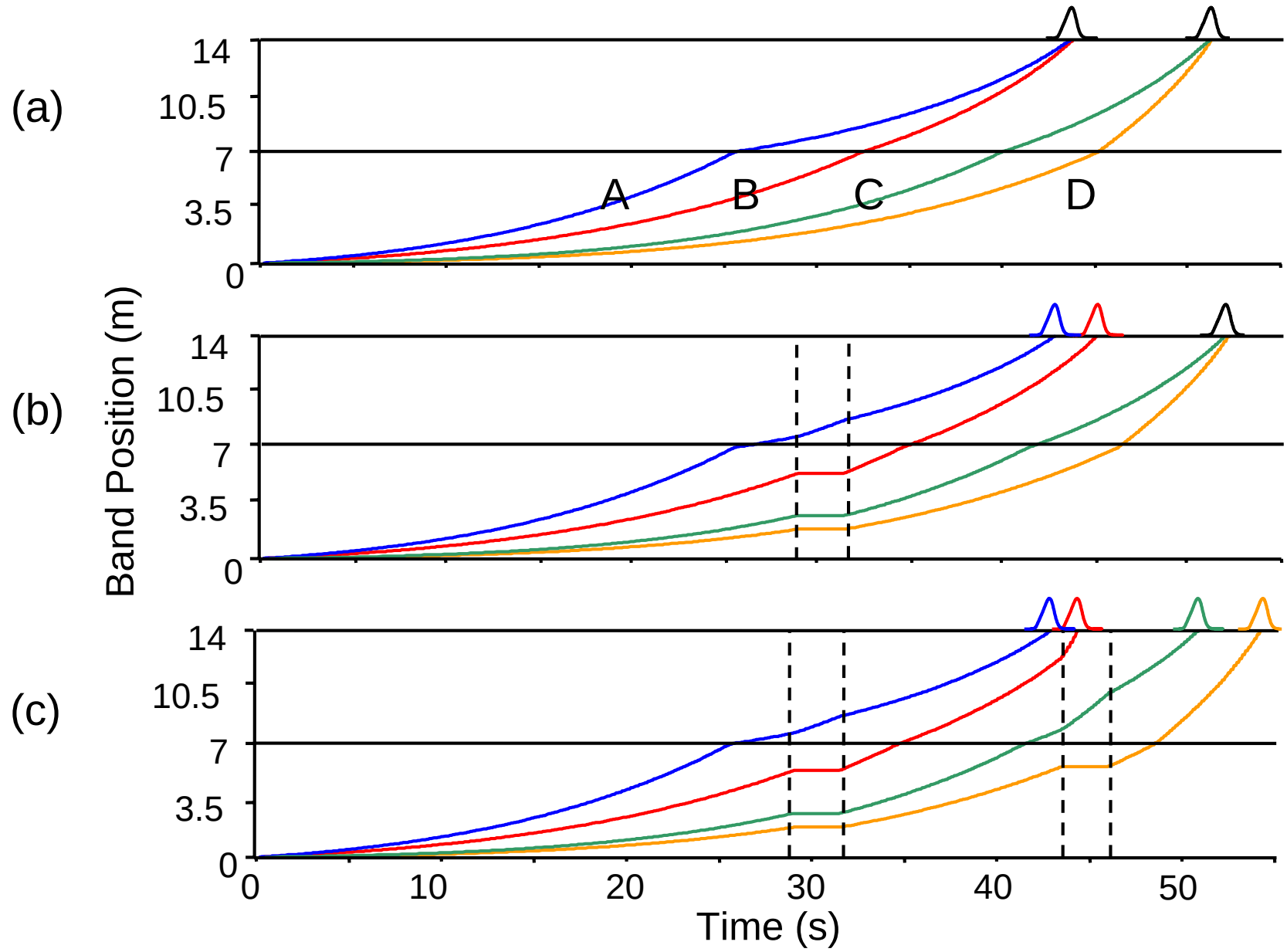


Figure courtesy of R. Sacks, University of Michigan

Figure 6. Volatile organic compounds, no stop-flow pulses.

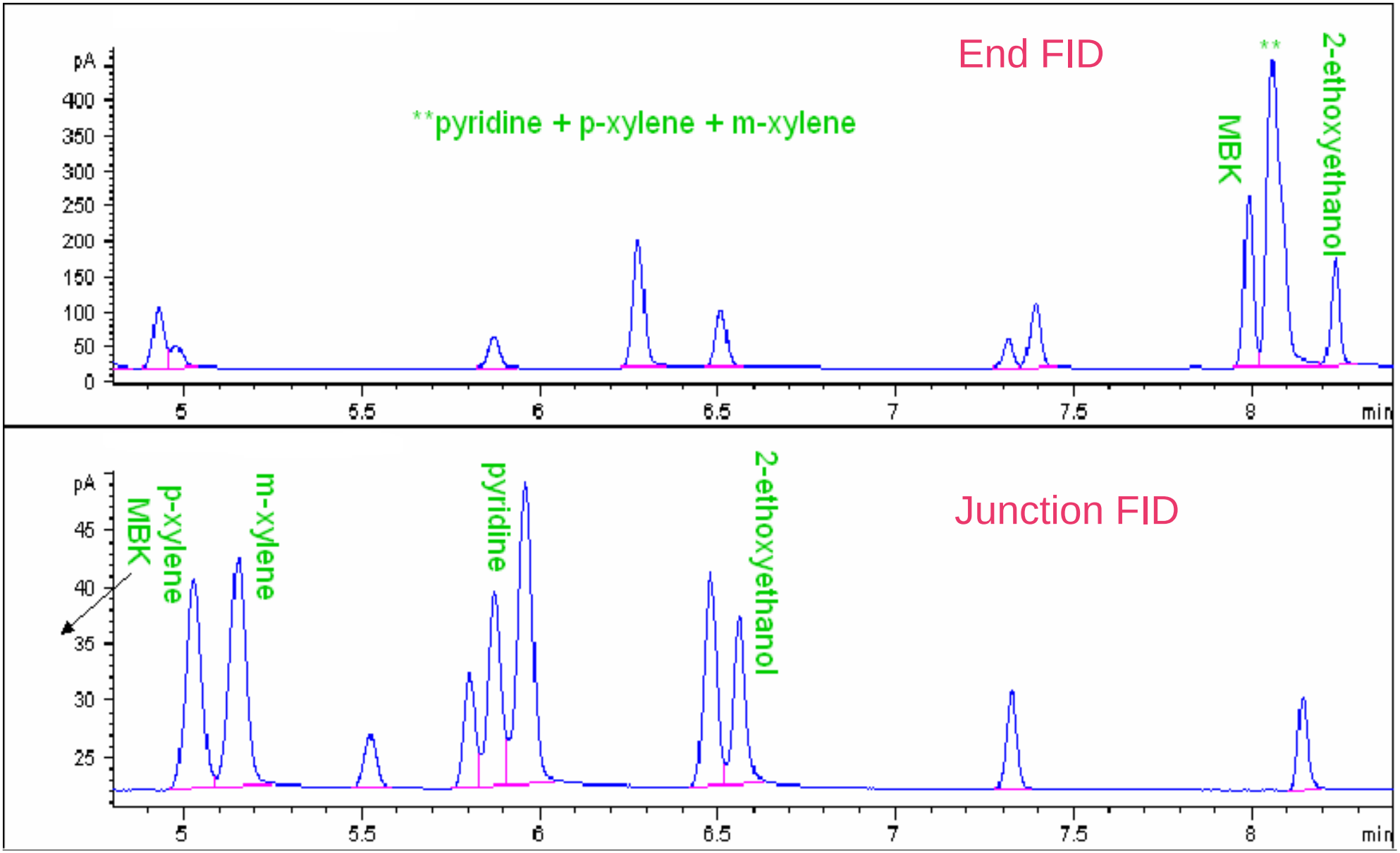


Figure 7. Volatile organic compounds, stop-flow pulses at 290, 330, & 346 sec.

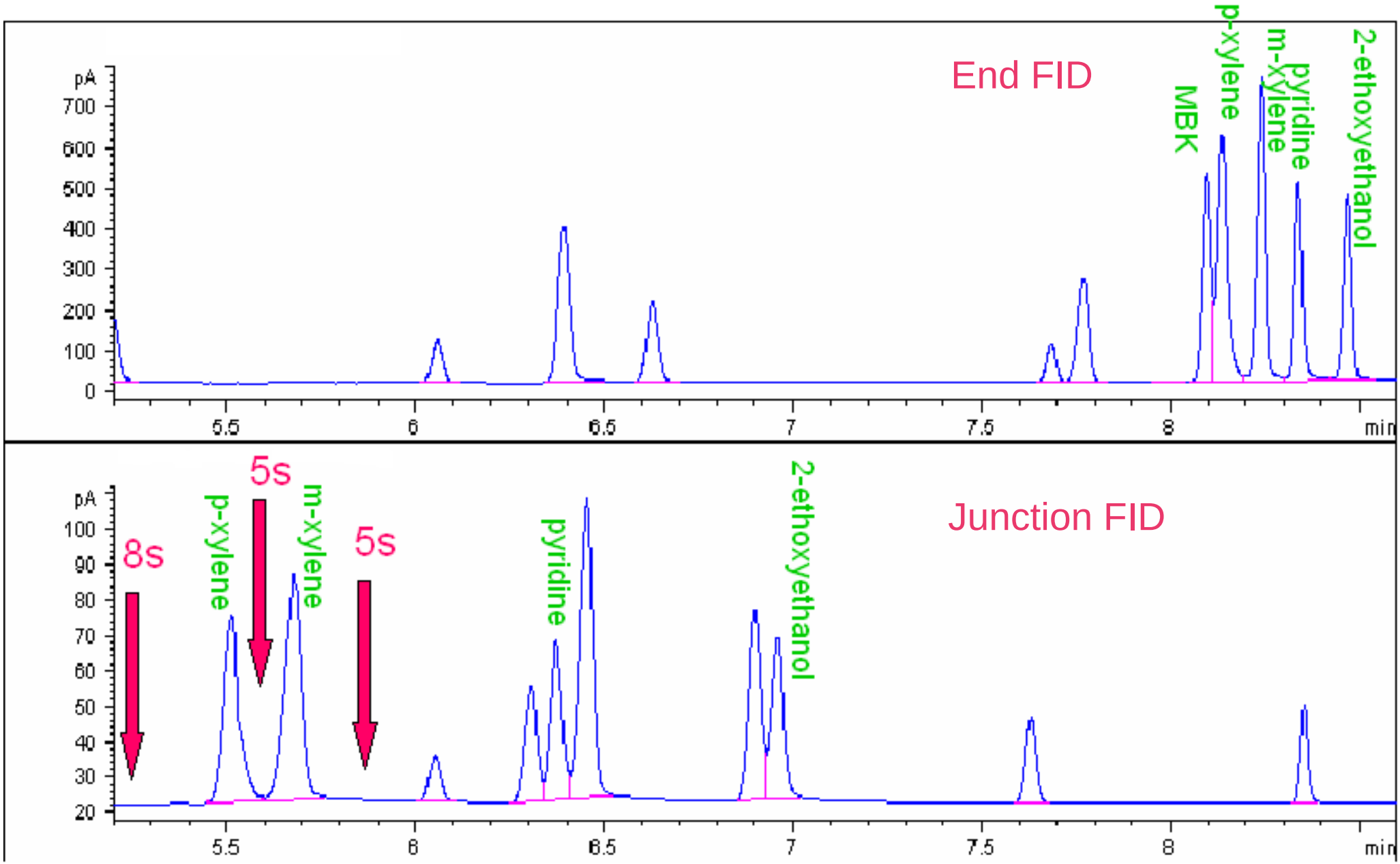


Table II. GC operating conditions for the volatile organic compound analysis in Figures 6-7.

Columns:	Rtx®-Stabilwax, 15m x 0.25mm x 0.5µm
	Rtx®-200, 30m x 0.25mm x 1.0mm
Injector:	230°C, 200:1 split
	0.2 µL injection
Carrier Gas:	hydrogen at 2.5 mL/min. (constant flow), 0-9.5 min.
	to 3.5 mL/min. at 10 min.
Oven:	40°C (1 min. hold)
	to 65°C at 6°C/min.
	to 100°C at 12°C/min.
	to 250°C at 70°C/min. (1.8 min. hold)
Detectors:	Dual FIDs @ 250°C
	Air @ 400cc/min.
	H2 @ 40cc/min.
	N2 @ 40cc/min.
Junction Pressure:	74 psig (59 psi headpressure)

Stop-Flow GC of an Essential Oil Standard

Essential oil samples are complex mixtures of organic compounds. A wide range of concentrations and boiling points typically are represented. The Fragrance Material Association (FMA) has proposed a test mix representative of the variety of compounds found in essential oil samples (see Table III). This mix was used to test the applicability of the stop-flow technique to this type of sample.

In Figure 8, the dual column analysis of the FMA test mix is shown, with no stop-flow pulses applied. The first column contains a polar polyethylene glycol stationary phase; the second column contains a non-polar 100% dimethylpolysiloxane stationary phase. GC conditions have been optimized to reduce the run time to approximately 10 minutes. At the end of the first column, thymol and cinnamyl acetate coelute; these compounds resolve at the end of the second column. Limonene and eucalyptol are adequately resolved on the first column; however, these compounds are not well resolved at the end of the second column. A 6 second stop-flow pulse at 187 seconds gives significantly better resolution of limonene and eucalyptol, as shown in Figure 9. In Figure 10, the effect of the length of the stop-flow pulse is shown.

Table II. GC operating conditions for the essential oil compounds analyzed in Figures 8-9.

Columns:	Rtx®-Stabilwax, 15m x 0.25mm x 0.5µm
	Rtx®-1, 30m x 0.25mm x 0.25µm
Injector:	230°C, 200:1 split
	0.2 µL injection
Carrier Gas:	hydrogen at 2.5 mL/min. (constant flow), 0-7 min.
	to 4.0 mL/min. at 7.4 min.
Oven:	40°C to 120°C at 20°C/min.
	to 250°C at 100°C/min. (4 min. hold)
Detectors:	Dual FIDs @ 250°C
	Air @ 400cc/min.
	H2 @ 40cc/min.
	N2 @ 40cc/min.
Junction Pressure:	87 psig (72 psi headpressure)

Figure 8. Analysis of an essential oil standard, using a dual column ensemble and no stop-flow pulses.

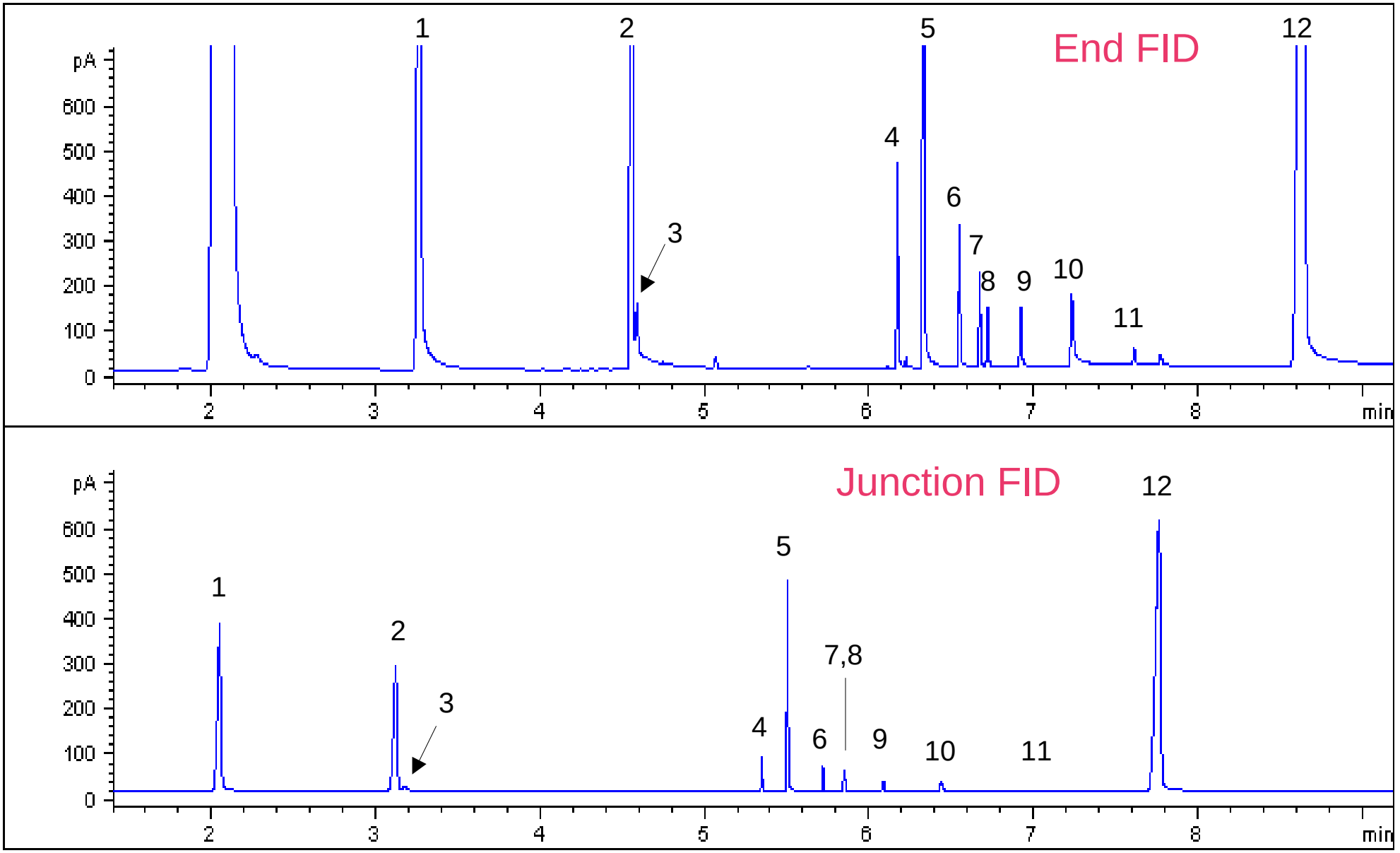


Table III. Compound list for the essential oil standard.

Compound #	*Compound Name*
1	Ethyl butyrate
2	Limonene
3	Eucalyptol
4	Geraniol
5	Hydroxycitronellal
6	Cinnamic aldehyde
7	Thymol
8	Cinnamyl acetate
9	Cinnamyl alcohol
10	Benzoic acid
11	Vanillin
12	Benzyl salicylate

Figure 9. Essential oil standard, with a 6 second pulse at 187 seconds. Note the improved resolution between limonene and eucalyptol.

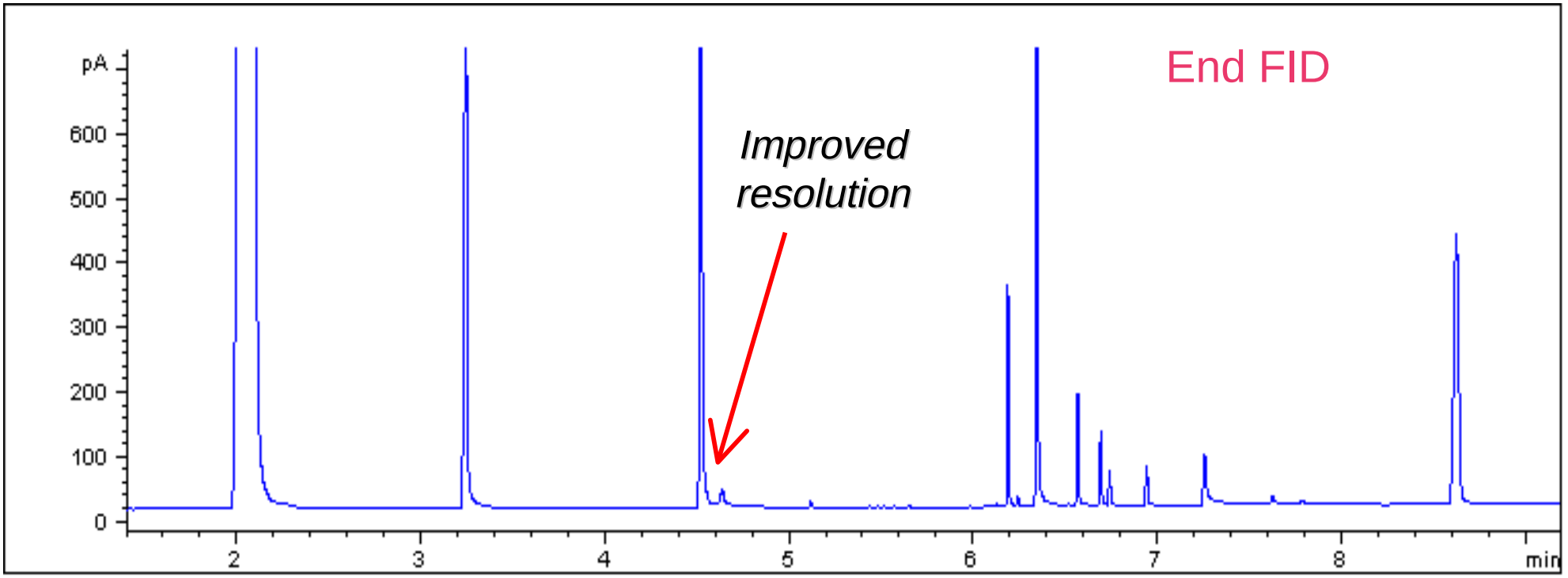
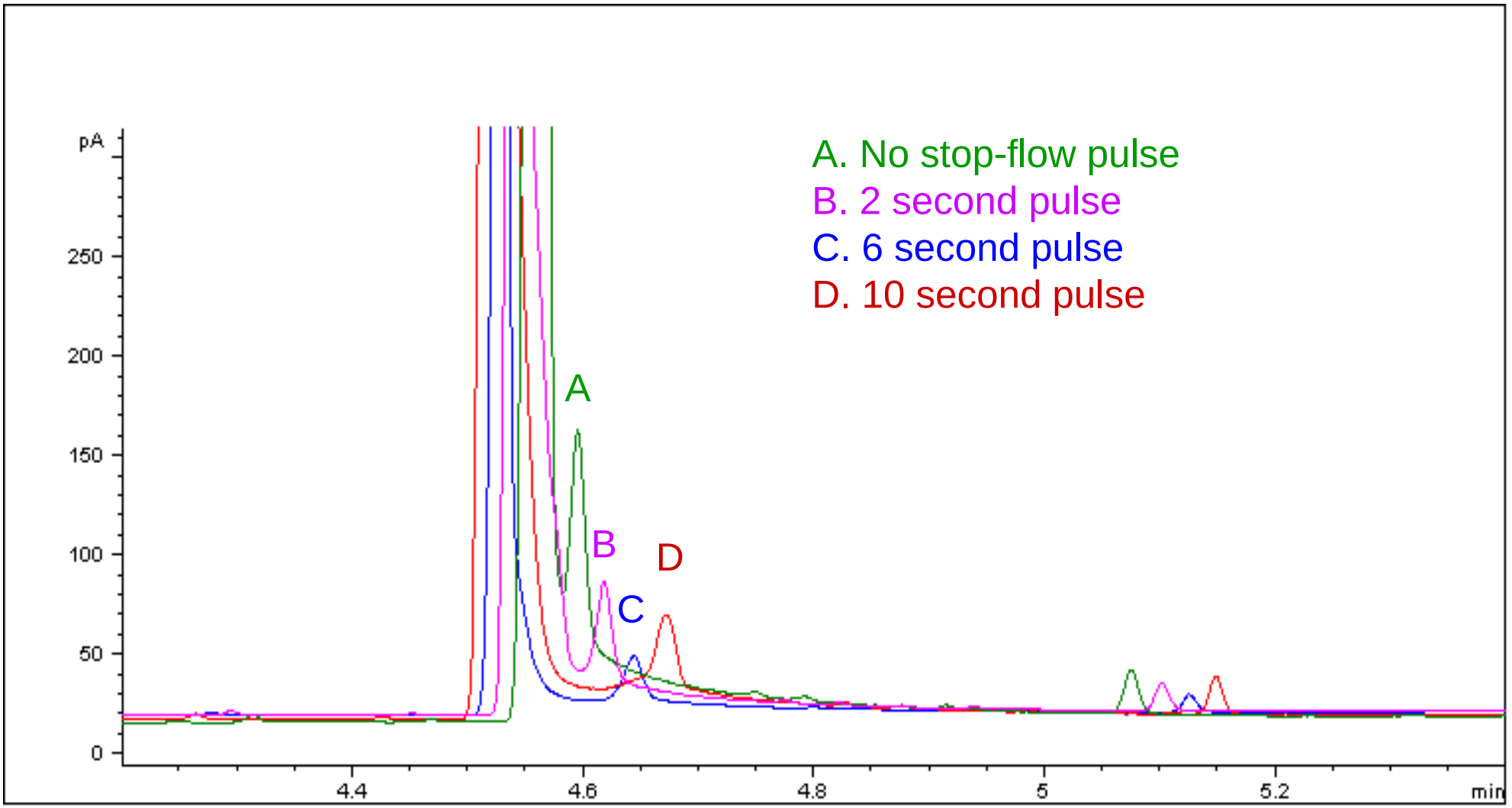


Figure 10. Separation of limonene and eucalyptol with increasing stop-flow pulse lengths.



Summary

GC analysis of flavor systems can be a time-consuming task. Because of the complexity of the separations, dual column systems often are required to resolve all of the target components. In addition, because of the wide range of boiling points, oven programs covering a wide range of temperatures often must be used. This work shows two approaches used to speed up chromatographic separations. Fast oven programs can be reproducibly maintained using an auxiliary heating unit. This can result in significant reductions in run time, as long as the resolution of critical component pairs can be maintained. For more complex systems that are difficult to completely separate on one type of stationary phase, Stop-Flow GC can be a powerful means of “tuning” the separations, with minimal hardware modifications required.

References

1. T. Veriotti and R. Sacks, *Anal. Chem.*, **2001**, *73*, 3045.
2. T. Veriotti and R. Sacks, *Anal. Chem.*, **2001**, *73*, 4395.