Resolution of Complex Volatile Organic Mixtures, Using Stop-Flow Modulation with Two Gas Chromatography Columns in Series

Rebecca E. Wittrig, Michael Goss, and Christopher English

Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

Introduction

Residual organic solvents are monitored in pharmaceutical products and packaging materials, and water supplies are tested for volatile organic contaminants. These compounds generally are amenable to analysis by gas chromatography (GC); however, because they encompass a wide range of functional groups, boiling points, and concentrations, analysis times can be quite long. Often, complete resolution of all target analytes requires analysis on two columns with dissimilar stationary phases. An ideal volatiles assay would be performed in a single chromatographic run, at minimal analysis time.

Two-dimensional GC techniques can significantly reduce analyses times for complex systems that require dual column analysis. Selectivity tuning, using flow modification and two capillary columns in series, will be discussed. In this technique, separation of target compounds is enhanced by modifying the flow through the columns, using a series of 'stop-flow' pulses. For compounds that are resolved on the first column, but co-elute at the end of the column pair, resolution can be greatly improved by increasing the separation of the compounds at the column junction.[1,2] The use of stop-flow GC in the analysis of volatiles, such as residual solvents commonly monitored in pharmaceutical products and packaging, will be described.

Stop-Flow GC

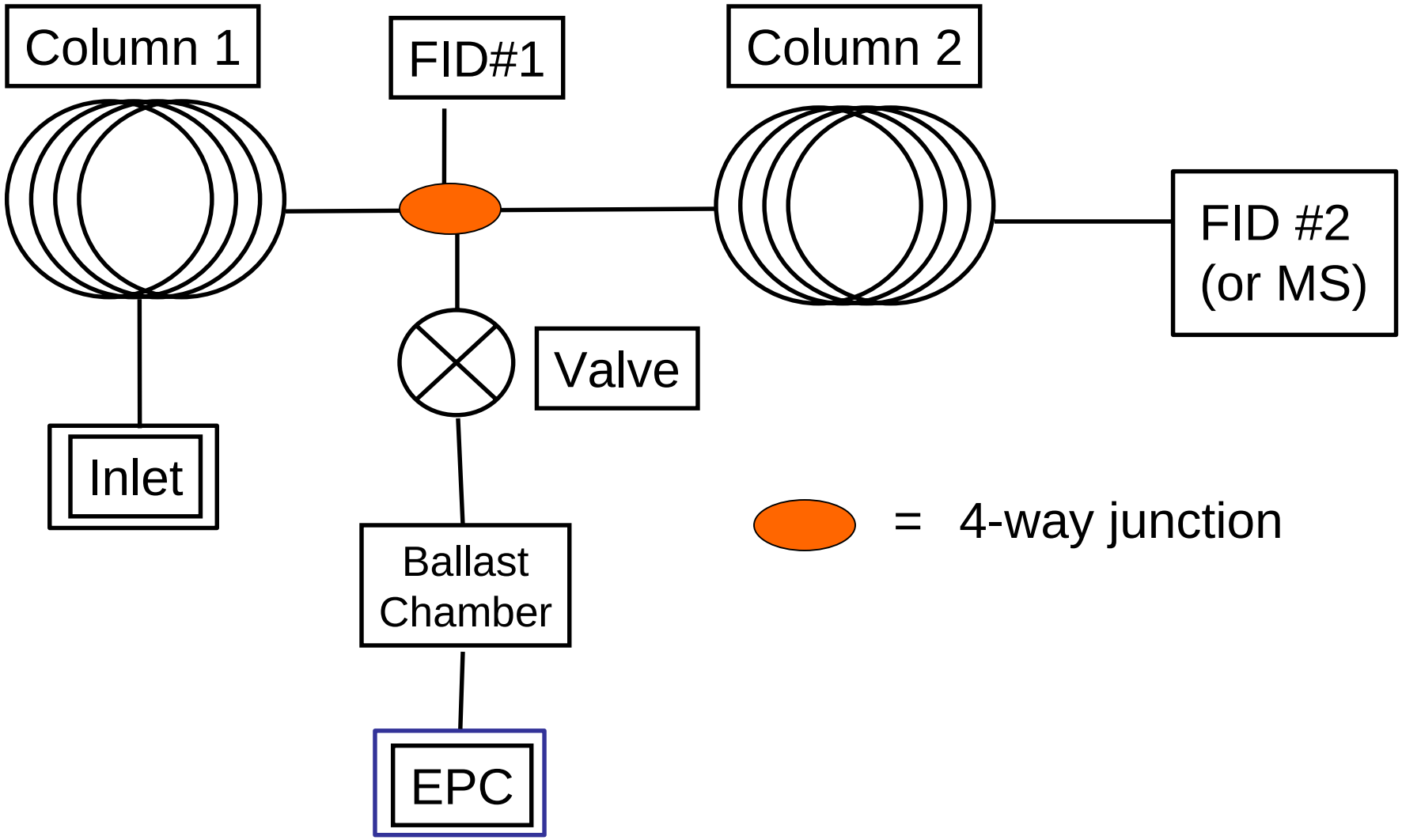
A diagram of the stop-flow system is shown in Figure 1. Two columns with dissimilar stationary phases are connected in series. At the column junction, an external source of carrier gas is connected; the flow of this gas to the junction is controlled by an air- or electronically-triggered valve.

When using the stop-flow column technique, there are four possibilities:

I. Compounds are resolved at the column junction and remain resolved at the end of the column pair. The separation can proceed normally.
II. Two or more compounds coelute at the junction, but are resolved on the second column. This separation also can proceed normally.
III. Compounds are resolved at the junction, but coelute at the end of the column pair. A stop-flow pulse is applied when the first compound has crossed the junction but the second compound is still in the first column, thus increasing the band spacing.
IV. The compounds coelute both at the junction and at the end of the column pair. Other stationary phase combinations should be investigated.

To use the stop-flow technique, as in (III), component bands must be completely separated by the first column if they coelute at the end of the column pair.

Figure 1. Diagram of the stop-flow GC system.



Application of Stop-Flow GC to Residual Solvent Assays

Residual solvent testing is a critical measure for manufacturers of pharmaceutical formulations. The International Conference on Harmonization (ICH) and the European Pharmacopoeia are among the regulatory agencies that have proposed guidelines for this testing.[3,4] The analytical challenges are significant, with over 60 compounds of regulatory interest. The solvents have been divided into 3 classes: solvents with unacceptable toxicities, which should be avoided (Class I); solvents with less severe toxicities, use of which should be limited (Class II); and less toxic solvents (Class III). An ideal residual solvent method would permit identification and quantification of the target components in a single analysis. For this study, Class I and Class II solvents listed in Table I were targeted.

Standard Preparation

The compound list was chosen to include solvents with unacceptable toxicities (Class I) and solvents that should be avoided (Class II). Neat materials were used to prepare three separate solvent-less mixes, which were combined to yield a concentration of 2.8% each component. The working standard components are shown in Table I.

Table I. Class I & class II residual solvents

Peak #	Compound	Peak #	Compound
1	2-methylpentane	19	1,2-dichloroethane (1,2-DCA)
2	hexane	20	2-hexanone (MBK)
3	methyl cyclopentane	21	*p*-xylene
4	1,1-dichloroethene (1,1-DCE)	22	*m*-xylene
5	methyl cyclohexane	23	nitromethane
6	*trans*-1,2-dichloroethene	24	2-methoxyethanol
7	carbon tetrachloride (CCl_4)	25	pyridine
8	1,1,1-trichloroethane (1,1,1-TCA)	26	*o*-xylene
9	methanol	27	chlorobenzene
10	1,2-dimethoxyethane	28	2-ethoxyethanol
11	methylene chloride (CH_2Cl_2)	29	1,1,2-trichloroethane (1,1,2-TCA)
12	benzene	30	dimethyl formamide (DMF)
13	*cis*-1,2-dichloroethene	31	N,N-dimethylacetamide (DMA)
14	trichloroethene (TCE)	32	1,2,3,4-tetrahydronaphthalene (THN)
15	acetonitrile (MeCN)	33	ethylene glycol (EG)
16	chloroform	34	1-methyl-2-pyrrolidinone (1-MP)
17	toluene	35	formamide
18	1,4-dioxane	36	sulfolone

Chromatographic Separation

36 residual solvents were analyzed using the conditions in Table II. The chromatogram at the junction FID is shown in Figure 2. The junction FID monitors the separation after the first chromatographic column. Multiple compounds coelute at the end of the column ensemble, as measured at the end FID (Figure 3). These include:

- hexane and 1,1-dichloroethene
- carbon tetrachloride and methylcyclohexane
- *cis*-1,2-dichloroethene and 1,2-dimethoxyethane
- acetonitrile and trichloroethene
- pyridine, *p*-xylene, and *m*-xylene
- ethylene glycol and 1,2,3,4-tetrahydronaphthalene

Note, however, that all compounds that coelute at the end FID (Figure 4) are resolved at the junction FID (Figure 3).

Table II. Conditions for the stop-flow residual solvent assay

Analytical Columns	Stabilwax®, 15m x 0.25mm, 0.5µm* Rtx®-200, 30m x 0.25mm, 1µm**
Oven Program	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)
Column Flow	2.5 mL/min. to 9.5 min. 3.5 mL/min. at 10 min.
Injector	230°C
Injection	0.2 mL headspace, 200:1 split
Detectors	Flame ionization detectors @ 250°C

*Polyethylene glycol stationary phase
**Trifluoropropyl stationary phase

Stop-Flow Pulses

Nine pulses, varying in duration from 2 - 8 seconds, were applied beginning 44 seconds after injection. The band for the first component in each critical pair has completely migrated to the second column when the valve is opened. The second compound in the pair stays on the first column until the end of the pressure pulse. The timing of the 9 pulses is shown in Figure 4, with the resulting chromatogram at the end FID in Figure 5. Pressure at the junction point was 74 psia, or 59 psig head pressure. At all times the junction head pressure was above the inlet head pressure, causing a slight reverse flow on the first column while the valve was open.

In some cases, multiple stop-flow pulses were used to "tune" the separation. Pyridine, *p*-xylene, and *m*-xylene all elute at 8.1 minutes, as shown in Figure 8. To resolve these components, a 3-pulse sequence was introduced, as shown in Figure 9. Figures 6-9 are enlarged views of the chromatographic resolution of several critical component pairs, without and with stop-flow pulses.

Figure 2. Residual solvents analyzed using conditions in Table II. Chromatogram at the junction FID, after the first (Stabilwax®) column.

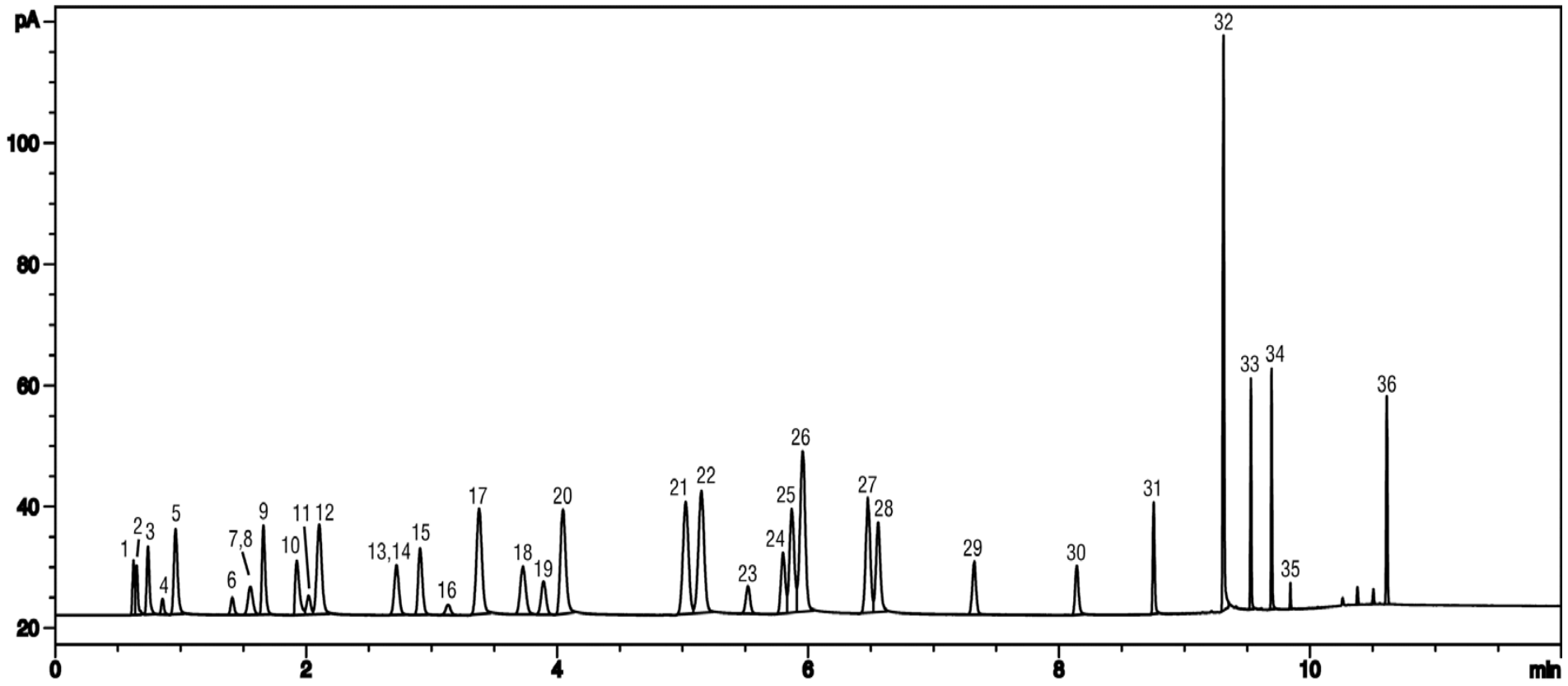


Figure 3. Residual solvents analyzed using conditions in Table II. Chromatogram at the end of the column ensemble.

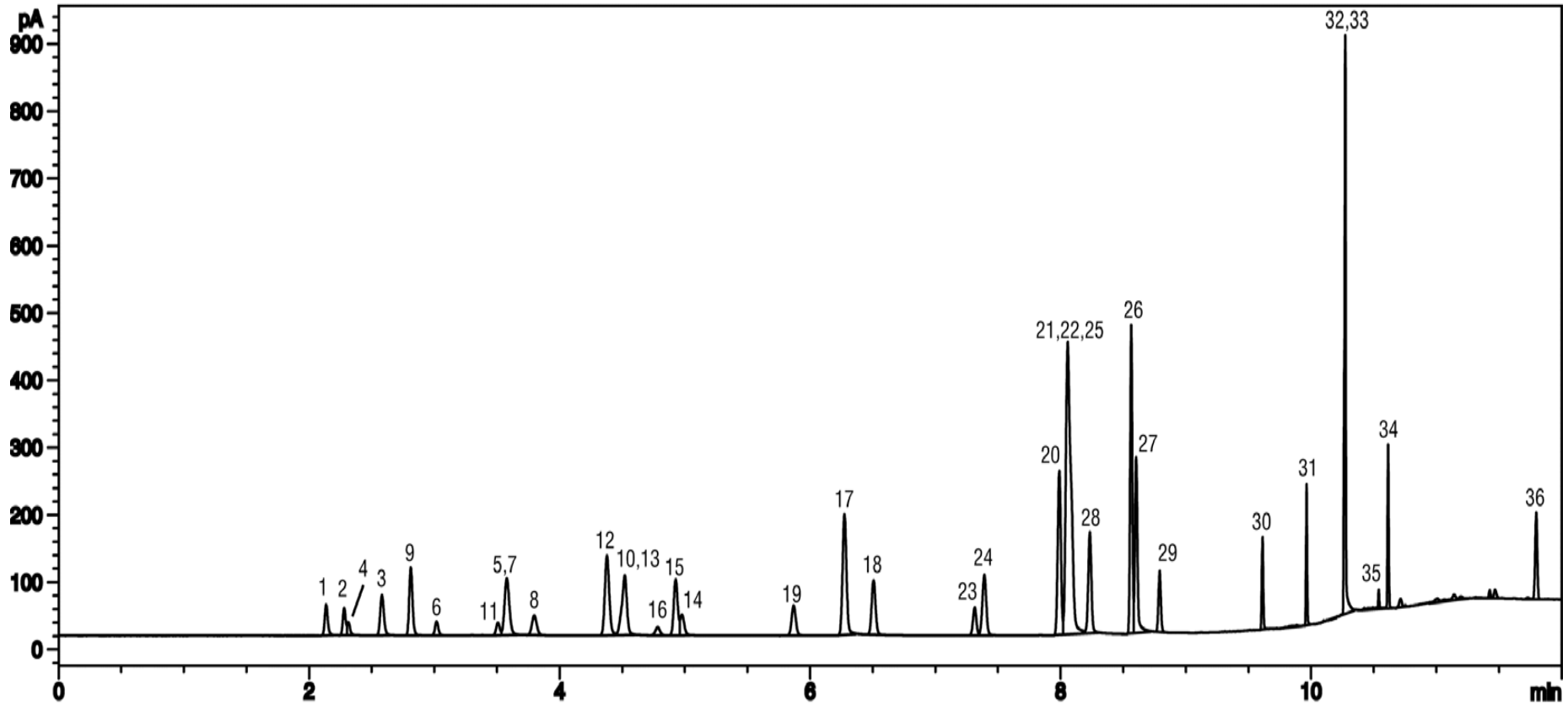


Figure 4. Chromatogram at the junction FID; arrows show locations and durations of 9 stop-flow pulses.

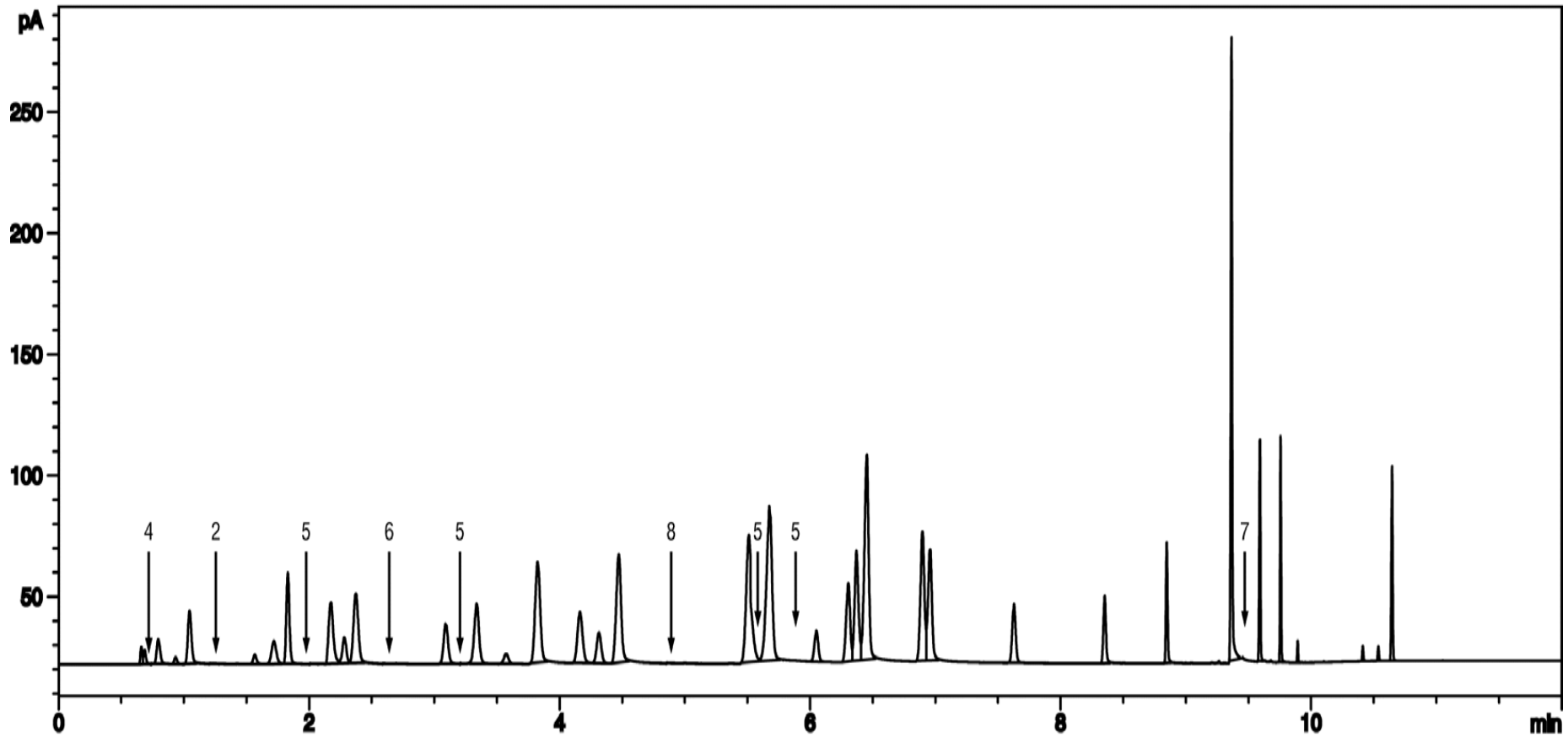


Figure 5. Chromatogram at the end of the column ensemble, using 9 stop-flow pulses to resolve all 36 volatile compounds.

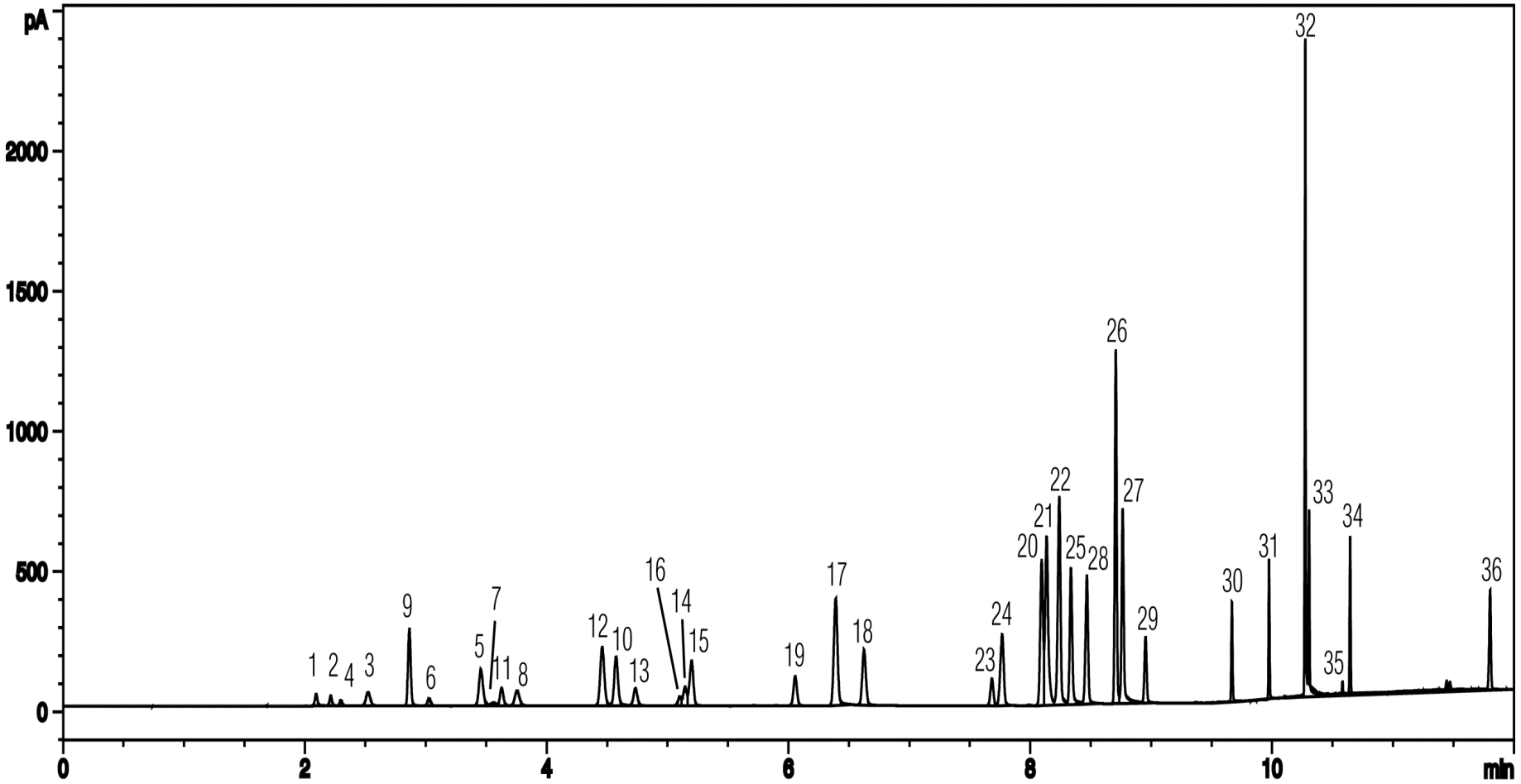


Figure 6. Enlargement of 1.3 – 4.0 minutes, no stop-flow pulses.

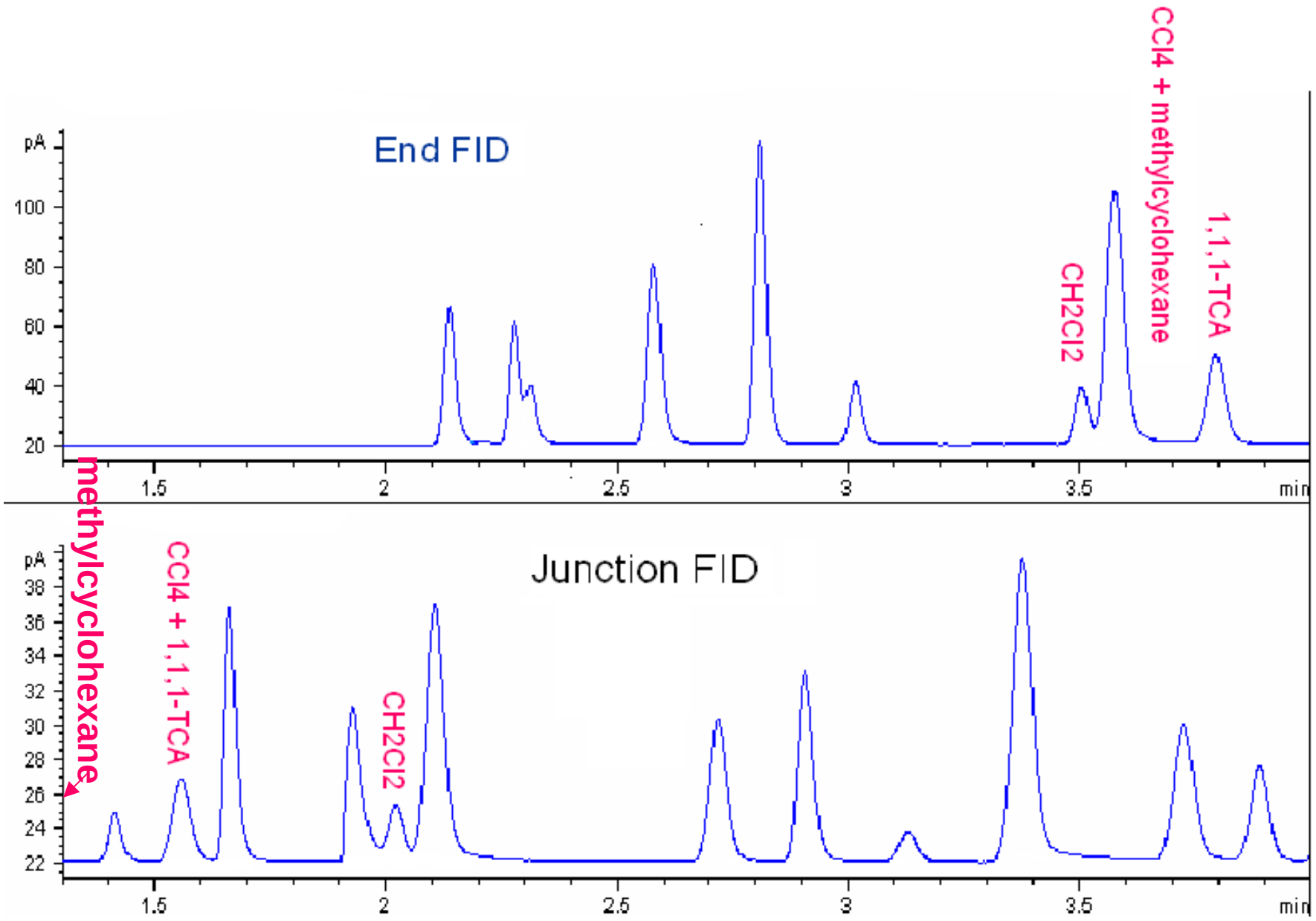


Figure 7. Enlargement of 1.3 – 4.0 minutes, stop-flow pulses initiated at 72 and 120 sec.

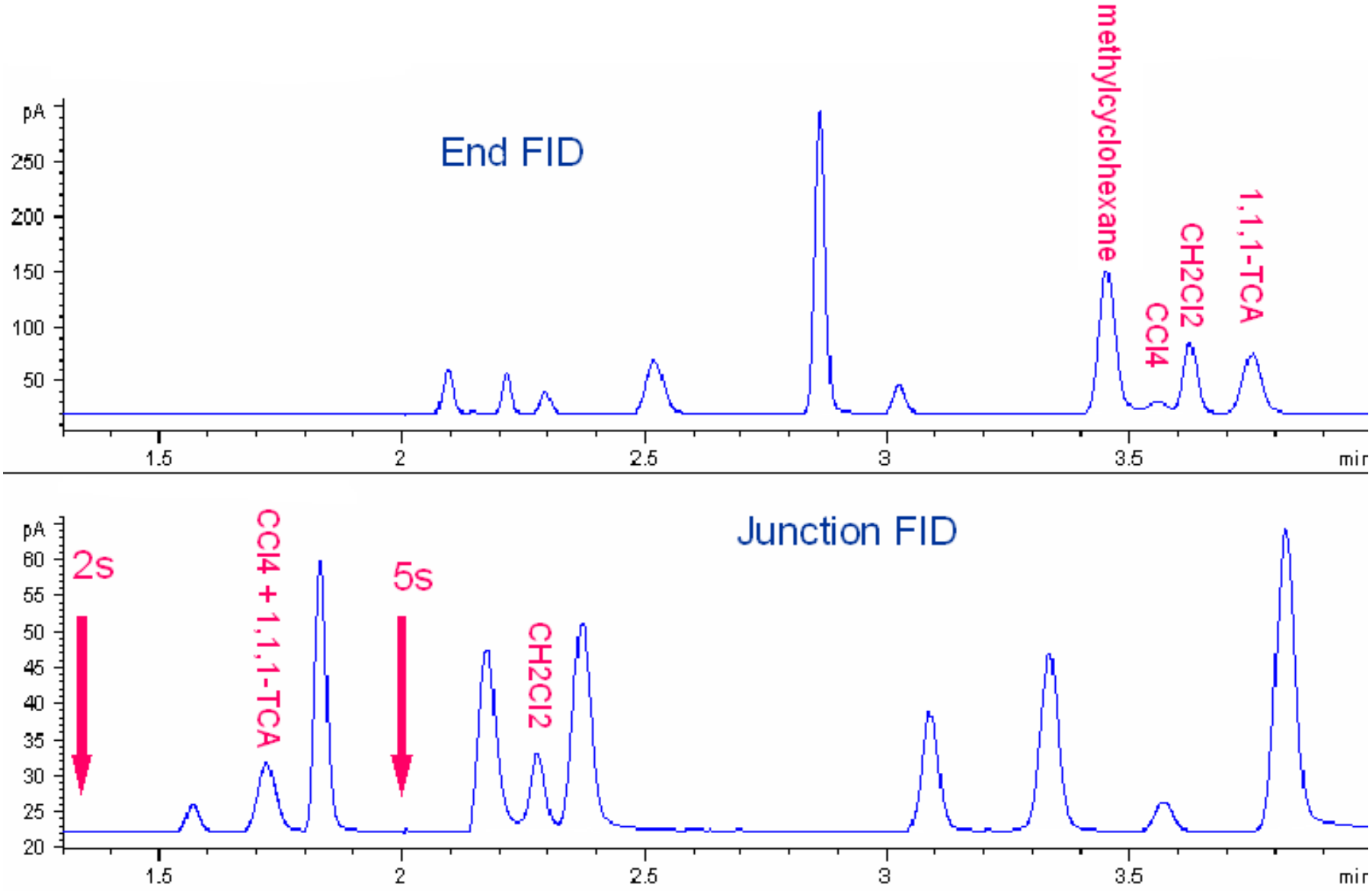


Figure 8. Enlargement of 4.3 – 8.5 minutes, no stop-flow pulses.

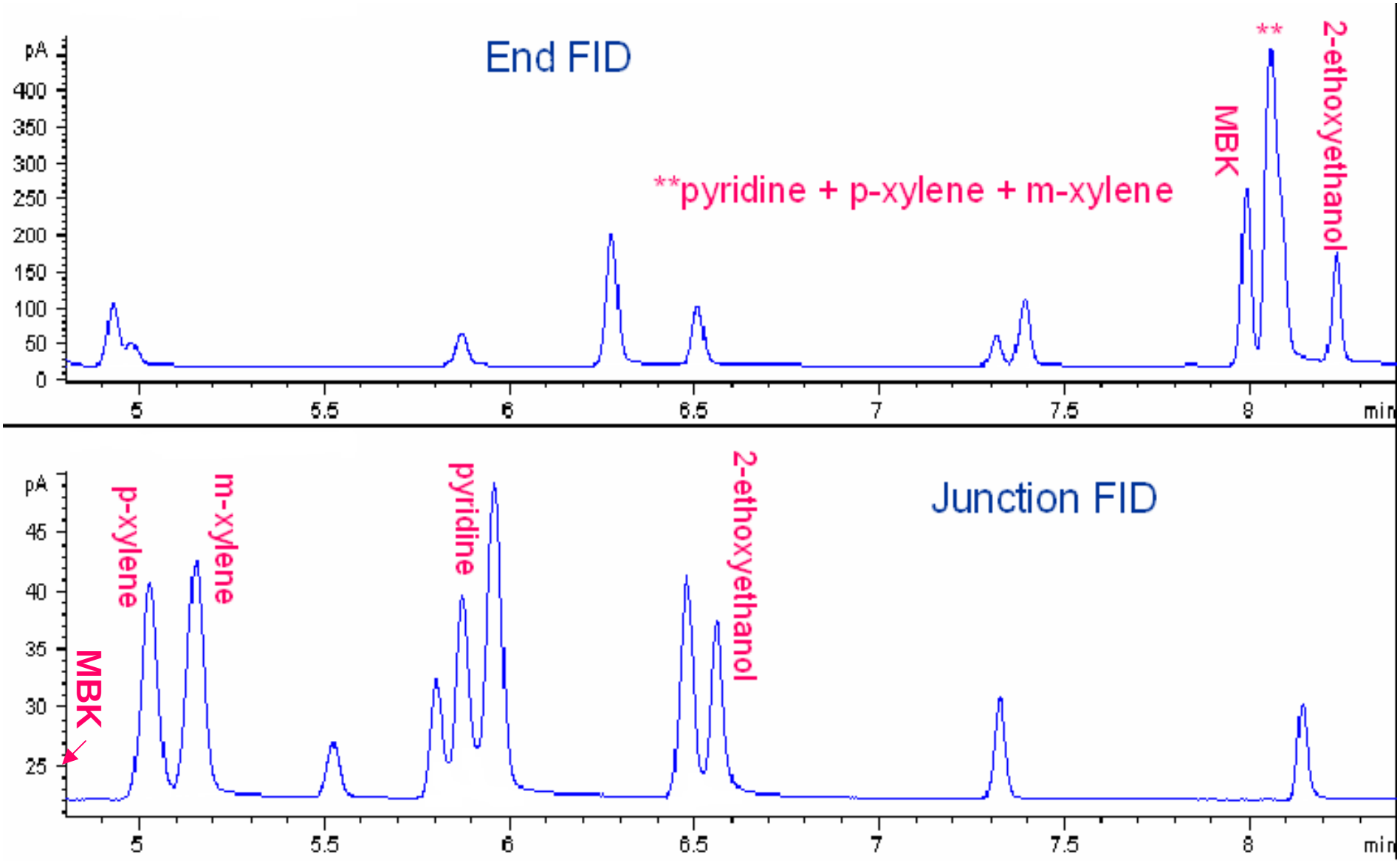


Figure 9. Enlargement of 4.3 – 8.5 minutes, stop-flow pulses initiated at 290, 330, and 346 sec.

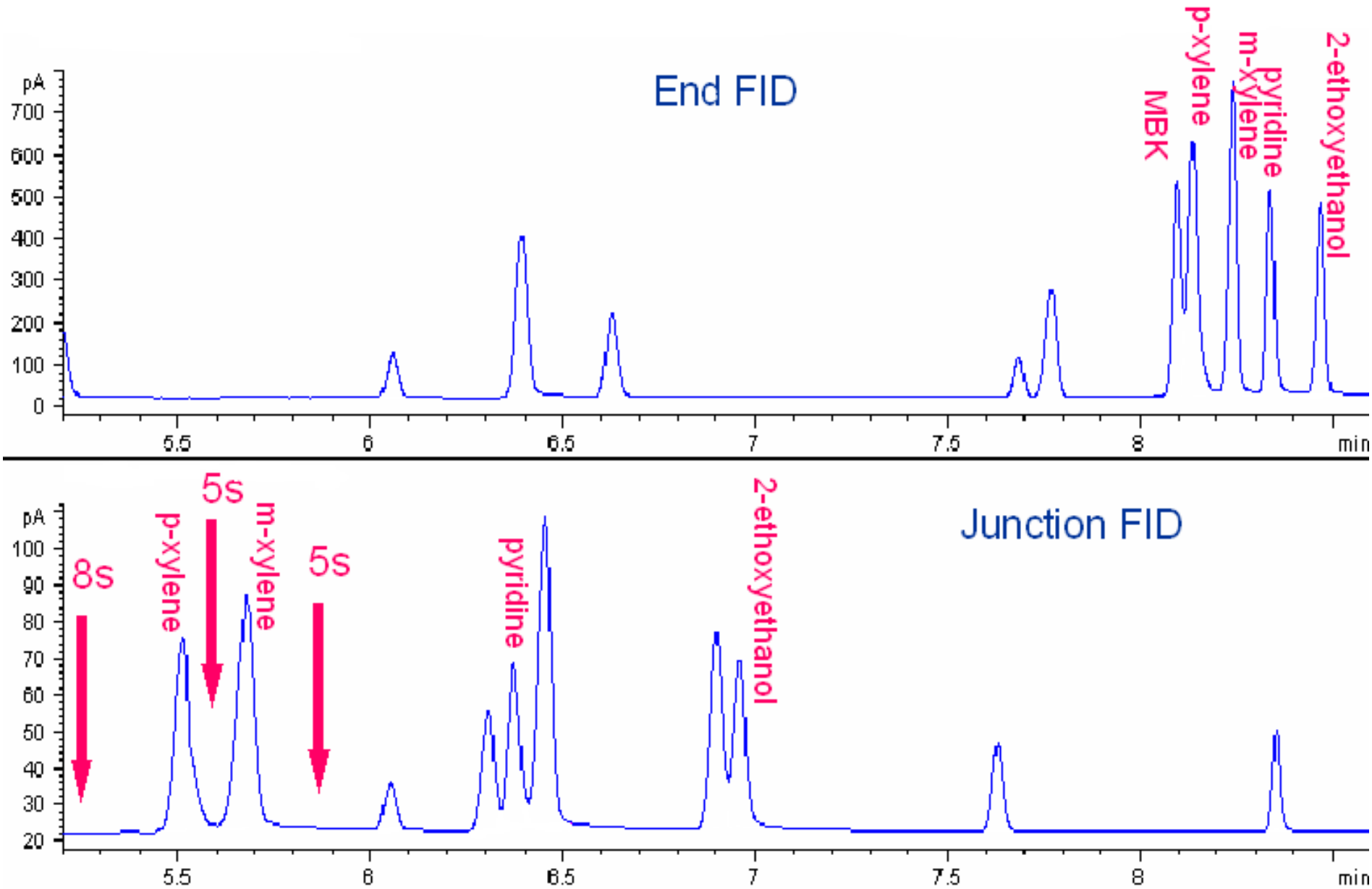


Figure 10. The stop-flow system connected to a gas chromatograph

Conclusions

High-speed separation of 36 residual solvents has been demonstrated in a single chromatographic run. Using a combination of a polyethylene glycol stationary phase and a trifluoropropyl stationary phase, this challenging separation was accomplished in 12 minutes. Resolution of coeluting or closely eluting components was substantially improved by introducing nine stop-flow pulses to "tune" the chromatographic separation.
The stop-flow GC accessory is shown in Figure 10.

Headspace analyses commonly are performed to achieve the desired detection limits for Class I and Class II residual solvents. This concentration step allows analytes to reach the capillary column with very little solvent interference. For this reason, we simulated a headspace analysis by combining our 36 components as neat analytes. Future work will combine stop-flow technology with headspace sampling to determine the achievable detection limits for each compound. The stop-flow GC technique, in combination with the proper choice of column stationary phases, can be used to dramatically improve difficult separations.

References

1. T. Veriotti and R. Sacks, *Anal. Chem*., **2001**, *73*, 3045.
2. T. Veriotti and R. Sacks, *Anal. Chem*., **2001**, *73*, 4395.
3. "Impurities: Residual Solvents" in *The ICH Harmonized Tripartite Guideline*, The Fourth International Conference on Harmonization, July 17, 1997.
4. *European Pharmacopoeia*, January 1999 supplement, 14-15.