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High-speed analysis of residual solvents by flow-modulation gas chromatography

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Abstract

High-speed gas chromatographic (GC) separation of residual solvents in pharmaceutical preparations, using a flow-modulation technique, is described. These volatile compounds are separated on a series-coupled (tandem) column ensemble consisting of a polyethylene glycol column and a trifluoropropylmethyl/dimethylpolysiloxane column. This column ensemble is operated in stop-flow mode to enhance, or "tune", the separation. A valve between the junction point of the tandem column ensemble and a source of carrier gas at a pressure above the GC inlet pressure is opened for intervals of 2–8 s. This stops or slightly reverses the flow of carrier gas in the first column. Stop-flow pulses are used to increase the separation of target analytes that overlap in the total ensemble chromatogram, compared to non-stop-flow, or conventional, operation. All 36 target compounds, based on ICH Classes I and II residual solvent lists, are resolved in 12 min using the stop-flow technique and a single chromatographic analysis.

Keywords: Gas chromatography, flow-modualtion; Pharmaceutical analysis

1. Introduction

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 [1,2]. The analytical challenges are significant, with over 60 compounds of regulatory interest. The solvents have been divided into three classes, including 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. Targets might include the Classes I and II solvents listed in Table 1, but resolving all of these components is extremely difficult, and typically must be accomplished using two or more chromatographic analyses with multiple stationary phase types.

Given the volume of residual solvent assays performed, analysis time also is very important. The chromatographic run time should not be shortened if doing so requires sacrificing resolution between compounds; the challenge is to design a method for performing this analysis in the minimum amount of time, while maintaining baseline or near-baseline separation of all analytes. Currently, in order to decrease analysis time significantly, while maintaining complete separation of the target compounds, instrument modification is necessary. Instrument modifications should be compatible with injection techniques (split, splitless, direct, and on-column) that are both appropriate for trace analysis and commercially available.

A flow-modulation technique has been described in detail by Sacks et al. [3–6], and has been applied to complex mixtures such as chlorinated pesticides and essential oils [7–9]. This technique is performed by programming the carrier gas pressure at the junction point of a series-coupled (tandem) ensemble of capillary columns that exhibit differing selectivity for the target compounds to be analyzed. By inserting a low dead-volume valve at the column junction, and connecting it to a source of carrier gas at or above the GC inlet pressure, flow programming can be accomplished [10]. When the valve is open, the carrier gas flow is stopped in the first column and is accelerated in the second column. Components that are separated by the first column in the

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Table 1
ICH Classes I and II residual solvents

Compound	Compound name	Class I or II
1	2-Methylpentane	II
2	Hexane	II
3	Methylcyclopentane	II
4	1,1-Dichloroethene (1,1-DCE)	I
5	Methylcyclohexane	II
6	*trans*-1,2-Dichloroethene	II
7	Carbon tetrachloride (CCl_4)	I
8	1,1,1-Trichloroethane (1,1,1-TCA)	I
9	Methanol	II
10	1,2-Dimethoxyethane	II
11	Dichloromethane	II
12	Benzene	I
13	*cis*-1,2-Dichloroethene (*cis*-1,2-DCE)	II
14	Trichloroethene	II
15	Acetonitrile	II
16	Chloroform	II
17	Toluene	II
18	1,4-Dioxane	II
19	1,2-Dichloroethane (1,2-DCA)	I
20	2-Hexanone (MBK)	II
21	*p*-Xylene	II
22	*m*-Xylene	II
23	Nitromethane	II
24	2-Methoxyethanol	II
25	Pyridine	II
26	*o*-Xylene	II
27	Chlorobenzene	II
28	2-Ethoxyethanol	II
29	1,1,2-Trichloroethane (1,1,2-TCA)	II
30	Dimethylformamide (DMF)	II
31	*N*,*N*-Dimethylacetamide	II
32	1,2,3,4-Tetrahydronaphthalene	II
33	Ethylene glycol	II
34	1-Methyl-2-pyrrolidinone	II
35	Formamide	II
36	Sulfolone	II

ensemble, but then co-elute from the second column, can be separated by the column pair if the valve is opened briefly when the band for one of the components has crossed the column junction point but the band for the other component is still in the first column. Pressure pulses at the junction of the column ensemble have been shown to be useful for increasing the separation between components without adversely affecting the separation of other components in the mixture [6–9]. Using series-coupled capillary columns with pressure switching techniques and fast oven temperature programming, it is possible to achieve resolution of 36 commonly analyzed organic volatile compounds in an analysis time of 12 min.

2. Experimental

2.1. Materials and procedures

An Agilent 6890 GC (Agilent Technologies, Wilmington, DE, USA) configured with a split/splitless inlet, electronic inlet pressure control (EPC), and dual flame ionization detection (FID) systems was used in this study. The pressure-programmable column ensemble consisted of two discrete capillary columns. The first column incorporated a 0.50 μm thick polyethylene glycol stationary phase (Rtx-Stabilwax, 15 m × 0.25 mm i.d., Restek C, Bellefonte, PA, USA). The second column incorporated a 1.0 μm thick trifluoropropylmethylpolysiloxane bonded stationary phase (Rtx-200, 30 m × 0.25 mm i.d., Restek). The columns were joined using a four-port Gerstel Graphpack 3D/2-Crosspiece (part GC09780 45, Gerstel, Baltimore, MD, USA), as shown in Fig. 1.

Pressure programming was provided by a pneumatically-operated, low-dead-volume valve (Model MOPV-1/50, SGE, Austin, TX, USA) connected between the junction of the column ensemble and a ballast chamber containing carrier gas at or above the GC inlet pressure, as depicted in Fig. 1. When the valve is open, and the carrier gas pressure at the column junction is equal to or greater than the GC inlet pressure, the carrier gas flow stops in the first column (stop-flow operation). Applying a pressure above the GC inlet pressure slightly reverses the analyte bands in the first column. The pneumatic valve is operated by a 50–55 psig compressed air source (1 psi = 6894.76 pa), connected through an electronically-actuated solenoid valve (Model GH3412, Precision Dynamics, Phoenix, AZ, USA). The configuration of the system is described in greater detail in [6]. Ballast chamber pressure is controlled by an electronic pressure controller (MKS Model 640 A, MKS Instruments, Andover, MA).

An Agilent FID system also was connected to the Gerstel connector, through 0.5 m × 0.05 mm i.d. deactivated fused silica tubing. The fourth port in the connector was used to connect the ballast chamber to the junction point. 0.05 mm i.d. deactivated fused silica tubing was used for this connection. The FID connected to the Gerstel Crosspiece was used to monitor the analytes as they eluted from the first column. Approximately 10% of the effluent from the first column was diverted to this detector. A second FID system, connected to the outlet of the second column, was used as the primary detector for the column ensemble; the majority of the effluent was sent to this detector.

The hydrogen carrier gas was purified using a UOP hydrogen purifier (part 22602, Restek) to remove water vapor, oxygen, and hydrocarbons. The GC system was operated in constant flow mode, with a flow program of 2.5 ml/min (9.5 min hold), to 3.5 ml/min (at 10 min). The inlet temperature was 230 °C. The oven temperature program was as follows: 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), for a total run time of 12 min. In order to program the oven temperature at faster rates, an auxiliary heating unit (GC Racer, Zip Scientific) was used. Injection volumes of 0.2 μl of the neat solvent mixture,

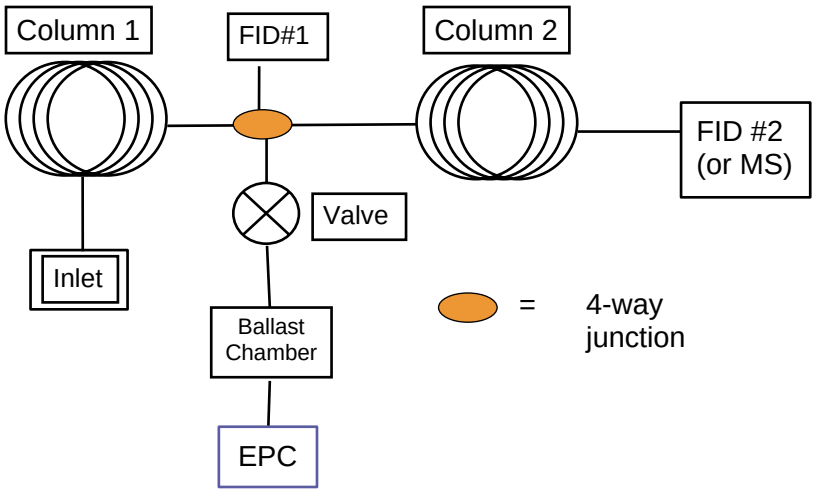

Fig. 1. Schematic of a stop-flow GC system. This system allows flow-modified selectivity tuning during the course of a chromatographic separation.

with a 200:1 split, were used. A 2 mm i.d. inlet liner was used (part 20712, Restek). The dual FID systems were set at 250 °C, with hydrogen flow at 40 ml/min, air at 400 ml/min, and helium (make-up) at 40 ml/min. The FID data collection rate was set at 100 Hz for both detectors.

Control of the ballast-chamber pressure and operation of the pneumatic valve was effected using a 330 MHz personal computer (OptiPlex GX1, Dell) and a 12-bit A/D board (DT-2801, Data Translation, Marlboro, MA, USA). The interface board was controlled with Labtech Notebook software (Laboratory Technologies, Wilmington, VA, USA). Agilent's Chemstation software was used for processing the FID chromatograms.

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 solventless mixtures. The first mixture contained 28 compounds that are stable for a period of at least 30 days at 0 °C. Seven nitrogen-containing compounds made up mixture 2. The 36-component residual solvent working standard was prepared by combining these two mixtures with 2-hexanone to a relative concentration of 2.8% for each component. The working standard components are shown in Table 1.

2.2. Chromatographic separation

When using the stop-flow column ensemble system, there are four chromatographic possibilities: (1) The compounds are resolved at the column junction and remain resolved at the end of the ensemble. For this case, the separation is allowed to proceed without stopping the flow. (2) The compounds coelute at the junction, but are resolved on the second column. For this case, separation also is achieved by the column ensemble, and therefore also is allowed to proceed as normal. (3) The compounds are resolved at the junction, but coelute at the end of the column ensemble. For this case, a stop-flow pulse is applied when one compound band has crossed the junction but the other compound band is still in the first column. The time of the pulse can be set to ensure that the two components stay separated in time on reaching the end of the column ensemble. (4) The compounds coelute both at the junction and at the end of the ensemble. In this case, other stationary phase compositions should be investigated to find a selectivity that would allow separation on one of the two columns.

3. Results and discussion

A mixture of 36 residual solvents was analyzed in the split mode using the parameters described. A number of compounds coelute at the end of the column ensemble, as can be seen in Fig. 2. These include: hexane and 1,1-dichloroethene; carbon tetrachloride and methylcyclohexane; *cis*-1,2-dichloroethene and 1,2-dimethoxyethane; pyridine, *p*-xylene, and *m*-xylene; and ethylene glycol and 1,2,3,4-tetrahydronaphthalene. To improve the chromatographic separation, pressure programming of the tunable column ensemble was used. In this study, pressure pulses of varying durations were used. The pressure at the junction point was set at 74 psia, or 59 psig head pressure. In constant flow mode, the inlet head pressure increased through the chromatographic run, as the oven temperature increased. However, 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 order to use the stop-flow technique to enhance the separation of a critical pair, it is necessary that the compo-

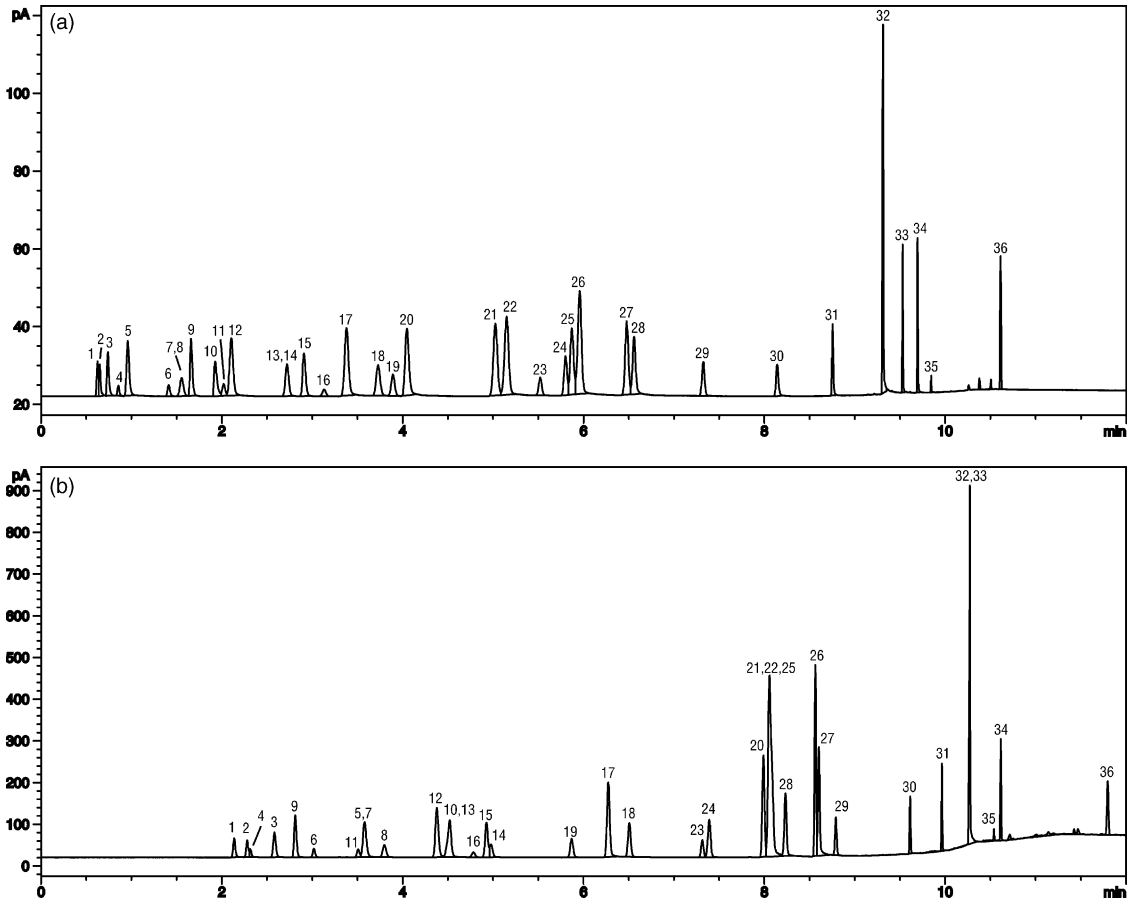

Fig. 2. Analysis of 36 ICH Classes I and II residual solvents. (a) FID chromatogram from column 1; (b) FID chromatogram at the end of the column ensemble, with no stop-flow pulse applied.

nent bands be completely separated by the first column in the ensemble. Fig. 3(a) shows the FID signal for the detector monitoring the effluent from the first column for a residual solvent sample, with the vertical arrow indicating the timing of the stop-flow pulses. Note that for all compounds that coelute at the end of the column ensemble, resolution is achieved at the junction point, as measured at the first FID system.

In the stop-flow experiment, a series of nine pulses, varying in duration from 2 to 8 s, were applied beginning 44 s after injection. Note that 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 resulting chromatogram at the end of the column ensemble is shown in Fig. 3(b). In some cases, multiple stop-flow pulses were used to "tune" the separation. For example, carbon tetrachloride and methylcyclohexane coelute in the original run. A 2 s pulse at 72 s was introduced to resolve these components, but caused carbon tetrachloride and dichloromethane to coelute. A 5 pulse at 120 s was then used to separate the carbon tetrachloride from the dichloromethane.

Pyridine, *p*-xylene, and *m*-xylene all elute at 8.1 min, as shown in Fig. 2(b). In order to resolve these components, a three-pulse sequence was introduced. An 8 s stop-flow pulse at 290 s moved *p*-xylene away from MBK, and a 5 s pulse at 330 s moved *p*-xylene away from *m*-xylene. Finally, a 5 s pulse at 346 s was used to separate *p*-xylene from pyridine. The significant improvement in the separation of these components could not be achieved by modifying the temperature program or the linear velocity.

Fig. 3(c–h) are enlarged views of the chromatographic resolution of several critical component pairs, without and with the introduction of one or more stop-flow pulses. With a series of nine stop-flow pulses, the 36 residual solvents can be resolved in 12 min. Most notably, the separation quality of coeluting or closely eluting compounds can be greatly improved. This is done without increasing the analysis time or sacrificing the separation of other components in the sample.

Headspace analyses commonly are performed to achieving the desired detection limits for Classes I and II residual solvents. This concentration step allows analytes to reach the capillary column with very little solvent interference.

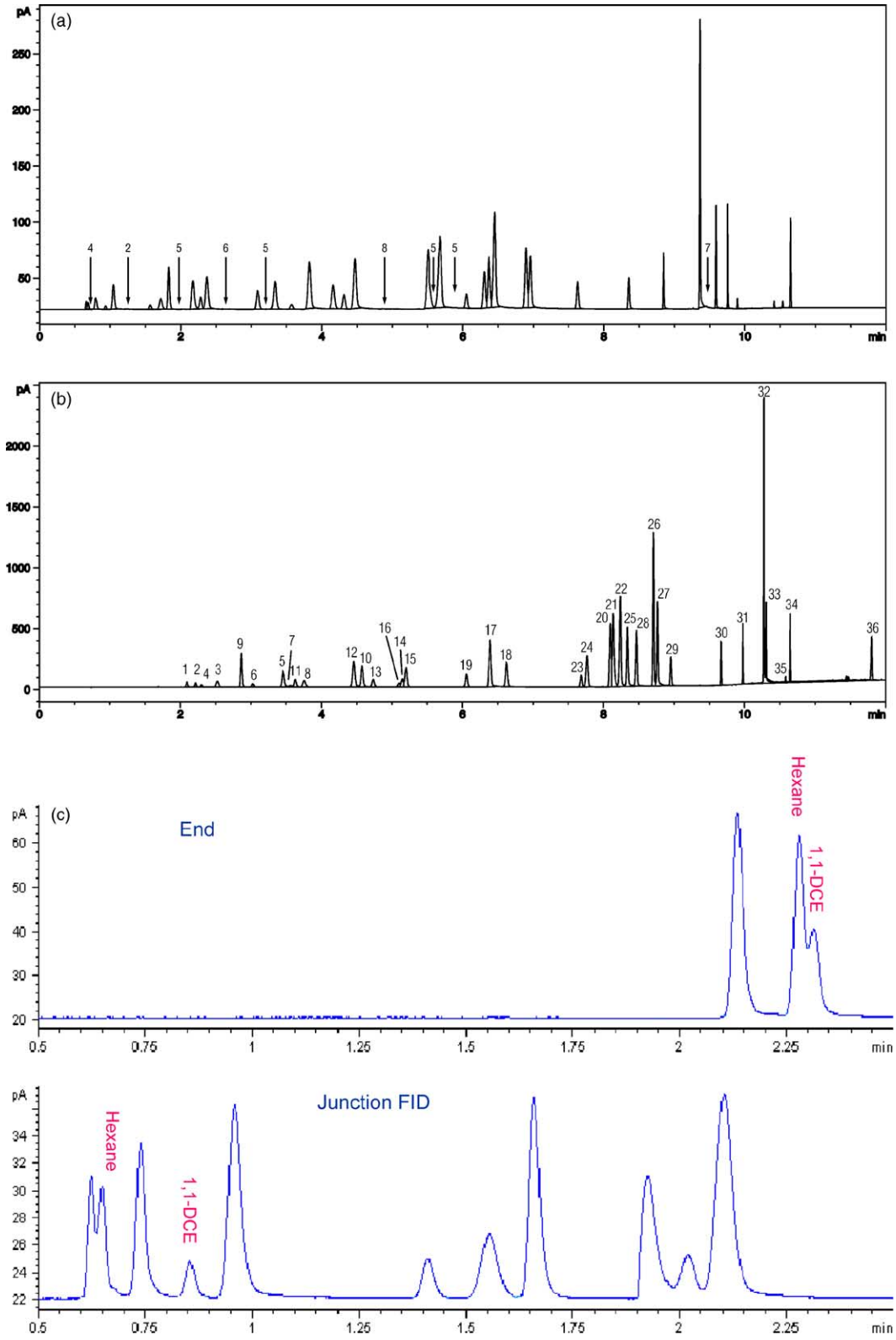

Fig. 3. Stop-flow GC for enhanced separation of coeluting or closely-eluting residual solvents. (a) FID chromatogram from column 1; arrows show times and durations of stop-flow initiation; (b) FID chromatogram from the end of the column ensemble, with nine stop-flow pulses as shown in (a); (c) enlargement of the region from 0.5 to 2.5 min, no stop-flow pulses; (d) enlargement of the region from 0.5 to 2.5 min, one stop-flow pulse applied at 44 s; (e) enlargement of the region from 1.3 to 4.0 min, no stop-flow pulses; (f) enlargement of the region from 1.3 to 4.0 min, two stop-flow pulses applied, at 72 and 120 s; (g) enlargement of the region from 4.8 to 8.4 min, no stop-flow pulses; (h) enlargement of the region from 5.2 to 8.6 min, three stop-flow pulses applied, at 290, 330, and 346 s.

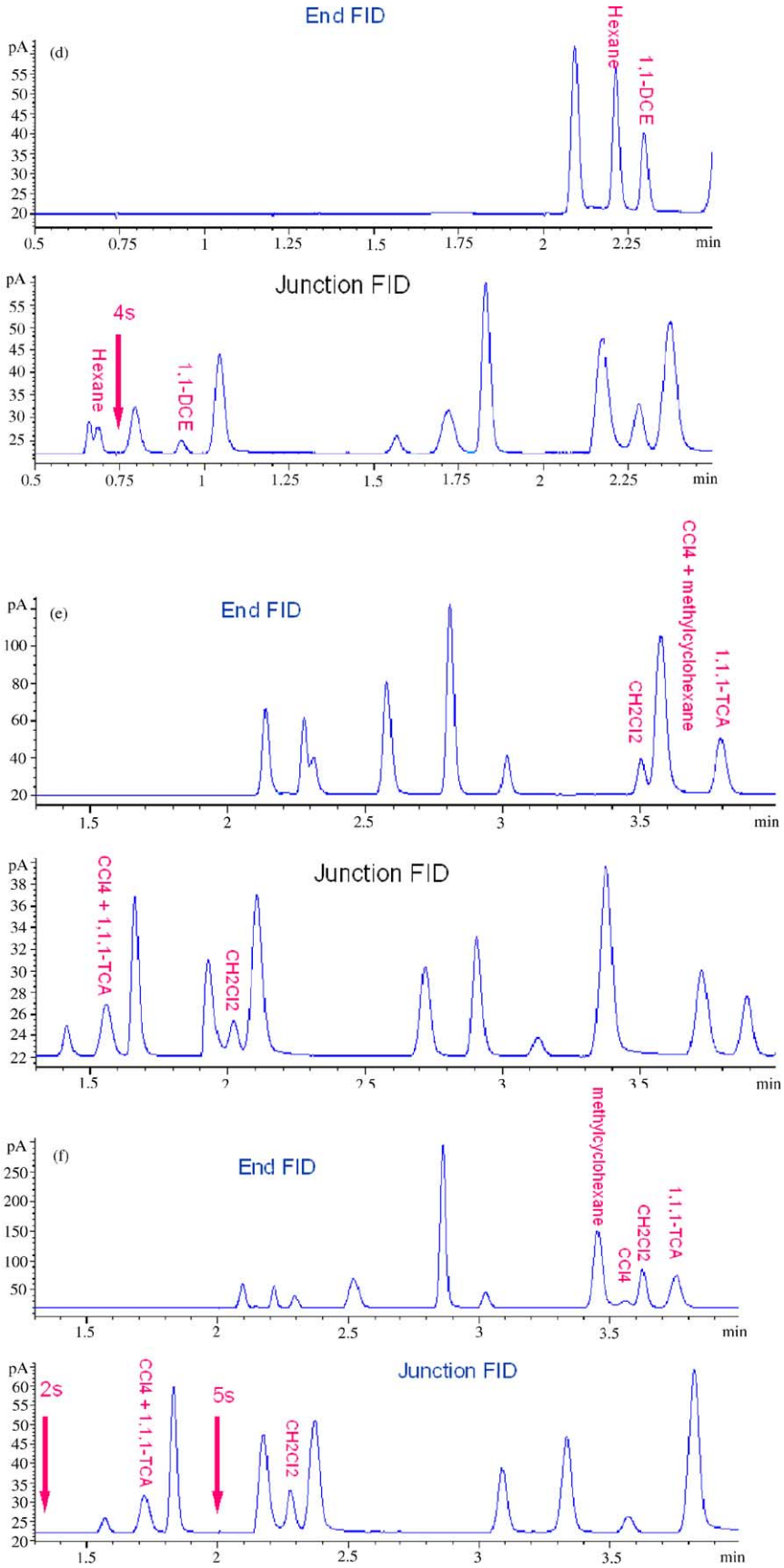

Fig. 3. (*Continued*).

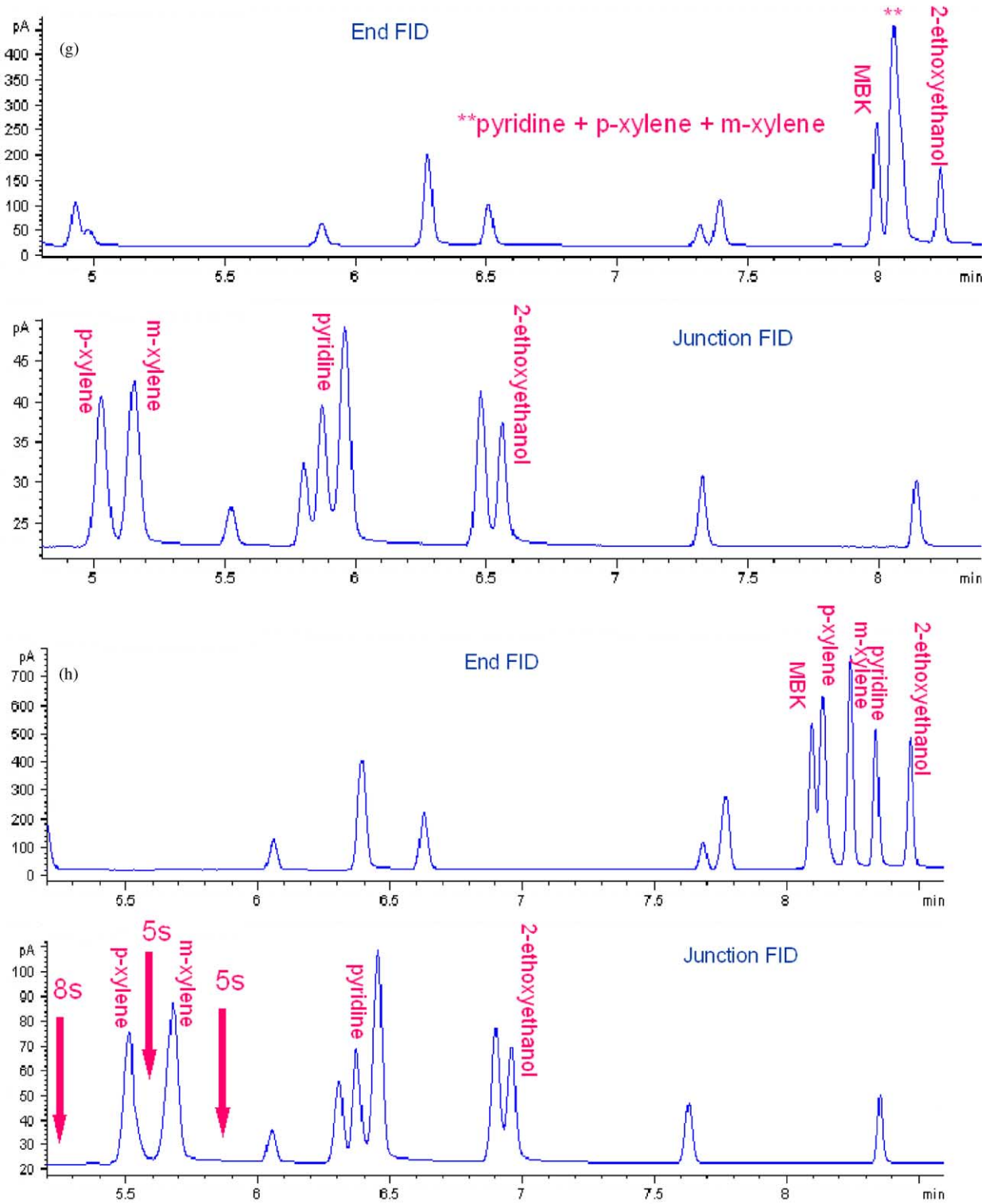

Fig. 3. (*Continued*).

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.

4. 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 min. Resolution between coeluting or closely eluting components was substantially improved by introducing nine stop-flow pulses to "tune" the chromatographic separation. The stop-flow GC technique, in combination with the proper choice of column stationary phases, can be used to dramatically improve other difficult separations.

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