ANALYSIS OF PERSISTENT ORGANIC POLLUTANTS (POPs) USING MICROBORE COLUMNS

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Introduction

Analysis of POPs (Dioxins, PCBs, PAHs, OC Pesticides) by GC/MS and GC/ECD have typically been carried out using 30M and 60M capillary columns. Converting analytical methods to shorter, narrower gas chromatographic columns can considerably reduce analysis times and costs, increase instrument analytical capacity and in some cases increase chromatographic selectivity. Converting methods to microbore columns can reduce analysis times by up to 80%.

Complete chromatographic separation is a prerequisite for most analytical determinations. Even with highly specific detection as in mass spectrometry, complete chromatographic separation prior to detection may be required for an accurate determination of analyte concentrations when isomers, congeners or structurally related compounds are analysed.

In order to separate analytes of interest on a GC column, each analyte passing through the column must interact with the stationary phase for a different amount of time than the other compounds that are to be separated. The degree to which a specific analyte (peak) is retained by the stationary phase depends upon the internal column diameter (id), stationary phase composition, film thickness, temperature, carrier gas and carrier gas flow rate. If the phase ratio (β - ratio of gas-phase to liquid-phase volumes) which is proportional to the ratio of the id to film thickness is kept constant, column temperature and carrier gas flow rates can be programmed so that relative retention times of the compounds of interest remain relatively constant from column to column[1]. Analyte retention times can therefore be shortened considerably by using narrower columns with thinner films while maintaining essentially the same chromatography. Using analyte specific stationary phase formulations can increase separation of the analytes of interest, thereby reducing retention times even further. The combination of analyte specific phases and Fast GC allows for the ultimate enhancement of chromatographic speed[2].

Gas chromatographs capable of running in the Fast GC mode (Column head pressures of >60 psi, temperature ramp rates of >75°C/min and injection volumes of less than 1 µL) have only been available for the past few years. Analytical run times can be reduced by a factor of 5 or more using Fast GC. Significant reductions in analysis times (25 to 50%) can also be achieved with conventional GCs by using higher column head pressures and shorter narrower columns. In order to obtain accurate and reproducible results with microbore columns, the complete chromatographic system from injection to detection must be optimized for Fast GC.

Methods and Materials
Original GC methods were translated for use on the corresponding shorter columns (40M, 20M, 10M) with Hewlett Packard Method Translation Software 1997. (Hewlett Packard - Palo Alto CA). Chromatography was then optimized using Pro EZ-GC (Restek Corporation -Bellefonte PA).

GC Conditions:
Dioxins: Hewlett Packard 6890 with Micromass Ultima HRMS @10,000RP. Injector: 280°C. Carrier gas: He
20M, DB-5, 0.1mm id, 0.1μm film thickness, column head pressure: 100psi. Initial temp:100°C, held 1 min, ramp to 200°C at 100°C/min, ramp to 235°C at 13°C/min, ramp to 300°C at 27°C/min and held 4 min. Injection volume: 0.2μL.
40M, DB-5, 0.18mm id, 0.18μm film thickness, column head pressure: 61psi. Initial temp:100°C, held 0.62 min, ramp to 200°C at 64.5°C/min, ramp to 235°C at 4.8°C/min, held 6.2 min, ramp to 300°C at 9.7°C/min and held 5.6 min. Injection volume: 1.0μL.

PAHs: Hewlett Packard 6890 with Micromass Ultima HRMS @10,000RP. Injector: 270°C. Carrier gas:He
20M, RTX-5, 0.1mm id, 0.1μm film thickness, column head pressure: 90psi. Initial temp:100°C, held 0.47 min, ramp to 125°C at 32°C/min, ramp to 225°C at 11°C/min, ramp to 300°C at 8°C/min and held 5 min. Injection volume: 0.2μL.
30M, RTX-5, 0.25mm id, 0.25μm film thickness, Constant flow mode at 1 mL/min. Initial temp: 100°C, held 1 min, ramp to 125°C at 15°C/min, ramp to 300°C at 5°C/min, held 10 min, Injection volume:1.0μL.

PCBs: Hewlett Packard 6890 with Micro ECD. 20M, DB-5 and DB1701, 0.1mm id, 0.1μm film thickness. Injector: 280°C, Detectors: 300°C. Carrier gas: He, at 0.4mL/min - column head pressure: 67psi. Initial temp: 90°C, ramp to 160°C at 35.5°C/min, held 0.28 min, ramp to 200°C at 71°C/min, held 0.28min, ramp to 275°C at 5.3°C/min and held 6.7 min. Injection volume: 0.2μL.

Results and Discussion
Chromatographic resolution and analysis times are dependant on column dimensions (length, id, phase thickness)[3]. Table 1 summarizes a number of column options for the analysis of PAHs and dioxins/furans. The conventional 5% phenyl columns (30M - PAH and 60M - dioxins) used are listed in the centre shaded columns of the table. Data in Table 1 shows that analysis times can be reduced considerably by using 10M or 20M columns for PAH analysis and 20M or 40M columns for dioxins. Accurate conversion of conventional methods to Fast methods is critical to ensure that chromatography is identical and analytes are identified correctly. In some instances, especially for isomers and similar compounds, peak shifting or reversal can occur when temperature ramps are modified. It is important that all components are checked separately to confirm retention times on the fast chromatographic system. Also, column performance criteria must be met and critical pairs for analysis must be resolved if a shorter column is used. For dioxin analysis using a 5% phenyl column, 2,3,7,8-TCDD must be separated from its nearest neighbours - the 1237/1238-TCDD unresolved pair eluting before and 1239-TCDD eluting after. The GC column must be capable of achieving about 175,000 theoretical plates to resolve 2378-TCDD from the 1237/1238-TCDD unresolved pair. This criterion can easily be met on both 40M and 60M columns, however, the analysis can be completed in 1/2 of the time on the 40M

column. The 20M column is also capable of meeting these requirements in about 1/4 of the time, however, there is little room for trimming the column when column performance begins to deteriorate with use. Analytical runs for PAH are typically 35 to 40 minutes long. Using a 5% phenyl column, Benzo[b]fluoranthene (B[b]F) and Benzo[k]fluoranthene (B[k]F), a critical pair for PAH analysis, must be separated. Figure 1 shows the mass chromatogram of B[b]F and B[k]F. The 20M column provides better chromatographic separation in about half the analysis time as compared to the 30M column.

TABLE 1: Comparison of Gas Chromatographic Columns

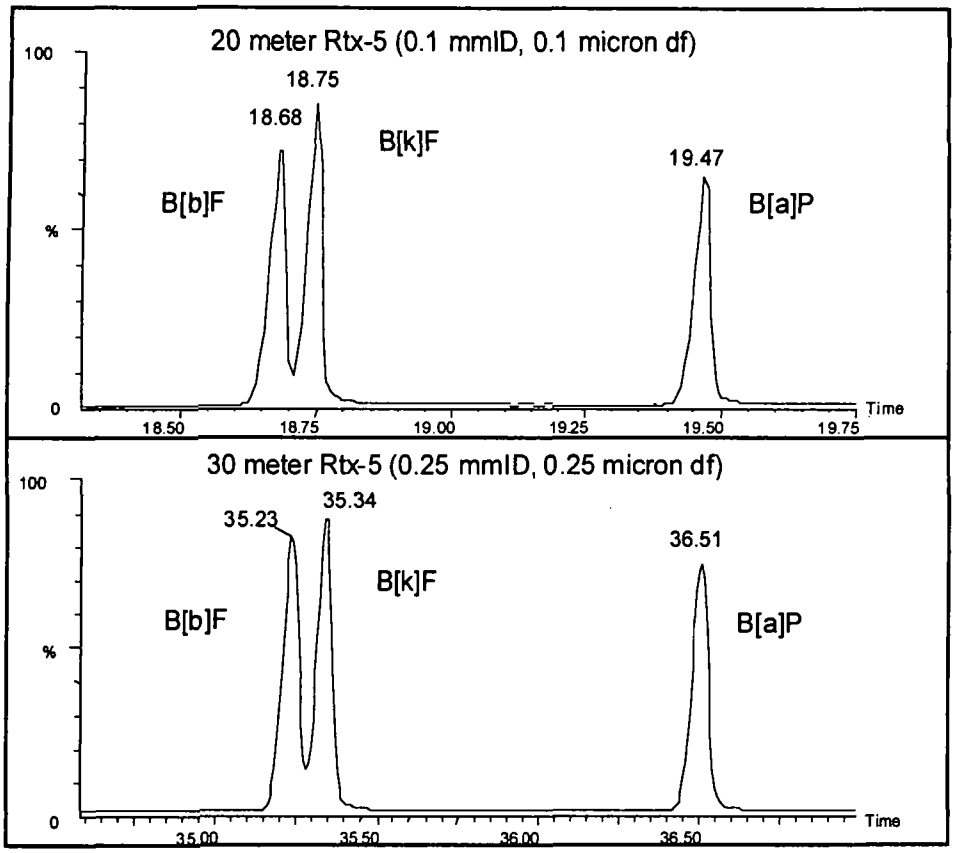
Analysis	PAH			DIOXIN		
Column Length (M)	10	30	20	20	60	40
i.d. (mm)	0.1	0.25	0.1	0.1	0.25	0.18
Film Thickness (μm)	0.1	0.25	0.1	0.1	0.25	0.18
Theoretical Plates / M	8,600	3,300	8,600	8,600	3,300	5,300
Theoretical Plates (Total)	86,000	99,000	172,000	172,000	198,000	212,000
Relative Column Efficiency	0.93	1	1.32	0.93	1	1.03
Relative Analysis Time	**0.38**	**1**	**0.50**	**0.25**	**1**	**0.51**

Table 2 contains results for the analysis of EC-3 reference material certified PCB congeners. The complete list of congeners analysed includes BZ#s:18, 19, 22, 28, 33, 37, 44, 49, 52, 54, 70, 74, 77, 81, 87, 95, 99, 101, 104, 105, 110, 114, 118, 119, 123, 126, 128, 138, 149, 151, 153, 155, 156, 157, 158, 167, 168, 169, 170, 171, 177, 178, 180, 183, 187, 188, 189, 191, 194, 199, 201, 202, 205, 206, 208 and 209. The conventional analytical run times using 60M columns were reduced from 80 minutes to 18 minutes on the 20M columns. Data reported for the most common certified congeners are listed and are in excellent agreement with the certified values.

TABLE 2: Analysis of PCB Congener Reference Sediment: EC-3

PCB CONGENER	EXPECTED VALUE (ng/g)	N	AVERAGE
18	9.0 ± 4.7	8	9.2
28	18.6 ± 8.6	8	14.7
52	35.6 ± 12.9	8	28.9
105	13.1 ± 4.3	8	18.9
118	28.5 ± 5.4	8	28.6
138	25.2 ± 6.3	8	26.0
153	24.2 ± 4.1	8	22.6
170	8.9 ± 1.3	8	9.6
180	15.4 ± 6.6	8	13.7

Figure 1: PAH Critical Pair



Conclusions:

Fast GC can be used for the analysis of a number of persistent organic pollutants (POPs) to significantly reduce analysis times with increased column efficiency in a number of instances. Temperature programs and methods can be converted from conventional 60M and 30M columns to 40M, 20M and 10M columns. Data quality (precision and accuracy) is not affected when using Fast GC. All areas/parameters of the chromatographic system must be adjusted and optimized to ensure proper chromatographic performance. Smaller injection volumes (0.2 - 0.5μL) and injection liners (1 - 2 mm) are critical to obtain optimum (reproducible) chromatography on 0.1 and 0.05mm id columns. Analysis times can also be reduced on older gas chromatographs that do not have electronic pressure programming or the ability to produce the fast temperature ramp rates required for Fast GC. Increasing column head pressures to above 30psi and using a 40M, 0.18μm, 0.18mm column can reduce analysis times by 50% when compared to 60M columns. Similar results can also be achieved by switching to 20M, 0.18μm, 0.18mm columns from 30M, 0.25μm, 0.25mm columns.

References

1. Hinshaw, J.V. (1995) LC-GC, Volume 13, Number 12, December, 994.
2. Dorman, F. (1998) The Advantage - Restek, Winter, 1.
3. Sellers, K. (2000) The Advantage - Restek, Winter, 13.