DEVELOPMENT AND EVALUATION OF AN AUTOMATED LIQUID CHROMATOGRAPHIC APPARATUS FOR ISOLATING PCDD/PCDF FROM COMPLEX SAMPLE MATRICES

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ABSTRACT

A computer-controlled low-pressure liquid chromatographic apparatus has been constructed to isolate polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) from sample extracts using a combination of silica, alumina and carbon columns. The automated procedures may be applied to many different types of samples and the prepared extracts often contain lower levels of extraneous compounds than manually prepared samples.

INTRODUCTION

Automated liquid chromatographic procedures for isolating PCDD/PCDF from sample extracts require fewer sample manipulations and are potentially more reproducible than manual preparation procedures. In addition, automated systems can be programmed to perform operations which would not be practical in manual procedures such as significantly extending a time-consuming elution to more thoroughly rinse unwanted matrix material from a column. An automated system for the preparation of biological samples for analysis of 2,3,7,8-TCDD only has been described previously.[1] As procedures are developed to quantify the entire set of PCDD/PCDF in a variety of sample matrices at concentrations in the part-per-quadrillion range, the higher efficiency and consistency afforded by automated liquid chromatographic procedures will be needed to reduce the levels of undesirable compounds resulting from the extraction of larger quantities of sample.

This paper describes development and testing of a computer-controlled low-pressure liquid chromatographic system which automates the manual liquid chromatographic procedures which are presently employed to isolate all PCDD/PCDF congeners from sample extracts.[2,3] Prior to applying these automated procedures, the sample is manually extracted, and the extract is washed with acid and base, dried over sodium sulfate, and concentrated. These initial operations ensure that a significant portion of the matrix material and water are removed from the sample extract, preventing blockage and deactivation of the columns in the automated system. As many as five concentrated sample extracts can be loaded into the automated system at one time, and a computer-controlled series of valve operations then allows the extracts to pass sequentially through a series of packed columns. The final toluene fraction containing the PCDD/PCDF is then manually concentrated for GC/MS analysis.

EXPERIMENTAL

A schematic diagram of the automated sample processing apparatus (FMS System PCDD/PCDF-A) which was developed in the present study is shown in Figure 1. This system is configured to sequentially chromatograph as many as five sample extracts through low pressure glass columns which are packed with silica gel, alumina and carbon. Separate silica gel and alumina columns are used to chromatograph each of the five samples while a single carbon column is automatically regenerated prior to use with each extract. The dimensions of the glass chromatography columns which were used in the initial tests of the automated system are the same as the dimensions of the gravity flow columns which are currently employed in typical manual preparation procedures.[2,3] Eight valve-control modules are used to direct the fluid flow through the fourteen three-port and six-port solenoid valves which are incorporated into the system. The valves shown in Figure 1 are labelled (M1 through M8) to indicate the control module which operates each valve. Where two valves are operated by a single module, such as M3, the identically numbered ports on both of these valves are opened simultaneously to create a flow path between these valves. Automated valves controlled by M1 are used in conjunction with manually-controlled valves A, B, and C1 through C5 to load the sample extracts into the sample loops. Automatically-controlled valves are then used to direct the samples through the columns into receiving vessels, pre-elute the silica and carbon columns, regenerate the carbon column, and control the direction of flow through the carbon column. A microprocessor system controls the eight valve modules and the flow rate

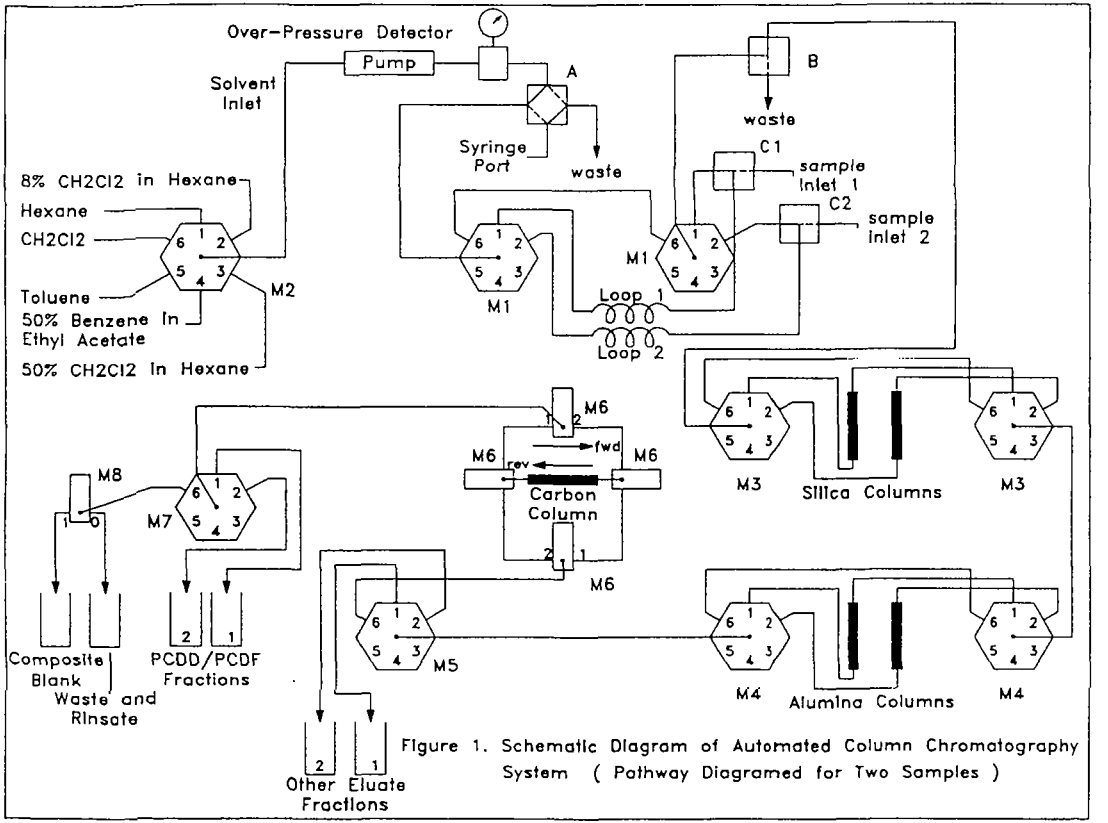

Figure 1. Schematic Diagram of Automated Column Chromatography System (Pathway Diagramed for Two Samples)

of the piston pump. While this microprocessor may be used alone to control the system, it may also be connected to a personal computer through a serial port. Programs containing information on flow rates, volumes, and valve positions which are stored on the computer are transferred to the microprocessor in the course of operating the system. The modular construction of the system and the flexibility of the programming allow the system to be adapted to accomodate virtually any desired cleanup protocol. In the present study, one set of programs has been developed to automate the isolation of the tetra- through octa-CDD/CDF isomers, and another set of programs has been developed to optimize the isolation of only the 2,3,7,8-TCDD and 2,3,7,8-TCDF isomers.

RESULTS AND DISCUSSION

Experiments Conducted to Develop and Evaluate the Automated System. In the course of manual liquid chromatographic cleanup of sample extracts during PCDD/PCDF analysis, the eluates from the silica column and the alumina column which contain these analytes are concentrated before proceeding with the carbon-column chromatographic separation. In order to simplify the design of the automated system, these concentration steps were omitted, and the activated alumina column directly receives the hexane eluate from the silica column, while the carbon column directly receives the 50% methylene chloride-in-hexane eluate from the alumina column. In order to demonstrate that PCDD/PCDF can be effectively chromatographed through the automated system without using the intermediate concentration steps, a solution containing each of the 2,3,7,8-substituted PCDD/PCDF isomers was loaded into Loop 1 of the automated system, while pure hexane solvent was loaded into Loop 2. The apparatus was then sequenced, after introducing the solution in Loop 1, and the hexane and 8% methylene chloride-in-hexane eluate fractions from the alumina column were collected separately. These were found to contain no detectable quantities of PCDD/PCDF. PCDD/PCDF isomers were also not detected in the 50% methylene chloride-in-hexane eluate fraction from the alumina column, after it had passed through the carbon column, indicating that the carbon column had retained all of the PCDD/PCDF isomers. The final 6 mL of toluene eluate from the carbon column, which was automatically collected after reverse elution at M7, was found to contain 70-97% of the several PCDD/PCDF isomers which had initially been introduced into Loop 1. This experiment verified that the intermediate solvent concentration steps were not essential to the cleanup process. However, when the hexane solvent in Loop 2 was subsequently chromatographed through the appartus, the final toluene eluate fraction from the carbon column was found to contain 2-9% of the PCDD/PCDF isomers which had been introduced via Loop 1, and were apparently still resident in the system when the hexane in Loop 2 was introduced.

It appeared that the most probable cause of the cross-contamination of processed samples which was observed in these experiments was the carbon column. Consequently, in a second series of tests, the automated program was modified to elute the PCDD/PCDF from the carbon column with 20 mL of toluene, but then the carbon column was purged with an additional 46 mL of toluene. The last portion of toluene was collected and analyzed separately from the initial toluene eluate. The initial toluene eluate fraction was found to contain 73-100% of the several 2,3,7,8-substituted PCDD/PCDF isomers which had been introduced into Loop 1. Moreover, the final toluene purge fraction, which had been used to ensure complete regeneration of the carbon column contained no detectable quantities of PCDD/PCDF, indicating that these compounds had been removed completely by the initial elution with 20 mL of toluene. The final carbon column eluate which was collected during the subsequent automated-system processing of the hexane solvent from Loop 2 however, still contained 0.2-1.1% of the PCDD/PCDF isomers from the initial injection with Loop 1. Obviously, holdup of PCDD/PCDF was occurring elsewhere in the apparatus.

In an effort to simplify the automated system and restrict the number of automated valves used, the initial configuration of the system, which was employed for the tests just described, incorporated two three-port valves which were connected to tees at each end of the carbon column to permit forward and reverse flows through this column. With this initial design, a single sample inlet port was provided on Valve B. Following the initial tests described above, the automated apparatus was modified by replacing the two tees with three-way solenoid valves, as shown in Figure 1, in an attempt to eliminate the possibility of solvents diffusing into unflushed sections of connecting tubing. The inlet system was also modified to provide separate manual three-way valves and inlet ports for each sample loop. The system configured as shown in Figure 1 was tested in a third set of experiments by again introducing the 2,3,7,8-substituted PCDD/PCDF isomer solution into Loop 1, and introducing pure hexane into Loop 2. When these two samples were sequentially chromatographed using the modified automated system, the final carbon-column eluate from chromatographing the Loop 1 solution was observed to contain all of the PCDD/PCDF isomers

which had been introduced into Loop 1, while the final carbon column eluate from chromatographing the pure hexane in Loop 2 contained no detectable PCDD/PCDF. On the basis of the prevailing limits of detection for PCDD/PCDF in these tests, the levels of these compounds in the second sample were lower by at least a factor of 300-500 than the levels detected in the first sample. It is clear that the modifications of the automated system described above eliminated cross-contamination of processed samples resulting from carryover of analytes. In still futher tests, extracts of actual samples (paper products and wastes) were successfully processed using the modified automated apparatus, with no carry-over problems. Additional tests of this system using complex flyash extracts are in progress.

Application of Automated Procedures to Sample Extracts. Prior to the inlet system modifications and the redirection of solvent flows around the carbon column which were just described, the automated system was employed to process an extract of a paper coffee filter sample. Before implementing the automated chromatographic cleanup, the sample was spiked, extracted, washed with acid and base, dried, concentrated, and loaded into Loop 1. The final eluate from the carbon column was collected, concentrated and analyzed by GC/MS. Another portion of the same filter sample had been previously prepared using the manual chromatographic procedures, and also analyzed by the same GC-MS techniques. PCDD/PCDF data obtained by using the automated cleanup methods are compared with corresponding data obtained using manual cleanup procedures in Table 1. These data are in reasonable agreement and essentially the same interferring compounds were observed in both types of samples following cleanup. However, these interferences were present in significantly higher concentrations in the sample prepared with the automated system. These interferences were most prominent in the chromatograms of the hexa-CDD/CDF isomers, causing distortion of the internal standard peaks and leading to detection limits as high as 68 ppt for 1,2,3,4,7,8-hexa-CDD. This suggested a need for increased cleanup capacity in the automated system.

In order to improve the cleanup efficiency of the automated system a larger activated alumina column (8 mm I.D. x 250 mm length), having a capacity of 12 g of alumina, was constructed and incorporated into the automated apparatus. The elution profile of this column was evaluated using all twenty-two TCDD and all thirty-eight TCDF isomers, along with all of the other 2,3,7,8-substituted PCDD/PCDF isomers. Using the higher capacity alumina column and the automated system configured as shown in Figure 1, a second coffee filter extract was processed. The automated cleanup of this sample extract and a blank each required approximately four hours to complete. In contrast to the sample extract prepared in the earlier experiment, the extract prepared using the higher capacity alumina column exhibited significantly lower levels of interferences in the mass chromatograms than the manually prepared sample. In additional experiments, the performance of the automated system has been evaluated with other sample matrices including pulp and paper industry products and sludges, flyash, aqueous effluents from waste processing, and environmental water samples. For each of these sample matrices, PCDD/PCDF data obtained for the samples prepared using the automated system have been compared with corresponding data obtained for samples prepared using manual techniques, and in all cases, the results from the automated cleanup are of superior quality.

Table 1
Comparison of Concentrations of PCDD/PCDF Congeners in Coffee Filter Extracts Prepared Using Manual and Automated Procedures

	Concentrations in pg/g	
PCDD/PCDF Congeners	Manual Procedure	Automated Procedure
Tetra-CDFs	38.6	30.6
Tetra-CDDs	5.41	6.76
Penta-CDFs	1.73	6.41
Octa-CDD	16.7	17.0

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