AN AUTOMATED SAMPLE CLEANUP APPARATUS USED IN THE PROCEDURE FOR POLYCHLORINATED DIBENZO-p-DIOXINS (PCDDs), DIBENZOFURANS (PCDFs), ORTHO-SUBSTITUTED (NON-PLANAR) AND ORTHO-UNSUBSTITUTED (PLANAR) BIPHENYLS (PCBs) IN SERUM AND ADIPOSE TISSUE

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ABSTRACT

A new automated sample cleanup apparatus for PCDDs, PCDFs, and PCBs in serum and adipose tissue has been developed. Compared to a prototype model, this versatile and easy to use device has reduced the time required to perform the first portion of the method from twenty to four hours.

INTRODUCTION

The accurate determination of PCDDs, PCDFs, and PCBs at the parts-per-quadrillion level (on a whole-weight basis) presents many analytical problems. Among the most difficult are the extraction of these compounds from a biological matrix and the removal of a wide variety of polychlorinated aromatic cocontaminants that could interfere with the analyses. The sample enrichment scheme selected by CDC and modified, as previously reported, for the analysis of human specimens (Patterson *et al.*, 1986, and Patterson *et al.*, 1987) was the five-column method of Smith, Stalling, and Johnson (1984). Isotope-dilution mass spectrometry is the basis of quantification in these methods. After spiking 50-100 g serum or 1-5 g adipose tissue samples with the appropriate carbon-13 labeled internal standard(s), the procedure is carried out in three parts, each having multiple steps: Part 1) extraction, cleanup and selective adsorption of PCDDs, PCDFs, and planar PCBs onto activated carbon; after elution from the carbon, Part 2) additional cleanup and fractionation of the residues using activated acid alumina; and Part 3) analysis of the analytes of interest in the enriched sample by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Initially, a week's time was required for an analyst to manually process a run of five samples (a blank, three unknowns, and a quality control sample) through Parts 1 and 2 of the method.

EXPERIMENTAL

Using Fluid Robotics technology, Fluid Management Systems, Inc. (FMS) assisted the CDC in upgrading its prototype automated extraction and enrichment system (Lapeza, Patterson, and Liddle, 1986). Figure 1 shows the new automated apparatus (FMS System 2378), which consists of a microprocessor, management module, valve module, self-calibrating pump, and pressure-protection

gauge. All fluid interconnections of the system are made of 1/8 in o.d. Teflon tubing and 1/4 in tube end fittings. Pump tubing is made of Masterflex Viton tubing, a solvent-resistant fluoroelastomer, and the only component of the system coming in contact with samples not made of glass or Teflon. In performing a run, five identical programs are sequentially executed, one for each sample. Each program consists of a series of nine tasks. These tasks are entered by keypad, via a menu-driven liquid crystal display. Programs are stored in the microprocessor and through the management module, selectively activate the five 6-way and two 2-way electric-solenoid valves on the valve module to control sample and solvent selection, direction and route of solvent flow, and the flow rate and volumes dispensed by the pump.

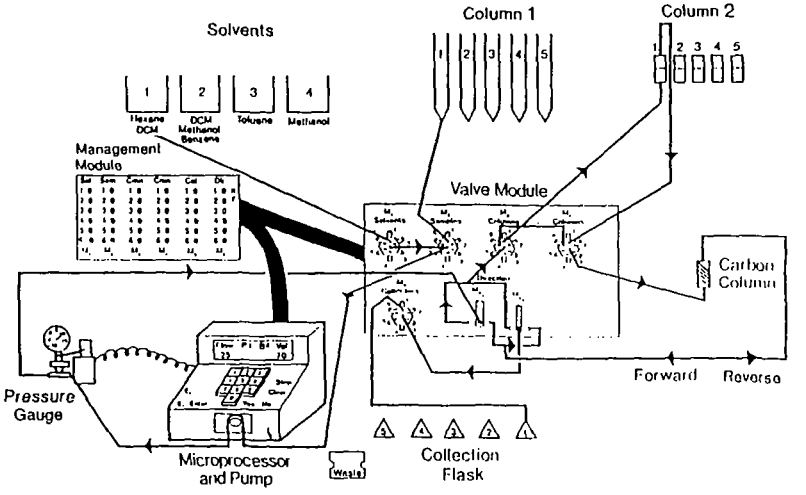

Fig. 1 Components of the FMS System 2378 automated sample enrichment apparatus for human serum and adipose tissue PCDDs, PCDFs, and PCBs.

In the automated component of the procedure (Part 1), the spiked samples are loaded onto individual columns and 50% dichloromethane (DCM)/hexane [v/v] added to a volume of 500 mL. The eluates are passed, in the same process, through the following adsorbents: a) Column 1 - potassium silicate, sulfuric acid impregnated silica gel, and silica gel; b) Column 2 - potassium silicate and silica gel; and c) Column 3 - activated carbon. During the first four tasks of a program, PCDDs, PCDFs and planar PCBs are extracted and selectively retained on Column 3. The remaining five tasks are involved in rinsing and regeneration of Columns 2 and 3. Column 1 is prepared fresh for each sample. The five Column 2's can be reused 2-3 times for adipose tissue and 3-5 times for serum samples. Column 3 may be utilized for an extended period of time, unless it becomes grossly contaminated.

The following nine tasks in Part 1 are executed, with the system operating at a flow rate of 25 mL/min: 1) 500 mL of the sample in 50% DCM/hexane are eluted from Column 1, through Columns 2 and 3 to waste; 2) Columns 2 and 3 are washed with 75 mL 50% DCM/hexane in the forward direction to waste; 3) Columns 2 and 3 are washed with 50 mL DCM/methanol/benzene (75:20:5,v/v) in the forward direction to waste; 4) 70 mL toluene is pumped through Column 3 in the reverse direction and the sample collected in a 125 mL round-bottom flask; 5) Column 3 is washed with 90 mL toluene in the reverse direction to waste; 6) Column 3 is washed with 100 mL methanol in the reverse direction to waste; 7) Column 3 is washed with 50 mL toluene in the reverse direction to waste; 8) Column 3 is washed with 50 mL 50% DCM/hexane in the reverse direction to waste; and 9) Columns 2 and 3 are flushed with 160 mL 50% DCM/hexane in the forward direction to waste. After completeion of Part 1, each of the samples (in toluene) are subjected to rotary evaporation and the second half the procedure is performed manually.

In Part 2, after a solvent exchange to hexane, the samples are manually applied to and eluted through two additional sets of columns, arranged in tandem. Column 4 contains cesium silicate and sulfuric acid impregnated silical gel. The eluate from this column flows directly onto Column 5, containing activated alumina on which the fractionation of residues is accomplished by elution with three solvents: hexane, followed by 2% DCM/hexane (v/v), and 50% DCM/hexane. PCDDs, PCDFs and planar PCBs are recovered in the last fraction. Solvent from these eluates is reduced in volume under a stream of nitrogen, transferred to 1 mL silanized glass vials, and allowed evaporate at room temperature. Finally, the vials are sealed using Teflon-faced silicone septa and crip-top aluminum seals. Samples are reconstituted with a carbon-13 labeled recovery standard solution prior to analysis by HRGC/HRMS.

An optional software package enables the System 2378 to be operated through a personal computer (via RS-232 interface, greatly expanding the number of programming steps available) making it possible, by modifing and increasing the number of the tasks in Part 1, to collect additional fractions containing non-planar PCBs (Patterson *et al.*, 1989).

RESULTS AND DISCUSSION

The FMS System 2378 has substantially reduced the time required to carry out Part 1 of the procedure from twenty hours with the prototype version, to four hours. One analyst can now process three to four runs (15-20 samples) through the entire procedure in a week. Utilizing six automated systems and four high-resolution mass spectrometers, it has been possible for CDC to analyze more than 6000 serum and adipose tissue samples from several large-scale epidemiologic studies for PCDDs, PCDFs, and PCBs in the past five years. An overview of laboratory quality assessment procedures and long-term method performance is presented in an accompanying paper in this issue (Turner *et al.*, 1990).

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