

An Improved SPE Extraction and Automated Sample Cleanup Method for Serum PCDDs, PCDFs, and Coplanar PCBs.

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1. INTRODUCTION

It is commonly recognized that sample cleanup and enrichment is critical to performing ultratrace analysis of 2,3,7,8-chlorine substituted PCDDs/PCDFs and coplanar PCBs (c-PCBs) by gas chromatography/mass spectrometry (GC/MS). The multi-column cleanup procedure of Smith, Stalling, and Johnson¹ (SSJ), incorporating both the specific adsorption properties of activated carbon for planar aromatic compounds and alumina chromatography to remove chlorinated organic interferences, has been one of the most widely used. Since its introduction in 1984, the SSJ method has been continually adapted and applied to a variety of sample matrices. In 1985, the five-column SSJ method was modified and semi-automated by the Centers for Disease Control and Prevention (CDC) for the analysis of these environmental toxicants in human serum and adipose tissue specimens.^{2,3} At *Dioxin '90*, Tiernan *et al.*^{4,5} of Wright State University (WSU) reported the development and evaluation of an automated liquid chromatographic apparatus for isolating PCDDs/PCDFs from complex sample matrices based, in principle, on the SSJ method, using Fluid Management Systems' (FMS) Fluid RoboticsTM technology. The following year, CDC made two fundamental changes to the WSU method. We eliminated the manual sample inlets and loops, thereby simplifying the procedure for our application to serum extracts; and the substitution of alumina A - Super I for the highly activated basic alumina originally used. We presented a preliminary evaluation of this modified FMS Dioxin Prep SystemTM at *Dioxin '92*.⁶ This year we further modified the Dioxin Prep System to accommodate five carbon columns. Each sample extract can now be processed through individual sets of silica, alumina, and carbon columns to eliminate cross-contamination among samples.

Although we have made progress in automating the CDC cleanup procedure, it still had several labor intensive manual steps. After serum samples were spiked with ¹³C-labeled internal standards and mixed, analytes had to be isolated by liquid-liquid extraction. The hexane extracts had to be recovered, combined, and washed 10 times with sulfuric acid, followed by three extractions with water. Subsequently, the hexane extracts had to be reduced in volume (from 200 mL to 10 mL) before the automated chromatography on the Dioxin Prep System. Also reported here is the development of a new semi-automated procedure for the initial extraction of these analytes from serum, using C₁₈-bonded-silica solid-phase-extraction (SPE) cartridges, based on the reverse phase extraction technique described by Chang *et al.*^{7,8}

2. EXPERIMENTAL

Dioxin Prep Sample Cleanup Systems. Figures 1 and 2 show plumbing diagrams for the one and five carbon column Dioxin Prep Systems, respectively. These Systems are computer controlled, and operate automatically and unattended after columns are attached. With both Systems, five hexane extracts are loaded, and pumped sequentially through five individual sets of multi-layer silica and alumina columns. Alumina columns are subsequently washed with additional hexane and 2% dichloromethane (DCM)/hexane, and the washes sent to waste. PCDDs/PCDFs and c-PCBs are eluted from alumina with 50% DCM/hexane and transferred to the carbon column(s). Eluates containing the PCDDs/PCDFS and c-PCBs are individually collected by reverse elution from carbon column(s) with toluene. In the one carbon column configuration, the single common carbon column is regenerated between samples and reused.

As isomer specific analysis for the PCBs has continued to develop, it appears that PCB isomers other than the non-*ortho* chlorine-substituted PCBs (c-PCBs) can contribute significantly to the overall total of dioxin toxic equivalents (TEQs) in some samples.⁹ In preliminary experiments we have demonstrated that the 2% DCM/hexane fractions can be diverted by the M7 and M8 valves on the Dioxin Prep System and collected separately for each sample, instead of being sent to waste. These fractions contain mono-*ortho*- and di-*ortho*-substituted PCBs.

Zymark TurboVap[®] II Concentration Workstation. We have replaced our vacuum-style rotary evaporators with Zymark TurboVap II Workstations. The micro-processor-controlled TurboVap II employs a nitrogen manifold that creates a solvent vortex that produces fast, efficient evaporation. The TurboVap II has optical sensors that monitor the concentration process and stops the evaporations at a 0.5 mL fixed end-point. The workstation automatically handles, unattended, up to six samples at a time, and signals when each sample evaporation is done. We now use the TurboVap II to concentrate both hexane extracts and toluene eluates. Concentrated toluene eluates are transferred from the TurboVap tubes to 1-mL silanized glass vials (to which 1 μ L of dodecane has been added) with three 0.5 mL washings of DCM and evaporated under a stream of nitrogen to a volume of $\approx$ 100 μ L. The remaining solvent is allowed to evaporate at room temperature until only the 1 μ L of 'keeper' remains. The vials are then sealed with Teflon-faced silicone septa and crimp-top aluminum seals and stored at room temperature until GC/MS analyses are performed.

Solid Phase Extraction (SPE) Method. A new SPE extraction method was developed to process five serum samples at a time. Weigh sera (5 to 100g) into 250 mL Teflon bottles, spike with ¹³C-labeled internal standards, and mix for 30 min on a wrist-action shaker. Add a volume of formic acid equal to the weight of the serum, swirl gently and allow the mixture to degas for 15 min. Add a volume of high-purity water to serum/formic acid mixture equal to that of the formic acid and swirl gently. Attach 10 g (75 mL) C₁₈ SPE cartridges [United Chemical Technologies, Inc. (#CEC18110-M75)] to peristaltic pump tubing [Cole-Parmer Instrument Co. Masterflex variable-speed drive (#07553-80), Ismatic minicartridge pump and cartridges (#07623-10 and #07624-65), and 2.79 mm O.D. Viton tubing]. Pump 100 mL methanol through SPE cartridge at 20 mL/min into waste container. Pump 100 mL high-purity water through cartridge at 20 mL/min into waste container. Pump the serum/formic acid/water mixture through the activated SPE cartridges at 5 mL/min into waste container. Do not let SPE cartridges pump to dryness

during these steps. Then rinse sample container with 10 mL water and add to SPE cartridge. Pump until all liquid is removed from the cartridge. Pump an additional 10 mL water at 5 mL/min until the cartridge is dry. Add 2 mL methanol to cartridges and pump the cartridge dry. Remove cartridges from pump and attach to a SPE vacuum manifold and dry for 1 hr under vacuum at -10 psi. Wash pump tubing with 25 mL 50% DCM/hexane, then 25 mL hexane at 20 mL/min into waste. Reattach cartridges to peristaltic pump tubing. Place exit end of pump tubing into labeled solvent rinsed 20x150 mm glass tube. Pump 12 mL hexane at 5 mL/min until cartridge is dry. Repeat with another 12 mL hexane. Pump an additional 5 mL hexane at 5 mL/min. Collect the three hexane eluates in a 20x150 mm tube. Remove any water from the bottom of the tube with Pasteur pipet and cap the tube with Teflon lined caps until ready to be processed on the Dioxin Prep System. We observed a notable improvement in the precision of our recoveries by performing the SPE elution steps using the flow control of the peristaltic pump compared to that afforded by the conventional SPE vacuum manifolds.

3. RESULTS AND DISCUSSION

After routinely using five of the one-carbon column Dioxin Prep Systems for over a year and five of the five-carbon column Dioxin Prep Systems for seven months, we estimate that our solvent usage had been reduced by half, compared to that of our original semi-automated version of the SSJ method. In addition, since the disposable pre-packed silica, alumina, and carbon columns made of Teflon are now commercially available from FMS, we were able to eliminate one full time staff position which had been dedicated solely to preparing the various adsorbents and packing columns. While we routinely achieved adequate recoveries for all analytes, we observed that about 0.5% carryover can occur between samples with the one carbon column systems. This amount of carryover is negligible for the low-level (fg/g) human samples we analyze from epidemiologic studies. This level of carryover could pose a problem with higher-level (pg/g to ng/g) samples from other sources. We have essentially eliminated cross-contamination among samples by using the five carbon systems, provided that the Teflon tubing on the Dioxin Prep Systems is adequately flushed with solvent between runs.

Replacing multiple rotary evaporations with the TurboVap II Concentration Workstation for simultaneous and automatic evaporation of toluene eluates has significantly increased the overall recovery of the analytes in this step of the procedure and freed sample-prep analysts' time to perform other tasks.

The the new semi-automated method for the initial extraction of these analytes from serum, using C_{18} bonded silica solid-phase extraction (SPE) cartridges takes about half the time to extract five samples using the SPE method, as compared to the manual liquid-liquid extraction procedure. In addition, the time required to clean Teflon labware as well as costs are reduced.

The method enhancements described above make the sample cleanup method easier to perform and more reproducible. One analyst can easily cleanup five samples a day and routinely achieve recoveries of 70-90% for all analytes.

4. REFERENCES

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Dioxin-Prep System (1 Carbon Column) Plumbing Diagram

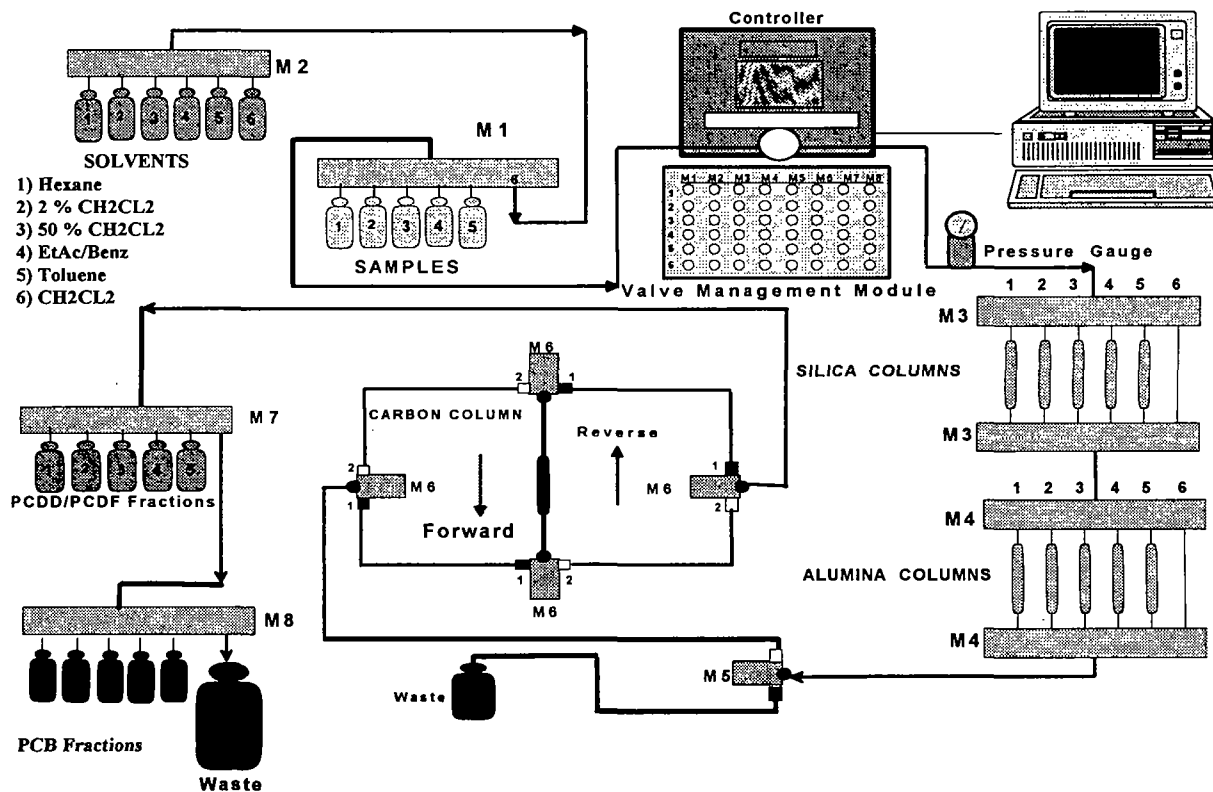


Figure 1. Plumbing diagram for one-carbon column FMS Dioxin Prep System

Dioxin-prep™ System (5 Carbon Column) Plumbing Diagram

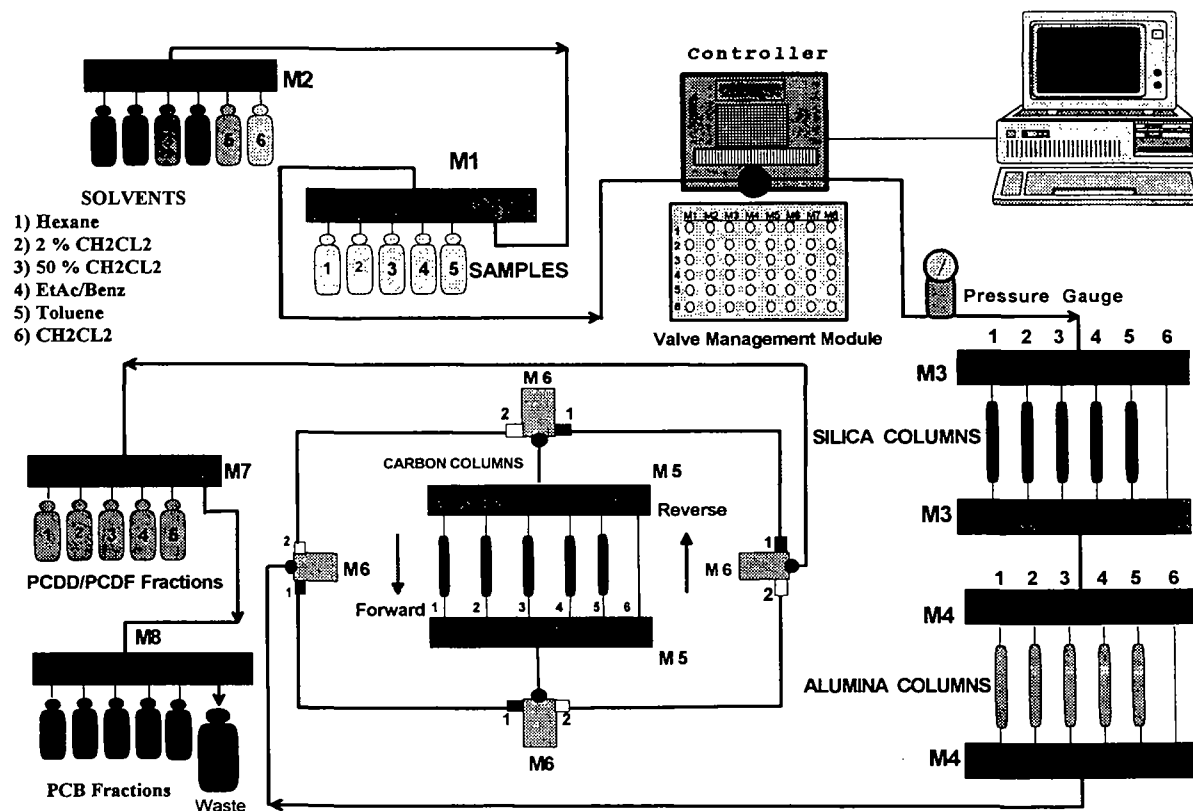


Figure 2. Plumbing diagram for five-carbon column FMS Dioxin Prep System