AN EVALUATION OF THE FMS DIOXIN PREP SYSTEM™ FOR AUTOMATED SAMPLE CLEANUP ADAPTED TO HUMAN SERUM

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ABSTRACT

The results of our preliminary evaluation of Fluid Management Systems'(FMS) Dioxin Prep System™, applied to the cleanup of human serum samples for PCDDs, PCDFs, and planar PCBs, is presented. This new automated low pressure chromatography system uses disposable pre-packed silica, alumina, and carbon columns made of Teflon and all its components are controlled by a personal computer. When all facets of sample cleanup (reagent, solvent, support preparation, sample extraction, and chromatography) are considered, we estimate that the new procedure takes about half the time to perform and uses about half the volume of solvents than our existing semi-automated method. Using a spiked human serum quality assurance pool, we found good agreement between the two methods for the eighteen 2,3,7,8-chlorine substituted PCDDs/PCDFs and four planar PCBs measured. We observed recoveries of 80-90% for all 22 analytes using the FMS System, compared to an average of 55-60% with our semi-automated procedure.

INTRODUCTION

Most of the evidence for the presence of PCDDs, PCDFs, and planar PCBs in samples of human origin has been found over the last 5-7 years, during which analytical techniques have been developed to a higher degree with regard to sample enrichment, availability of standards, detection limits, and specificity[1]. In 1985, Albro *et al.*[2] reported the results of an inter-laboratory study of eight cleanup methods and concluded that there was no one method clearly and conspicuously "better" than all other methods in regard to this particular set of analytes. However, since Albro's report the multi-column cleanup procedure of Smith, Stalling, and Johnson[3] (SSJ), incorporating both the specific adsorption properties of activated carbon for planar aromatic compounds and alumina chromatography to remove numerous chlorinated organic interferences, has been one of the most widely used, best documented, and extensively validated methods for cleanup of human samples.

In 1985, the five-column SSJ method was modified and semi-automated by the Centers for Disease Control (CDC) for the analysis of human serum and adipose tissue specimens[4,5]. Since then we have used this semi-automated cleanup procedure to analyze more

than 5000 serum and adipose tissue specimens for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and over 1000 other serum specimens for TCDD and 17 additional 2,3,7,8-chlorine substituted PCDDs/PCDFs and four planar PCBs from various large-scale epidemiologic studies. In general, epidemiologic studies of human exposure have been limited in some egard in their exposure assessment and/or statistical power. This arises in part from the time and expense involved in the generation of actual body burden data. Providing reliable quality exposure assessment data using less sample, on a more timely, and/or cost effective basis is a challenge that requires laboratories to continually evaluate new methods and changes in related technology. The results of our preliminary evaluation of a new, commercially available, automated sample cleanup apparatus, is presented.

EXPERIMENTAL

Outline of CDC semi-automated procedure[4,5]. After a serum sample is spiked with ^{13}C-labeled internal standard(s) and manually extracted, our cleanup procedure is carried out in two parts: Part 1 - (Automated) The sample extract in 50% dichloromethane (DCM)/hexane is pumped in the forward direction serially through Column 1 [silica gel, acid silica gel, and potassium silicate], Column 2 [potassium silicate and silica gel], and Column 3 [AX-21 carbon]. After several washings, the sample is eluted from the carbon column in the reverse direction with toluene. Part 2 - (Manual) After evaporation of toluene, the sample is redissolved in hexane and passed serially through Column 4 [acid silica gel and cesium silicate] and Column 5 [acid alumina]. After removing Column 4, Column 5 is washed with 2% DCM/hexane and the eluate discarded. The enriched sample is eluted from alumina with 50% DCM/hexane and the solvent evaporated before analysis by HRGC/HRMS.

Evaluation and modification of the WSU automated procedure. At Dioxin '90 in Bayreuth F.R.G, Tiernan *et al.*[6,7] of Wright State University (WSU), in collaboration with FMS, reported the development and evaluation of a totally automated method for isolating PCDDs/PCDFs from a variety of complex sample matrices. We elected to evaluate the WSU method, using serum extracts prepared by the CDC procedure. Although, the CDC method and that of WSU employ essentially the same chromatographic materials and solvents, there are several differences between them: a) the order of columns through which a WSU sample is sequentially eluted - silica column [silica gel, potassium silicate, silica gel, acid silica gel, and silica gel], alumina column, and carbon column; b) the amounts of silica-based supports are considerably less than used by CDC; c) the WSU method uses 12 g of basic alumina instead of 3.65 g of acid alumina; d) the WSU method uses 22 mg of AX-21 carbon, whereas the CDC method uses 70 mg; e) the WSU method does not use cesium silicate; f) the WSU method uses basic instead of acid alumina; and g) and all steps in the WSU method are automated using the expanded memory of a personal computer connected through a serial port in the microprocessor/pump module. Similarities between the two methods include: a) the first two eluates from the alumina column are discarded, b) the 50% DCM/hexane alumina eluate containing PCDDs/PCDFs/PCBS is retained; c) the congeners of interest are eluted from the carbon column in the reverse direction with toluene; and d) up to five manually prepared sample extracts can be processed at one time.

RESULTS AND DISCUSSION

Because FMS had previously assisted us in automating Part 1 of our cleanup procedure, we were already familiar with the Fluid Robotics™ technology used in the WSU method. We made our preliminary evaluation of the WSU automated method after making two modifications: manual sample inlets and loops were eliminated, and the piston pump head was replaced by a peristaltic pump head. The manual sample inlets and loops were eliminated, thereby simplifying the WSU method, because fewer congeners are usually found in human serum extracts and are lower in concentration than those in environmental samples such as flyash or sludge, for which the WSU method first applied. Initially our evaluation experiments were made using solvents and glass columns packed as originally described in the WSU method. However, in our first experiments we failed to recover any of the congeners from either basic or acid alumina. We determined that all of the congeners were being prematurely eluted from alumina columns by the 8% DCM/hexane wash used in the WSU method, and therefore bypassing the carbon column. This finding was consistent with the original SSJ method which used 8% DCM/hexane to elute PCDDs/PCDFs from alumina, following hexane and 2% DCM/hexane washes of the alumina. After replacing 8% DCM/hexane with 2% DCM/hexane, the WSU method performed as described. Subsequently we further modified the WSU method by using disposable pre-packed silica, alumina, and carbon columns made of Teflon, manufactured by FMS according to WSU specfications, with one exception, alumina A - Super I is substituted for basic alumina.

Table 1

Comparison of PCDD/PCDF and Planar PCB Congener Concentrations in Spiked Human Serum Extracts Prepared Using the CDC and modified-WSU Methods

Congener	CDC Method [n=72] Mean (fg/g)	Modified-WSU Method [n=8] Mean (fg/g)
TCDD	151.2	155.7
PeCDD	143.6	144.8
HxCDDs	875.9	889.4
HpCDDs	751.8	746.3
OCDD	6806.3	7397.0
TCDF	322.8	298.1
PeCDFs	360.1	353.8
HxCDFs	685.0	730.7
HpCDFs	612.2	629.2
OCDF	203.3	167.9
Planar PCBs	2613.6	2665.4

When all facets of the sample cleanup procedure (reagent, solvent, support preparation, sample extraction, and chromatography) are considered, we estimate that the modified-WSU procedure takes half the time to perform as our existing semi-automated method. We also observed that the modified-WSU system uses about half the volume of solvents as the CDC procedure and generates substantially less waste. We speculate that we could cut the cost of

sample cleanup in half by performing the modified-WSU cleanup method instead of our semi-automated method. Table 1 shows that we found good agreement, using 50 g samples of a spiked human serum quality assurance pool, between the CDC and modified-WSU methods for the 22 congeners measured. In these experiments, we observed recoveries of 80-90% for all analytes by the modified-WSU method, compared to average recoveries of only 55-60% by the CDC method. When 50g aliquots of a background level serum pool was spiked at 12 levels for all congeners (from 250 to 30,000 fg/g, depending on the congener), excellent linearity was noted for all congeners when observed versus expected concentrations were plotted. Using 50 g samples, we estimate that about 0.5% carryover occurs between samples, and is negligible for low-level (fg/g) samples, but could pose a problem with high-level (pg/g to ng/g) samples. Increasing the volume of toluene used to wash and regenerate the carbon column after a sample is eluted in the reverse direction did not appear to reduce the carryover, suggesting that the cross-contamination was not coming from the common carbon column. We speculate that this residual cross-contamination is possibly due to "dead volume" and inadequate flushing of the tubing between samples, and related to the smaller volumes of solvents used in the modified-WSU method compared to the CDC method. Studies are underway to resolve this problem. Mass chromatograms of enriched samples prepared by the modified-WSU method appear to have less baseline noise and fewer extraneous interference peaks than the same samples prepared by the CDC method.

REFERENCES

1. Ryan JJ and Norstrom RJ. Occurrence of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in humans and major exposure routes. In: Rappe C, Buser HR, Dodet B, and O'Neill IK, eds. *Environmental Carcinogens Methods of Analysis and Exposure Measurement. Vol. 11 -- Polychlorinated Dioxins and Dibenzofurans*. IARC Scientific Publications No. 108, 1991: 51-104.
2. Albro PW, Crummett WB, Dupuy AE, *et al.* Methods for the quantitative determination of multiple, specific polychlorinated dibenzo-p-dioxin and dibenzofuran isomers in human adipose tissue in the parts-per-trillion range. An interlaboratory study. *Anal Chem* 1985; 57:2717-2725.
3. Smith LM, Stalling DL, and Johnson JL. Determination of polychlorinated dibenzofurans and dioxins in environmental samples. *Anal Chem* 1984; 56:1830-1842.
4. Patterson DG Jr., Isaacs SG, Alexander LR, Turner WE, Hampton L, Bernert JT, and Needham LL. Method 6: Determination of specific polychlorinated dibenzo-*p*-dioxins and dibenzofurans in blood and adipose tissue by isotope-dilution high-resolution mass spectrometry. In: Rappe C, Buser HR, Dodet B, and O'Neill IK, eds. *Environmental Carcinogens Methods of Analysis and Exposure Measurement. Vol. 11 -- Polychlorinated Dioxins and Dibenzofurans*. IARC Scientific Publications No. 108, 1991: 299-342.
5. Turner WE, Isaacs SG, Patterson DG Jr., and Needham LL. Method 7: Enrichment of biological samples by the semi-automated Smith, Stalling, and Johnson method: Human serum and adipose tissue. In: Rappe C, Buser HR, Dodet B, and O'Neill IK, eds. *Environmental Carcinogens Methods of Analysis and Exposure Measurement. Vol. 11 -- Polychlorinated Dioxins and Dibenzofurans*. IARC Scientific Publications No. 108, 1991: 343-355.
6. Tiernan TO, Wagel DJ, Sloch JG, Garrett JH, VanNess GF, and Shirkhan H. Development and evaluation of an automated liquid chromatographic apparatus for isolating PCDD/PCDF from complex sample matrices. In: Hutzinger O, and Fiedler H, eds. *Dioxin '90 EPRI-Seminar Short Papers*. ECO-Informa Press, Bayreuth, F.R.G, 1991: Vol 2:229-232.6.
7. Tiernan TO, 1991. Personal communication.

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