

CONTRIBUTIONS TO AUTOMATION AND ENRICHMENT METHODS FOR MEASURING TRACE ENVIRONMENTAL POLLUTANTS SPAN FOUR DECADES

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Introduction

In 1968, I entered the field of environmental chemistry as chief chemist at the National Fisheries Research Laboratory, Fish and Wildlife Service, US Department of Interior, Columbia, MO, greatly benefitting from the public's support of legislation brought about by the publication of Silent Spring (1). Challenges faced by the analytical residue chemist were legion in measuring chlorinated persistent chemical residues in biota at parts-per-trillion concentrations prior to 1980 (2). At that time, methods employed by the analyst generally most frequently required sensitivity measurements for residue levels of chlorinated pesticides such as p-, p'-DDT, at low ug/g of tissue (3). Following concerns with p-, p'-DDT and other persistent organochlorine pesticides and their impact on the environment, the complexity of residue method needs increased to accommodate measurement of residues of polychlorinated PCBs, a significant contaminant problem of the 70's (4). We were soon to realize the full complexity of halogenated persistent environmental contaminants in the environment.

Only a decade later, the first international conference on chlorinated dioxins was held in Rome in 1980 (5). This conference presented a clear need for sensitive and isomer specific measurement at tissue concentration approaching 10^{-12} g/g. Although the matter of dioxin contaminants in Agent Orange had been established (6), issues related to 2,3,7,8 tetrachloro-dibenzofuran and -dibenzo-p-dioxin and related toxic congeners emerged to occupy prominence on the public's stage of environmental concerns; these matters received world-wide scientific attention with a focus on the requirement for sensitive isomer specific methods (7-9).

Sample preparation methods that provided rejection of matrix co-extractives concomitant with selective functional group or class enrichment were highly desired tools supporting these difficult isomer specific analyses (10). Sample preparation instrumentation and techniques suitable for automation were few, leading to a personal research goal to approach automation via the development of modular elements that could be integrated into automated methods that allowed faster, cheaper, and more easily automated methods of determination and sample preparation. One of the first automated sample preparation methods and instrument we developed employed gel permeation chromatography (GPC) (11-13).

Materials and Methods

The introduction of an automated cleanup enrichment method and instrument by Tindle and Stalling (4,11,12) demonstrated that multiple sample extracts could be processed sequentially with minimal cross contamination using a single gel permeation chromatography column. This technique achieved ~100 fold enrichment of contaminants (small molecule entities) through removal of co-extracted lipids or sulfur related sediment co-extractive components. Toluene and polycyclic aromatic hydrocarbons (PAHs), naphthalene, anthracene, pyrene, and perylene, were retained in the order of increasing ring number; nitro-substituents increased retention; and chlorinated-dibenzo-p-dioxins and -dibenzofurans were more strongly retained than their parent compounds. PCB congeners with no chlorine in the *ortho*, *ortho'*-positions are co-planar and are strongly retained; for these isomers, retention increased with higher chlorination. This method also provided a framework to contemplate integration and further automation of modular elements using carbon-based and other adsorption columns (14-20). GPC as an automated cleanup method, was adopted widely into environmental contaminant regulatory methods by the USDA, FDA, CDC and other researchers and international entities (17,22,23).

Automation of the GPC system was extended by the development and automation of solvent removal (18) and integration with automated GPC with solvent exchange (AccuVap®, 20); subsequent developments include automated integrating solvent exchange to allow automation of subsequent separations using carbon or other adsorbents (21-23). Another GPC enhancement was the development of alternative high pressure ABC-GPC EnviroSep columns, reducing the time and solvent required for sample analysis in an effort related to the Consortium on Automated Analytical Systems (CAALs) (19).

The 1974 IUPAC Third International Congress of Pesticide Chemistry, Helsinki (21) afforded the opportunity to meet and establish collaboration with Dr. Christoffer Rappe, University of Umea. Significant contributions to this methodology were also made by Dr. Hans-Rudi Buser, Swiss Federal Research Station, Wädenswil, Switzerland. The result was improved isomer specific methods to address the spectrum of persistent environmental contaminants using GPC and subsequent enrichment techniques combined with isomer separation with high resolution GC coupled to low resolution MS detection. This collaboration first documented the occurrence of various PCDFs residues in fish and other sample types, including a human liver sample (9). Collaboration with Dr. Rappe also resulted in collaboration with Dr. Svante Wold, University of Umea and Dr. William Dunn, III, University of Illinois, Chicago Medical Center on the use of SIMCA pattern recognition in the study of PCB

congeners (26). Requirement for determining the isomeric distribution of PCB residues in fish tissue brought improved methods for separating PCBs from chlorinated pesticides and led to the use of a capillary GLC columns and development of a database for calculating concentrations of congeners.

Because of the wide variation in toxicity of the individual congeners, analyses methods were required that provided isomer specific detection and a high level of enrichment of PCDDs and PCDFs, a sample preparation method based on GPC separations of co-extractives followed by retention of planar chlorinated PCBs and chlorinated-dibenzo-furans and -dibenzo-*p*-dioxins on carbon mini-cartridges was developed (24). The planar constituents were recovered by reverse elution of the carbon with toluene. The use of carbon cartridges was also integrated with direct extraction of tissue admixed and ground with anhydrous Na₂SO₄ (14).

The evaluation of planar absorption of various carbons was facilitated by measurement of their aromatic character by Magic Angle Spinning through the assistance of Dr. Jake Shaffer, Monsanto Co. Prior to selection of Amoco PX-21, coconut charcoal was the most active carbon (Personal Communication). The selection and adoption of PX-21 as the preferred carbon improved the selectivity and recovery of the planar contaminants (NYAS). A later investigation on the use of fullerenes amino linked to polystyrene polymers absorbent material as an alternate separation material showed promise for retention of the planar contaminants; however, this material did not exhibit long-term stability, thereby limiting its utility (27).

Alternative carbon dispersion approaches included integration of PX-21 admixed with polystyrene-divinyl benzene resin (GPC resin) or polyurethane as a dispersant (patent). A byproduct of the carbon enrichment research was a patent and method proposed for removal of dioxin moieties from Herbicide Orange using coconut charcoal (30); this technique was abandoned in favor of incineration of the material by the Vulcanis (31).

Future

One can speculate that the recent studies on nano-materials including graphene (32) could be exploited to provide new means of chromatographic separation and/or detection of halogenated planar compounds. Their integration with “lab on a chip” technologies can continue to ease the effort required for trace analyses of hazardous materials.

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GPC AutoPrep 1002B with AS2000 AutoVap



J2 Scientific PrepLine Platform with GPC, AccuVap w/3 SPE Column Modules

Example Automated Systems: Early GPC AutoPrep w/AutoVap (ABC Instruments) and Current PrepLine™ Automated GPC+Sequential Adsorbent Systems with Solvent Exchange (J2 Scientific).

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"Stephen H. Safe **DIOXIN - 1980 - A Beginning**. On October 22, 1980, approximately 100 scientists assembled at the Istituto Superiore di Sanita in Rome to discuss a topic entitled "Chlorinated Dioxins and Related Compounds". This was an area of research interest to a broad range of scientists due to a number of industrial accidents involving these compounds, rice oil contamination in Japan and Taiwan, Agent Orange, and 2,4,5-T incidents and, of course, Seveso. Not surprisingly, many of the reports at this conference were focused on the contamination and health effects at Seveso where high levels of TCDD were emitted from the Icmesa factory and areas around Seveso were evacuated. Scientists from several countries presented fifty-two papers in the areas of (a) analytical methods, (b) environmental fate and levels, (c) the incineration story, (d) biochemical toxicology and metabolism, (e) animal toxicology, and (f) observations in man."
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