HRGC/HRMS ANALYSIS OF CHLORINATED DIOXINS AND FURANS, 209 PCB CONGENERS AND CHLORINATED PESTICIDES IN A SINGLE SAMPLE ALIQUOT: APPLICATION TO PLASMA SAMPLES.

M. Coreen Hamilton, Douglas J. Weir and Brian R. Fowler

Axys Analytical Services Ltd. PO Box
2219, 2045 Mills Road, Sidney, B.C. Canada V8L 3S8

Introduction

Many analytical methods have been published for the analysis of chlorinated organic compounds such as dioxins, furans, PCBs and pesticides in a variety of sample matrices. Because of the toxic nature of most of these chemicals, their persistence in the environment and their ability to accumulate in lipid-rich biological materials, the focus has shifted to methods which provide excellent specificity and superior detection limits. High resolution gas chromatography with high resolution mass spectrometric detection (HRGC/HRMS) has become the method of choice for this purpose. When coupled with an extensive extract cleanup scheme, superb method performance can be achieved.

EPA Method 1613B [1] for polychlorinated dioxins and furans and EPA Method 1668 draft [2] for PCB congeners are examples of such state-of-the-art methods. They are used routinely for the analysis of aqueous, solid, biological, and other sample matrices with low pg/L or ng/kg detection limits. The disadvantage of this approach is that each of the methods is targeted towards only one group of chlorinated compounds such as the dioxins and furans. In the process of extract cleanup for dioxin/furan analysis by Method 1613B, most of the PCB congeners are discarded before the GC/MS analysis. However, the coplanar PCB congeners are not separated from the PCDD/PCDF and, with the addition of the appropriate labelled coplanar PCBs as surrogate standards, Method 1613B can be easily modified to include the coplanar PCB congeners (IUPAC # 77, 126, 169). However, if information is desired for total PCBs or for all 209 PCB congeners, a new aliquot of sample must be taken and the analysis conducted by a full congener PCB method such as 1668. Analysis for DDTs and other chlorinated pesticides would require yet another analysis procedure. Each of these analyses consumes sample and wastes the information on the groups of organochlorine compounds that are not targeted in the particular method being carried out. This can become a serious concern when analyzing samples with limited quantities available, such as fish, avian or other animal organ tissues or blood. Extensive splitting of a small sample among various analytical procedures is required, and the detection limits will be adversely affected.

In this paper a procedure will be described for the determination of all three groups of chlorinated organic compounds, dioxins/furans, organochlorine pesticides and 209 PCB congeners, with a single workup on a single aliquot of sample. We refer to this in house method as the DOCP method. The protocols are entirely compliant with both Methods 1613B and 1668 and no compromise on detection limits or any other data quality criterion is required. The protocol also includes an isotope dilution (where standards are available) or internal standard HRGC/HRMS method of a full suite of chlorinated pesticides.

Materials and Methods

All reagents and materials are either pesticide grade or pre-extracted and proofed before use. Glassware baked at 300°C in a forced air oven and rinsed with solvent before use Cut points for target compounds of all LC columns (i.e. GPC, Florisil, alumina and activated APX carbon on Celite) were optimised prior to use. Details of cleanup columns are summarised in table 1.

LC column	Packing and column diameter
GPC	BioRad Biobead SX-3200-400, 60g, 3 cm id.
Carbon	Anderson AX-21 4.5% on Celite 545, 0.22 g , 0.4 cm id
FlorisilFisher	2.1% water deactivated, 60-100, 8 g, 1 cm id
Alumina	Fisher (1.0 % water deactivated) 60-325, 6 g 1 cm id

The sample workup and analysis protocol is outlined in Figure 2. An extensive suite of 13C labelled surrogate standards is added to each sample before analysis. These include $^{13}C_{12}$ labelled PCBs-3,15,28,77, 105, 118, 126, 156, 157, 167,169, 180, 189, 194, 206 and 209 for extended Method 1668, and $^{13}C_{12}$ labelled analogues of the seventeen 2,3,7,8 substituted dioxins and furans specified in Method 1613B. For the pesticides, 13C labelled hexachlorobenzene, gamma-HCH, PCB-101, p,p'-DDE, p,p'-DDT as well as deuterium labelled ($^2H_4$) alpha-endosulphan are added. Additional labelled cleanup standards are added after extraction but before the extract cleanup process begins. After sample workup is complete, but before GC/MS analysis, additional labelled "instrument internal standards" are added to each fraction of the extract collected. These standards are specified in Methods 1613B and 1668.

Results and Discussion

The method performance was demonstrated by analyzing plasma samples. This is a complex matrix requiring extensive cleanup and often with only very small samples available for analysis. In such a situation the combined analysis protocol reported here becomes a necessity.

The results for four spiked plasma replicates are reported below. Each consisted of a one-gram plasma sample spiked with target dioxins, PCB congeners and pesticides. Labelled dioxin, furan and PCB standards were added according to Method 1613B and 1668 specifications. Labelled pesticide standards were also added to each sample and the samples were then taken through the entire workup procedure. The Florisil F1 + F2 fraction (Figure 2) was analyzed for the less polar pesticides, the Florisil F3+F4 fraction was analyzed for the polar pesticides and the E2 fraction was analyzed for dioxins and furans according to Method 1613B specifications. The E2 fraction also includes the coplanar PCB congeners IUPAC# 77, 126 and 169 which may be conveniently determined with the dioxin/furans by a minor extension of the 1613B HRMS scan descriptor. These congeners are also determined in the final combined extract by Method 1668.

The F1 + F2 fraction of Florisil and E2 fractions of the carbon column are then combined and analyzed for PCB congeners by the full congener version of Method 1668[3]. Features were added to the Draft 1668A to extend its scope from the 13 toxic congeners to all 209 congeners. Several of these modifications have subsequently been incorporated into EPA Method 1668A [4].

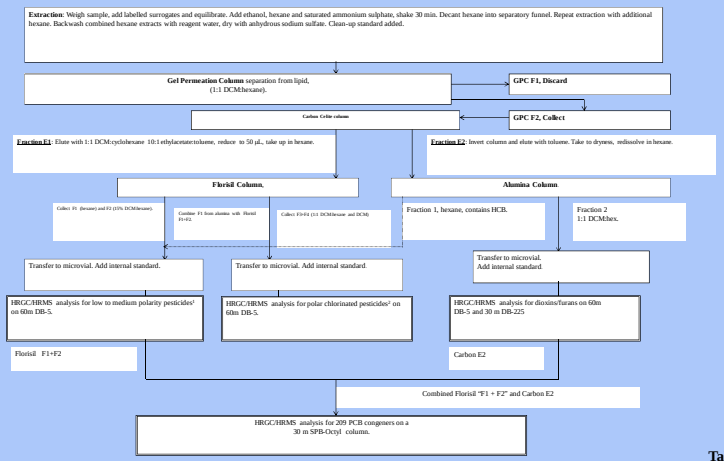
Pesticide spiking levels were in the range 4.2 to 41.6 ng. Blanks and unspiked plasma samples showed no significant pesticide levels. The spiked sample analysis results showed good reproducibility with relative standard deviations (RSD) less than 15% for all pesticides for the four replicate samples. Absolute spike recoveries were in the range of 65-120%.

The dioxins were spiked at 200 pg for 2,3,7,8-TCDD and TCDF, 2000 pg for OCDD and OCDF and 1000 pg for other congeners. Blanks produced OCDD and OCDF values <10 pg, TCDF and TCDD were <0.3 pg and all other dioxins and furans were present at < 2.0 pg. All dioxin surrogate and authentic recoveries fell within the limits allowed for Method 1613B. The samples also showed good reproducibility with RSDs for the authentic dioxin/furans being 3.5% for all but two compounds (OCDF and 1,2,3,7,8,9-HxCDD each had an RSD of 10%).

After recombination of the Florisil F1 + F2 and E2 portions, the extract was analyzed for all 209 PCB congeners. The spiking levels ranged from 25 ng for mono-, di- and trichloro- congeners, 50 ng/g for tetra-, penta-, hexa- and hepta- and 75 ng/g for octa-, nona- and decachloro congeners. Blanks had levels of less than 10 pg per congener with the exception of PCB15 which was at ~20 pg. The initial performance, calibration and surrogate recovery criteria of Draft EPA Method 1668 were met.

To illustrate the effectiveness of the method, a 1.7-gram sample of eagle blood was extracted and analyzed. This single sample's extract provided results for the Method 1613B dioxin/furans, all 209 PCB congeners as well as 27 pesticides. The results for selected parameters are shown in **Table 1**.

Figure 2: Schematic of Combined Dioxin/Furan, PCB Congener and Chlorinated Pesticide Method for Blood Plasma.



Each step of the extract cleanup procedure outlined in Figure 2 was adapted from EPA Method 1613B or 1668 by optimisation of the collection/discard points to maximize recovery of the target compounds while excluding as much interfering material as possible.

This DOCP method was been validated by the analysis of chicken plasma samples unspiked and plasma fortified with target analytes at several levels. Chicken blood was obtained from a slaughterhouse and centrifuged to separate the plasma. The plasma was stored at –20°C until analyzed. Spiked plasma samples were prepared for analysis by adding aliquots of authentic dioxins, furans, PCBs and pesticides to a weighed amount of plasma.

Instrumental analysis were conducted by HRGC/HRMS systems in mass-locked voltage selected ion recording mode (V-SIR) at 10,000 mass resolution using Micromass VG70-VSE and Autospec Ultima magnetic sector high resolution mass spectrometers. Each system was equipped with an HP 5890/6890 gas chromatograph, and CTC autosampler. For the PCB congener analysis, an SPB-Octyl (30 m, 0.25 mm i.d. 0.1 µm film) chromatography column was employed. For the pesticide and PCDD/PCDF analysis, a DB-5 (60 m, 0.25 mm i.d., 0.1 µm film thickness) chromatography column was employed. A DB-225 (30 m, 0.25 mm i.d., 0.15 µm film thickness) column was employed where confirmation of the concentration of 2,3,7,8-TCDF was required. HRGC/HRMS V-SIR instrumental methods, similar to methods 1613 and 1668, were developed for the analysis of the F1+F2 and F3+F4 chlorinated pesticide fractions which were determined by two separate and additional HCGC/HRMS instrument runs.

Sample and instrumental QA/QC protocols defined in Methods 1613B and 1668 were adopted for the PCDD/F and PCB congener analyses combined procedure described here. Calibration of the 209 congener PCB method is described in Method 1668A [4].

Table 1 . Selected Dioxin/Furan and toxic PCB Congener concentrations from a 1.7 g sample of Avian Blood Plasma

Dioxins	Conc. (pg/g)	SDL (pg/g)		Selected PCB Congeners	Conc. (pg/g)	SDL (pg/g)
2,3,7,8-TCDF	1.8	0.30		PCB-77	96	3.3
1,2,3,7,8-PeCDF	0.54	0.23		PCB-123	78	7.9
2,3,4,7,8-PeCDF	0.64	0.23		PCB-118	3400	7.7
1,2,3,4,7,8-HxCDF	0.56	0.20		PCB-114	100	9.0
1,2,3,4,6,7,8-HpCDF	0.99	0.20		PCB-105	1200	7.0
2,3,7,8-TCDD	0.60	0.40		PCB-126	19	7.4
1,2,3,7,8-PeCDD	0.48	0.26		PCB-167	200	3.6
1,2,3,6,7,8-HxCDD	0.45	0.27		PCB-156/157	460	4.6
1,2,3,4,6,7,8-HpCDD	0.81	0.20		PCB-169	11	3.3
OCDD	2.14	0.47		PCB-180/193	3300	0.51
				PCB-170	1000	0.58
				PCB-189	54	1.1

Table 2. Selected Pesticide Concentrations for a 1.7 g sample of Avian Blood plasma

Pesticide	Conc (pg/g)	SDL (pg/g)		Pesticide	Conc (pg/g)	SDL (pg/g)
HCB	630	1.3		o,p'-DDT	120	6.2
Mirex	1200	0.41		p,p'-DDT	380	8.1
α-HCH	120	7.5		Heptachlor Epoxide	470	3.4
γ- HCH	11	6.6		α-Endosulphan	ND	41
Heptachlor	2.6	1.8		Dieldrin	1600	5.7
Aldrin	ND	1.8		Endrin	150	16
Oxychlordane	452	6.9		o,p'-DDE	38	15
trans-chlordane	110	5.1		p,p'-DDE	15000	18
cis-chlordane	554	5.9		o,p'-DDD	110	4.7
				p,p'-DDD	1300	5.9

The results for this sample demonstrate that the presence of the coplanar PCBs and their surrogates had no observable effect on the ability to quantify the compounds in the dioxin fraction, even at low dioxin levels. Indeed the sample met all the QC criteria for a typical Method 1613B sample. Likewise the presence of the dioxins, pesticides, their surrogates and recovery compounds did not have any discernable effect on the ability to quantify the 209 PCB congeners by the protocols of draft Method 1668. Used with appropriate toxicity equivalents factors (TEFs) for PCDD/Fs and 12 WHO toxic PCBs provides a TEQ value for these classes. In conclusion, the method provides an effective extraction and analysis protocol for determination of a large suite of persistent chlorinated organic contaminants in samples of limited size.

Acknowledgement: The authors gratefully acknowledge the staff of Axys Analytical Services who participated in the development of this protocol, those who assisted with the extension of draft method 1668 to a full congener method and the development of HRMS procedures for pesticide analysis.

References

1. EPA Method 1613, Revision B, Tetra- Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, September 15, 1997
2. EPA Draft Method for the Measurement of Toxic PCB congeners By Isotope Dilution HRGC/HRMS, October 4, 1995 Draft Revision
3. Draft Method for the Measurement of Toxic PCB congeners By Isotope Dilution HRGC/HRMS, October 4, 1995 and Modified EPA Method 1668 incorporating additional protocols developed by Axys for quantification of 209 congeners
4. Method 1668, Revision A. Chlorinated Biphenyl Congeners in Water, Soil, Sediment and Tissue by HCGC/HRMS. December 1999. EPA No. EPA-821-R-00-002