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

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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 toxicity of these chemicals, best practices in the environment and their ability to accumulate in lipid-rich biological materials, the focus has shifted to methods which provide excellent specificity and sensitivity detection limits. High resolution gas chromatography (HRGC) with high resolution mass spectrometric detection (HRMS) has become the method of choice for this purpose. When coupled with an extensive extract cleanup scheme, superb method performance can be achieved.

EP A Method 616B [1] for polychlorinated dibenzodioxins and furans and EPA Method 616A [2] for PCB congeners are examples of such types of the methods. They are used routinely for the analysis of aqueous, solid, biological, and other sample matrices with low ppt, or ng/l detection limits. The disadvantage of this type of approach is that in each of these methods compounds are only a group of chlorinated compounds such as the dioxins and furans. In the process of extract cleanup for dioxin fraction analysis by Method 161B, most of the PCB congeners are discarded before the GC/MS analysis. However, the coplanar PCB congeners are not resolved from the FPD/MS and, with the addition of the appropriate labelled coplanar PCBs as surrogate standards, Method 616A/B can be easily modified to isolate the coplanar PCB congeners (IUPAC # 77, 126, 169). However, if it is desired to isolate total PCBs or all 209 PCB congeners, a new scheme of samples must be taken and the analysis conducted by small capillary GC/MS method such as 1666A. Analysis of DDEs and other chlorinated pesticides would require yet another analysis procedure. Each of these analyses consumes a sample and wastes the information on the groups of compounds that are not unique to the particular method being used. This can become a serious concern when analyzing samples with limited quantities available, such as lab, onsite or other animal tissues and blood. Extracting a small sample amount among various analytical procedures can be overwhelming.

In this paper a protocol will be described for the determination of all groups of chlorinated organic compounds, dioxin/furans, organochlorine pesticides, chlorinated pesticides and PCB congeners. This protocol, with a single working day, is a single aliquot of sample. We refer to this in house method and method for 209 congeners. The protocols are entirely compliant with both Methods 1613B and 1666A and no compromise on detection limits or any other data quality criteria is required. The protocol also includes an isolation (where standards are available) or internal standard HRGC/HRMS method of full a suite of chlorinated pesticides.

Materials and Methods

All reagents and materials are either pesticide grade or pre-extracted and freed before use. Glutaraldehyde buffer at 100 mM in a formalin based sample and utilized as the solvent buffer in the extraction protocol for major columns i.e. PCBs. Fluorisil alumina and activated APS on C18 were optimized prior to use. Details of cleanup columns are summarized in Table 1.  

Table 1 . Selected Dioxin/Furan and toxic PCB Congener concentrations from a 1.7 g sample of human plasma.

<table>
<thead>
<tr>
<th>Congener</th>
<th>470</th>
<th>491</th>
<th>492</th>
<th>524</th>
<th>525</th>
<th>526</th>
</tr>
</thead>
<tbody>
<tr>
<td>470</td>
<td>0.61</td>
<td>0.64</td>
<td>0.70</td>
<td>0.72</td>
<td>0.74</td>
<td>0.75</td>
</tr>
<tr>
<td>491</td>
<td>0.62</td>
<td>0.65</td>
<td>0.70</td>
<td>0.72</td>
<td>0.74</td>
<td>0.75</td>
</tr>
<tr>
<td>492</td>
<td>0.62</td>
<td>0.65</td>
<td>0.70</td>
<td>0.72</td>
<td>0.74</td>
<td>0.75</td>
</tr>
<tr>
<td>524</td>
<td>0.72</td>
<td>0.74</td>
<td>0.76</td>
<td>0.78</td>
<td>0.80</td>
<td>0.82</td>
</tr>
<tr>
<td>525</td>
<td>0.73</td>
<td>0.75</td>
<td>0.77</td>
<td>0.79</td>
<td>0.81</td>
<td>0.83</td>
</tr>
<tr>
<td>526</td>
<td>0.74</td>
<td>0.76</td>
<td>0.78</td>
<td>0.80</td>
<td>0.82</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Each step of the extract cleanup procedure outlined in Figure 2 was adopted from EPA Method 1613B or 1666A by optimization of the collected/isolated pairs to maximize recovery of the target compounds while excluding as much interfering material as possible. 

The DOPCP method was validated by the analysis of chicken plasma samples spiked and plasma isolated with target analytes at several levels. Chicken blood was obtained from a slaughterhouse and considered to be the poultry. The samples were at 1000 ppm concentrations. Prepared samples were prepared for analysis by adding aliquots of authentic dioxins, furans, PCBs and pesticides to a weighted amount of plasma. Instrumental analyses were conducted by HRGC/HRMS systems in mass locked voltage selected ion recording (MS/MS) at 3000 mass resolution using a MicroMass VG 70-70 and Autoion. Ultimate magnetic sector high resolution mass spectrometers. Each system was equipped with an HP 1090/5989 chromatograph, and CTMS auto-sampler. For the PCB congener analysis, an SPB-5 (5% Phenyl/95% Dimethylpolysiloxane) was employed. For the pesticide analysis, a DB-5 (5%) dimethylpolysiloxane column was employed. Confirmation of the concentration of the 2,3,7,8-TCP was required. HRGC/HRMS (HRM) instrumental methods, similar to methods 1613 and 1666, were developed for the analysis of the PeCDF and PePFB chlorinated pesticide fractions which were determined by two separate and additional HRGC/HRMS instrumental runs.

Sample and Instrumental Optimization: PCB congeners definitions in Methods 1613B and 1666A were adopted for the TCD/HRMS and PCB congener analyses combined procedure described here. Calibration of the 209-congener PCB method is described in Method 1666A.

The results for this sample demonstrate that the presence of the coplanar PCBs and their congeners did not observable effect on the ability to quantify the compounds in the dioxin fraction, even at low detection levels. Indeed the sample met all the QC criteria for a typical Method 1613B sample. Likewise the presence of the dioxins, pesticides, their congeners and recovery compounds did not have any discernible effect on the ability to quantify the 289 PCB congeners by the protocols of draft Method 1666A. Used with appropriate sensitivity factors (ISFs) for PCDD/Fs and CWB toxic PCBs provides a TSB 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 creation of draft Method HEB to a full congener method and the development of the HRM protocol for plasma samples.

References

1. EPA Method 1613. Revision B: Timo-Through Oxid Chlorinated Dioxin and Furans by Isotope Dilution HRGC/HRMS, September 15, 1997
2. EPA Draft Method for the Measurement of Toxic PCBs by Isotope Dilution HRGC/HRMS, October 4, 1995 Draft Revision
3. Draft Method for the Measurement of Toxic PCBs by Isotope Dilution HRGC/HRMS, October 4, 1995 and Modified EPA Method 1666 incorporating additional protocols developed by Axys for quantification of 289 congeners

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