ANALYSIS OF POPS FROM 50 µL DRIED BLOOD SPOTS

Vining B[1]*, Hart J[1], Boivin L[1], Saul D[1], Appele R[1], Nichols J[2]

[1]SGS Environmental Services, 5500 Business Dr., Wilmington, NC, USA; [2]General Electric Company
One Research Circle, CEB 154, Niskayuna, NY, USA

Introduction

Assessment of the blood levels of persistent organic pollutants (POPs) in humans has traditionally required venipuncture and collection of relatively large sample volumes (as much as ~40 mL whole blood). These large volumes are required to obtain adequate detection limits given the relatively low concentrations of POPs in human blood. Such large collection volumes present a number of difficulties. Amongst those difficulties are: 1) Reluctance on the part of subjects to participate in resampling should analytical difficulties occur and 2) extreme difficulty in field sampling.

Collection of blood spots for analysis overcomes these difficulties readily. First, each sample is a small (~1 cm diameter) spot of blood on a piece of paper. Multiple samples may be collected in a very short time, avoiding the need for resampling in the case of analytical difficulties. Additionally, field sampling becomes far more viable, as all that is needed in the field are paper cards, lances, and sterilization materials (such as alcohol soaked wetnaps).

Analysis of blood spots for POPs, however, presents a new set of difficulties. Some initial work has been performed along these lines[1], but the field is still a burgeoning one. Most notably, the detection limits required for POPs analyses are much more difficult to attain when using a sample whose volume is nominally 50 µL. Additionally, the extremely low detection limits make background contamination (especially from the paper cards themselves) a potential problem that would not be observed when using larger sample volumes.

We present here the successful development and validation of a method for the analysis of five POPs from ~50 µL dried blood spots that we used to analyze 72 samples from humans.

Materials and methods

Each blood spot was analyzed for PCBs 101, 105, and 138, as well as PBB-153 and lindane. All five compounds were analyzed using isotope dilution with single ion monitoring high resolution gas chromatography mass spectrometry (ID-SIM-GC/HRMS). Each of the five target analytes was measured relative to a ^{13}C labeled version of itself (hereafter called extraction standards).

Dried blood spots were received at the laboratory already cut away from any other paper. The spots had been cut into strips before being sent to the laboratory.

Each dried blood spot was spiked with 10 pg of the ^{13}C labeled extraction standards (ES). The samples were then allowed to equilibrate for one hour. A formic acid/acetone mixture was used to disrupt protein binding, after which the spots were sonicated to remove the blood from the paper. A liquid-liquid extraction of the formic acid solution using dichloromethane and hexane was then used. After concentration of the extract and addition of ^{13}C PCB-128 as an injection (recovery) standard (JS), the extracts were ready for analysis at a final volume of 10 µL in nonane.

Analysis took place on a Waters™ Autospec Premier. A 30m DB-5 MS GC column was used, with a GC temperature ramp starting at 120ºC, rising to 325 ºC over a 29 minute run time. The injector temperature was set at 280ºC. The transfer line (between the GC and HRMS system) turned out to be critical to balancing the sensitivity of response of the five compounds and was optimized at 260ºC. A 4 mL GC liner without glass wool was used, and an injection volume of 2 µL was necessary to obtain the desired detection limits.

The instrument was calibrated with eight calibration standards. More standards that might normally be used were utilized to ensure adequate coverage of the low range of the calibration curve. Table 1 shows the concentrations of the calibration standards for each target analyte.

Table 1. Concentrations of calibration standards used in the analysis of POPs from Dried Blood Spots

	CS1	CS2	CS3	CS4	CS5	CS6	CS7	CS8
Analyte	pg/µL	pg/µL	pg/µL	pg/µL	pg/µL	pg/µL	pg/µL	pg/µL
PCB 101	0.0125	0.025	0.05	0.1	0.2	0.4	0.8	1
PCB 105	0.0125	0.025	0.05	0.1	0.2	0.4	0.8	1
PCB 138	0.0125	0.025	0.05	0.1	0.2	0.4	0.8	1
BB 153	0.03125	0.0625	0.125	0.25	0.5	1	2	2.5
Lindane	0.1	0.2	0.4	0.8	1.6	3.2	6.4	8

As Table 1 shows, the calibration extended as low as 12.5 fg/µL, at which we were able to obtain signal-to-noise ratios of 10:1. Maximization of duty cycles on the native (unlabeled) m/zs monitored assisted in making this achievement possible. Further, the m/z monitoring functions were chosen to allow maximization of response sensitivity. RSDs for the relative response factors obtained at each calibration standard level for each compound ranged from 5.1% to 6.5%, indicating excellent linearity over the range indicated, as well as sufficiently strong response at the low end to prevent degradation of the signal.

The method was validated through the analysis of blood spots spiked with known amounts of native analyte prior to shipment to the laboratory. For the laboratory, these were blind QC samples. That is, the laboratory did not know what the concentrations were supposed to be. Initially, four replicate samples were sent to the laboratory along with an equal number of blank paper samples. In a second round of validation a triplicate calibration curve in blood was sent to the laboratory. In addition, several types of blank paper were sent to the laboratory to test various paper backgrounds with and without cleaning.

Results and discussion

In the first validation experiment, two pairs of spiked samples were sent to the laboratory. Each pair was at the same spiked concentration. Although the spike was evident (see Figure 1), the background from the paper was sufficiently high as to make accurate determination of the spiked concentration impractical.

In particular, the PCBs can be observed at particularly high levels (relative to the samples) in the paper blanks. The method blank (which consisted of only solvent without paper) showed that the laboratory contributed very little to this paper blank background. Only PBB-153 was at a low enough level in the paper blanks and a high enough level in the samples to be possibly quantitated with accuracy.

As a consequence, before the second set of validation samples were sent, the paper cards were cleaned using a carbon dioxide cleaning regimen.

Another observation from this first study was that the sonication step had to be carefully controlled. The original extraction protocol did not call for the use of a sufficiently large volume of the formic acid solution to cover the blood spot. As a consequence, the labeled standard recoveries for most of the compounds was poor, running around 20% or less. Only lindane was recovered well.

The second set of validation samples consisted of a triplicate calibration curve (again sent blind to the laboratory) as well as a large number of blank samples to evaluate different kinds of paper and the efficacy of the carbon dioxide cleaning.

The results from these samples indicated that the method is capable of generating reliable quantitative results. Mean labeled standard recoveries ranged from 51% (lindane) to 100% (PBB 153). Recoveries were generally quite consistent, with the exception of lindane, which sometimes failed to recover at all.

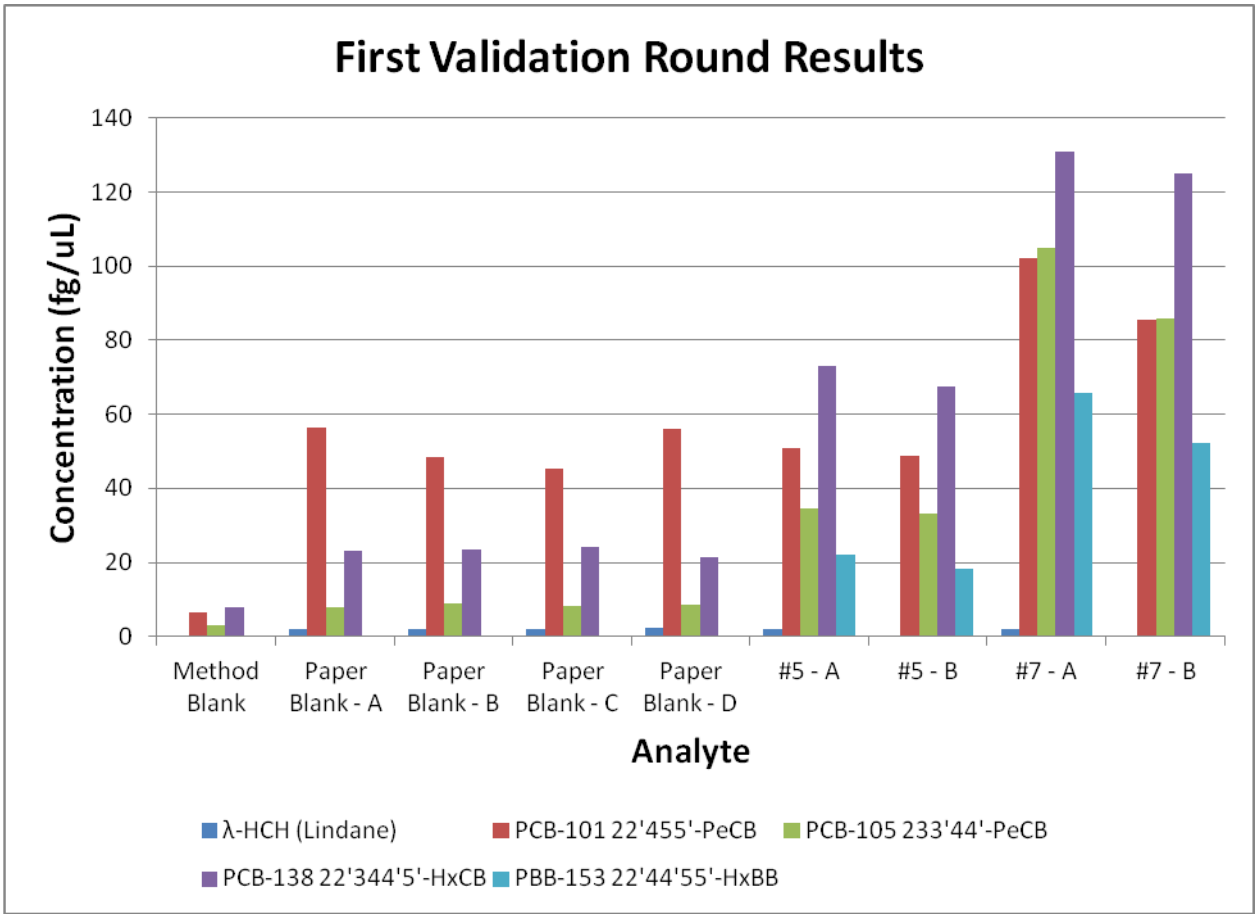

Figure 1. Results of the first round of validation testing. The method blank consisted of just solvent without paper. Although it is clear that the PCBs and PBB were spiked into the samples, the background in the paper blanks is too large to allow for reliable quantitation of the target analytes, with the possible exception of the PBB.

The PCBs and PBB 153 all extracted reasonably well, although the PCBs showed a non-linear response with concentration (Figure 2). The reason for this non-linearity is not immediately evident. PBB 153, however, shows linear response with concentration. These results may suggest that the PCBs engage in stronger protein binding than the PBBs, but these data do not provide sufficient information to reach that conclusion.

Both classes of compound also show an apparent measurement at less than the nominal concentration. We are currently investigating the source of this discrepancy to determine if it is due to error in the preparation of the matrix-matched samples. The special nature of isotope dilution should prevent the emergence of such artifacts, provided spiking was performed correctly.

Finally, the blank data show that the carbon dioxide cleaning was not highly effective in reducing the paper background.

We are continuing to explore the unexpectedly low measured concentrations. These results suggest that integration of the labeled standard spike with a dried blood spot represents a special analytical challenge that will require additional effort to overcome. Possible avenues to resolution of this problem include digestion with carboxypeptidase or some means of disaggregating protein clusters formed during the drying of the blood.

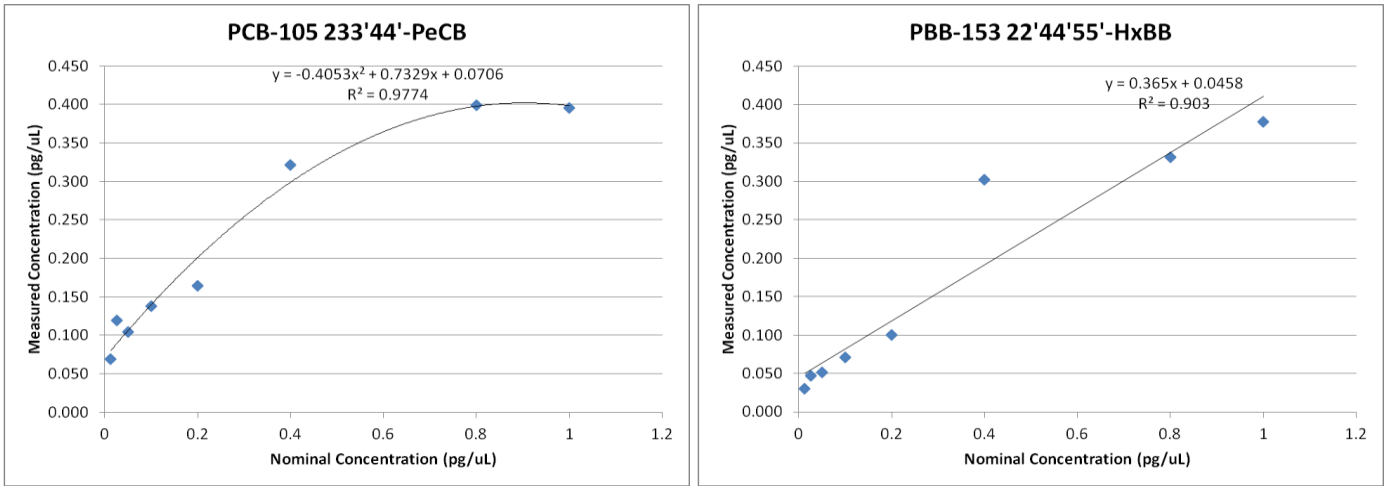

Figure 2. Curves showing measured concentration versus nominal concentration. For PCBs, the response is non-linear, while it is linear for PBB-153. Both curves, however, show less than the nominal concentration being measured.

References:

1. Lu D, Wang D, Ip HSS, Barley F, Ramage R, She J; (2012) *Journal of Chromatography B* 891-892: 36-43