An attempt to validate serum and plasma as sample matrices for analyses of polychlorobiphenylols

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Introduction

Polychlorinated biphenyls (PCBs) form hydroxylated metabolites (OH-PCBs), as reported both from wildlife and from experimental animal studies already in the early 1970s' [1-10]. However, the interest increased in OH-PCBs from the mid 1990s' depending on the discovery that some OH-PCB congeners are strongly retained in the blood of birds, fish and mammals, including humans [11,12]. The interest is linked to the fact that OH-PCBs is strongly, but reversibly, bound to the blood protein transthyretin (TTR) [12,13]. It is reasonable to believe that the strong TTR binding may have toxicological impact, probably related to endocrine type effects [13]. Importantly, OH-PCBs are present in blood at far higher concentrations than in any other compartment in the body [14], which is dependent on the physico-chemical characteristics of the phenols. Analyses of OH-PCBs have thus been concentrated to whole blood, plasma or serum [15-17]. Still there is no comparison between the three sample types even though it is clear that whole blood is not optimal due to the large proportion of haemoglobin in the sample that make the clean up more difficult than if plasma or serum is selected for analysis.

In the present study we have addressed two questions: First we have looked at any potential differences in the analytical results of OH-PCBs when using serum and plasma for extraction and clean up; Second, the serum and plasma applied in the validation has been unfrozen, frozen (at -20 ºC) for two months and frozen for twenty months, respectively.

Material and Method

All solvents are of pesticide analysis reagent grade. Methyl-*tert*-butyl ether (MTBE) and 2-propanol were glass-distilled prior to use. Silica gel (0.063-0.200 mm) was activated in an oven at 300 ºC over night before the gel was used. Gas chromatography (GC) was performed on a Varian 3400 (J&W Scientific), with an ECD detector. A DB-5 capillary column (30 m, 0.1 i.d., 0.1 µm film; J&W Scientific) with helium as carrier gas. The column oven temperature was programmed: 80 ºC, (2 min), 200 ºC (50 °/min), 230 ºC (1 °/min), 330 ºC (30 °/min, 3min). Data were collected and processed using PC-based ELDS Win version 1.

Serum and plasma were obtained from the personnel at the Department of Environmental Chemistry, Stockholm University, in 2001, representing 8 individuals.

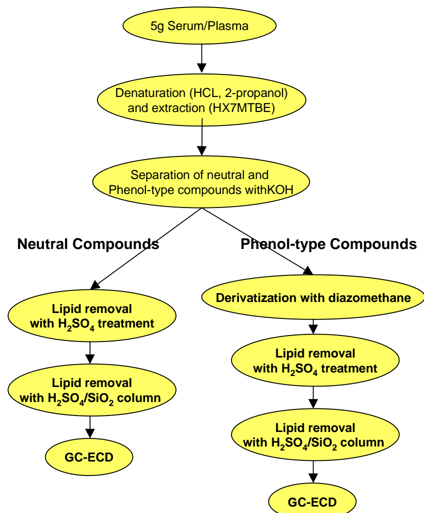

Figure 1. Method of analysis.

An equal amount of serum and plasma from these persons, both men and women, were pooled and thoroughly stirred. Thirty sub-samples of each matrix were prepared (5 gram) in glass tubes and 20 of these samples were kept frozen at -20 ºC until analysed. Ten samples of serum and 10 samples of plasma were immediately analysed representing the unfrozen, fresh samples (t = 0). Serum and plasma samples, 10 of each, were taken out after frozen for 2 months (t = 2) and 20 months (t = 20), respectively, for analysis. The serum and plasma samples were all analysed as described in detail elsewhere[18] and as outlined in Figure 1. The samples were analysed for 4-OH-CB107, 4-OH-CB146 and 4-OH-CB187.

Results and Discussion

The results that were obtained after 20 months (Figure 2) indicated that the OH-PCB concentrations increased to twice the original concentration, which is very unlikely. Hence it was necessary to investigate sources of errors. It was determined, unfortunately, that the surrogate standard used was from different batches at each analysis occasion. It was not possible to control which batch was wrong. Instead it was decided that all samples should be spiked with another volumetric internal standard, all samples at the same time, to eliminate another possible source of error, and then re-analysed.

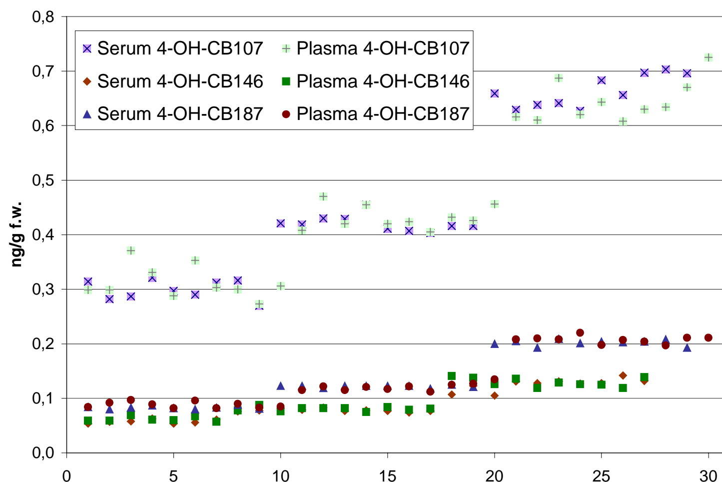

Figure 2. First concentration (ng/g f.w.) result obtained according the OH-PCB in the serum (s) and plasma (p) samples at t = 0 (nr 1-10), t = 2 (nr 11-20) and t = 20 (nr 21-30).

That means that the recovery will not be taken into consideration at this time of analysis.

Bartlett's test [19] showed that the variance within the serum analysis of 4-OHCB107 & 4-OHCB187 gave significantly deviating results. Therefore a Non-parametric Multiple Comparison was carried out by means of the STP method [19]. There was a significant concentration difference, both for plasma and serum samples, between group 1 (t = 0) and 3 (t = 20) for the 4-OH-CB107 and between groups 1 and 2 (t =2) and 1 and 3 for the 4-OH-CB146. No significant differences were found between the groups for 4-OH-CB187. Single classification ANOVA was also applied giving essentially the same results.

In the plasma samples the last group contains only 9 samples. For groups with unequal sample sizes the non-parametric method is not recommended. Furthermore the plasma-groups can be considered homoscedastic. Therefore, single classification ANOVA was applied, using the T'-method [20] as a post-hoc test following the ANOVA. There was a significant difference between group 1 and 2, and 1 and 3 for the 4-OH-CB107 and the 4-OH-CB146. No significant differences were found between groups for 4-OH-CB187.

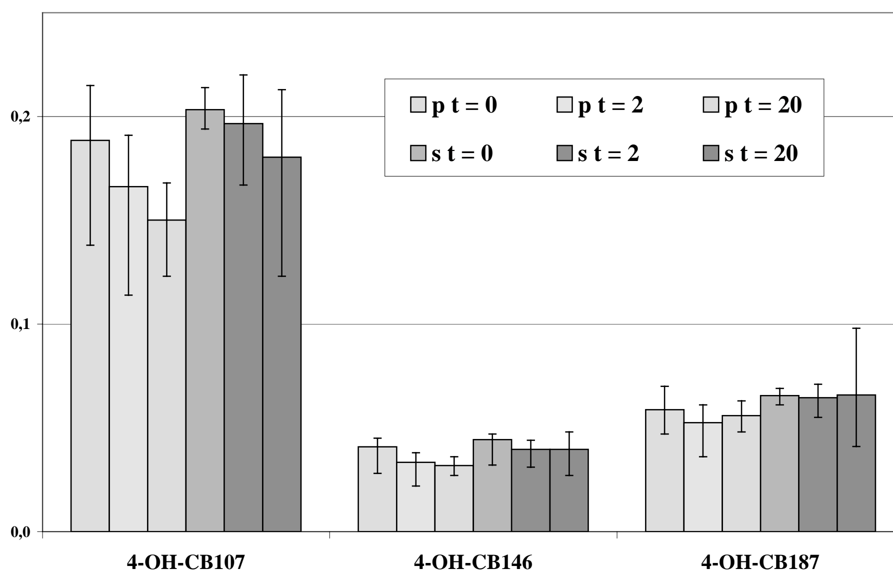

Figure 3. OH-PCB concentrations (ng/g fresh weight (f.w.)) found in serum (s) and plasma (p), analysed as unfrozen (t = 0), after 2 months (t = 2) and after 20 months frozen (t = 20). The error bars indicate the maximum and minimum levels determined.

At the second occasion of analysis it was indicated that the recovery was lower for the plasma samples than the serum samples. This was tested with a T-test and the hypothesis that serum and plasma concentration was equal at every occasion when analysed (t = 0, 2 and 20) was rejected, except between the 4-OH-CB146 analysis at t = 0. This is not surprising since plasma contains more proteins and this might influence the extraction of the analytes. At the first time of analysis such a differences was not seen since recovery compensation was performed when the analytes were calculated in comparison with an internal surrogate standard.

What also might be noticed is that the standard deviation was rather large for some of the analysed batches at the second time point of analysis. Coefficient of variance (CV) ranged from 3 to 22%. This might be due to different recoveries between samples. At the first time of analysis (t = 0) the CV varied between 1 and 10%.

It is important to emphasise that the OH-PCB decrease is within an accepted variation, 20 percent. That means that the decrease indicated here might be due to a normal variance. It is clear that the study can be optimised but it must then be repeated.

The cholesterol and triglyceride content was also measured enzymatically [4] at t = 0, 2 and 20. The measurements were repeated 5 times at every occasion and the variation was 2%. The results are given in Figure 4. It is indicated that the triglyceride is increasing and the cholesterol decreasing. This may be due to a variance of the measurement method and/or instruments used.

Triglycerides and cholesterol are presumed to be stable in a minus 20 freezer (personal com. Åke Norström, Clinical Pharmacology, Umeå University Umeå, Sweden).

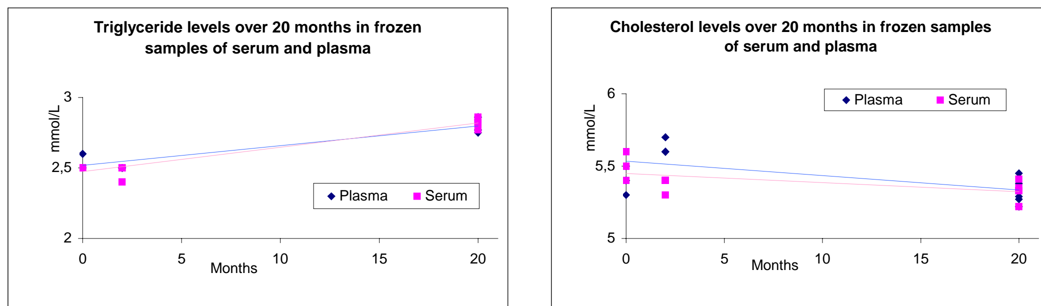

Figure 4. Triglyceride and cholesterol (mmol/L) measured in serum and plasma (n = 1, repeated 5 times) at t = 0, 2 and 20.

Conclusion

This study had sources of errors from the beginning and the result should be understood as indications.

It is indicated that 4-OH-CB107 and 4-OH-CB146 concentrations decreases in both serum and plasma over time. If this is not caused by the variation within the clean-up method, is it likely caused by decomposition, but this should be further investigated. On the other hand, 4-OH-CB187 seems to be stable over time, or is easier to extract and therefore have a better reproducibility.

The extraction of OH-PCBs in plasma has a lower recovery than serum samples. This is most likely due to that plasma is a more protein rich matrix and other studies has experienced clotting of the proteins before extraction when samples were taken after being frozen for a longer period of time.

This study should be regarded as a pilot study indicating several pitfalls and areas of particular interest. Consequently, additional work has to be performed to be able to draw any firm conclusions.

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