SEMI-AUTOMATED METHOD FOR THE DETERMINATION OF 150 PERSISTENT ORGANIC POLLUTANTS IN HUMAN SERUM USING GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) WITH SIMULTANEOUS DR-CALUX ASSAY

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Introduction

Analytical laboratories involved in human monitoring of persistent organic pollutants (POPs) face challenges such as the limited amount of biological sample available (serum or plasma) and the need to quantify not only more and more compounds but also their metabolites as the latter are often the more toxicologically relevant molecules.

The measurement of dioxin-like compounds (DLCs) in biological samples is expensive and, therefore, not feasible to perform on large numbers of samples. The DR-CALUX (dioxin responsive chemically-activated luciferase expression) assay has however been shown to be a reliable and cheaper alternative for screening of dioxin-like activities in human plasma samples.

To face these requirements we developed a semi-automated method that can simultaneously monitor 150 POPs or their metabolites in human plasma or serum by GC-MS using only 5 ml of sample. In our procedure, we also included the possibility of using part of the extract for dioxin-like compound determination with the DR-CALUX assay.

The list of compounds monitored includes 45 polychlorinated biphenyls (PCB) congeners, 32 hydroxylated PCBs (HO-PCBs), 22 methylsulfonyl PCBs ($MeSO_2$-PCB), 16 halogenated phenolic compounds (HPCs), 29 organochlorine pesticides (OCPs), 5 brominated flame retardants (BFRs) and 2 of their hydroxylated metabolites (HO-BFRs).

Materials and Methods

The analytical protocol is summarised in Figure 1. Extraction and purification were completed on a Rapidtrace Automated SPE workstation (Caliper Life Science Hopkinton, MA, USA) and evaporation was performed on a Labconco evaporator (Labconco Corp., Kansas City, MO).

Plasma samples were extracted on an Oasis HLB (540 mg; Waters Corp.) solid phase extraction (SPE) column according to the method describe by Sandau[1]. A mixture of internal standards, plasma sample, formic acid and deionised water was slowly applied to the column. After drying the column with pressurized nitrogen, the sample was extracted using methanol/dichloromethane (15 ml; 1:9). The sample was evaporated to dryness before it was dissolved in n-hexane (1 ml). The extract was then divided in two equal parts: one for POPs determination and the other for the DR-CALUX assay.

The extract for POPs analysis was eluted through a column containing activated Florisil (1 g). The first fraction (F1) containing the non-polar compounds (PCBs, OCPs, PBDEs) was eluted using hexane/dichloromethane (9 ml; 5:1). The second fraction (F2) containing $MeSO_2$ PCBs was eluted using hexane/acetone (9 ml; 4:1). The third fraction (F3) containing HPCs and HO-PCBs was eluted using dichloromethane/methanol (13 ml; 5:1).

After evaporation to dryness the compounds in F3 were derivatised using fresh diazomethane in hexane according to the method described by Sandau[2]**.** The derivatised fraction was then combined with F2, evaporated and cleaned up

on an activated silica/acidic silica column and compounds were eluted with dichloromethane (19 ml). The extract for the DR-CALUX assay was also cleaned up on an activated silica/acidic silica column and dioxin-like compounds were eluted with dichloromethane (8 ml).

F1 was evaporated, taken up in 20 µl of hexane and analysed for PCBs, OCPs and PBDEs on an Agilent (Wilmington, DE, USA) 6890 Network gas chromatograph (GC) equipped with an Agilent 7683 series automatic injector and an Agilent 5973 Network mass spectrometer (MS). The GC was fitted with an Agilent 60 m XLB column (0.25 mm i.d., 0.25 mm film thickness). The carrier gas was helium, and all injections were 2 µL in splitless mode. The mass spectrometer was operated in selected ion monitoring (SIM) mode, using electron capture negative ionisation (ECNI) with methane (99.97%) as the reagent gas or with electronic impact ionisation (EI) depending on which gave better sensitivity.

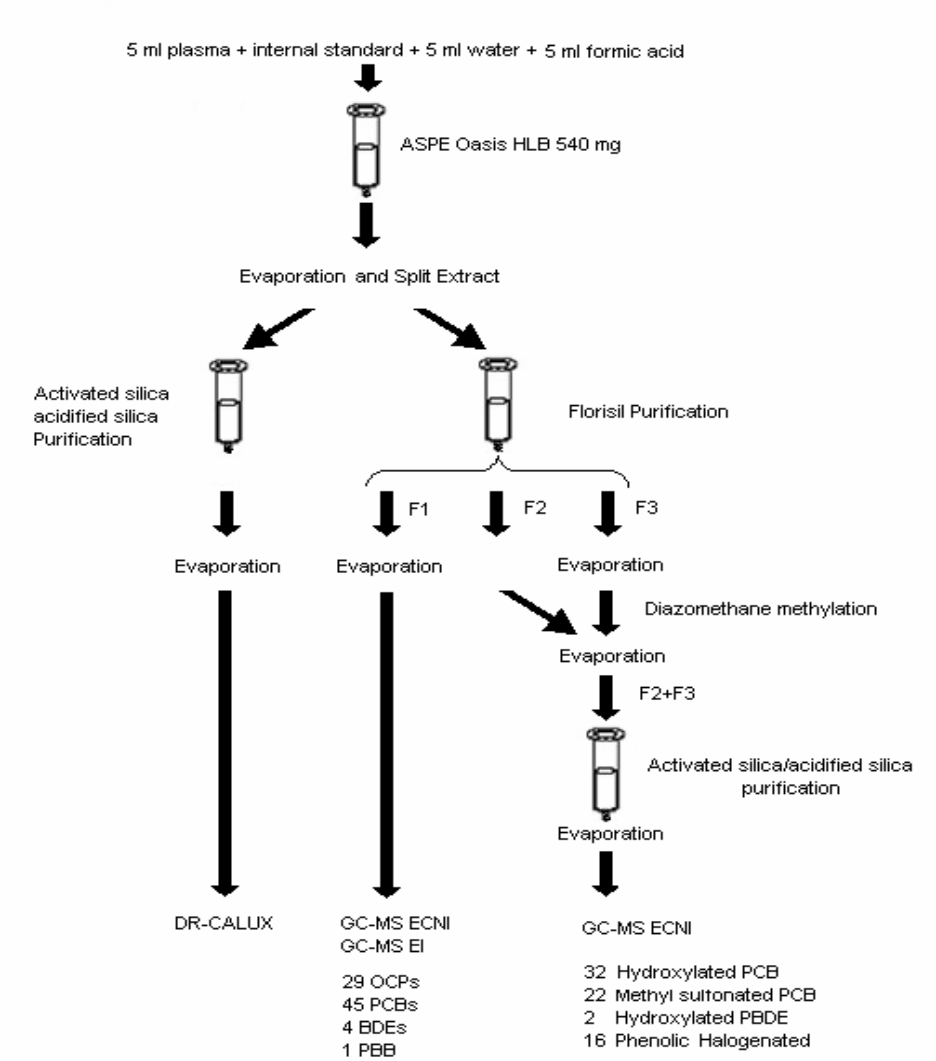

Figure 1 POPs in human plasma procedure scheme

The combined F2+F3 fraction was evaporated, taken up in 20 µl of hexane and analysed for HPCs, HO-PCBs and $MeSO_2$-PCBs on a Hewlett Packard (HP) 5890 Series II Plus gas chromatograph (GC) equipped with an HP G1512A automatic injector and a HP 5890B mass spectrometer (MS) (Agilent, Wilmington, DE, USA). The GC was fitted with a 30 m DB-5 column (5 % phenyl-methylpolysiloxane; 0.25 mm i.d., 0.25 mm film thickness) from

J&W Scientific (CA, USA). The carrier gas was helium, and all injections were 2 µL in splitless mode. The mass spectrometer was operated in selected ion monitoring (SIM) mode, using electron capture negative ionisation (ECNI) with methane (99.97%) as the reagent gas.

The CALUX fraction was evaporated to dryness and then reconstituted with 5 µl of dimethylsulfoxide before being tested for its dioxin-like activity using the H4IIE-Luc cell line (kindly donated by prof. A. Brouwer). The cells were plated at a density of $8x10^4$ cells/well in 24-well plates. After 5h, the 2,3,7,8-TCDD standards and plasma extracts were added on the cells for 24 hours. The cells were then washed in PBS and lysed in lysis buffer (Promega). The luciferase activity was determined with a luminometer (LMax Molecular Devices).

Results and Discussion

The analytical performance evaluated from the validation process according to ISO 17025 criteria is summarised in Table 1.

Table 1 Analytical method performance summary

Compound	**LOD Range (ng/l)**	**Working Range (ng/l)**	**Precision Same day n=10 At 20 ng/l**	**Precision Day to day n=10**	**Recovery % For 200 ng/l Spiked level**
PCBs	1-50	2-5000	±2-10%	±5-15%	89-105%
OCPs	1-200	2-25000	±4-18%	±3-30%	60-115%
HPCs	2-100	2-5000	±5-30%	±6-30%	30-85%
OH PCBs	2-10	2-5000	±4-15%	±9-20	60-108%
$MeSO_2$ PCBs	2-5	2-5000	±4-18%	±1-20	45-95%
BFRs	4-8	4-5000	±6-10%	±5-15%	94-106%
CALUX TEQ	0.03	0.03-0.3	±8%*	±21%	75% **

- *at 0.01 ng TEQ TCDD/l of plasma
- **for a spiked level of 0.322 ng TEQ TCDD/l

The accuracy of the method was tested on the certified reference material NIST 1589 and a good agreement was obtained on consensus values. Participation in international inter laboratory comparison programs also indicates that the method performance is highly reliable. Scores between 80 and 90% were obtained for the 2005 AMAP (Arctic Monitoring and Assessment Program) ring tests and the performance was comparable to reference methods.

We also noted strong correlations between PCB congeners and their metabolites as expected. Examples of correlations are presented in Figures 2 and 3 which include measurements for 50 plasma samples obtained from a population highly exposed to PCBs.

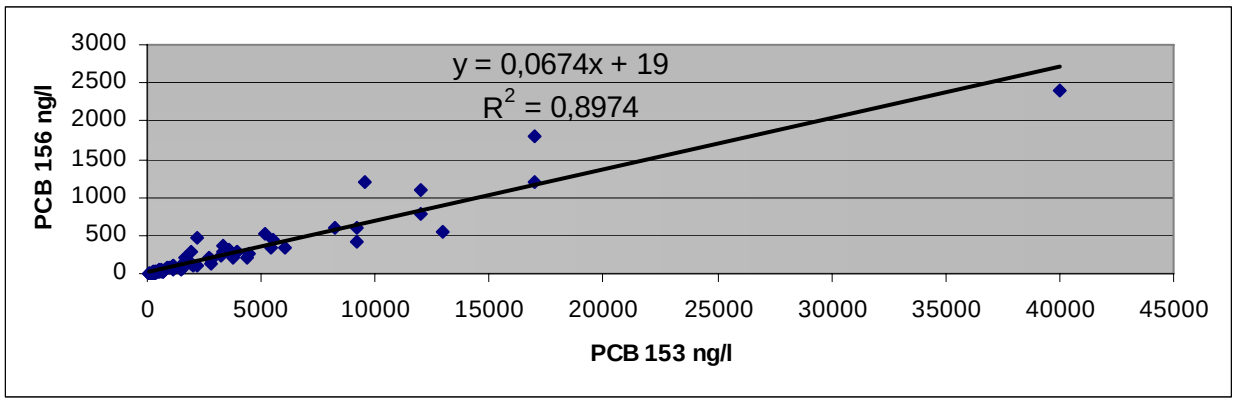

Figure 2: Correlation between PCB 153 and PCB 156 in human plasma samples (n=50)

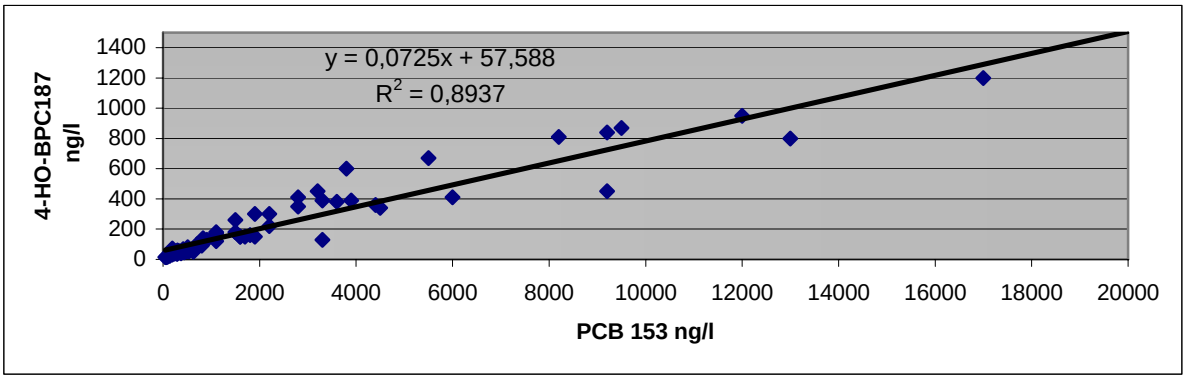

Figure 3: Correlation between PCB 153 and 4-HO-PCB 187 in human plasma samples (n=50)

A strong correlation was also observed between PCBs and the DR-CALUX response (Figure 4) showing the great potential of the DR-CALUX assay, which for the first time was performed using an automated solid phase extraction and purification procedure. These preliminary results suggest that PCBs are a major constituent of dioxin-like compounds in these samples. This is still under investigation.

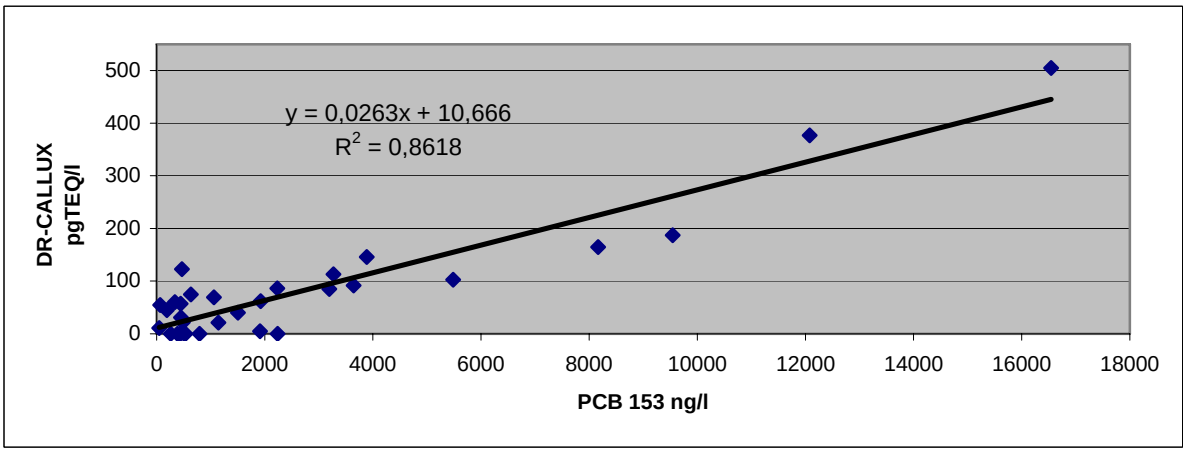

Figure 4: Correlation between PCB 153 and the DR-CALUX response in human plasma sample (n=32)

The preparation and analysis of 40 samples can be performed in 5 days with reduced technical manipulations compared to non-automated methods. Five more days are needed to complete the interpretation of the results. The method produces sensitive, precise and accurate data with a minimum volume of sample. This method can provide valuable information to researchers involved in the monitoring of POPs in human plasma.

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References

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2. Sandau C.D. (2000) Analytical Chemistry of hydroxylated Metabolites of PCBs and other Halogenated Phenolic Compounds in Blood and Their Relationship to Thyroid Hormone and Retinol Homeostasis in Humans and Polar Bears, PhD Carleton University.