IN-HOUSE VALIDATION OF A TIME- AND COST- SAVING METHOD FOR THE DETERMINATION OF INDICATOR PCBs AND ORGANOCHLORINATED PESTICIDES IN HUMAN SERUM

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Introduction

Human biomonitoring has been widely recognised as the most powerful tool to characterize human exposure to environmental contaminants, especially in the case of substances that are not single molecules but a family of congeners, like many persistent organic pollutants (POPs). Among human tissues recognized to be good indicators of POP body burden (blood, milk, and adipose tissue), blood is the matrix most frequently analysed in biomonitoring. To meet the increasing demand of biomonitoring data in epidemiology/risk assessment studies, and the associated need of large databases on POP levels in human blood, it is necessary to use rapid and economic analytical methods suitable to provide highly reliable congener-specific analytical data.

The purpose of this study was to optimize and test the analytical method routinely used in our laboratory for the determination of indicator PCBs, PCB 118, hexachlorobenzene (HCB), and *p,p*'-DDE in human serum. Because of its characteristics (it is rapid and economic, and requires a small sample size), the method is suitable for the analysis of a high number of samples in reasonably short times. The in-house validation scheme agreed with the International Organization for Standardization (ISO) 17025[1] criteria.

Materials and Methods

Sample preparation

The sample used in this study was an aliquot (200 mL) of a 700 mL human serum homogeneous bulk sample (pool) made with individual specimens with background contamination, not fortified with standards. The pool was prepared by mixing 100 individual serum specimens collected between 2000 and 2004 from Italian male and female donors. Homogenization was performed in a 1 L Sovirel glass bottle, by stirring the pool for 1 hour on a magnetic stirrer. The pool was divided into two aliquots, one (200 mL) was used for this study and the other (500 mL) was frozen and stored in order to be used as a control sample. The subsample used for validation (S) was divided into 19 10 mL aliquots (S1-S19). Six aliquots (S1-S6) were used to estimate the repeatability, eight aliquots (S7-S14) to estimate ruggedness and intra-laboratory reproducibility, and the last five aliquots (S15-S19) were sent to an accredited external laboratory in order to estimate trueness (certified reference materials were not available).

Analysis

According to the isotope dilution technique, serum subsamples S1-S6 were fortified with a mixture of ^{13}C-labelled PCBs (28, 52, 101, 118, 138, 153, 180) and pesticides (HCB and *p,p*'-DDE) and allowed to rest overnight. After spiking, the samples were added with a mixture of formic acid and 2-propanol, sonicated, and extracted by manual shaking with *n*-hexane. The organic phase was removed after centrifugation. The extraction process was performed two times. The *n*-hexane extracts were added with concentrated sulfuric acid, shaked, separated by centrifugation and then concentrated and transferred to 1 mL autosampler vials and quantified. Instrumental analysis was carried out by ion trap mass spectrometry (Thermofinnigan Polaris Q) in the MS-MS mode. Lipid determination (cholesterol, phospholipids, and triglycerides) was carried out by enzymatic methods.

Serum subsamples S7-S14, used for ruggedness evaluation, were also analyzed by the method reported above except for the minor deviations from the experimental conditions deliberately introduced to evaluate their effect on results. These deviations from the standard procedure were related to the factors (A-G) identified as potentially able to significantly influence the analytical results. They were: change of the operator (A), addition of a multilayer column purification step after the standard acidic treatment (B), change in shaking time during extraction (C), change in the number of extractions after the acidic treatment (D), addition of an alumina column

purification step after the acidic treatment (E), change in the number of *n*-hexane extractions (F), and change in *n*-hexane volume used for extraction (G). The ruggedness test was carried out using the Youden's approach[2].

In every sample batch (S1-S6 and S7-S14), a blank (distilled water extracted three times with *n*-hexane) was added and analyzed together with the samples.

Results and Discussion

Repeatability

The results of the repeatability test are shown in Table 1.

Table 1. Mean results (pg/g fresh weight) of the repeatability test on human serum subsamples S1-S6.

Analyte	Concentration	SD_r[a]	RSD_r[a] (%)	Recovery (%)
PCB 28	<17[b]	—	—	70
PCB 52	<17	—	—	73
PCB 101	<6.3	—	—	77
PCB 118	77.7	6.5	8.4	78
PCB 138	200	19	9.6	79
PCB 153	410	42	10	79
PCB 180	354	13	3.7	82
HCB	168	23	13	84
p,p'-DDE	1250	93	7.4	95
Fat percentage	*0.370*	*0.003*	*7.4*	—

a) SD_r and RSD_r are repeatability standard deviation and repeatability relative standard deviation.

b) <0.017. Sign < indicates limit of quantification (LOQ)

In the absence of a specific legislation setting requirements to be met by the analytical procedure for NDL-PCBs in human serum, the values of the estimated parameters (RSDr and recoveries) were compared with those required for confirmatory methods for the analysis of PCDDs, PCDFs, and dioxin-like PCBs in food[3]. All RSDr values were ≤ 15 %, and mean recovery rates were between 70 and 95 %, thus meeting the criteria set by Commission Directive 2002/69/CE. Table 1 shows the results of the enzymatic lipid determination in terms of fat. RSDr of the six independent lipid determinations was 7.4%.

Ruggedness

The parameters estimated in order to verify the ruggedness of the method are reported in Table 2.

Table 2. Statistical evaluation of the results of the ruggedness test.

Analyte	D_A[a]	D_B	D_C	D_D	D_E	D_F	D_G	SD_{Di}	F[b]
PCB 118	-3.4 10-4	7.4 10-4	-1.4 10-2	3.6 10-3	6.8 10-4	3.4 10-3	1.1 10-2	7.5 10-3	1.3
PCB 138	-1.6 10-2	3.5 10-3	-8.5 10-3	1.5 10-2	-6.1 10-3	8.9 10-3	-1.6 10-4	1.1 10-2	0.30
PCB 153	2.5 10-2	4.0 10-3	-9.4 10-3	3.6 10-2	4.8 10-2	1.3 10-2	2.4 10-2	1.9 10-2	0.21
PCB 180	-4.1 10-3	-5.4 10-2	-2.6 10-2	1.9 10-2	5.7 10-2	2.1 10-2	2.6 10-2	3.7 10-2	7.8
HCB	-7.2 10-3	1.4 10-2	5.6 10-4	6.8 10-3	-1.4 10-2	-4.7 10-3	-1.2 10-2	1.0 10-2	0.20
p,p'-DDE	-1.8 10-2	6.5 10-2	-4.9 10-2	-1.1 10-2	-3.9 10-1	5.7 10-2	-1.1 10-1	1.6 10-1	2.8

a) D_i are the differences between the average of the four measurements with no variation in factor i and the four measurements with the variation in the standard procedure of the same factor set by the ruggedness test.

b) Fisher test result. $F = SD_{DI}^2/SDr^2$.

As showed in the table, no D_i value was appreciably higher than the differences for the other factors for all the analytes, with the exceptions of D_C and D_G for PCB 118 and D_E for *p,p*'-DDE. Nevertheless, the Fisher

test did not show a significant difference between the two variances SD_{DI}^2 and SDr^2 (F values < F tabulated), this leading us to consider the method robust against all the tested factors.

Within-laboratory reproducibility

Results of ruggedness test carried out on S7-S14 subsamples were also used to estimate within-laboratory reproducibility and are reported in Table 3.

Table 3. Mean results (pg/g fresh weight) of the estimation of within-laboratory reproducibility on human serum subsamples S7-S14.

Analyte	Concentration	SD_R^a	RSD_R^a (%)	Recovery (%)
PCB 28	<17[b]	—	—	70
PCB 52	<17	—	—	70
PCB 101	<6.3	—	—	75
PCB 118	85.9	9.9	11	74
PCB 138	206	14	6.8	75
PCB 153	410	38	9.2	70
PCB 180	353	49	14	66
HCB	259	14	5.3	68
p,p'-DDE	1630	220	14	91
Fat percentage	*0.324*	*0.016*	*4.9*	—

a) SD_R and RSD_R are within-laboratory reproducibility standard deviation and within-laboratory reproducibility relative standard deviation.

b) <0.017. Sign < indicates limit of quantification (LOQ)

RSDr values were between 5.3 and 14 %, and mean recovery rates were between 70 and 91 %. These values meet the criteria set by Commission Directive 2002/69/CE. Within-laboratory reproducibility standard deviations were used as the estimation of uncertainty of measurement.

Trueness

For every analyte, trueness of the method was estimated as the ratio between the mean of the results of the determinations carried out in our laboratory (S1-S14) and the mean of those obtained by the external accredited laboratory (S15-S19), expressed as a percentage. Trueness values (together with a synopsis of the other validation parameters estimated in this study and LOQ values) are reported in Table 4.

Table 4. Summary of the results of the estimation of validation parameters on human serum subsamples S1-S14.

Analyte	Uncertainty of measurement (%)	Trueness (%)	Recovery[a] (%)	LOQ[b] (ng/g fw)
PCB 28	—	—	70	1.7E-02
PCB 52	—	—	71	1.6E-02
PCB 101	—	—	76	6.0E-03
PCB 118	11	93	76	9.4E-03
PCB 138	6.8	60	77	8.6E-02
PCB 153	9.2	86	74	5.9E-02
PCB 180	14	89	73	1.3E-02
HCB	5.3	76	75	8.8E-03
p,p'-DDE	14	74	93	6.4E-02
Fat percentage	*4.9*	*110*	—	—

a) Mean recoveries of the repeatability and ruggedness tests (S1-S14)

b) For a 10 g sample 1 μL of 200 μL injected.

All values were satisfactory (between 74 and 93 %; 110 % for lipid determination), apart from PCB 138 for which a value of trueness of 60% was observed.

LOQ estimation

Repeatability, within-laboratory reproducibility and trueness were not evaluated for those congeners (PCB 28, 52, and 101) always under the limit of quantification (LOQ). LOQ values were determined for each analyte as the level of the analogous ^{13}C-labelled analyte necessary to generate a signal-to-noise ratio ≥ 3 in the injected extract.

In conclusion, the analytical method resulted to be characterized by good repeatability, within laboratory reproducibility and trueness. Moreover, the method resulted to be robust with respect to the factors tested in the study.

References

1. ISO/IEC 17025, General Requirements for the Competence of Testing and Calibration Laboratories, ISO, Geneva, 2005.
2. Youden W.J. and Steiner E.H. *Statistical Manual of the AOAC–Association of Official Analytical Chemists*, AOAC-I, Washington DC, 1975.
3. Commission Directive 2002/69/CE laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs.