QUALITY ASSURANCE IN PLANAR PCBs ANALYSIS – VALIDATION OF THE METHOD AND RESULTS FROM FRESHWATER FISH ANALYSIS

Pavel Gregor[1], Jana Hajšlová[1], Vladimír Kocourek[1] and Jacob de Boer[2]

[1]Department of Food Chemistry and Analysis, Institute of Chemical Technology, Technická 3, 166 28 Prague 6, Czech Republic
[2]Netherlands Institute for Fisheries Research (RIVO-DLO), Haringkade 1, P.O. BOX 68, 1970 IJmuiden, the Netherlands

Introduction

Nowadays there are a lot of well-established methods for routine analysis of *non*-ortho PCB congeners (No. 77, 81, 126 and 169) in fish [1,2,3]. Although these coplanar congeners are much less toxic than usually monitored polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs), their occurrence in more than three order of PCDD/Fs levels made these analysis greatly worthwhile for the survey of toxic equivalent concentration of 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD (TEQ) [4,5].
The aims of this study were: (i) optimization of extraction step for lipid portion isolation (together with studied compounds), which may be considered as one of the most critical step in analysis [6], (ii) validation of ultra-trace analytical method for *non*-ortho PCBs determination and interlaboratory comparison, and (iii) survey for planar PCBs levels in freshwater fish from Czech Republic to assess health risk due to dietary intake of these compounds, in terms of TEQ.

Methods and Materials

For the extraction step optimization the homogenized fish muscle material was prepared: chub (*Leuciscus cephalus*) and barbel (*Barbus barbus*), 2:1 (w/w). Different extraction techniques and solvent mixtures (or pure solvents) were tested: ultrasonic extraction (hexane:acetone, 2:1, v/v) after and without 3 hours dessication after Nasulphate mixing, Soxhlet extraction (pentane:dichloromethane, 1:1, v/v, and hexane:dichloromethane, 1:1, v/v), extraction with Soxtec apparatus (hexane EN 1528, diethylethere EN 1528, hexane CSN 570146 – as dried material, and hexane ISO 1443 – after hydrolysis). Extracted lipids were determined gravimetrically.

The validation of ultra-trace method for planar PCBs determination was carried out as a part of homogeneity testing of candidate reference material chub (*Leuciscus cephalus*) muscle (from the bottom reaches of Elbe river) in CHRONO project: "*Chub and herring as reference materials for ortho and non-ortho chlorobiphenyls*", and consisted of following parts:
(a) "between-batch variance" homogeneity testing: 20 analysis from 20 tins (each selected from 150 pieces batch)
(b) "within-batch variance" homogeneity testing: 5 analysis from pooled content of 3 tins
(c) "clean-up error" testing: 5 analysis from pooled extract of 3 tins
(d) "GC error" testing: 10 analysis of *non*-ortho PCB standard solution

(e) interlaboratory comparison of planar PCB determination in candidate reference material chub and other RIVO-DLO intralaboratory materials (cod liver, eel muscle).

Fish tissue samples (20 g fish muscle dessicated with 50 g of anhydrous sodium sulphate) were extracted with Soxhlet extractor (170 ml of mixture pentane: dichloromethane, 1:1, v/v, for 7 hours). Before extraction samples were spiked with ^{13}C isotopes (each 3.2 ng), incubation time was 16 hours. Lipid removal was carried out with disposable silica columns (30 g of SiO_2, 10% H_2O), analytes were eluted with 100 ml of hexane. Separation of *non*-ortho PCB group was done with the HPLC-PYE system, on Cosmosil PYE column (2-/1-pyrenyl/ethylsilica, 250×4.6 mm, 5 µm particles, operated at 0°C with hexane as eluent, 0.5 ml.min^{-1}). Fraction of monitored analytes (9-18 ml) was collected, and after addition of internal standard (16 ng of ^{13}C PCB 101) evaporated and transferred to 200µl of isooctane. Final identification and quantification was carried out by HRGC-LRMS-NCI (HP 6890 coupled to HP 5973, ionization gas methane) on DB-5ms column (60m×0.25mm×0.25µm phase).

Fish samples for planar PCB survey in Czech freshwater ecosystem were collected in summer 1999 from four localities on major rivers: Dříteč-Němčice (Elbe river), Všenory-Jíloviště (Berounka river), Štekeň-Radomyšl (Otava river) and Uherské Hradiště-Jarošov (Morava river). The whole set consisted of 17 samples and included these different species: chub (*Leuciscus cephalus*), barbel (*Barbus barbus*), perch (*Perca fluviatilis*) and bream (*Abramis brama*).

Results and Discussion

The comparison of studied extraction techniques is summarized in Figure 1. As can be seen from picture, the highest amounts of lipids were obtained when extraction was performed with Soxtec apparatus, but unfortunately due to the limited sample size this technique is not suitable for planar PCB isolation. The best choice is using Soxhlet extraction with mixture pentane:dichloromethane (1:1, v/v) for the almost identical boiling points of these solvents.

From the planar PCB method validation there were obtained satisfactory low values of uncertainty associated with clean-up or GC error in determination of these compounds, as same as regarding between or within batch variance, see Table 1. Recoveries of ^{13}C labelled PCB analogues were in the acceptable range of 77-89%, with rsd 3.0-3.7%.

Table 1: Comparison of relative standard errors in planar PCBs analysis

RSD [%]	PCB 77	PCB 81	PCB 126	PCB 169
$rsd_{between\ batch}$	4.2	8.3	4.8	9.4
$rsd_{within\ batch}$	2.2	5.3	6.9	7.0
$rsd_{inhomogeneity}$*	3.6	6.4	0.0	6.3
$rsd_{clean\text{-}up\ error}$	4.8	8.4	3.0	6.9
$rsd_{GC\ error}$	4.0	4.0	3.4	3.2

* $rsd^2_{inhomogeneity} = rsd^2_{between\ batch} - rsd^2_{within\ batch}$

Results of interlaboratory comparison of planar PCB determination in chub are summarized in Table 2. (observed values from RIVO-DLO were taken from initially stability testing of candidate reference material at t=0). Excellent agreement in determined *non*-ortho PCB levels as well as

very low values of standard deviations were found except for PCB 169 (probably due to greatly difficult determination at this concentration level).

Table 2: Interlaboratory comparison of planar PCB determination in chub material (in ng/kg tissue)

	PCB 77	PCB 81	PCB 126	PCB 169
ICT, CZ (n=20)	160.9	5.4	21.4	2.5
stdev	6.8	0.4	1.0	0.2
RIVO-DLO, NL (n=5)	177	5.9	22	10
stdev	1.2	0.2	1.3	0.4

Results from analyzed Czech freshwater fish samples (including *non-* and *mono-*ortho PCB) were expressed as TEQ equivalents (using currently valid TEFs for human exposure [5]) with values in range of 1-12 ng/kg. Contributions from *non*-ortho PCB to TEQ value were higher than from *mono*-ortho CBs, the major ones resulted from CB 126, CB156 and CB 118. The highest levels of planar PCBs were detected in barbel samples, less in chub and bream and lowest values were obtained in perch samples (due to very low amount of lipids, i.e. typically < 1%). Obtained TEQ values are comparable with reported data from fish caught from heavily polluted rivers in Western Europe [1,7,8].

Acknowledgments

The autors wish to thank for financial support from the project „Monitoring of Contaminants in Food Chains" funded by Czech Ministy for the Environment (reg. No. VAV/340/2/99).

References

1. de Boer J., Stronck C. J. N ., Traag W. A. and van den Meer J. (1993) Chemosphere 26, 1823.
2. Hess P., de Boer J., Cofino W. P., Leonards P.E.G. and Wells D.E. (1995) J. Chrom. A 703, 417.
3. Kuehl D.W., Butterworth B.C., Libal J. and Marquis P. (1991) Chemosphere 22, 849.
4. Alcock R.E., Behnisch P.A., Jones K. C. and Hagenmaier H (1998) Chemosphere 37, 1457.
5. van der Berg M., Birnbaum L., Bosveld A.T.C., Brunström B., Cook P., Feeley M., Giesy J.P., Hanberg A., Hasegava R., Kennedy S.W., Kubiak T., Larsen J.Ch., van Leeuwen F.X.R., Liem A.K.D., Nolt C., Peterson R.E., Poellinger L., Safe S., Schrenk D., Tillitt D., Tysklind M., Younes M., Wærn F., Zacharewski T (1998) Environ. Health Persp. 106, 775.
6. de Boer J (1988) Chemosphere 17, 1803.
7. Lulek J., Sauvain J.J., de Alencastro L.F., Grandjean D., Tarradellas J (1997) Organohalogen Compounds 32,344.
8. Malisch R (1996) Organohalogen Compounds 28, 271.

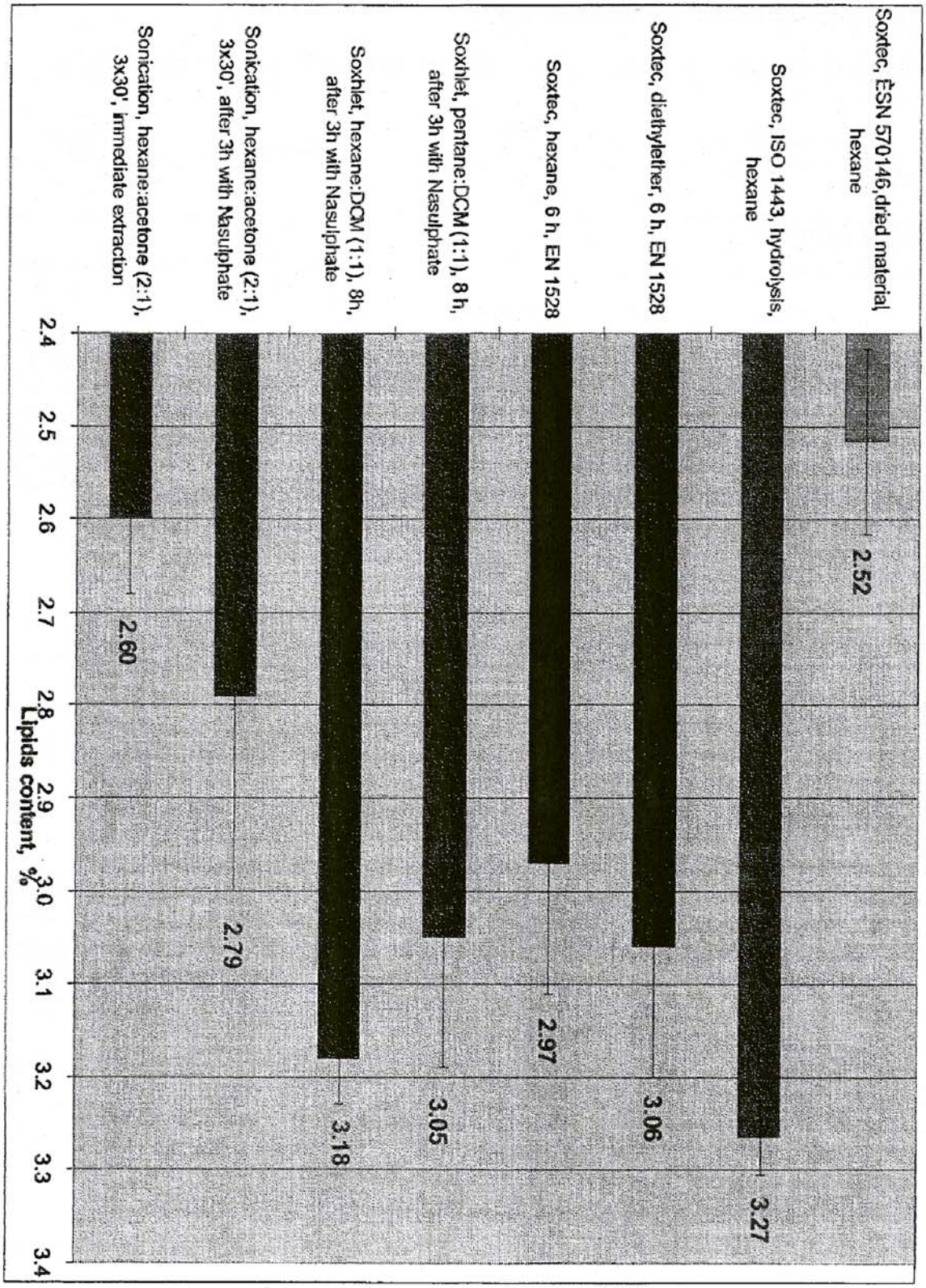

Figure 1: Comparison of different extraction methods for the isolation of lipids from fish muscle (n=6)