Improved determination of selected POPs in human serum using solid-phase disk extraction and dual-column CGC-ECD

Adrian Covaci, An Pauwels and Paul Schepens

Toxicological Center, Antwerp University (UA), Universiteitsplein 1, B-2610 Wilrijk, Belgium

Introduction

Chlorinated biphenyls (CBs) and organochlorine pesticides, two groups of persistent organic pollutants (POPs), are an important topic of public health concern (1-4). The requirements for risk assessment in epidemiological studies have created the need for efficient, fast and less-costly analytical methods. Monitoring of human exposure to POPs is most conveniently performed by analysis of the blood plasma or blood serum (5-8).

At Dioxin'98, we reported for the first time the use of solid phase disk extraction (SPDE) technology for analysis of POPs from human serum (9,10). The procedure involved denaturation of serum proteins with formic acid, solid-phase extraction using C_{18} Empore^{TM} disk cartridges, followed by elimination of lipid interferences using a sulphuric acid wash of the eluate. Use of SPDE improved assay throughput and allowed reduced volumes for elution. However, due to residual interferences, identification and quantitation was only possible for major CBs and *p,p'*-DDE, and the method did not allow the determination of minor CB congeners, which contribute substantially to total dioxin-like toxicity. Hereby, we present an improvement of the method by using an additional clean-up step on silica gel and a dual-column configuration to improve selectivity and a more accurate quantitation.

Materials and Methods

Sample preparation and clean-up. Human serum was provided by the University Hospital of Antwerp. Blood was centrifuged (15 min, 2000g) within 24 hr after collection. The serum was kept frozen at -20^{o}C until analysed.

To 10 ml of human serum, 12.5 ng of CB 46 and CB 143 were added as surrogate standards. The serum was then mixed with 10 ml formic acid, 500 µl acetonitrile and 100 µl triethanolamine and was equilibrated in an ultrasonic bath for 30 min.

The sample was applied in parallel to two activated C_{18} disk cartridges (Empore^{TM}, 10mm/6ml from 3M Company, St. Paul, USA) to avoid overloading and breakthrough of the analytes. After sample loading, each cartridge was rinsed with deionized water and the sorbent bed was dried thoroughly under a nitrogen stream and by centrifugation. Each cartridge was eluted with 1.5 ml hexane and the eluates combined. One ml concentrated sulphuric acid was added to the combined eluate and mixed for 3 min. After centrifugation, the organic layer was removed and further purified on a silica gel cartridge (100 mg/1ml from Supelco, Bellefonte, USA) topped with Na_2SO_4. The eluate was concentrated under a gentle nitrogen stream at room temperature to approximately 50 µl and transferred to a vial. The internal standard TCN (1,2,3,4-tetrachloronaphthalene) was added to the final concentrate, prior to GC analysis.

Instrumental analysis. A Hewlett Packard 5890 series II GC-ECD was used with helium as carrier gas and Ar/CH_4 (95:5) as make-up gas (flow 55 ml/min). Two µl were injected in splitless mode with the split outlet opened after 2 min. Injector and detector temperatures were set at 260^{o}C and 300^{o}C, respectively. Two different capillary GC columns were applied: a 50m x 0.25 mm, df=0.10

μm, CP-Sil 5/C18 column, with 10% C18 incorporated in the stationary phase (Chrompack, Middelburg, The Netherlands) and a DB-XLB column, 60m x 0.25 mm, df=0.25μm (J&W Scientific, Folsom, USA).
The temperature program of the CP-Sil 5/C18 column was set to 80°C for 0.5 min and then increasing the temperature with 15°C/min to 180°C, kept for 5 min and further by 2°C/min to 260°C kept for 10 min. The temperature program of the other column DB-XLB, was starting from 80°C, kept for 0.5 min and then increasing the temperature with 15°C/min to 220°C, stay 5 min and further by 2°C/min to 275°C kept for 25 min. The inlet pressures were 20 psi and 23 psi for the CP-Sil 5/C18 column and the DB-LXB column, respectively.
Multi-level calibration curves (r^2 > 0.97) were created for the quantification using standard solutions in iso-octane, covering the entire range of expected values for each congener. Method limits of detection range between 5 and 10 pg/ml serum (whole weight) using the above mentioned analytical conditions.

Results and discussion
Solid-phase extraction. The protein denaturation method without precipitation has been evaluated because of the possible loss of analytes by occlusion in the precipitate (9,10). Acetonitrile and reduced pH due to formic acid inhibit protein binding and increase the extraction efficiency of the analyte by the C_{18} sorbent.
Diffusion distance between particles is minimized in the Empore™ membrane (90% sorbent, 10% PTFE matrix), adsorption is more efficient and extraction can be accomplished using less sorbent mass. The C_{18} disk cartridge employed for sample clean-up and analyte enrichment has a non-polar character in order to retain organochlorine and other non-polar compounds. It also has a size exclusion function to eliminate macromolecular interferences (such as serum proteins) in biological extracts. The small bed volume disk approach to SPE reduces elution volume from 5-10 ml (usually used in traditional SPE) to 500 μl, thus making this method more attractive.
Removal of lipids and other interfering compounds from the SPE eluate is achieved by using a concentrated sulphuric acid wash and by silica gel column chromatography. Additional sample clean-up on silica gel resulted in a lower background, which facilitated peak identification of minor congeners and provided better instrumental performance and longer column lifetime (traces of sulphuric acid are retained on the silica gel cartridge).

Recoveries. Recoveries for selected POPs ranging from 62 to 74%, and a good reproducibility (RSD < 14%) are depicted in Figure 1. There were no differences in recovery between CB congeners with a low or high degree of chlorination or with respect to the degree of chlorination in *ortho*-position.

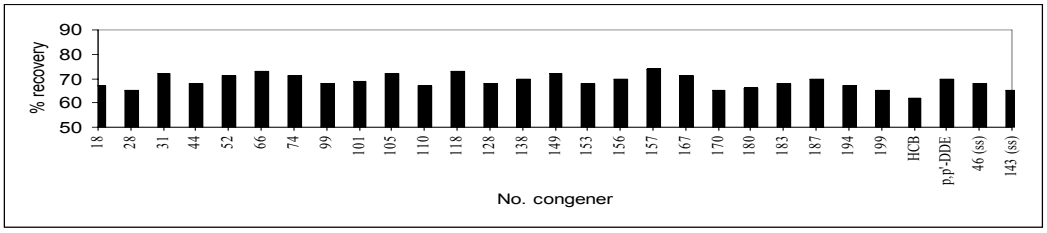

Figure 1. Recoveries of selected POPs from human serum.

Recoveries from pooled serum (n=5) for surrogate standards were 68 ± 8% for CB 46 and 65 ± 9% for CB 143, and they were considered satisfactory for our protocol.

GC separation. The separation of individual CBs on each type of stationary phase was previously investigated (11, 12), but none of these two columns is routinely used in CB analysis. When compared to the most used GC capillary column in CB analysis - DB-5 (5% phenyl stationary phase) or equivalent - these two columns offer better separation and avoid co-elution of interesting congeners. Typical ECD chromatograms of a serum sample analysed on the CP-Sil5/C18 and DB-XLB columns are shown in Figure 2.

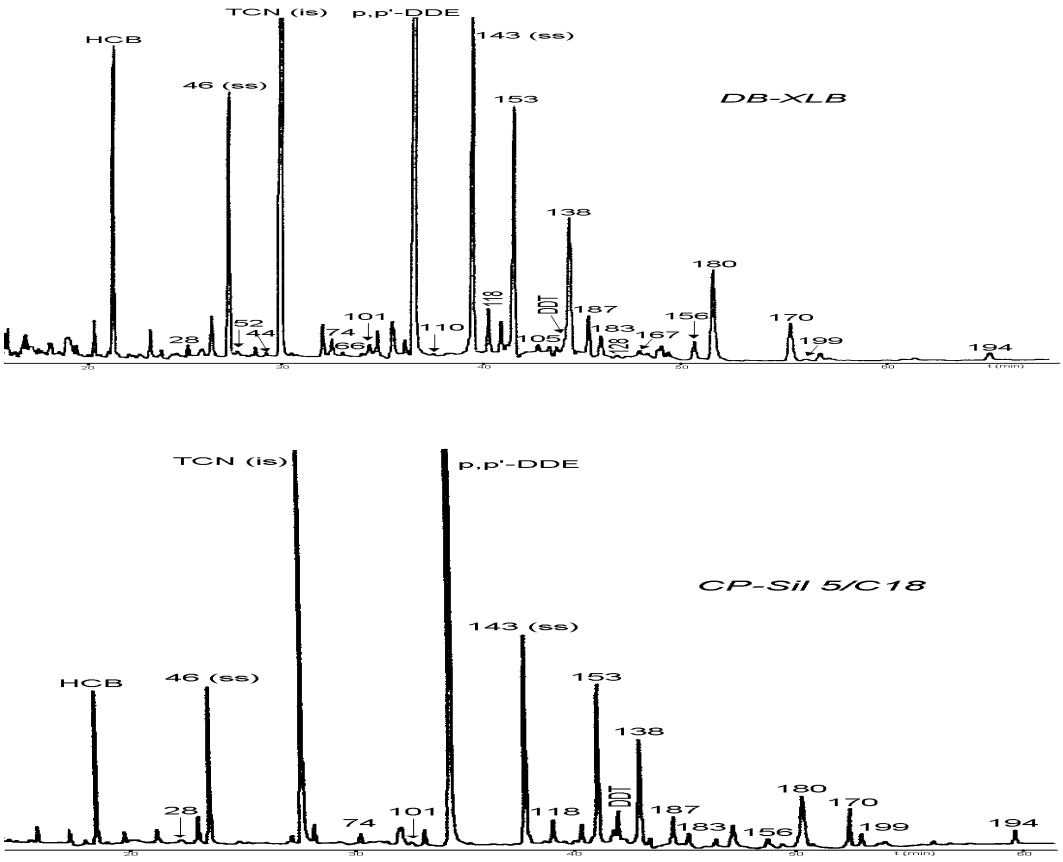

Figure 2. GC-ECD chromatograms of a serum sample successively analysed on a 60 m DB-XLB column and a 50 m CP-Sil 5/C18. Individual CBs are identified by their IUPAC numbers.

Both columns resolve the CB pairs 170/190, 105/153, 118/149, 28/31 and 77/110. Additionally, the CP-Sil 5/C18 column separates *p,p'*-DDT from CB 138, reported to co-elute on XLB column, but fail to separate CB 156 from CB 157, though this separation can be achieved on XLB. The pair CB 128/183 had different order of elution on the two columns. CBs 118 and 167 were better quantified on XLB column, due to a co-eluting interference on CP-Sil 5/C18, while HCB was

better quantified on CP-Sil 5/C18. Considering these arguments, a good reproducibility (RSD<18%) of the quantification using both columns is achieved.

Analysis of serum samples. The method was validated on several individual serum samples obtained from the Municipal Hospital of Timisoara, Romania. Good conformity in the relative concentration profile of the CBs was demonstrated (Figure 3). In general, these levels found are somewhat higher than the residues observed in Western Europe populations (2,5). This can be explained by the fact that DDT and old electrical equipment may still be in use in Romania.

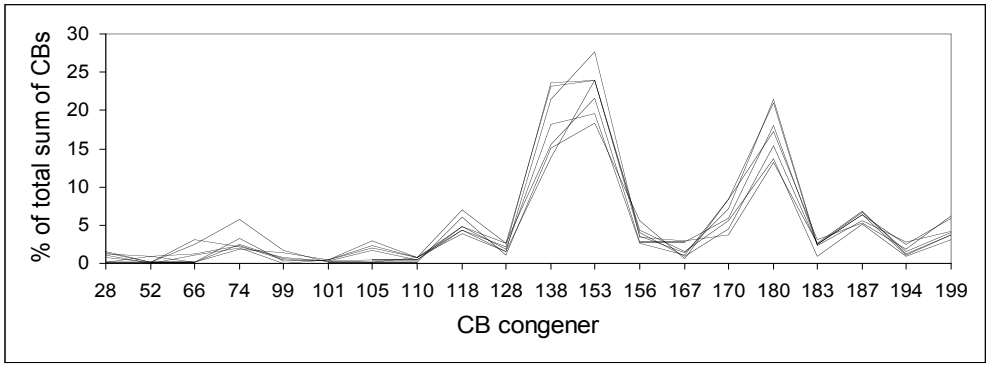

Figure 3. Profile showing the percentage of individual CBs of the total sum of CBs in serum samples (n=7) from Romania. The sum of all congeners range between 3.7 and 16.5 ng/ml whole weight.

In summary, the present work provides a reliable, simple, rapid and sensitive methodology for the routine congener specific analysis of CBs.

References

1. Anderson HA, Falk C, Hanrahan L, Olson J, Burse VW, Needham L, Paschal D, Patterson Jr. D and Hill Jr. RH, *Environ. Health Perspect*, **106**, 279-289 (1998).
2. Grimvall E, Rylander L, Nilsson-Ehle P, Nilsson U, Strömberg U, Hagmar L, and Östman C, *Arch. Environ. Contam. Toxicol*, **32**, 329-336 (1997).
3. Johansen RH, Alexander J, Rossland OL, Planting S, Lovik M, Gaarder PI, Gdynia W, Bjerve KS, and Becher G, *Environ. Health Perspect*, **104**, 756-764 (1996).
4. Cogliano VJ, *Environ. Health Perspect*, **106**, 317-323 (1998).
5. Grimvall E, Östman C, and Nilsson U, *J. High Resolut. Chromatogr*, **18**, 685-691 (1995).
6. Gill US, Schwartz HM, and Wheatley B, *Chemosphere*, **32**, 1055-1061 (1996).
7. Greizerstein HB, Gigliotti P, Vena J, Freudenheim J, and Kostyniak PJ, *J. Anal. Toxicol*, **21**, 558-566 (1997).
8. Brock JW, Burse VW, Ashley DL, Najam AR, Green VE, Korver MP, Powell MK, Hodge CC, and Needham LL, *J. Anal. Toxicol*, **20**, 528-536 (1996).
9. Pauwels A, Wells DA, Covaci A, and Schepens PJC, *Organohalog. Comp*, **35**, 167-170 (1998).
10. Pauwels A, Wells DA, Covaci A, and Schepens PJC, *J. Chromatogr. B*, **723**, 117-125 (1999).
11. Frame GM, *Fresenius J. Anal. Chem.* **357**, 701-722 (1997).
12. Ballschmiter K, Mennel A, and Buyten J, *Fresenius J. Anal. Chem*. **346**, 396-402 (1993).