Journal of Chromatography A, 1216 (2009) 897–901

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A simple and fast liquid–liquid extraction method for the determination of 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (*p*,*p*′-DDE) from human serum for epidemiological studies on type 2 diabetes

Panu Rantakokko[a,*], Hannu Kiviranta[a], Lars Rylander[b], Anna Rignell-Hydbom[b], Terttu Vartiainen[a]

[a] *National Public Health Institute, Department of Environmental Health, P.O. Box 95, FIN-70701 Kuopio, Finland*
[b] *Division of Occupational and Environmental Medicine, Department of Laboratory Medicine, University Hospital, 221 85 Lund, Sweden*

ARTICLE INFO

Article history:
Received 22 September 2008
Received in revised form 1 December 2008
Accepted 5 December 2008
Available online 10 December 2008

Keywords:
Liquid–liquid extraction
Human serum
Type 2 diabetes
Persistent organic pollutants (POPs)
PCB-153
p,*p*′-DDE

ABSTRACT

A simple and fast method is presented to be used for example in studies on the relationship between serum levels of persistent organic pollutants and type 2 diabetes mellitus. Method is based on liquid–liquid extraction and gas chromatography coupled with high-resolution mass spectrometry. In the sample pre-treatment special attention was paid to minimize the number of sample manipulation steps and the amounts of organic solvents needed. Compounds analyzed were 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153) and 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene (*p*,*p*′-DDE), the major metabolite of DDT. The method included extraction and cleanup of 0.2 ml of serum in a single test tube and subsequent analysis of the extract from 0.2 ml final volume. Validation was conducted to explore the performance of the method. The limits of detection for *p*,*p*′-DDE and PCB-153 based on the standard deviation of the blank samples were 4.3 and 3.1 pg/ml, respectively. Repeatability was less than 2.5% at three concentration levels tested and recovery from Certified Reference Material SRM 1589a was 84% for *p*,*p*′-DDE and 87% for PCB-153 of the certified values, respectively. Serum samples from the AMAP intercalibration round 2008-2 were also analyzed, and results were 101–116% of the assigned values. The presented method was used for an epidemiological study with more than 700 serum samples from a type 2 diabetes cohort from Sweden.

1. Introduction

Evidence is accumulating that persistent organic pollutants (POPs) are positively associated with type 2 diabetes mellitus (T2DM) and also with pre-diabetic stages. The first evidence about positive association between POPs (mainly related to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD) and T2DM originated from studies conducted after accidental, occupational or otherwise unusual exposure conditions [1–11]. Most of these early studies assessing the POP-T2DM relationships have serious limitations. In many cases there was a long period between exposure and the POP measurements. Also, the risk assessments made in the occupational settings may be confounded by the much higher simultaneous exposure to the main chemical (e.g. herbicide) in which the POP of interest occurred as impurity [12].

A number of studies with subjects more representative of general population have also been conducted. In a study from Belgium a highly significant elevation of serum levels of dioxins and polychlorinated biphenyls (PCBs) among patients with T2DM was found [13]. When a group of pesticide users was compared with an unexposed group it was found that subjects with T2DM had higher blood levels of dichlorodiphenyl trichloroethane (DDT) and its major metabolite 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (*p*,*p*′-DDE) [9]. In Sweden, a positive association between T2DM and exposure levels of 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) and *p*,*p*′-DDE have been found [14,15]. In Michigan, US, in 1973 a brominated flame retardant, polybrominated biphenyl (PBB) accidentally contaminated domestic animal feed resulting in the exposure of hundreds of people. Serum levels of PBBs and also PCBs were measured at the enrolment of this prospective Michigan cohort study. Thirty years later the risk of T2DM was 2.3 times higher among women that had the highest levels of PCBs at enrolment [16]. So far the strongest epidemiological evidence regarding the positive association between POPs and T2DM among the general population was a result of a cross-sectional cohort study in the National Health and Nutrition Examination Survey (NHANES) in the US ($n = 2016$). In this study, depending on the adjustment for confounding factors, the risk for T2DM in the most exposed decile of the study population was 16–38 times higher when compared to the least exposed group [17]. Strikingly, obesity was found to be a risk factor for T2DM

* Corresponding author. Tel.: +358 17 201 395; fax: +358 17 201 265.
E-mail address: Panu.Rantakokko@ktl.fi (P. Rantakokko).

0021-9673/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2008.12.013

and also for insulin resistance only when concentrations of POPs got higher [17,18].

These associations call for a rapid and sensitive analytical method that could determine selected POPs from small volumes of human serum. We have chosen to use CB-153 as a biomarker for POP exposure, because it correlates very well ($r \geq 0.98$) with total PCB concentration in plasma and serum from Swedish subjects [14,15]. Another relevant exposure biomarker is the anti-androgenic compound *p,p′*-DDE.

Direct headspace solid phase microextraction (HS-SPME) with automated instrumentation is a feasible method for the analysis POPs in human serum because it has little manual sample preparation, extraction is solvent free, and has no cleanup steps. HS-SPME applications for human serum have been published for PCBs and organochlorine pesticides (OCPs) [19–21]. The main drawback of this technique for POPs is the lengthy extraction time (typically 30–50 min) that limits the sample throughput per instrument. However, possibly due to lack of proper instrumentation, most laboratories analyzing POPs from human serum still use different solid phase extraction (SPE), and to a lesser extent, liquid–liquid extraction (LLE) plus cleanup methods.

SPE has gained popularity in the analysis of POPs from serum during the last decade. Manual SPE methods with off-line cleanup [22,23] and even without cleanup steps [24] have been developed for PCBs and OCPs. For PCB and PBDEs, a simple manual SPE method with on-line cleanup has been presented [25]. SPE methods with varying level of automation have been developed for PCBs and other POPs [26–28]. SPE methods are generally considered to have higher sample throughput, be less labor intensive, faster, and consume less solvents than LLE methods.

As a result, not much effort has been put to new LLE methods during the last years and published methods using LLE have focused more on other methodological aspects, like improvements in the GC [29] and cleanup [30] or have included phenolic POPs in addition to neutrals where also LLE is useful [31,32].

Contrary to this SPE dominant trend, we present here a novel LLE method for *p,p′*-DDE and PCB-153 in human serum, which was used in an epidemiological study on type 2 diabetes. Method is very simple and fast, and uses very small amounts of solvents. Extraction and cleanup procedures take place inside a regular 8 ml glass test tube, from where the extract is transferred to GC autosampler vial for final GC-HRMS measurement. To the best of our knowledge, a method combining efficient extraction and cleanup to a single test tube has not been published previously for the determination of POPs from serum samples.

2. Materials and methods

2.1. Chemicals

Analytical grade hexane, toluene, silica gel 60 and sulphuric acid were from Merck (Darmstadt, Germany). High-purity ethanol was obtained from Altia (Rajamäki, Finland). Sulphuric acid impregnated silica was prepared by mixing 500 g of silica gel and 76.5 ml of sulphuric acid for 24 h in an overhead mixer.

Individual standards of native *p,p′*-DDE and PCB-153 were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Internal standards ^{13}C- *p,p′*-DDE and ^{13}C-PCB-153, and recovery standard ^{13}C-PCB-128 were purchased from Cambridge Isotope Laboratories (Andover, MS, USA). From these chemicals six level calibration solutions for *p,p′*-DDE and PCB-153 between 25 and 10,000 pg/ml in toluene were prepared. An internal standard working solution containing 10 ng/ml of ^{13}C-*p,p′*-DDE and 2 ng/ml of ^{13}C-PCB-153 was prepared, and used for both calibration solutions and real samples.

2.2. Extraction and cleanup procedure

Serum samples (200 μl) were pipetted into 8 ml glass test tubes (10 mm outer diameter). Internal standards in 0.2 ml of toluene were added (2.0 ng of ^{13}C-*p,p′*-DDE and 0.4 ng of ^{13}C-PCB-153) followed by 0.5 ml of ethanol. Samples were sonicated for 5 min to precipitate the proteins and equilibrate the internal standards. To extract the analytes, 2 ml of hexane was added to the serum–ethanol mixture and samples were shaken at 2000 rpm for 10 min with Vibramax 110 vortex shaker from Heidolph (Schwabach, Germany). To cleanup the samples, 1.0 ml of 15% sulphuric acid silica was added to the serum–ethanol–hexane mixture, and samples were shaken with Vibramax 110 at 2000 rpm for another 5 min. After this, test tubes were centrifugated for 2 min at 3500 rpm. A solid precipitate containing water, ethanol, proteins, fat and other biogenic compounds adsorbed to 15% sulphuric acid silica was packed to the bottom of the test tube. Clear hexane from the top was poured to another test tube. Samples were evaporated to about 0.5 ml with gentle stream of nitrogen, and transferred to autosampler vials. The recovery standard, 200 μl of ^{13}C-PCB-128 solution was pipetted to GC autosampler vials, and samples were evaporated to 200 μl of toluene as the final volume.

2.3. Instrumental determination with GC-HRMS

The gas chromatograph used was a Hewlett–Packard 6890 (Avondale, PA, USA) equipped with Combi PAL autosampler from CTC Analytics (Zwingen, Switzerland). GC system was connected to Autospec Ultima high-resolution mass spectrometer from Waters (Manchester, UK) operating in the selected ion monitoring mode with a resolution of 8000. The column used was a DB-5MS capillary column (30 m, 0.25 mm ID, 0.25 μm film) from J&W Scientific (Folsom, CA, USA). Two microliters of the final extract was injected to S/SL injector having a temperature of 280 °C. Temperature program of the GC oven was: 140 °C for 2 min, 20 °C/min to 190 °C, 8 °C/min to 260 °C, 40 °C/min to 300 °C, hold for 4 min. Flow rate of helium carrier gas was 1.0 ml/min. Temperature of the transfer line from GC to MS was 280 °C and temperature of the ionization chamber was 270 °C. An ionization energy of 35 eV, trap current of 600 μA, and accelerating voltage of 8 kV were used. Two most abundant ions from the molecular ion pattern were monitored for each ^{12}C and ^{13}C compound. The masses of the ions were: 315.9380 and 317.9352 for *p,p′*-DDE, 359.8415 and 361.8385 for PCB-153, 327.9783 and 329.9753 for ^{13}C-*p,p′*-DDE, and 371.8818 and 373.8788 for ^{13}C-PCB-153 and ^{13}C-PCB-128. Dwell time of the ions was 50 ms.

2.4. Method validation

Linearity of the GC-HRMS determination was checked with the calibration solutions mentioned above. Limits of detection (LoDs) and limits of quantification (LoQs) were determined by analyzing five blank samples per day during 3 consecutive days. Total variance (V_{tot}) was calculated by the analysis of variance and LoD as $V_{tot} \times 3$ and LoQ as $V_{tot} \times 8$.

Human serum samples of three levels (low, medium and high) were analyzed during 3 consecutive days, five samples per day from each level. Low- and medium-level serums were prepared in-house by pooling human serum from several individuals of similar concentrations. High-level serum was Standard Reference Material 1589a from National Institute of Standards and Technology (Gaithersburg, MD, USA). For *p,p′*-DDE low-, medium- and high-level serums covered approximately a concentration range from 100 to 10,000 pg/ml, and for PCB-153 a range from 50 to 1000 pg/ml. To calculate the precision of the method on each level, total variance was calculated with the analysis variance. Method trueness was obtained by comparing the average of the measured concentrations

of *p,p′*-DDE and PCB-153 from SRM 1589a with the certified concentrations. Additional human serum samples for the evaluation of method trueness were obtained from the AMAP interlaboratory exercise 2008-2 (ring test for persistent organic pollutants in human serum, National Institute of Public Health, Quebec, Canada). The organizing institute delivered serum samples at three different concentration levels of POPs. These intercalibration samples were analyzed during the time when real serum samples were analyzed.

2.5. Analysis of real samples

The presented method was applied for the determination of 745 human serum samples used in the epidemiological study on POP-T2DM relationship. These samples were obtained from a population based study performed during 1995–1999, the Women's Health in Lund Area (WHILA) in Sweden. At baseline examination in the WHILA study, blood samples were collected and stored in −80 °C. The obtained samples for POP analyses were prepared in batches of 40 samples including 2 blank samples and 2 serum control samples (in-house medium level serum and high-level serum SRM 1589a). Quality control data from control serum samples is also presented to evaluate the long-term precision and accuracy of the method.

3. Results and discussion

3.1. Optimization of the extraction procedure

As a starting point for method development, it was estimated that LoD (S/N = 3) for *p,p′*-DDE and PCB-153 with our GC-HRMS instrument would be somewhere around 5 pg/ml. Based on recent Swedish data on these POPs [14,15], this level was considered low enough so that there was no need to concentrate the samples, only efficient extraction, sufficient cleanup, and return to original sample volume in organic solvent was needed. This was found to be true as *p,p′*-DDE and PCB-153 were present at levels higher than 50 pg/ml in the serum of all participants in the Swedish T2DM study cohort, WHILA.

The separation of liquid layers is often the most laborious stage of LLE [30], and one aim in this method was to speedup this stage by making the extraction solvent pourable after LLE. As a starting point for extraction procedure, we adopted from our previous LLE method for POPs [33] protein precipitation with ethanol and subsequent extraction with hexane. In the initial experiments we also tried protein precipitation with methanol, but recoveries of ^{12}C-compounds tended to be lower as compared with ethanol. As 0.2 ml of serum was available from each subject of the study cohort, 0.5 ml of ethanol was chosen to ensure efficient denaturation and good equilibration of internal standards to serum sample during the 5 min period in the ultrasonic bath. To extract simultaneously larger number samples, a vortex shaker Vibramax 110 with a capacity of 49 test tubes of 10 ml diameter was used. A vortex frequency of 2000 rpm for 10 min provided good mixing of aquatic and organic phases. Following extraction, the cleanup sorbent was added, and the resulting slurry was again vortexed at 2000 rpm for 5 min. The volume of hexane and type and volume of cleanup sorbent were optimised simultaneously. Two ml of hexane together with 1 ml of 15% sulphuric acid silica gave 5–10% higher recoveries of internal standards and clearly less interferences in the chromatogram than 1 ml of hexane with 1 ml of 15% sulphuric acid silica. Activated silica was also tried as a cleanup sorbent, but recoveries of internal standards were about 15–20% lower than with sulphuric acid impregnated silica. The volume of the 15% sulphuric acid silica added was also critical. Too low amount did not bind all water and ethanol, leaving a non-pourable organic-aquatic mixture after sentrifugation. Too much sorbent adsorbed also analytes, which was observed as the decreased recovery of internal standards and increased LoDs. A volume of 1.0 ml added conveniently with a household measuring spoon was found to be optimal. Finally, the shaken slurry was centrifugated for 2 min at 3500 rpm, and clean hexane phase was poured away and concentrated to a final volume of 0.2 ml for GC-HRMS analysis. The chromatograms resulting from this sample treatment were clean and free of non-separable interferences in the channels of the measured compounds. Fig. 1 shows a typical chromatogram from the WHILA cohort for both ions of PCB-153, one ion of ^{13}C-PCB-153 and the lock mass of this channel. The consumption of solvents per sample was very low, only about 3 ml in total. However, the method could be further simplified by downscaling the volume of serum sample, and pouring the subsequently smaller volume of the extract directly to autosampler vial.

3.2. Results of the validation

The R^2 values for solvent standards were better than 0.9997 in every series of samples in the validation. LoD and LoQ were 4.3 and 11.5 pg/ml for *p,p′*-DDE, and 3.1 and 8.2 pg/ml for PCB-153, respectively. There was a small, but repeatable blank in every sample used

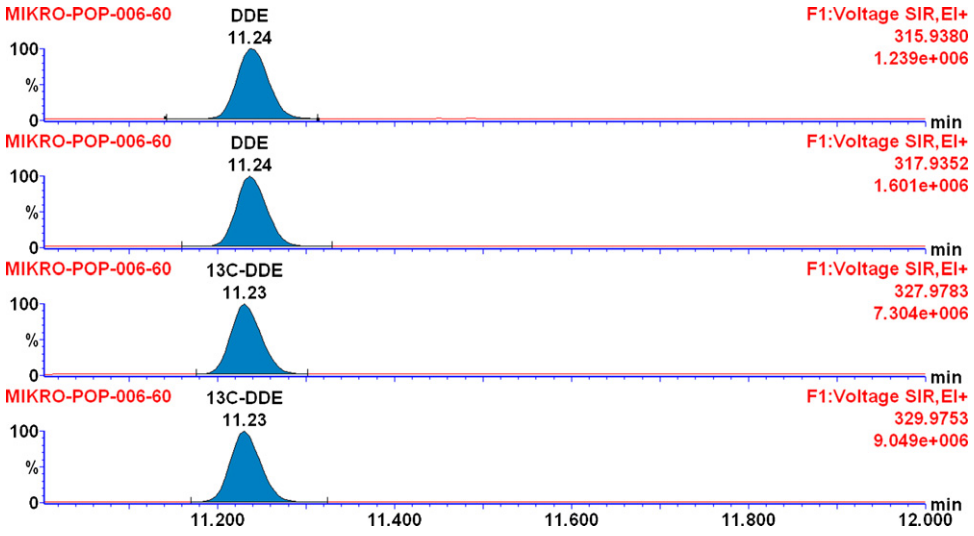

Fig. 1. A typical chromatogram of a serum sample from the WHILA cohort containing 1870 pg/ml of PCB-153. Only one ion of the respective internal standard ^{13}C-PCB-153 is shown. The ion 380.9760 is the lock mass of PCB-153's function.

Table 1
Results for precision and trueness. Precision was calculated as the percent total variance (V_{tot}) by the analysis of variance from low, middle and high (SRM 1589a) serums. Trueness was calculated as the percent recovery from high (SRM 1589a) serum and three AMAP round 2008-2 intercalibration serum samples (W-08-04, W-08-05, and W-08-06). Method as described in the text.

Parameter/sample	Measured (pg/ml)	V_{tot} (%)	Certified/assigned concentration (pg/ml)	Recovery (%)
Low serum[a,b]				
p,p′-DDE	87	2.5	[a]	[a]
PCB-153	60	2.5	[a]	[a]
Middle serum[a,b]				
p,p′-DDE	1,066	2.3	[a]	[a]
PCB-153	485	1.4	[a]	[a]
High serum (SRM 1589a)[b]				
p,p′-DDE	9,703	1.2	11,500	84.3
PCB-153	809	1.9	936	86.5
AMAP W-08-04				
p,p′-DDE[c]	21,299	–	21,000	101.4
PCB-153[c]	2,207	–	2,000	110.4
AMAP W-08-05				
p,p′-DDE[c]	8,457		8,100	104.4
PCB-153[c]	1,248		1,100	113.5
AMAP W-08-06				
p,p′-DDE[c]	6,243	–	5,900	105.8
PCB-153[c]	2,777	–	2,400	115.7[d]

[a] Low- and medium-level serums were prepared in house by pooling human serum from several individuals of supposedly similar concentrations. As a result, no certified or assigned concentrations are available for these serums.
[b] Average of 15 measurements.
[c] Result of one measurement.
[d] Organiser had spiked the sample with 1900 pg/ml PCB congener PCB-132 that closely co-eluted with PCB-153 and interfered with integration.

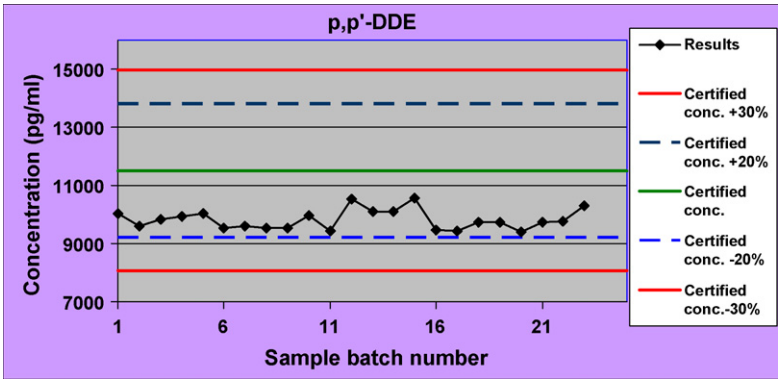

Fig. 2. Control chart of *p,p′*-DDE results from SRM 1589a analyzed during 23 batches of samples.

for the calculation of LoD and LoQ. As a result, LoD and LoQ were not significantly higher than they would have been based on S/N = 3 or S/N = 8, respectively. Results for precision and trueness are presented in Table 1. Total variance was very low at all three levels tested, from 1.2% to 2.5% indicating very good short-term precision of the method. Also the trueness was good, from 84.3% to 115.7% from SRM 1589a and AMAP intercalibration samples, respectively. Good precision and accuracy at different levels of target analytes makes the method useful for epidemiological studies.

3.3. Results of the real samples and quality control data

Average concentrations in the study cohort of 745 subjects were quite high, 3930 pg/ml for *p,p′*-DDE and 1453 pg/ml for PCB-153. LoQ of the method was low enough, since the minimum concentrations in the cohort were 63 pg/ml for *p,p′*-DDE and 88 pg/ml for PCB-153, respectively. Maximum concentration of *p,p′*-DDE was 39,770 pg/ml, higher than the upper end of the tested linear range (10,000 pg/ml) and higher than the highest AMAP serum (21,000 pg/ml), but it was still well within the known linear range of the HRMS instrument. For 95% of the real samples recoveries of ^{13}C-labelled internal standards were from 70% to 100% indicating efficient and robust extraction procedure. Medium- and high-level (SRM 1589a) serums were used also as control samples during the analysis of real samples. As an example, Fig. 2 shows the control chart of *p,p′*-DDE of SRM 1589a from 23 separate batches of samples, 21 of which belonged to WHILA study cohort. Average recoveries as the percentage of certified values and relative standard deviations from SRM 1589a were 85.3% and 3.5% for *p,p′*-DDE and 91.0% and 2.7% for PCB-153, respectively. This data was collected during a time period of 10 weeks, indicating also very good long-term precision of the method. RSDs from the medium level serum also used as quality control sample were 3.5% for *p,p′*-DDE and 2.6% for PCB-153, respectively.

4. Conclusions

The method developed was simple, sensitive enough, had good trueness and excellent precision, and it was successfully applied for a timely preparation of more than 700 serum samples for an epidemiological study on POP-T2DM relationship. One technician can easily prepare a batch of 40 samples a day without any special

automated instrumentation and have still time for the paperwork needed. By far the most labour intensive step in the analysis was the final transfer and evaporation of the 2 ml hexane extract to 200 μl of toluene in GC autosampler vial. By simple downscaling of the volumes, this step will be further quickened in the future.

The main novelty aspect of the method lies in the simple but effective combination of extraction and cleanup. It is fair to say that our procedure is as simple as any manual SPE method that require balancing of the internal standards to the sample, conditioning of the cartridges, loading of the sample, washing of the impurities, elution of the analytes, and solvent evaporation. Part of the simplicity of our LLE method is attributable to high sensitivity of the HRMS instrument that requires no concentration of the serum samples to reach sufficiently low LoDs. However, even widely used GC-ECD and GC-MSD instruments are nowadays quite sensitive and reach reasonable LoDs. Furthermore, new GC-MS/MS instruments that are made for routine laboratories and have sensitivity comparable to HRMS, have been introduced recently [34]. Taken together, this may signify a new coming of very simple LLE methods, like the one presented here, for the analysis of many groups of POPs in cases where manual, non-robotic, sample preparation methods are needed.

Acknowledgement

We thank Tuula Rissanen for her invaluable help and ideas in the development of the method.

References

[1] P.A. Bertazzi, D. Consonni, S. Bachetti, M. Rubagotti, A. Baccarelli, C. Zocchetti, A.C. Pesatori, Am. J. Epidemiol. 153 (2001) 1031.
[2] A.C. Pesatori, C. Zocchetti, S. Guercilena, D. Consonni, D. Turrini, P.A. Bertazzi, Occup. Environ. Med. 55 (1998) 126.
[3] G.L. Henriksen, N.S. Ketchum, J.E. Michalek, J.A. Swaby, Epidemiology 8 (1997) 252.
[4] M.P. Longnecker, J.E. Michalek, Epidemiology 11 (2000) 44.
[5] J.E. Michalek, F.Z. Akhtar, J.L. Kiel, J. Clin. Endocrinol. Metab. 84 (1999) 1540.
[6] G.M. Calvert, M.H. Sweeney, J. Deddens, D.K. Wall, Occup. Environ. Med. 56 (1999) 270.
[7] J. Pazderova-Vejlupkova, E. Lukas, M. Nemcova, J. Pickova, L. Jirasek, Arch. Environ. Health 36 (1981) 5.
[8] J. Vena, P. Boffetta, H. Becher, T. Benn, H.B. Bueno-De-Mesquita, D. Coggon, D. Colin, D. Flesch-Janys, L. Green, T. Kauppinen, N. Littorin, E. Lynge, J.D. Mathews, M. Neuberger, N. Pearce, A.C. Pesatori, R. Saracci, K. Steenland, M. Kogevinas, Environ. Health Perspect. 106 (1998) 645.
[9] D.P. Morgan, L.I. Lin, H.H. Saikaly, Arch. Environ. Contam. Toxicol. 9 (1980) 349.
[10] K. Steenland, L. Piacitelli, J. Deddens, M. Fingerhut, L.I. Chang, J. Natl. Cancer Inst. 91 (1999) 779.
[11] A. Zober, M.G. Ott, P. Messerer, Occup. Environ. Med. 51 (1994) 479.
[12] R.B.J. Remillard, N.J. Bunce, Environ. Health Perspect. 110 (2002) 853.
[13] S. Fierens, H. Mairesse, J.F. Heilier, C. De Burbure, J.-F. Focant, G. Eppe, E. De Pauw, A. Bernard, Biomarkers 8 (2003) 529.
[14] A. Rignell-Hydbom, L. Rylander, L. Hagmar, Hum. Exp. Toxicol. 26 (2007) 447.
[15] L. Rylander, A. Rignell-Hydbom, L. Hagmar, Environ. Health: Glob. Access Sci. Source 4–11 (2005) 1, doi:10.1186/1476-069X-28.
[16] O. Vasiliu, L. Cameron, J. Gardiner, P. DeGuire, W. Karmaus, Epidemiology 17 (2006) 352.
[17] D.H. Lee, I.K. Lee, K. Song, M. Steffes, W. Toscano, B.A. Baker, D.R. Jacobs, Diab. Care 29 (2006) 1638.
[18] D.H. Lee, I.K. Lee, S.H. Jin, M. Steffes, D.R. Jacobs, Diab. Care 30 (2007) 622.
[19] J. Beltran, E. Pitarch, S. Egea, F.J. Lopez, E. Herndndez, Chromatographia 54 (2001) 757.
[20] P.E. Hernandez, F. Beltran, F.J. Lopez, J. Chromatogr. B 769 (2002) 65.
[21] R. López, F. Goñi, A. Etxandia, E. Millán, J. Chromatogr. B 846 (2007) 298.
[22] K. Conka, B. Drobná, A. Kocan, J. Petrík, J. Chromatogr. A 1084 (2005) 33.
[23] F. Goñi, R. López, A. Etxeandia, E. Millán, P. Amiano, J. Chromatogr. B 852 (2007) 15.
[24] S.E. Sundberg, J.J. Ellington, J.J. Evans, J. Chromatogr. B 831 (2006) 99.
[25] J.J. Ramos, B. Gómara, M.A. Fernández, M.J. González, J. Chromatogr. A 1152 (2007) 124.
[26] J.-F. Focant, G. Eppe, A.-C. Massart, G. Scholl, C. Pirard, E. De Pauw, J. Chromatogr. A 1130 (2006) 97.
[27] A. Sjödin, R.S. Jones, C.R. Lapeza, J.-F. Focant, E.E. McGahee, D.G. Patterson, Anal. Chem. 76 (2004) 1921.
[28] C. Thomsen, V.H. Liane, G. Becher, J. Chromatogr. B 846 (2007) 252.
[29] T. Matsumura, M. Morita, H. Miyata, N. Suzuki, M. Watanabe, Y. Ishiguro, T. Iida, Abstracts of Papers, Dioxin 2007, Tokyo, Japan, September 2–7, 2007.
[30] K. Kitamura, A. Mochizuki, J.W. Choi, Y. Takazawa, S. Hashimoto, H. Ito, Y. Fujimine, M. Morita, Analyst 129 (2004) 789.
[31] R. Cariou, J.-P. Antignac, P. Marchand, A. Berrebi, D. Zalko, F. Andre, B. Le Bizec, J. Chromatogr. A 1100 (2005) 144.
[32] L. Hovander, M. Athanasiadou, L. Asplund, S. Jensen, E. Klasson Wehler, J. Anal. Toxicol. 24 (2000) 696.
[33] H. Kiviranta, T. Vartiainen, J. Tuomisto, Environ. Health Perspect. 110 (2002) 355.
[34] Agilent Technologies, see e.g. http://www.chem.agilent.com/en-US/Products/Instruments/ms/7000atriplequadrupolegcms/Pages/default.aspx.