DISTURBANCE OF PCDD/PCDF ANALYSIS CAUSED BY IMPURITIES FROM COMMERCIAL ^{13}C-LABELLED PCB-STANDARDS

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Introduction

Simultaneous determination of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) has gained increased importance in the past few years because of the inclusion of PCBs into the scheme of toxic equivalency factors (TEFs) for dioxins and dioxin-like compounds (1,2). Moreover, the revised tolerable daily intake (TDI) for dioxins recommended by the World Health Organization (WHO) in 1998 is no longer based on PCDDs/PCDFs only, but in addition on certain PCB congeners (3). This new approach requires a reliable and more detailed PCB analysis as a number of non-ortho and mono-ortho PCBs have to be determined besides the indicator PCB congeners 28, 52, 101, 138, 153 and 180.

Analytical methodologies using combined high resolution capillary gas chromatography and high resolution mass spectrometry (HRGC/HRMS) are nowadays state of the art. The advantages of this technology are increased sensitivity and specificity and the ability to use deuterated or ^{13}C-labelled compounds as ideal internal standards. Therefore, HRGC/HRMS procedures were introduced in the three above laboratories for the simultaneous determination of PCDDs/PCDFs and PCBs in food and human milk samples. During the validation processes of the new methods two problems were observed. Firstly, a number of additional signals which also fitted the theoretical isotope ratios for dioxins and furans occured in the traces for some ^{13}C-labelled PCDDs/PCDFs. Secondly, certain ^{13}C-labelled PCDFs had apparently false recoveries far above 100 %.

Materials and Methods

All ^{13}C-labelled PCDDs/PCDFs and ^{13}C-labelled PCBs were manufactured by Cambridge Isotope Laboratories (USA) and purchased from Promochem (Germany). The purity of the standards was generally specified as 98-99%. Standard solutions were prepared separately for PCDDs/PCDFs, non-ortho, mono-ortho and di-ortho PCBs. The analytical methods comprise:

- extraction of fat,
- spiking with up to seventeen ^{13}C-labelled PCDDs/PCDFs, three ^{13}C-labelled non-ortho PCBs, four ^{13}C-labelled mono-ortho PCBs and six ^{13}C-labelled di-ortho PCBs,
- various clean up steps on different columns, including Florisil and carbon columns, enabling separation of non-ortho PCBs from other PCB congeners and from PCDDs/PCDFs,
- separation by capillary gas chromatography and determination by high resolution mass spectrometry (HRMS) at a resolution of R=10,000 in the selected ion recording mode (SIR)

Results and Discussion

When samples were spiked with ^{13}C-labelled dioxins, furans and PCBs simultaneously, the chromatograms for ^{13}C-labelled PCDDs/PCDFs looked different from earlier chromatograms of specimens which were spiked with ^{13}C-labelled PCDDs/PCDFs solely. Some ^{13}C-labelled PCDDs/PCDFs with 2,3,7,8-chlorine substitution were definitely superimposed, and some congeners without 2,3,7,8-chlorine substitution were detected additionally, as depicted in figures 1 and 2. Figure 1 illustrates these observations for pentachlorodibenzofurans in human milk. The upper traces A1 and A2 show chromatograms for PeCDFs in a human milk sample which was solely spiked with ^{13}C-labelled PCDDs/PCDFs. The lower traces B1 and B2 show chromatograms of the same sample, however, after addition of ^{13}C-labelled PCDDs/PCDFs and PCBs at the beginning of the analysis. It became evident that ^{13}C-2,3,4,7,8-PeCDF was superimposed, and that a number of non 2,3,7,8-chlorine substituted ^{13}C-labelled PeCDFs are detectable, as well.

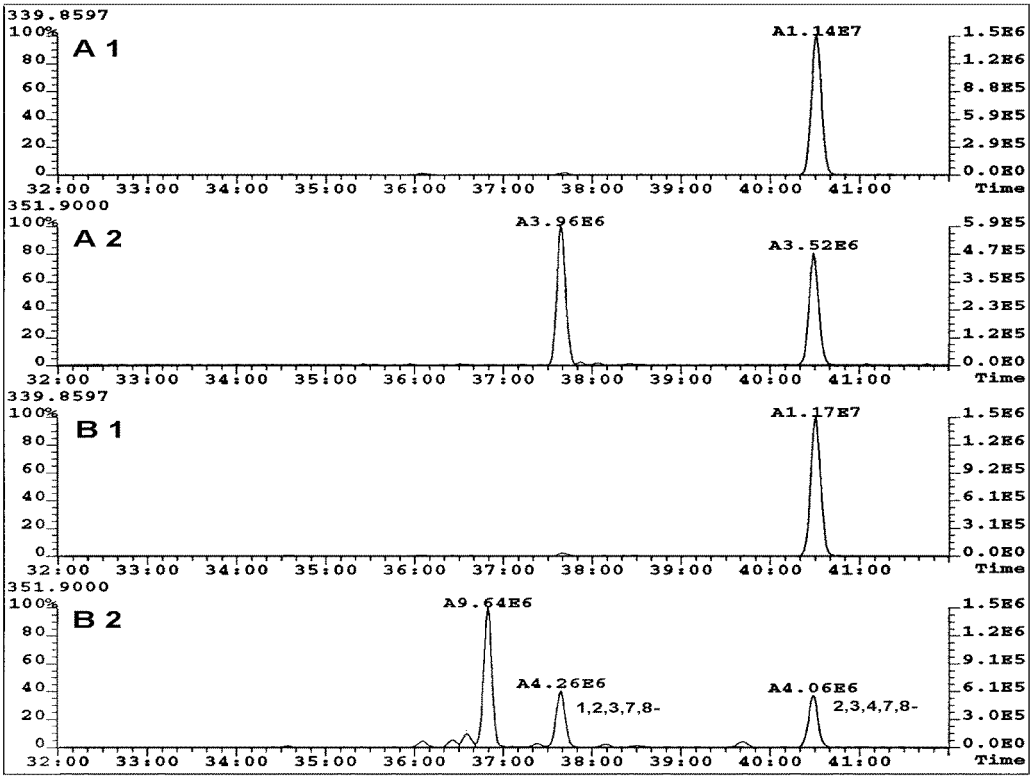

Figure 1: Determination of pentachlorodibenzofurans in human milk
Fragmentograms for native (A1) and ^{13}C-labelled PeCDF (A2) after spiking with ^{13}C-labelled PCDDs/PCDFs solely
Fragmentograms for native (B1) and ^{13}C-labelled PeCDF (B2) after spiking with ^{13}C-labelled PCDDs/PCDFs and PCB

Further investigation showed that the unknown substances were indeed ^{13}C-labelled pentachlorodibenzofurans. The isotope ratios met the theoretical value for ^{13}C-labelled PeCDFs, and the superimposing peak was confirmed on different columns (DB-5 and DB-Dioxin) to be ^{13}C-labelled 2,3,4,7,8-PeCDF. Figure 2 illustrates the same for hexachlorodibenzofurans. A1 and A2 show the traces for native and labelled HxCDFs of a human milk extract, solely spiked with ^{13}C-labelled PCDDs/PCDFs. B1 and B2 represent the HxCDF traces of the same sample, which in contrast was spiked with ^{13}C-labelled PCBs additionally to PCDDs/PCDFs. When comparing the two fragmentograms for the internal standards, it can be clearly seen that 2,3,4,6,7,8-HxCDF is superimposed.

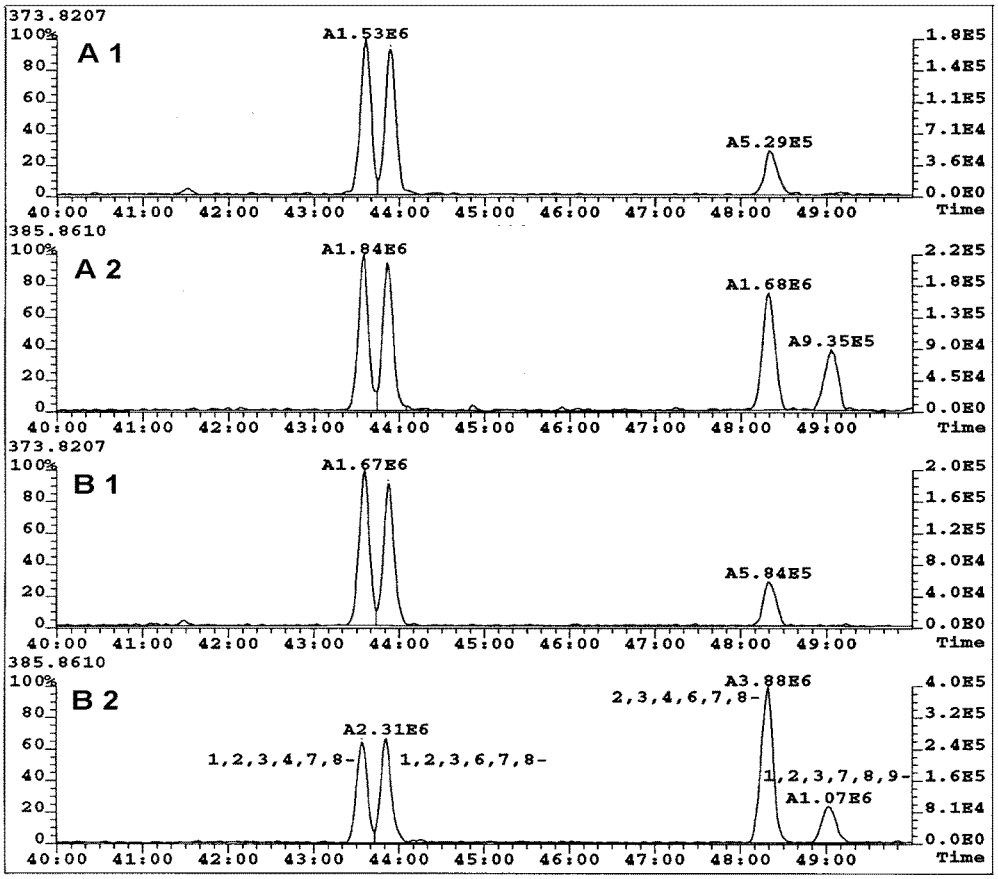

Figure 2: Determination of hexachlorodibenzofurans in human milk
Fragmentograms for native (A1) and ^{13}C-labelled HxCDF (A2) after spiking with ^{13}C-labelled PCDDs/PCDFs solely
Fragmentograms for native (B1) and ^{13}C-labelled HxCDF (B1) after spiking with ^{13}C-labelled PCDDs/PCDFs and PCB

It must be mentioned that non-ortho, mono-ortho and di-ortho PCBs are present in food and human milk samples at levels that differ tremendously. The di-ortho congeners PCB 138, 153 and 180 are usually 10-100 times higher than the di-ortho congeners PCB 52 and 101 or the mono-ortho congeners PCB 28, 105, 118, 123, 156, 157, 167, 189 and roughly 10,000 times higher than the non-ortho PCBs 77, 126 or 169 and all PCDDs/PCDFs of interest. Consequently, the internal standards have to be spiked at adequate levels, ranging from a few picogramm to several nanogram per compound. To meet this specific requirements, various ^{13}C-labelled PCDD/PCDF and PCB spiking solutions were prepared with appropriate concentrations.

Some ^{13}C-labelled PCB standard mixtures as well as a number of individual congeners were checked for impurities. These analyses revealed a lot-dependent contamination of certain ^{13}C-labelled PCBs with ^{13}C-labelled PCDDs/PCDFs, e.g.:

- ^{13}C-labelled PCB 180 contained about 0.16 % ^{13}C-1,2,3,4,6,7,8-HpCDF,
- ^{13}C-labelled PCB 153 contained about 0.17 % ^{13}C-2,3,7,8-TCDF and additionally ^{13}C-1,2,3,6,7,8-HxCDF and ^{13}C-1,2,3,4,6,7,8-HpCDF,
- ^{13}C-labelled PCB 138 disturbed the determination of 2,3,7,8-TCDD,
- ^{13}C-labelled PCB 194 disturbed the determination of 2,3,4,6,7,8-HxCDF

Other disturbances could be attributed to ^{13}C-labelled PCBs 118, 194 and 209. In contrast, the lots of ^{13}C-PCBs 28, 52 and 101 analysed, seemed to be free of disturbing impurities at the level of interest. But the impurities of ^{13}C-PCBs listed above cause elevations of some ^{13}C-PCDDs/ PCDFs resulting in wrong calculations for native PCDDs/PCDFs, e.g. native 2,3,4,7,8-PeCDF and 1,2,3,4,6,7,8-HpCDF concentrations are only one third of the real value. The following apparently false reductions for native congeners were observed: 2,3,7,8-TCDF > 90 %, 2,3,4,7,8-PeCDF 75 %, 1,2,3,7,8-PeCDD 40 %, 1,2,3,6,7,8-HxCDF 25 %, 1,2,3,4,6,7,8-HpCDF 70 %.

In summary, it can be concluded that the simultaneous determination of dioxins, furans and PCBs in food and human milk samples can result in false levels for PCDDs/PCDFs due to impurities from ^{13}C-labelled PCBs. Because of the wide spiking range needed for the different analytes of interest, even a specified purity of 98-99% for the internal PCB standards seems to be too low for a simultaneous PCDD/PCDF and PCB analysis of food and human milk samples. As a result, the manufacturer of the standards must include further steps which remove the disturbing ^{13}C-PCDD/ PCDF impurities from the ^{13}C-PCBs. This can e.g. easily be achieved on a Florisil column, as performed in one of our laboratories. On the other hand, these investigations demonstrate the need for routinely analysing quality control samples in order to early recognize problems and changes in the analytical methodology.

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