DEVELOPMENT AND APPLICATION OF A QUANTITATIVE METHOD FOR SIMULTANEOUS PCDD/F AND PCB DIOXIN-LIKE IN FEED AND IN ANIMAL ORIGIN FOOD

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Introduction

Foods are the main source of human and animals exposure to persistent organic pollutants (POPs). We have been dedicated to the development of a rapid analytical methods for the measurement of dioxins in various types of (food and feed) matrices. Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (dl-PCBs, i.e. polychlorinated non-ortho and mono-ortho biphenyls) are compounds present in samples at part-per-billion (ppb) or part-per-trillion (ppt) level. Their measurement requires the use of very sensitive analytical methods. We have used the EPA 1613b method as reference and afterward modified. Accurate measurement of dioxins and related compounds requires high standard analytical strategies since their low level in food. Those multistep strategies include efficient sample extraction, rapid sample clean-up procedure and accurate analyte measurements under strict quality assurance/quality control (QA/QC) criteria. Several non-instrumental and instrumental automated approaches are available for both extraction and clean-up step.
Recoveries data were in the range established by the EPA as acceptable.
Further studies are in progress to improve and develop the precision of this method.

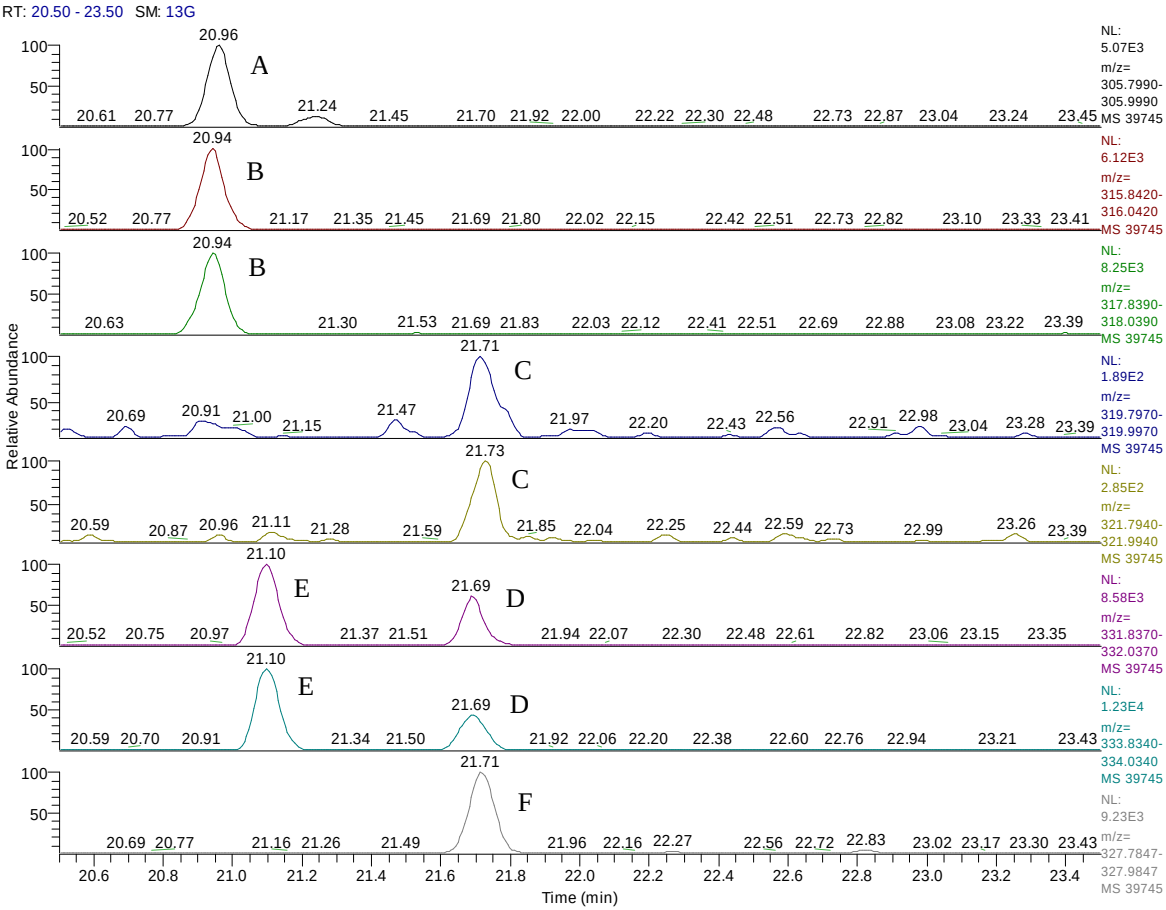
Materials and Methods

Chemicals: ^{13}C-labeled (EDF-8999-4) diluted 1:50, clean-up (EDF-6999), internal standard spiking (EDF-5999) diluted 1:100, ^{13}C-WHO-PCB mixtures (EC-4937) and ^{13}C PCB-180 (EC-1407) of EPA method 1613 were purchased from Cambridge Isotope Laboratory (Andover, MA, USA) and ^{13}C PCB-79 (mbt-79) Wellington Laboratories. For the quantification of low concentrations of PCDDs/Fs, calibration standards CS1 through CS5 (EDF-9999) 1:5 dilute were used.
Equipment: High-resolution gas chromatography coupled to high-resolution mass spectrometry (HRGC/HRMS) Thermo Finnigan MAT95XP. Pressurized Solvent Extration (PSE) - Applied Separation. Automated-PowerPrep System - Fluid Management System, Syncor Polyvap – Buchi.
Kind of Specimen: different type of feedstuff, hen eggs, meat and meat products, milk and milk products, fish and animal fat.
Sample Extraction: Ground sample spiked with ^{13}C-labeled PCDD/F and WHO-PCB mixtures was freeze-dried. Fat was extracted by PSE system from lyophilized sample using hexane:acetone 80:20. Extracted solvent was dried and redissolved in hexane.
Delipidation and Sample Clean-up: Delipidation was carried out loading the extract on the mixed silica/H_2SO_4 column. Hexane was used to elute compounds from column and dried to 10 ml. Sample spiked with clean-up solution was loaded on the automatic Power Prep. Two fraction were obtained, dioxin and pcb.
Pcb's fraction containing pcb non dioxin like too, therefore was loaded onto a cartridge of carbon (Supelco) and eluted with a different mix of solvent [(1)].
HRGC/HRMS: The extracts after cleanup were analyzed by high-resolution gas chromatography (HRGC)/high-resolution mass spectrometry (HRMS) using an isotope dilution method. The average recoveries of 17 labeled congeners in the spiked samples at 2.5-20 pg/g were ranged between 70 and 96%.

Result and Discussion

This simple and automated multi-analyte clean-up procedure enables the separation and the analysis of PCDD, PCDF, coplanar PCB and mono-*ortho* PCB. The method furnishes clean extracts with good recovery rates.
Robustness, repeatability and reproducibility during quality control and for a variety of types different samples were satisfactory for all the 29 selected analytes. This enables estimation of the TEQ including not only PCDD/F, but also PCB by use a single sample clean-up step. Although the last step on the mixed silica/H_2SO_4 column is functional and of straightforward execution, further improvements are ongoing for a faster delipidation with similar performance. Enhancement forecast a freeze delipidation[(2)].
Further clean-up step was inserted to separate coplanar PCB and mono-ortho PCB from ortho PCB. That rapid step has been established essential to bound chromatographic interference due to considerable ortho PCB presence.
Furthermore this framework should provide a validation scheme including reproducibility, recovery, detection limit and method blanks and set thresholds for these parameters which a method has to pass.

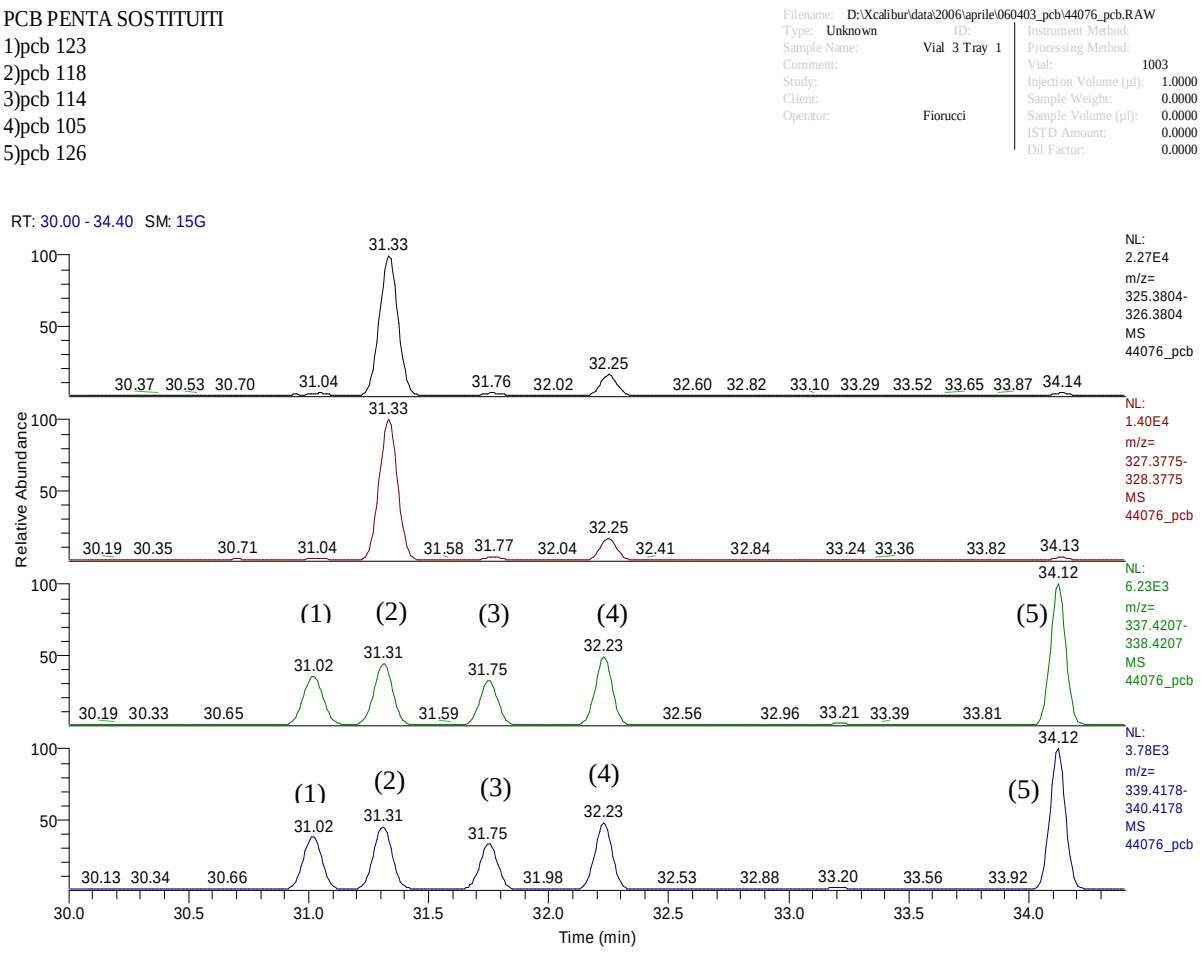
A real sample of fish flour



A **2-3-7-8- TCDF**
B **$^{13}C_{12}$- 2,3,7,8- TCDF**
C **2,3,7,8 – TCDD**
D **$^{13}C_{12}$- 2,3,7,8- TCDD**
E **$^{13}C_{12}$- 1,2,3,4- TCDD**
F **^{37}Cl – 2,3,7,8 - TCDD**

A real sample of bovine tissue

PCB PENTA SOSTITUITI
1)pcb 123
2)pcb 118
3)pcb 114
4)pcb 105
5)pcb 126



References

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EPA Method 1668, Revision A: Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS

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(2) Yun Gyong Ahna, Jungju Seo a, Jeoung Hwa Shin a, Jeehyeong Khimb, Jongki Hongc, Analytica Chimica Acta (2006) - Article in Press