DIOXIN SCREENING IN FISH MEALS BY PATTERN RECOGNITION OF FATTY ACID PROFILES

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Introduction

The general cause of high levels of persistent lipophylic organic pollutants (PLOP's), such as dioxin, in human tissue is primarily caused by bioaccumulation through the food chain in the lipid phase [1,2,3]. Food, especially fish products, is the main cause of exposure. Accordingly, reliable, time- and cost-effective PLOPs screening methods are required in foods and feeds for effective reduction of this intake through the nutritional pathways. This paper describes and applies s method for dioxin screening, based on chemometrics and employing fatty acid pattern recognition [4] to a large number of samples: above 200 fishmeal samples.

Material and Methods

Sampling

236 fishmeals samples from two Danish fishmeal manufacturers were collected in a five year period.

Fat assessment, reference analysis for dioxin (TEQ-PCDD/F -WHO) and fatty acid determination

These analyses were performed according to previous publication[4].

Data analyses

GC-FID data: automatic peak integration

Warping [5] was performed to correct retention time shift. After removal of injection peak, the chromatogram dataset was reduced in time-resolution using every eighth time point, thereafter simple linear interpolation was employed between the first and last peak to stretch all the chromatograms to the same length, and to provide suitable data for warping. The warping procedure employed a segment of 10 points and a slack of 1, for a total chromatogram length of 8601 time points. The chromatograms were segmented according to baseline delimited common signal zone. Thereafter each chromatogram was submitted to automated peak detection and area calculation through gauss curve fitting algorithm, to be available in the next release of Eigenvector Research Inc.'s PLS Toolbox, vers. 4.00, for MatLab. At last the obtained integrated peak areas were associated in 80 time intervals providing a total of 80 variables (fig. 1).

Calibration

The 80 variables expressed (as % total area) resulting from the integrated GC data and the lipid weighted dioxin content were used as independent variables to build calibration models for dioxin.

Preprocessing

The samples were ordered according to the dioxin content. From Principal Component Analyses, 30 obvious outliers with high T2 and residual values above the sum of the median and the standard deviation) were removed. 10% of the samples were kept aside as an independent validation set covering the whole dioxin range, and the remaining 90% were used for calibration. This calibration set was divided in three groups of equivalent size covering similar dioxin ranges for variable selection by iterative backward PLS[6]. The variables commonly removed from the three groups by this variable selection procedure, were removed for further PLS calibration.

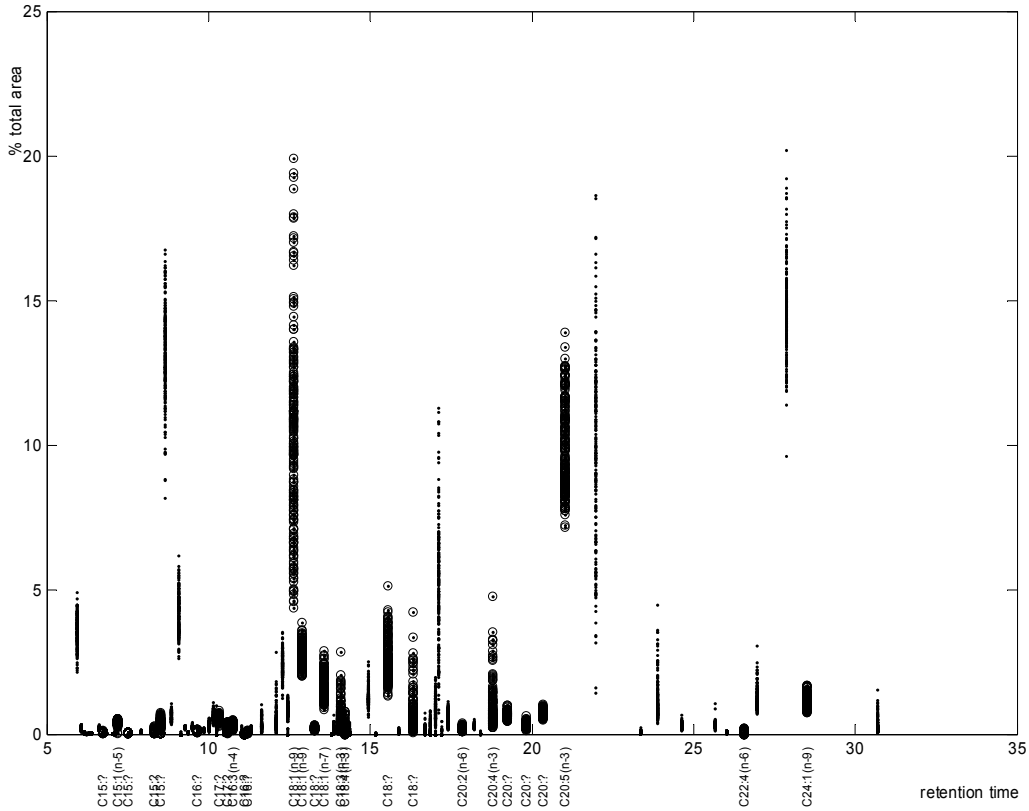

Fig. 1. Illustration of the 206 samples and their respective fatty acid profiles expressed as % area of 80 detected fatty acids (.). The area of fatty acids marked with ⊙ were the variables selected by the backwards interval PLS variable selection.

PLS and PCA calibration

Partial Least Squares regression was performed on the auto-scaled data: the data were autoscaled. This multivariate regression was performed using the Eigenvector Research Inc.'s PLS_Toolbox (vers. 3.50) for MatLab (vers. 7.1, MathWorks, Inc.).

Results and Discussion

An alternative, cost- and time-effective dioxin screening method relying on lipid biomarkers was employed. The investigated 206 fishmeal samples provided a dioxin range varying from 1 to 50 pg PCDD/F TEQ-WHO / g lipid. The method was based on multivariate data analyses using patterns of fatty acid composition monitored by GC-FID for predicting the dioxin content. Raw GC-FID data were successfully transformed by baseline correction, automatic peak alignment and integration, enabling automatic extraction of the respective area of 80 peaks from the gas chromatograms. The PLS results are summarized in fig.2.. Variable selection reduced the X variables from 80 to 27, without affecting the calibration and the prediction ability. This prediction error of 2.6 pg PCDD/F TEQ- WHO /g lipid validates previous calibration errors[4]. The employed GC FID data analyses is cost and time saving due to automation of the main steps.

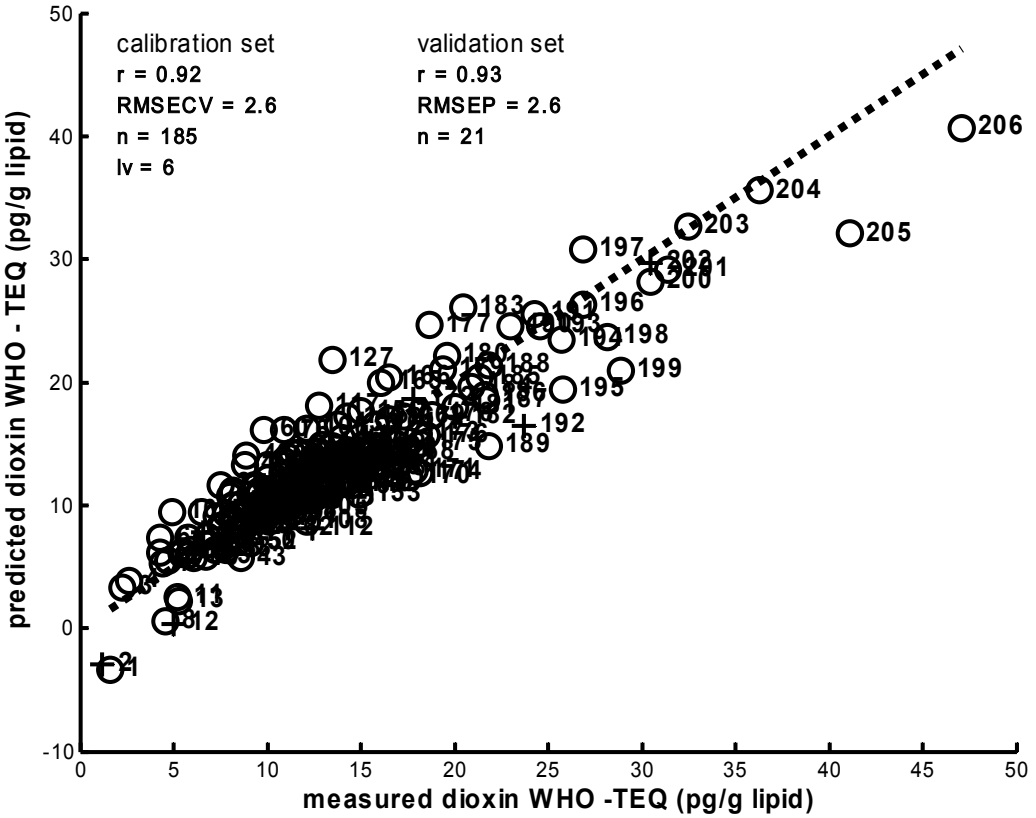

Fig. 2 Fatty acid PLS (lv=6) predicted dioxin values versus dioxin reference method (confirmatory method, 2002/70/EC). The ideal prediction is symbolized by the diagonal line. The samples from the calibration (O) and the validation (+) set are presenting the same prediction ability.

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