DETERMINATION OF DIOXINS AND FURANS FROM EGGS AND OILS, THROUGH HIGH RESOLUTION GAS CHROMATOGRAPHY IN COMBINATION WITH HIGH RESOLUTION MASS SPECTROMETRY

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Abstract. *Dioxins (polychlorinated dibenzo-p-dioxins – PCDD) and furans (polychlorinated dibenzofurans – PCDF) represent a group of chemical substances with high toxicological potential, which are persistent within environment and which can be accumulated within organisms through food chain. In this paper we present the results of the performed researches for dioxins and furans determination in eggs and oils, through high resolution gas chromatography in combination with high resolution mass spectrometry. Egg and oil samples available in commerce were analyzed. Within the performed experiments, some steps were taken: sample preparation for testing, fat extraction, extract clean-up and concentration, separation, identification and quantification of different native compounds of dioxins and furans.*
Fat extraction from eggs was achieved in many more steps with organic solvents. Extracts cleanup was achieved on multiple columns, using different absorption materials (silica gel, aluminium oxide, florisil). Concentration of cleaned extract was achieved under nitrogen flow, at 40ºC and pressure 5 psi.Separation, identification and quantification of different compound PCDDs/PCDFs were achieved by complex equipment: a system of two high resolution gas chromatographs coupled with high resolution mass spectrometer. In the case of analysed oil samples, no item of the native congener of dioxins and furans was detected. The total concentration of dioxins and furans in the analysed egg samples was expressed in toxic equivalents (TEQ) and was in the range: 0.0216 – 0.034 pg WHO-PCDD/PCDF-TEQ/g fat, being under maximum allowed limit by Regulation of the European Commission 1881/19 of December 2006 (3 pg WHO-PCDD/PCDF-TEQ/g fat).

Keywords*: high toxicological potential, furans, fat extraction*

Introduction

Dioxins (polychlorinated dibenzo-*p*-dioxins – PCDD) and furans (polychlorinated dibenzofurans – PCDF) represent a group of chemical substances with high toxicological potential, which are persistent within environment and which can be accumulated within organisms through food chain. Decomposition of dioxins in the external environment is extremely slow, so that dioxins can be accumulated in food chain, animals having in their bodies (through bioaccumulation) higher concentrations (hundreds and thousands times) than plants, water and soil [1].
Dioxins have 75 congeners, of which 7 are the most toxic, and furans have 135 congeners with variable toxicity. Among these, the compound with the highest toxicity is: 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (2,3,7,8-TCDD) [2].
Because of their extreme toxicity, dioxins and furans, at European and international level, are given special attention on the

monitoring of these contaminant concentrations, both in environment (water, air, soil) and in foods.

Analytical methods for determination of dioxins and furans in foods use *high resolution gas chromatography in combination with high resolution mass spectrometry, present high sensitivity and selectivity*. The first method for determination of dioxins and furans through *high resolution gas chromatography in combination with high resolution mass spectrometry,* was elaborated by *U.S. Environmental Protection Agency Office of Water-Engineering and Analysis Division,* under the coordination of professor dr. William A. Telliard in 1994 [3].

In this paper we present the results of the performed researches for dioxins and furans determination in eggs and oils, through high resolution gas chromatography in combination with high resolution mass spectrometry. Egg and oil samples available in commerce were analyzed.

Experimental

We determined the contamination degree by dioxins and furans of eggs and oils, through *high resolution gas chromatography in combination with high resolution mass spectrometry*, within the performed experiments. We analyzed samples available in commerce.

Within the performed experiments more steps, were taken: preparation of test sample, fat extraction, extract cleanup and concentration, separation, identification and quantification of different compound PCDDs/PCDFs [3].

Fat extraction from eggs was achieved in many more steps with organic solvents (ethyl alcohol HPLC grade (99.7%, v/v), diethylether pico grade, n–hexane pico grade).

Extracts cleanup was achieved on multiple columns, using different absorption materials (acid silica gel, aluminium oxide, florisil activated with ultra pure water). The concentration of cleaned extract was achieved under nitrogen flow, at 40ºC and pressure 5 psi, about 15-18 minutes.

In the case of oil samples, cleanup and, subsequently concentration of cleaned extract were achieved through the same procedures, as in the case of egg samples.

Separation, identification and quantification of different compound PCDDs/PCDFs were achieved by a complex equipment: *System of two high resolution gas chromatographs in combination with high resolution mass spectrometer (Capillary column* - 5% phenyl – 95% dimethylpolysiloxane, 5MS, L = 30 m, d_i = 0.25 mm, thickness film = 0.1 µm; *Carrier gas* = He 6.0, *Flow of carrier gas* = 15 mL/min; *High resolution mass spectrometer – Ionization type* = EI+; *Ionization energy* = 30–50 eV; *Resolution* = 10,000; *Source temperature* = 260ºC).

In order to achieve calibration curves of those 17 native congener of dioxins and furans from the analyzed egg samples, we used standard solutions S1, S2, S3, S4, S5 (solutions certified BCR – 614, LGC Promochem, Wesel, Germany). We also used the following internal standards: *standards for verification of extraction efficiency–S6, recovery standards–S8, quantification standards–S7.*

Results and discussion

Within the performed experiments, we made a calibration curve for each interest compound, thus obtaining 17 calibration curves. The compounds for which the calibration curves were made are the following: 2378-TetraCDD, 12378-PentaCDD, 123678-HexaCDD, 123789-HexaCDD, 123478-HexaCDD, 1234678-HeptaCDD, OctaCDD, 2378-TetraCDF,

23478-PentaCDF, 12378-PentaCDF, 1234678-HeptaCDF, 123789-HexaCDF, 123478-HexaCDF, 234678-HexaCDF, 123678-HexaCDF, 1234789-HeptaCDF, OctaCDF.

The calibration curve is used to calculate relative response factor for each congener of interest. Relative response factors are used with $^{13}C_{12}$-labeled dioxins and furans congeners, which are added into sample, in order to determine the mass of native congeners of interest through isotope dilution.

Figures 1 and 2 show the calibration curves for 2378-TetraCDD and 12378-PentaCDF.

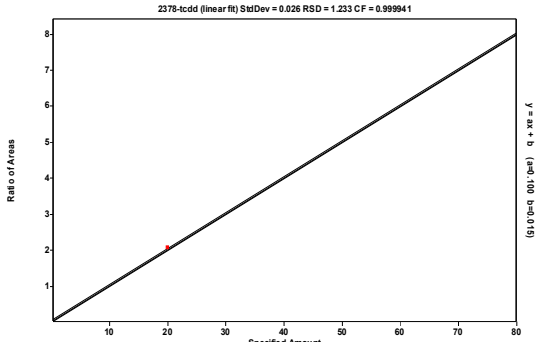

Figure 1. Calibration curve for 2378-TetraCDD

The relative response factor for each congener is defined and calculated by the following equation (1):

$$rrf = \frac{(A1_n + A2_n) \times C_l}{(A1_l + A2_l) \times C_n} \quad (1)$$

where:

rrf – relative response factor of the native CDD/CDF compound against the labelled CDD/CDF compound

$(A1_n+A2_n)$ – the areas of the two strongest ions (m/z) in the molecular ion cluster, for native CDD/CDF compound in the standard solution

$(A1_l+A2_l)$ – the areas of the two strongest ions (m/z) in the molecular ion cluster, for the labelled CDD/CDF compound in the standard solution

C_n – the concentration of the native compound in the calibration standard

C_l – the concentration of the labelled compound in the calibration standard

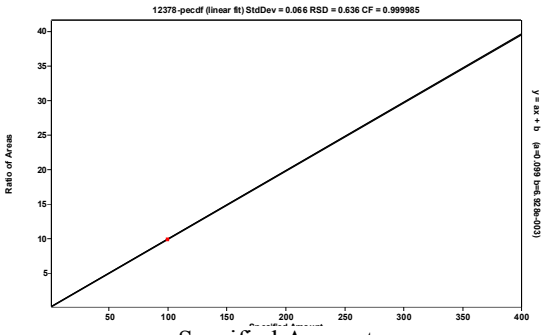

Figure 2. Calibration curve for 12378-PentaCDF

Isotope dilution quantization - We added a known amount of labelled compounds (surrogates) to every sample prior to extraction and correction for recovery of the PCDDs/PCDFs because the native and their labelled analogues exhibit similar effects upon extraction, concentration, and gas chromatography. Using the surrogate responses from the sample run, and the RRF values, recovery corrected concentrations of PCDDs/PCDFs are calculated directly.

The concentration of the native congener i, in sample is calculated using the following equation (2):

$$C_{ex} = \frac{[(A1ex_n + A2ex_n) \times Cs_l]}{[(A1ex_l + A2ex_l) \times rrf} \quad (2)$$

where:

C_{ex} – the concentration of the native CDD/CDF in extract

$(A1ex_n + A2ex_n)$ – the areas of the two strongest ions (m/z) in the molecular ion cluster for the native CDD/CDF surrogate compound in the sample extract

$(A1ex_l + A2ex_l)$ – the areas of the two strongest ions (m/z) in the molecular ion cluster for the labelled CDD/CDF surrogate compound in the samples extract

Cs_l – concentration of the labelled compound in the sample extract
rrf – relative response factor, response factor of unlabelled relative to the $^{13}C_{12}$-labeled internal standard
Recovery ratios of the internal standards are calculated using the equation (3):

$$R = \frac{[(A1ex_l + A2ex_l) \times (A1_n + A2_n)]}{[(A1_l + A2_l) \times (A1ex_n + A2ex_n)]} \times 100 \quad (3)$$

where:
($A1ex_l$ +$A2ex_l$) – the areas of the two strongest ions (m/z) in the molecular ion cluster for the labelled CDD/CDF compound in the sample extract
($A1_n$ + $A2_n$) – the areas of the two strongest ions (m/z) in the molecular cluster of the performance (recovery) internal standard in the standard injection
($A1_l$ + $A2_l$) – the areas of the two strongest ions (m/z) in the molecular ion cluster for the labelled CDD/CDF compound in the samples extract
($A1ex_n$ +$A2ex_n$) – the areas of the two strongest ions (m/z) in the molecular ion cluster of the performance (recovery) internal standard in the sample injection
The concentration of the native congener i, in sample is corrected by recovery factor.
As food samples contain, in generally, complex mixtures of different congeners of dioxins, ***the concept – toxic equivalency factors*** **(TEF**) was developed, in order to facilitate the evaluation of their risk on human body. Toxic equivalency factors **(TEF**) to evaluate human risk were established by specialists in the field, based on the conclusions of the World Health Organization, at Stockholm, Sweden, on 15-18 of June 1997 (Table 1).

Table 1.
Toxic equivalency factors of CDD/PCDF

No.	Congener PCDD/PCDF	TEF (WHO 1997)
1.	2378-TetraCDD	1.0
2.	12378-PentaCDD	1.0
3.	123478-HexaCDD	0.1
4.	123678-HexaCDD	0.1
5.	123789-HexaCDD	0.1
6.	1234678-HeptaCDD	0.01
7.	OctaCDD	0.0001
8.	2378-TetraCDF	0.1
9.	12378-PentaCDF	0.05
10.	23478-PentaCDF	0.5
11.	123478-HexaCDF	0.1
12.	123678-HexaCDF	0.1
13.	123789-HexaCDF	0.1
14.	234678-HexaCDF	0.1
15.	1234678-HeptaCDF	0.01
16.	1234789-HeptaCDF	0.01
17.	OctaCDF	0.0001

The concentrations of each native congener of dioxins and furans in each analyzed egg sample, are multiplied through own toxic equivalency factor and, then, are summarized, giving the total concentration of dioxins and furans, expressed in toxic equivalents (TEQ).

$$TEQ = (Conc.\ X_i \times TEF) \quad (4)$$

where:
TEQ – toxic equivalents
Conc. X_i – concentration of the native congener i, of dioxins or furans
TEF – toxic equivalency factor

In the case of the analyzed egg samples, total concentration of dioxins and furans was also expressed in the following forms:

Upper Bound WHO TEQ (A), in pg/g fat - ***"superior limit of TEQ"*** (TEQ-value calculated by including the full value of the LOQ for non detected congeners)
Lower Bound WHO TEQ (A), in pg/g fat - ***"inferior limit of TEQ"*** (TEQ-value calculated by including quantified congeners only)
In the case of those 6 analyzed egg samples, the only detectable native congener was 2378-TCDF, its concentration being in the range 0.22 – 0.34 pg/g fat (figure 3).

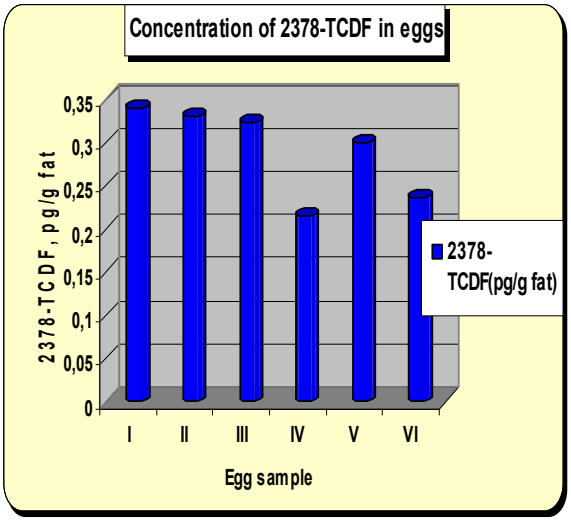

Figure 3. Concentration of 2378-TCDF in eggs

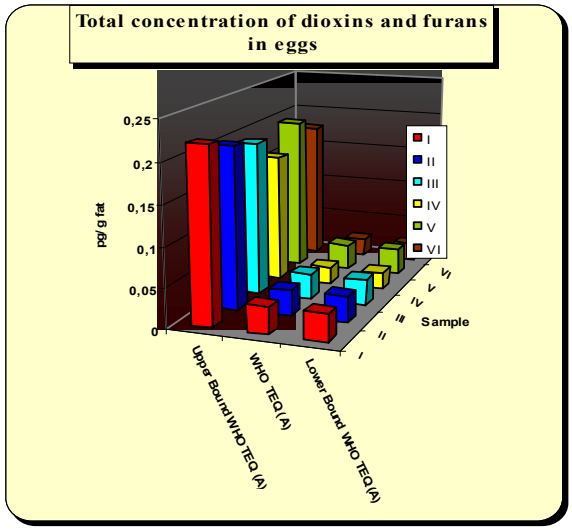

Figure 4. Total concentration of dioxins and furans in eggs

In the case of those 6 analyzed egg samples, the total concentration of dioxins and furans, expressed in toxic equivalents (WHO-TEQ(A)) also varied in the range: 0.0216 – 0.034 pg WHO-PCDD/PCDF-TEQ/g fat, being under the maximum allowed limit by Regulations of the European Commission 1881/19 December 2006 (3 pg WHO-PCDD/PCDF-TEQ/g fat).

The "Superior limit of TEQ" (*Upper Bound WHO TEQ (A)),* was in the range 0.1627 – 0.2191 pg WHO-PCDD/PCDF-TEQ/g fat, being under the maximum allowed limit by Regulations of the European Commission 1881/19 December 2006 (3 pg WHO-PCDD/PCDF-TEQ/g fat).

Figure 4 shows the total concentration of dioxins and furans for each analyzed egg sample, expressed in toxic equivalents (TEQ (A)), as well as *Upper Bound WHO TEQ (A), Lower Bound WHO TEQ (A)* respectively.

When determining dioxins and furans in egg samples, the average recovery factors of the used internal standards are in the following ranges:

- ✓ 83.67% - 88.67% (in the case of internal standards used for control of extraction efficiency-**S6**)
- ✓ 56.83% - 99% (in the case of quantification standards-**S7**)
- ✓ 100% (in the case of recovery standards–**S8)**

Within the performed experiments, the detection limit of the native congeners of dioxins and furans varied in the range 0.0231 – 0.3734 pg/g fat. The minimum value of this range is the detection limit of congener 1234678-HpCDF, and the maximum one represents the detection limit of congener OCDF. Also, in the case of those 6 egg samples, the detection limit of the compound with the highest toxicity among dioxins and furans, **2, 3, 7, 8-TCDD,** is in the range: 0.0395 – 0.0478 pg/g fat. For all the native congeners of dioxins and furans, the detection limit was calculated at a *"signal-to-noise ratio"* (S/N = 2.5).

Also, within the performed experiments, the limit of quantification of the native congeners of dioxins and furans varied in the range 0.0924 – 1.4936 pg/g fat. The minimum value of this range is the limit of quantification of the congener 1234678-HpCDF, and the maximum one represents limit of quantification of the congener OCDF. Also, in the case of those 6 egg samples, the limit of quantification of the compound with the highest toxicity among dioxins and furans, **2,3,7,8-TCDD,** is in the range: 0.158 – 0.1912 pg/g fat.

For all the native congeners of dioxins and furans, the detection limit was calculated at a *"signal-to-noise ratio"* (S/N = 10).

In the case of those 8 analyzed samples of sun flower oil, with addition of 5% olive oil, none of the native congeners of dioxins and furans was detected (so, there are under the limit of detection - LOD). Therefore, for all these samples, the total concentration of dioxins and furans, expressed in toxic equivalents (TEQ), was 0.

In the case of those 8 analyzed samples of sun flower oil, with addition of 5% olive oil, the "superior limit of TEQ" (*Upper Bound WHO TEQ (A)),* was in the range 0.15550 – 0.1801 pg WHO-PCDD/PCDF-TEQ/g fat, being below the maximum allowed limit by Regulations of the European Commission 1881/19 December 2006 (0.75 pg WHO-PCDD/PCDF-TEQ/g fat).

When determining dioxins and furans in samples of sun flower oil, with addition of 5% olive oil, the average recovery factors of the used internal standards are in the following ranges:

- ✓ 60.50% - 91.00% (in the case of quantification standards-**S7**)
- ✓ 100% (in the case of recovery standards–**S8)**

In the case of those 8 analyzed samples of sun flower oil, with addition of 5% olive oil, the detection limit of the native congeners of dioxins and furans was in the range 0.0314 – 0.283 pg/g fat. The minimum value of this range is the detection limit of congener 1234678-HpCDF, and the maximum one represents the detection limit of congener OCDF. The detection limit of the most toxic compound among dioxins and furans, **2,3,7,8-TCDD** is in the range: 0.0366 – 0.0512 pg/g fat. In the case of all native congeners of dioxins and furans, the detection limit was calculated at a *"signal-to-noise ratio"* (S/N = 2.5).

In the case of those 8 analyzed samples of sun flower oil, with addition of 5% olive oil, the limit of quantification of the native congeners of dioxins and furans was in the range 0.1257 – 1.1321 pg/g fat. The minimum value of this range is the limit of detection of congener 1234678-HpCDF, and the maximum one represents the limit of detection of congener OCDF. The limit of quantification of the most toxic compound among dioxins and furans, **2,3,7,8-TCDD,** is in the range: 0.1465 – 0.2048 pg/g fat. For all native congeners of dioxins and furans, the limit of detection was calculated at a *"signal-to-noise ratio"* (S/N = 10).

Conclusions

1. In this paper we presented some performed experiments to determine dioxins and furans in eggs and oils, using high resolution gas chromatography in combination with high resolution mass spectrometry.
2. In the case of those 6 analyzed egg samples, the only native congener detectable was 2378-TCDF, which concentration was in the range 0.22 – 0.34 pg/g fat.
3. In the case of those 8 analyzed samples of sun flower oil, with addition of 5% olive oil, none of the native congeners of dioxins

and furans was detected (so, they are below the limit of detection - LOD).

4. In the case of those 6 analyzed egg samples, the total concentration of dioxins and furans, expressed in toxic equivalents (WHO-TEQ(A)), was in the range: 0.0216 – 0.034 pg WHO-PCDD/PCDF-TEQ/g fat, being below the maximum allowed limit by Regulations of the European Commission 1881/19 December 2006 (3 pg WHO-PCDD/PCDF-TEQ/g fat).

4. In the case of those analyzed egg samples, within the performed experiments, the limit of detection of the native congeners of dioxins and furans was in the range 0.0231 – 0.3734 pg/g fat. The minimum value of this range is the limit of detection of the congener 1234678-HpCDF, and the maximum one represents the limit of detection of the congener OCDF.

5. In the case of those 8 analyzed samples of sun flower oil, with addition of 5% olive oil, the limit of detection of the native congeners of dioxins and furans was in the range 0.0314 – 0.283 pg/g fat. The minimum value of this range is the limit of detection of congener 1234678-HpCDF, and the maximum one represents the limit of detection of congener OCDF.

6. In the case of those 6 analyzed egg samples, within the performed experiments, the limit of quantification of the native congeners of dioxins and furans was in the range 0.0924 – 1.4936 pg/g fat. The minimum value of this range is the limit of quantification of congener 1234678-HpCDF, and the maximum one represents the limit of quantification of congener OCDF.

7. In the case of those 8 analyzed samples of sun flower oil, with addition of 5% olive oil, the quantification limit of the native congeners of dioxins and furans was in the range 0.1257 – 1.1321 pg/g fat. The minimum value of this range is the limit of detection of congener 1234678-HpCDF, and the maximum one represents the limit of detection of congener OCDF.

Acknowledgments

The experiments were performed within the contract no. 51-050/18.09.2007, financed by the Programme 4 "Partnerships in priority S&T Domains" 2007 – 2013 – National Centre for Projects Management.

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