VILNIUS UNIVERSITY
THE CENTER FOR PHYSICAL SCIENCES AND TECHNOLOGY

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RESEARCH OF PERSISTENT ORGANIC POLLUTANTS IN FOOD AND FEED USING MASS SPECTROMETRY METHOD

Summary of doctoral disertation

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FIZINIŲ IR TECHNOLOGIJOS MOKSLŲ CENTRAS

RASA GODLIAUSKIENĖ

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Introduction

Various groups of persistent organic pollutants such as polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), dioxin like polychlorinated biphenyls (DL-PCB), non dioxin like polychlorinated biphenyls (non DL-PCB), and polybrominated diphenyl ethers (PBDE) are known as xenobiotics, characterized by not only bioaccumulative features but also toxicological, carcinogenic, neurotoxicological, and teratogenic influence. Undoubtedly, food contaminated by these kind of pollutants may cause a range of various illnesses (porphyria, chloracne, dermatitis, disorders of both immune and neurological systems) including different types of cancers which in fact are prevailing. The group of PCDD/PCDF contains 210 isomers where 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (tetraCDD) can be distinguished as the most toxic one. Polychlorinated biphenyls (PCB) is a category of 209 organic compounds which differ in two benzene rings connected by C-C link. 209 brominated diphenyl ethers are aromatic compounds in which 1-10 hydrogen atoms are replaced by bromine. Polybrominated diphenyl ethers (PBDE) considering bromine atoms from 2 to 10 are used in commercial fire retardant and it is divided into tri (3), tetra (4), penta (5), hexa (6), hepta (7), octa (8), nona (9) and decabrom ether. The latter, which are incorporated into polymers as fire retardants reduce the risk of ignition. Increasing environmental pollution was caused by the chemical stability of POP. For this reason, industrial production of these compounds was gradually rejected. However, the pollution remains to be a huge problem even nowadays.

Since these persistent organic pollutants may provoke negative effects on human beings even with extremely low concentrations like 10^{-12}g/g or 10^{-15}g/g, in this case precise, sensitive and reliable methods of analysis are necessary in order to identify these negative effects. For the last 30 years, gas chromatography mass spectrometry method (GC-MS) has been used for the qualitative and quantitative identification of dioxins and furans. Nevertheless, because of decreasing regulated maximum allowable concentration, the methods of high-resolution mass spectometry analysis became irreplaceable due to both high sensitivity and ability to identify low concentrations.

Due to the toxicity of low concentrations of persistent organic pollutants, not only complex instrumental analysis is required, but also thorough methods of sample

preparation. In recent years, more than one instrument which is able to automatize a part of sample preparation procedure has been invented. It reduces the duration of analysis, and the level of pollution and increases the sensitivity. Nevertheless, the manual preparation method which has been used for a long time still holds its position comparing with the latter since it meets all the requirements of European Commission for POP analysis.

Even 90% of pollutants get into the human body together with nutrition, especially with the food of animal origin. Therefore, it is of great importance to estimate and control the tolerated daily limit of POP. Moreover, large quantities which have been accumulated during several decades are stored in sediments of the bottom of the sea which is essential for Lithuania which possesses the seashore of the Baltic sea.

The aim of gathered scientific research in this doctoral thesis is to examine the possibilities and to establish an identification procedure of persistent organic pollutants (PCDD/PCDF, DL-PCB, non DL-PCB and PBDE) assigned to the analysis of food and feed with the help of high-resolution gas chromatography mass spectrometry method.

In this dissertation the purpose of summaried research work is to develope, optimise and validate the persistent organic pollutants (PCDD/PCDF, DL-PCB, non DL-PCB and PBDE) method of analysis in food and feedingstuffs using high resolution gas chromatography-mass spectrometry method.

The objectives of the doctoral dissertation:

1. To investigate and optimize the conditions of persistent organic pollutants fat extraction in food and feed.
2. To investigate and optimize the cleaning procedure of persistent organic pollutants while using both manual and automatic sample preparation techniques.
3. To optimize the conditions of chromatographic separation and mass spectrometric detection of PCDD/PCDF, DL-PCB, non DL-PCB and PBDE while using the method of high-resolution gas chromatography mass spectrometry.
4. To evaluate the analytical characteristics of the method with reference to EU requirements.
5. To set the tolerable daily intakes of persistent organic pollutants in different food groups in Lithuania in 2007-2014.

6. To investigate and summerize the level of pollution of persistent organic pollutants in Lithuanian market of food and feed.

The scientific novelty of the work:

There were analyzed 55 persistent organic pollutants in this research. Tests were carried out for the first time, which have contributed to compare different methods of sample preparation and clean-up. Not only in Lithuania, but also in the world, due to the high toxicity and low concentrations of POPs, it is the need to use sensitive analytical methods, therefore, one of the objectives of the dissertation was to create and offer the right methodology. Such an extensive research is applied for food and feedingstuffs, so certainly has great practical significance. Polybrominated diphenyl ethers are compounds, whose levels are not yet regulated by EU documents. The dissertation provides a number of new data, and characteristics of the method of analysis, that may have an effect for further POP research and regulation in Lithuania.

The statements to be defended:

1. The methods of optimized POP fat extraction in food and feed are reliable and appropriate for the analysis of actual objects.
2. The methods of analysis using both manual and automatic sample preparation techniques meets the requirements of EU, but the automatic sample preparation technique is more effective and accurate.
3. The method of high-resolution gas chromatography mass spectrometry is sufficiently sensitive and able to determine persistent organic compounds with the concentrations of 10^{-12}g/g or even 10^{-15}g/g in food and feedingstuff.
4. The pollution of persistent organic pollutants in food products in Lithuania does not have any negative effect for health except some Baltic Sea fish species and fish products.

Abbreviations

POP – Persistent Organic Pollutants;

PCB – Polychlorinated biphenyls;

PBB – Polybrominated biphenyls;

DDT – 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane;

HCB – Hexachlorobenzene;

PCDD – Polychlorinated dibenzo-*p*-dioxins;

PCDF – Polychlorinated dibenzofurans;

TCDD – Tetrachlorodibenzo-*p*-dioxin;

PBDE – Polybrominated diphenyl ethers;

BDE – Brominated diphenyl ethers;

HBCD – Hexabromocyclododecane;

TBBPA – Tetra Bromo Bisphenol A;

UV – Ultraviolet;

PCDD/PCDF, PCDD/F – Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans;

DL-PCB – Dioxin-like polychlorinated biphenyls;

Non DL-PCB – Non dioxin-like polychlorinated biphenyls;

ML – Maximum limit;

TEQ – TEQ - Toxic Equivalent;

TEQ_{2005} PCDD/PCDF – Toxic equivalent of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans using toxic equivalency factors of 2005;

TEQ_{1998} PCDD/PCDF – Toxic equivalent of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans using toxic equivalency factors of 1998;

TEQ_{2005} PCDD/PCDF, PCB – Toxic equivalent of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and dioxin-like polychlorinated biphenyls using toxic equivalency factors of 2005;

TEQ_{1998} PCDD/PCDF, PCB – Toxic equivalent of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and dioxin-like polychlorinated biphenyls using toxic equivalency factors of 1998;

S/N – Chromatographic signal-to-noise ratio;

MS – Mass spectrometry;

GC – Gas chromatography;

GC-MS – Gas chromatography - mass spectrometry;

ABS –Acrylonitrile-butadiene-styrene;

IUPAC – International Union of Pure and Applied Chemistry;

BZ - Ballschmiter and Zell applied numeration;

Aroclor, Kaneclor – Mixtures of polychlorinated biphenyls;

WHO – The World Health Organization;

AhR – Aryl hydrocarbon receptor;

CYP1A1 – Cytochrome P450 1A1;

CYP1A2 – Cytochrome P450 1A2;

IARC – International Agency for Research on Cancer;

T2 – 3,5-diodotironine;

T3 – 3,3,5-triodotironine;

T4 – 3,3,5,5-tetraiodotironine;

U.S. EPA – United States Environmental Protection Agency;

ECD – Electron capture detection;

HRMS – High-resolution mass spectrometry;

SFE – Supercritical fluid extraction;

SFE – Solid phase extraction;

m/z – Mass-to-charge ratio;

GC-ICP-MS - Gas chromatography - inductively coupled plasma mass spectrometry;

GC×GC-HRMS – Tandem gas chromatography - inductively coupled plasma mass spectrometry;

GC×GC-ECD – Tandem gas chromatography - electron capture detection;

GC×GC-TOF-MS – Tandem gas chromatography – time-of-flight mass spectrometry;

GC×GC-MS/MS – Tandem gas chromatography – tandem mass spectrometry;

GC-MS-MS – Gas chromatography - tandem mass spectrometry;

GC-QISTMS/MS – Gas chromatography - quadrupole ion trap tandem mass spectrometry;

TOF-MS – Time of flight mass spectrometry;

QIT – Quadrupole ion trap;

TDI – Tolerable daily intake;

TWI - Tolerable weekly intake;

TMI - Tolerable monthly intake;

PFK – Perfluoro Kerosene;

The structure of the doctoral dissertation

The dissertation is written in Lithuanian language and it consists of eight parts: introduction, theoretical overview, the methodology of the experiment, results and its review, conclusions, the list of researchers connected to the dissertation, the curriculum vitae of the author of the dissertation, and references.

1. Theoretical overview

First of all, the theoretical overview includes the whole information related to the structure and the characteristics of persistent organic pollutants, the methods of sample preparation and its qualitative and quantitative analysis while using different analytical instruments. Moreover, the toxicological significance of the pollutants is summarized in the work. The scientific work related to the global issues of polychlorinated dibenzo-*p*-dioxin (PCDD), polychlorinated dibenzofurans (PCDF), dioxin like polychlorinated biphenyls (DL-PCB), non-dioxin-like polychlorinated biphenyls (non DL-PCB), and polybrominated diphenyl ethers which have been written by other authors are also reviewed and discussed in the dissertation. Last but not least, the safety of food in Lithuania is also discussed.

2. The methodology of the experiment

The methodology of the work consists of several separate parts: equipment (2.1.), reagents and solutions (2.2.), the procedures of sample preparation (2.3.), the conditions of separation and setting (2.4.), and the procedures of the confirmation of methods (2.5.).

Part 2.1. describes the equipment used in the experiment. The instrumental analysis was executed with Agilent 6890 N (Santa Clara, USA) gas chromatograph which comprises CTC Analytics, Swiss injection system, thermostatically controlled columns, and magnetic sector high-resolution mass spectrometer Micromass Autospec Premier (Manchester, UK) with positive ionization.

One of the extraction methods used in the experiment is automatic extraction method with the use of high pressure extractor ASE 200 (Eng. Accelerated Solvent

Extraction, DIONEX ASE 200 with DIONEX solvents controller, Dionex company, USA).

Automatic sample preparation is conducted using DEXTech (LCTech GmbH, Dorfen, Germany).

Part 2.2. includes the description of all the solutions and reagents used in the experiment. The list of all standard materials used in the work and manufacturers of these materials is also provided in the work. Moreover, all of the concentrations of working standard solutions used in the experiment are also presented in the dissertation.

Part 2.3. characterizes both manual and automatic procedures of sample preparation.

Part 2.4. provides both the characteristics of gas chromatography and the mass spectrometric analysis and quantitative calculation of analytes.

Part 2.5. defines the procedures of the analytical method validation of POP.

3. Results and discussions

3.1. Sample preparation and extraction

The major aim of this stage is fat extraction since POP is soluble in fat.

10 to 50 g of samples are usually used for analysis. The samples are respectively prepared before the extraction, i.e. the samples are dewatered using either an anhydrous sodium sulfate or a polyacrylic acid polymer.

Several methods of extraction are used in the work:

- ‘‘Cold‘‘extraction
- Twisselmann extraction
- Soxhlet extraction
- High-pressure extraction method (Accelerated Solvent Extraction);

The effectiveness of the extraction is estimated according to all the recoveries of internal standards of individual analytes $^{13}C_{12}$. Yet, TCDD has been selected as an example because of being one of the most toxic compounds. The comparison of $^{13}C_{12}$

TCDD recoveries in various matrixes using different methods of extraction is demonstrated in Figure 3.1.

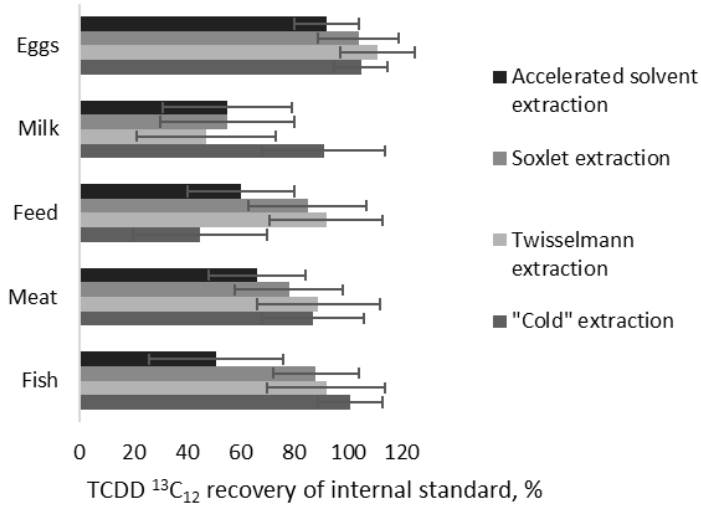

Figure 3.1. The comparison of average recoveries of TCDD $^{13}C_{12}$ in different matrixes using various extraction methods (n=13).

The automatic high pressure extraction method can be applied for both matrixes of meat and eggs. As for the other matrixes, TCDD recoveries of internal standard are below 60%, which does not meet European Commission requirements for analytical methods (according to the requirements, the recoveries must be in the range of 60-120%). The methods of Soxhlet and Twisselmann extractions are appropriate for all of the matrixes except for milk. The recovery of TCDD in the matrix of the latter hardly reaches 50%. However, these extraction methods possess one disadvantage (duration). The extraction is conducted at least 12h. On the other hand, this is the only appropriate fat extraction method for the feed matrix. The traditional cold extraction is appropriate for the matrixes of fish, meat, milk, and eggs.

3.1.1. „Cold“ extraction

The method of the „cold“ extraction is applicable for the samples of fish, meat, eggs, and milk. The extraction is executed with the help of chromatographic columns. The samples are mixed with dewatering reagent and placed into the columns. POP are eluted with 250ml of fish, meat and eggs matrixes whereas the samples of milk are

eluted with 500ml solution of cyclohexane/dichloromethane (1/1(v/v)). The amount of fat is set by gravimetric technique. Figure 3.2. presents the dependence of extraction effectiveness on the amount of solvent being eluted.

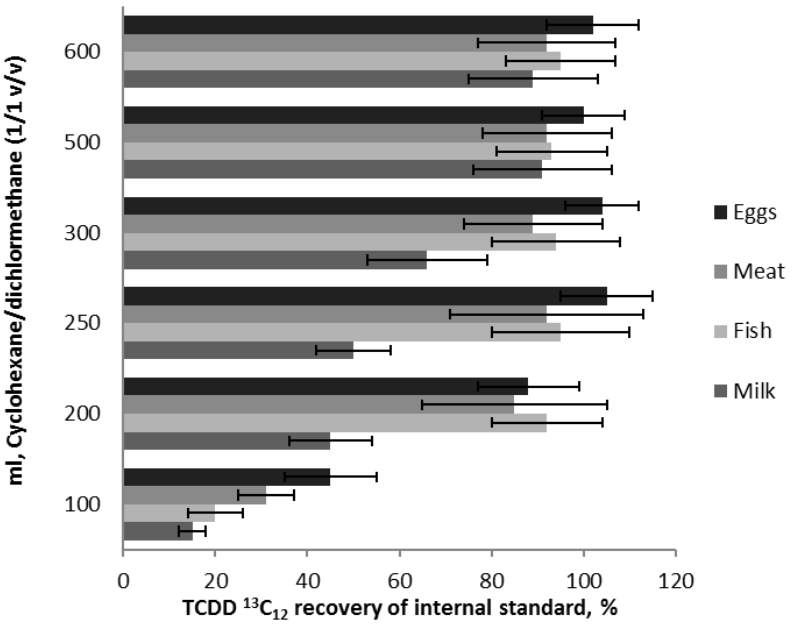

Figure 3.2. The average TCDD $^{13}C_{12}$ internal standard recovery dependence on the amount of solvent being eluted (n=5).

3.1.2. Soxhlet and Twisselmann extractions

The method of Soxhlet and/or Twisselmann extractions is applied for the fat extraction of feed and it's raw materials since the largest recovery of $^{13}C_{12}$ internal standard is obtained. $^{13}C_{12}$ TCDD, as being one of the most toxic, is selected as an example (see Figure 3.1.). The principle of the extraction method of the latter is boiling of the sample in particular solvents in a particular time. The samples of feed are boiled with different reagents at least 6h.

1. 3500ml of cyclohexane toluene (1/1 (v/v)) at least 6h.

2. 350ml of ethanol/toluene (7/3 (v/v)) at least 6h.

The quantity of fat is determined in a gravimetric method.

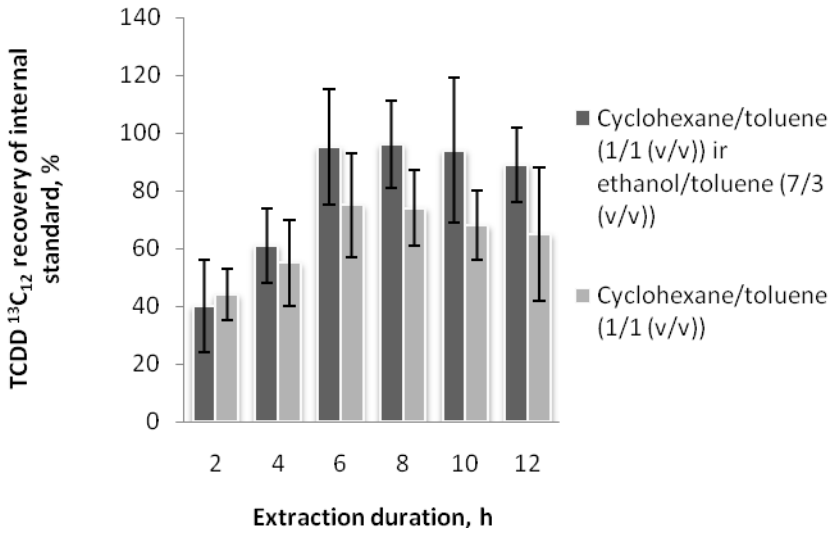

Figure 3.3. The recovery of TCDD $^{13}C_{12}$ internal standard and the duration of extraction dependence in feed (n=5).

3.1.3. Extraction with the use of high pressure

This is the method when the extraction of high pressure fat from the matrix is used. This extraction method possesses both advantages and disadvantages, the considerable risk of cross pollution remains and some of the matrixes are blocked in the cells. Despite fast and cheap fat extraction from the matrix, the method is quite inconvenient since the size of cells restricts the quantity of the sample. This factor is of great importance in order to reach an appropriate analytical sensitivity considering that POP concentrations in the samples reach even 10^{-15} g/g. Figure 3.1. discloses that a lower TCDD internal standard recovery is obtained than using other extraction methods.

3.2. The purification and fractionation of the samples

Further preparation of the sample is executed during the stages of purification and fractionation of the fat obtained. The execution of this stage can be done in both manual and automatic methods. The very principle of the process is identical in both methods except for several fundamental differences: the duration of analysis, the price, the limit of determination of individual analytes, and the level of pollution of empty samples. The purification and fractionation of the samples include several stages: fat clearing using layered acidified silica gel column, florisil and carbon columns.

3.2.1. The purification and fractionation of the samples using the automatic sample preparation system

In contradistinction to manual method, the advantage of the automatic method is that all of the columns are produced and there is no need to prepare it. Layered acidified silica gel and florisil columns are one-time while carbon columns can be used up to 20 times. The whole duration of analysis including the conditioning of columns takes only 70 min and this process is sustained which is important for the analysis of PDBE for the pollution of samples.

In part 3.1. the obtained fat (the capacity of layered acidified silica gel column is up to 5g) are diluted with 7 ml of n-hexane and 2 ml of toluene and injected into the injection loop. The fat in acidified silica gel column is detained. PCB separates from PCDD/PCDF in florisil column. After the purification of carbon columns these fractions are obtained:

1. Non DL-PCB, DL-mono orto-PCB, PBDE;
2. DL-non orto-PCB;
3. PCDD, PCDF.

3.2.2. The purification and fractionation of the samples using the manual method of sample preparation

The stages of the manual method of sample preparation are homogeneous like using the automatic sample preparation system. The method consists of these stages: the purification of fat using layered acidified silica gel column, the separation of fractions using florisil column, and the purification using carbon/celit columns. The fractions are obtained after the procedure:

1. Non DL-PCB, DL-mono orto-PCB, PBDE;
2. DL-non orto-PCB;
3. PCDD, PCDF.

The disadvantage of this method is the duration of analysis which requires two weeks of work. The whole procedure is presented in Figure 3.4.

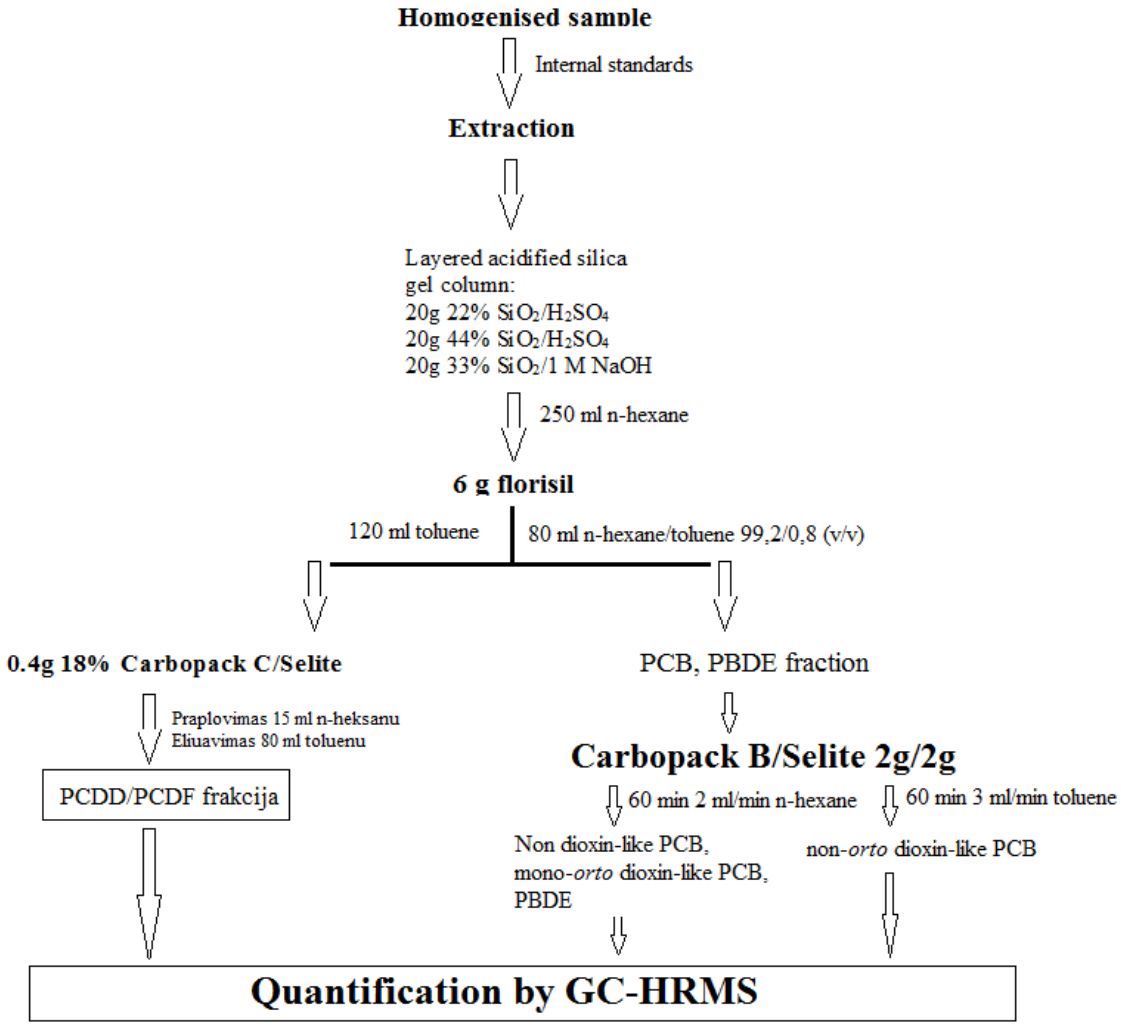

Figure 3.4. The scheme of the manual sample preparation procedure.

The samples are eluted with 250ml of n-hexane through the layered acidified silica gel column. The solvent is evaporated after the elution to ~1ml and the next stage of sample purification is executed using florisil column. The dioxin type non-orto, mono-orto dioxin type polychlorinated biphenyls, non dioxin like biphenyls and PBDE are eluted with 80ml of n-hexane/toluene (99,8/0,2 (v/v)).

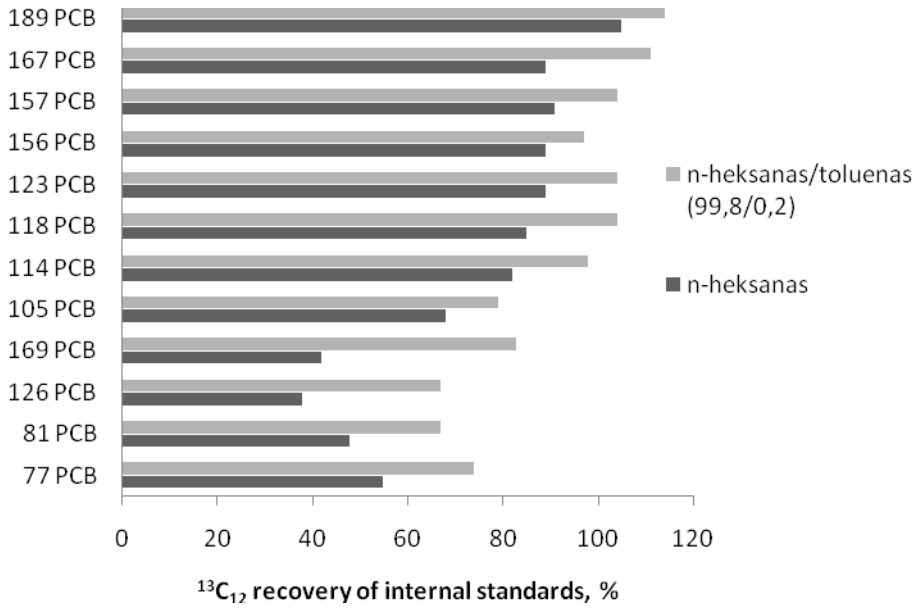

Figure 3.5. The dependence of the $^{13}C_{12}$ internal DL-PCB standards of the constitution of eluted solvent.

Figure 3.5. indicates, that using only n-hexane for the elution of PCB, the recoveries are smaller, especially of DL-non orto-PCB. For this reason, the problem was solved by adding 0,2% of toluene. The recoveries improved significantly, therefore for the analysis of PCB, PBDE the elution using 80ml of n-hexane/toluene has been selected (99,8/0,2 (v/v)). The fraction of dioxins and furans is eluted with 120ml of toluene.

The analysis of PCDD/PCDF is continued to be performed with 0,4g (carbopack C) 1/4,5 parts, that is to say 18% (v/v) of the mixture. The process of purification:

1. 5ml of toluene (conditioning);

2. 15ml of n-hexane (conditioning);

3. On the top of the column, the obtained PCDD/F fraction after florisil column is spread.

4. The column is washed with 15ml of n-hexane, the eluted is poured out.

5. Eluting with 80ml of toluene, PCDD/F fraction is gathered.

PCB fractionation is executed using celit/carbopack B (60/80 porosity) in the proportion of 1/1 (1g/1g). It is intensive preconditioned with n-hexane. This column is beneficial for the separation of mono-orto and non-orto PCB fractions in order to achieve a better sensitivity and individual separation of PCB.

1. The conditioning of the column 5ml/min with n-hexane around 1h;
2. Automatic injection;
3. A non-dioxin type PCB are eluted for the first 10 min;
4. Eluting DL-mono orto-PCB, PBDE with n-hexane 2ml/min for 30min;
5. Eluting DL-non orto-PCB with toluene 3ml/min for 45min;

DL-mono orto-PCB, PBDE, non DL-PCB and DL-non orto-PCB fractions are obtained.

PCDD/PCDF fraction are dissolved in 10 µl 1,2,3,4-TCDD $^{13}C_{12}$ 10 ng/ml concentration recovery standard, while DL-PCB and non DL-PCB are dissolved in 100 µl 1,2,3,4-TCDD $^{13}C_{12}$ 10 ng/ml concentration recovery standard, PBDE are dissolved in 50 µl of toluene when the concentration of recovery standard ($^{13}C_{12}$ 77 PBDE) is 20 ng/ml.

The qualitative and quantitative PCDD/PCDF, DL-PCB and PBDE setting using high-resolution gas chromatography mass spectrometry method follow next.

3.3. The selection of conditions of chromotographic separation

The chromatographic separation of persistent halogenated pollutants is executed using gas chromatograph Agilent 6890N (Santa Clara, USA). After the theoretical overview, DB-5MS (fenil aryl polymer, which is equivalent to 5 % fenil methylpolysiloxane) has been chosen for the chromatographic separation of POT. The separation of PCDD/PCDF, PCB is executed using DB-5MS 60 m × 0,251 mm × 0,10 µm column, PBDE - DB-5MS 30 m × 0,251 mm × 0,10 µm and PBDE 209 15 m × 0,251 mm × 0,10 µm (Agilent Technologies, Belgium). Helium is the carrier gas. During the process of the experiment, the appropriate temperature gradient has been in search of. Since the final extract is diluted with standard solution produced in toluene solvent which $t_{boiling}$ is 110,6^{o}C, the initial tempereture of 100-120^{o}C has been chosen in order to evaporate grouted sample volume.

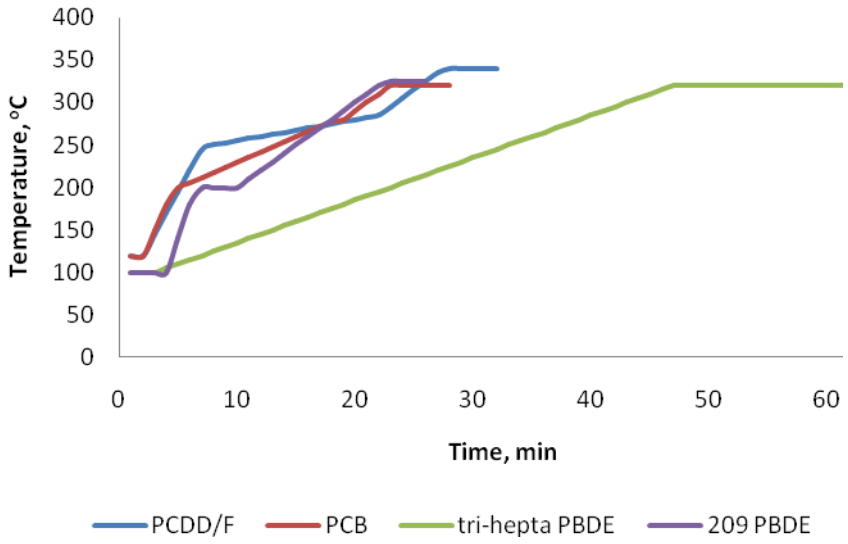

Figure 3.6. The formation of POP gas chromatography temperature gradient.

The latter figure presents PCDD/F, PCB and PBDE temperature gradients. It can be clearly seen that the duration of PCDD/F, PCB and 209 PBDE chromatograms reach 30min while tri-hepta PBDE – 60min.

During the experiment, it was found out that during the procedure of sample preparation carbon column must be used for the separation of analytes (see Figure 3.7.).

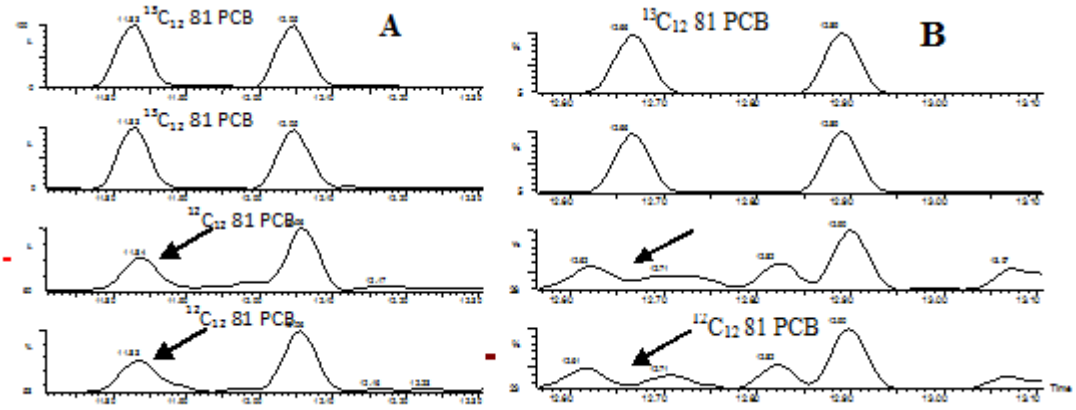

Figure 3.7. For the chromatograms of $^{12}C_{12}$ 81 PCB ir $^{13}C_{12}$ 81 PCB GC-HRMS. A - the manual sample preparation method using carbon column and B - the manual sample preparation method not using carbon column.

Figure 3.7. demonstrates that using the manual sample preparation method without the use of carbon column, i.e. not separating DL-mono orto-PCB, PBDE and DL-non orto-PCB fractions, not all biphenyls separate. In B chromatograms where

carbon column has not been used and the sample has been analyzed immediately after florisil column, it can be clearly noticed that $^{12}C_{12}$ PCB 81 does not separate. In A chromatograms using carbon column, it is evident that $^{12}C_{12}$ 81 PCB separates from extraneous compounds. This means that during the process of sample preparation the carbon column must be used for the separation of some individual analytes. What is more, this column is a must for the sensitivity improvement of $^{12}C_{12}$ PCB 126 and PCB 169, i.e. for the better detection limit. The toxic counterpart factors of these biphenyls are quite high (126 PCB TEF_{2005} is 0,1 and 169 PCB – 0,03), therefore they make a huge impact on toxic equivalent.

The separation of some furans, such as 1,2,3,4,7,8 HexaCDF and 1,2,3,6,7,8 HexaCDF applying gas chromatography is complicated. According to the regulations, the separation of these compounds between peaks must be < 25 % and this is proved in Figure 3.8. which demonstrates that the separation of peaks reaches ≈ 13 %.

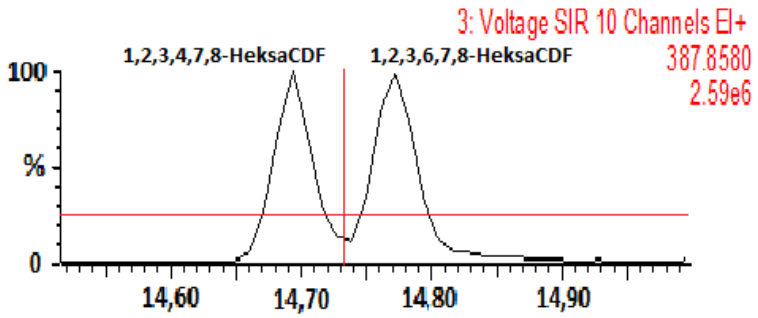

Figure 3.8. The chromatographic separation of $^{13}C_{12}$ 1,2,3,4,7,8-HexaCDF and 1,2,3,6,7,8-HexaCD peaks.

3.4. The selection of mass spectrometric conditions

According to the requirements of the European Commission, the confirmed methods of POP analysis must be analyzed using all 17 PCDD/F 2,3,7,8 internal standards changed in their positions with marked $^{13}C_{12}$ atoms and all 12 dioxin like PCB internal standards with marked $^{13}C_{12}$ atoms. Since the European Commission has not regulated the requirements for PBDE analysis, at least one internal standard with marked $^{13}C_{12}$ atom for the homologous row has been chosen for the experiment. Table 3.1. presents scanned masses of polyhalogenated organic pollutants, the periods of measurement and skips, isotope ratio which is analyzed.

Table 3.1. The scanned masses of polyhalogenated organic pollutants, the periods of measurement and skips, isotope ratio.

Analyte	$^{12}C_{12}$ mass (m/e)	m/z	Isotope ratio	$^{13}C_{12}$ mass (m/e)	m/z	Isotope ratio
PCDD/PCDF						
TetraCDF	303.9016 305.8987	$[M]^+$ $[M+2]^+$	0,77	315.9419 317.9389	$[M]^+$ $[M+2]^+$	0,77
TetraCDD	319.8965 321.8936	$[M]^+$ $[M+2]^+$	0,77	331.9368 333.9339	$[M]^+$ $[M+2]^+$	0,77
Fixation mass	330.9792	-	-	330.9792	-	-
PentaCDF	339.8597 341.8586	$[M+2]^+$ $[M+4]^+$	0,65	351.9000 353.8970	$[M+2]^+$ $[M+4]^+$	0,65
PentaCDD	353.8576 355.8546	$[M]^+$ $[M+2]^+$	0,65	365.8978 367.8949	$[M]^+$ $[M+2]^+$	0,65
Fixation mass	366.9792	-	-	366.9792	-	-
HexaCDF	373.8207 375.8178	$[M+2]^+$ $[M+4]^+$	0,81	385.8610 387.8580	$[M+2]^+$ $[M+4]^+$	0,81
HexaCDD	389.8156 391.8127	$[M+2]^+$ $[M+4]^+$	0,81	401.8559 403.8530	$[M+2]^+$ $[M+4]^+$	0,81
Fixation mass	380.9760	-	-	380.9760	-	-
HeptaCDF	407.7818 409.7788	$[M+2]^+$ $[M+4]^+$	0,95	419.8220 421.8191	$[M+2]^+$ $[M+4]^+$	0,95
HeptaCDD	423.7767 425.7737	$[M+2]^+$ $[M+4]^+$	0,95	435.8169 437.8140	$[M+2]^+$ $[M+4]^+$	0,95
Fixation mass	430.9728	-	-	430.9728	-	-
OCDF	441.7428 443.7398	$[M+2]^+$ $[M+4]^+$	0,89	453.7830 455.7801	$[M+2]^+$ $[M+4]^+$	0,89
OCDD	459.7348 461.7320	$[M+4]^+$ $[M+6]^+$	0,65	471.7750 473.7721	$[M+4]^+$ $[M+6]^+$	0,65
Fixation mass	454.9728	-	-	454.9728	-	-
Dioxin like PCB						
TetraCB	289.9223 291.9194	$[M]^+$ $[M+2]^+$	0,77	301.9626 303.9597	$[M]^+$ $[M+2]^+$	0,77
Fixation mass	318.9792	-	-	318.9792	-	-
PentaCB	325.8804 327.8775	$[M+2]^+$ $[M+4]^+$	1,55	337.9206 339.9178	$[M+2]^+$ $[M+4]^+$	1,55
Fixation mass	330.9292	-	-	330.9292	-	-
HexaCB	359.8415 361.8385	$[M+2]^+$ $[M+4]^+$	1,24	371.8817 373.8788	$[M+2]^+$ $[M+4]^+$	1,24
Fixation mass	368.9760	-	-	368.9760	-	-
HeptaCB	393.8025 395.7995	$[M+2]^+$ $[M+4]^+$	1,05	405.8428 407.8398	$[M+2]^+$ $[M+4]^+$	1,05
Fixation mass	404.9760	-	-	404.9760	-	-
PBDE						
DiBDE	325.8942 327.8921	$[M]^+$ $[M+2]^+$	0,51	337.9344 339.9324	$[M]^+$ $[M+2]^+$	0,51
Fixation mass	292.9824 330.9792	-	-	292.9824 330.9792	-	-
TriBDE	405.8027 407.8002	$[M+2]^+$ $[M+4]^+$	1,03	417.8429 419.8409	$[M+2]^+$ $[M+4]^+$	1,03
Fixation mass	392.9755 430.9723	-	-	392.9755 430.9723	-	-
TetraBDE	483.7132 485.7111	$[M+2]^+$ $[M+4]^+$	0,7	497.7514 499.7493	$[M+4]^+$ $[M+6]^+$	1,54
Fixation mass	480.9696 504.9696	-	-	480.9696 504.9696	-	-
PentaBDE	563.6216 565.6296	$[M+4]^+$ $[M+6]^+$	1,03	575.6619 577.6598	$[M+4]^+$ $[M+6]^+$	1,03
Fixation mass	580.9633 630.6216	-	-	580.9633 630.6216	-	-
HexaBDE	641.5322 643.5302	$[M+4]^+$ $[M+6]^+$	0,77	655.5704 657.5683	$[M+6]^+$ $[M+8]^+$	1,37

Fixation mass	630.9601 666.9601	-	-	630.9601 666.9601	-	-
HeptaBDE	721.4406 723.4386	$[M+6]^+$ $[M+8]^+$	1,03	733.4809 735.4788	$[M+6]^+$ $[M+8]^+$	1,03
Fixation mass	716.9569 735.4788	-	-	716.9569 735.4788	-	-
$^{12}C_{12}$ – OctaBDE	639.5165 641.5144	$[M-2Br+4]^+$ $[M-2Br+6]^+$	0,77	733.4809 735.4788	$[M-2Br+4]^+$ $[M-2Br+6]^+$	0,77
Fixation mass	630.9601 666.9601	-	-	630.9601 666.9601	-	-
DecaBDE	797.3355 799.3334	$[M-2Br+6]^+$ $[M-2Br+8]^+$	0,82	809.3757 811.3737	$[M-2Br+6]^+$ $[M-2Br+8]^+$	0,82
Fixation mass	630.9601 666.9601	-	-	630.9601 666.9601	-	-
Non dioxin like PCB						
TriCB	255.9613 257.9584	$[M]^+$ $[M+2]^+$	1,03	268.0016 269.9986	$[M]^+$ $[M+2]^+$	1,03
TetraCB	289.9224 291.9194	$[M]^+$ $[M+2]^+$	0,77	301.9626 303.9597	$[M]^+$ $[M+2]^+$	0,77
Fixation mass	304.9824	-	-	304.9824	-	-
PentaCB	325.8804 327.8775	$[M+2]^+$ $[M+4]^+$	1,55	337.9206 339.9178	$[M+2]^+$ $[M+4]^+$	1,55
Fixation mass	330.9792	-	-	330.9792	-	-
HexaCB	359.8415 361.8385	$[M+2]^+$ $[M+4]^+$	1,24	371.8817 373.8788	$[M+2]^+$ $[M+4]^+$	1,24
Fixation mass	330.9792	-	-	330.9792	-	-
HeptaCB	393.8025 395.7995	$[M+2]^+$ $[M+4]^+$	1,04	405.8428 407.8398	$[M+2]^+$ $[M+4]^+$	1,04
Fixation mass	330.9792	-	-	330.9792	-	-

During each analysis, the chromatographic sequence of solutions, forms, empty samples, and quality control samples is created. The latter is created in this manner in chronological order: the standard points of calibration curve, blank (toluene), empty sample, samples (blank form is inserted in every third sample), quality control sample, the third point of the calibration curve. Figure 3.9. presents the chromatographic separation of $^{13}C_{12}$ and $^{12}C_{12}$ TCDD and TCDF after eliminating a part of the column, changing injector insert and septa (A), and when the column is polluted (B).

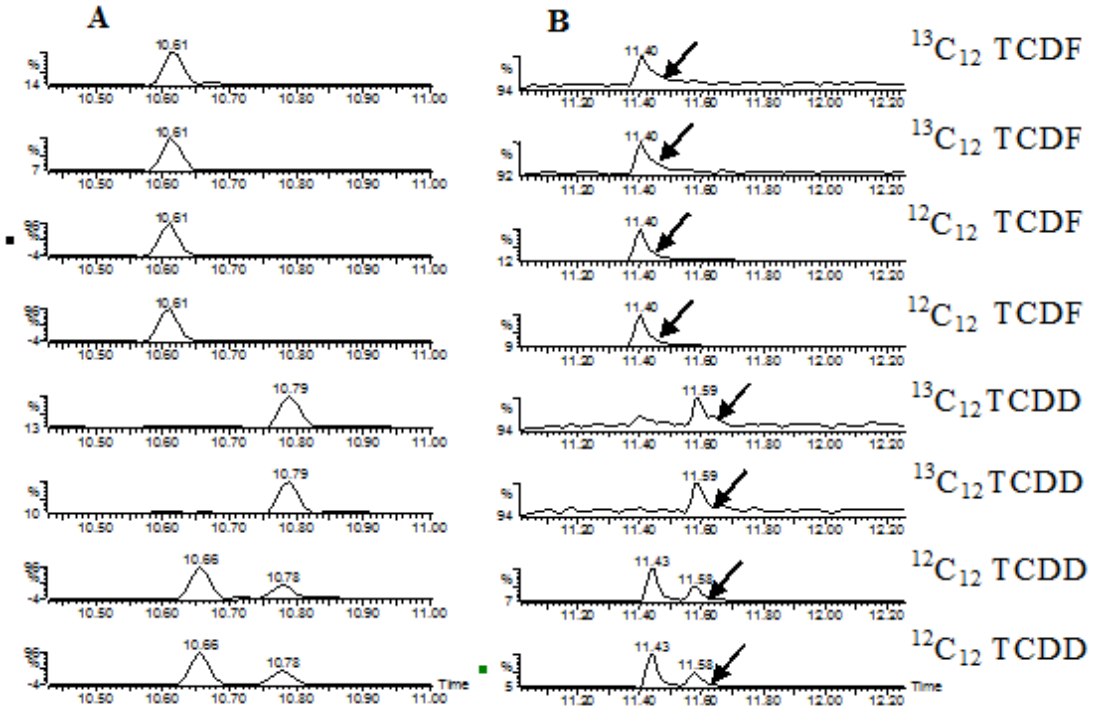

Figure 3.9. The chromatographic separation of $^{13}C_{12}$ ir $^{12}C_{12}$ TCDD and TCDF after eliminating a part of the column, changing injector insert and septa (A), and when the column is polluted (B).

Figure 3.9. discloses that analytical signal intensity is decreasing when the chromatographic column is polluted and the peak asymmetry is increasing.

3.5. The analytical characteristics of the method

The analytical characteristics of the method has been selected in accordance with the Commission regulation (EU) No. 589/2014 in food products and the Commission regulation (EU) No. 709/2014 in feed and its raw materials with 0,5/1/2 the maximum allowable concentration (next - ML), however each matrix has different ML (Tables 1.10. and 1.11.). Using the manual sample preparation method, the analytical conditions has been checked in fish and its products (with TEQ PCDD/F 3,40 pg/g, TEQ PCDD/F, PCB 8,09 pg/g), meat and its products (with TEQ PCDD/F 0,76 pg/g, TEQ PCDD/F, PCB 3,42 pg/g), and feed and its raw materials (with TEQ PCDD/F 2,28 pg/g, TEQ PCDD/F, PCB 7,5 pg/g feed of animal origin and TEQ PCDD/F 1,14 pg/g, TEQ PCDD/F, PCB 3,75 pg/g feed of vegetable origin). Using automatic sample preparation method, the confirmation is executed combining all matrixes of adipose products and

feed. The concentrations of method confirmation using the automatic sample preparation method are presented in Table 3.2.

Table 3.2. POP method validation matrixes and concentrations.

Analyte	**TEF_{2005}**	**Concentration in the sample 0,5/1/2 ML, pg/g**	**TEQ 0,5/1/2 ML, pg/g**
2,3,7,8-tetraCDD	1	0,05/0,2/0,5	0,05/0,2/0,5
1,2,3,7,8-pentaCDD	1	0,25/1,0/2,5	0,25/1,0/2,5
1,2,3,4,7,8-hexaCDD	0.1	0,25/1,0/2,5	0,025/0,1/0,25
1,2,3,6,7,8-hexaCDD	0.1	0,25/1,0/2,5	0,025/0,1/0,25
1,2,3,7,8,9-hexaCDD	0.1	0,25/1,0/2,5	0,025/0,1/0,25
1,2,3,4,6,7,8-heptaCDD	0.01	0,25/1,0/2,5	0,0025/0,01/0,025
OktaCDD	0.0003	0,5/2,0/5,0	0,0002/0,0006/ 0,0015
2,3,7,8-tetraCDF	0.1	0,05/0,2/0,5	0,005/0,02/0,05
1,2,3,7,8-pentaCDF	0.03	0,25/1,0/2,5	0,0075/0,03/0,075
2,3,4,7,8-pentaCDF	0.3	0,25/1,0/2,5	0,075/0,3/0,75
1,2,3,4,7,8-hexaCDF	0.1	0,25/1,0/2,5	0,025/0,1/0,25
1,2,3,6,7,8-hexaCDF	0.1	0,25/1,0/2,5	0,025/0,1/0,25
1,2,3,7,8,9-hexaCDF	0.1	0,25/1,0/2,5	0,025/0,1/0,25
2,3,4,6,7,8-hexaCDF	0.1	0,25/1,0/2,5	0,025/0,1/0,25
1,2,3,4,6,7,8-heptaCDF	0.01	0,25/1,0/2,5	0,0025/0,01/0,025
1,2,3,4,7,8,9-heptaCDF	0.01	0,25/1,0/2,5	0,0025/0,01/0,025
OktaCDF	0.0003	0,5/2,0/5,0	0,0002/0,0006/ 0,0015
TEQ_{2005} PCDD/PCDF			**0,57/2,28/5,7**
3,3‘,4,4‘-tetraCB (77 PCB)	0.0001	5/60/110	0,0005/0,006/ 0,011
3,4,4‘,5-tetraCD (81 PCB)	0.0003	5/60/110	0,0015/0,018/ 0,033
3,3‘,4,4‘,5-pentaCB (126 PCB)	0.1	5/60/110	0,5/6/11

3,3‘,4,4‘,5,5‘-hexaCB (169 PCB)	0.03	5/60/110	0,15/1,8/3,3
2,3,3‘,4,4‘-pentaCB (105 PCB)	0.00003	5/60/110	0,00015/0,0018/ 0,0033
2,3,4,4‘,5-pentaCB (114 PCB)	0.00003	5/60/110	0,00015/0,0018/ 0,0033
2,3‘,4,4‘,5-pentaCB (118 PCB)	0.00003	5/60/110	0,00015/0,0018/ 0,0033
2‘,3,4,4‘,5-pentaCB (123 PCB)	0.00003	5/60/110	0,00015/0,0018/ 0,0033
2,3,3‘,4,4‘,5-hexaCB (156 PCB)	0.00003	5/60/110	0,00015/0,0018/ 0,0033
2,3,3‘,4,4‘,5‘-hexaCB (157 PCB)	0.00003	5/60/110	0,00015/0,0018/ 0,0033
2,3‘,4,4‘,5,5‘-hexaCB (167 PCB)	0.00003	5/60/110	0,00015/0,0018/ 0,0033
2,3,3‘,4,4‘,5,5‘-heptaCB (189 PCB)	0.00003	5/60/110	0,00015/0,0018/ 0,0033
TEQ_{2005} PCDD/PCDF, PCB			**1,22/10,12/20**
2,4,4'trichlorobiphenyl (28 PCB)	-	1,5/15/50	1/15/50
2,2',5,5' tetrachlorobiphenyl (52 PCB)	-	1,5/15/50	1/15/50
2,2',4,5,5' pentachlorobiphenyl (101 PCB)	-	1,5/15/50	1/15/50
2,2',3,4,4',5' hexachlorbiphenyl (138 PCB)	-	1,5/15/50	1/15/50
2,2',4,4',5,5' hexachlorbiphenyl (153 PCB)	-	1,5/15/50	1/15/50
2,2',3,4,4',5,5' heptachlorbiphenyl (180 PCB)	-	1,5/15/50	1/15/50
Total non DL-PCB			9/90/300

According to the concentration intervals given in Table 3.2., the confirmation of the method has been carried out in reproducibility conditions. During the experiment, these have been evaluated: sensitivity of the method (detection limit), linearity of the method (the calibration curves), the recoveries of internal standard $^{13}C_{12}$ in reproducibility conditions, the relative standard deviations, veracity.

3.5.1. The minimum POP measurement quantities

During the experiment, 1, 2 and 3 µl injection volumes have been checked using standard POT solutions (the lowest point of the calibration curve). Figure 3.10. presents $^{12}C_{12}$ TCDD chromatograms with different injections.

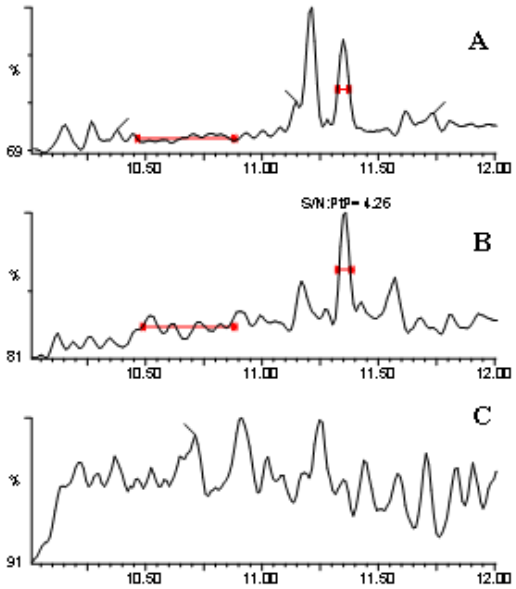

Figure 3.10. TCDD resolution capacity with different injections: A – 3 µl, B – 2 µl, C – 1µl.

The chosen PCDD/PCDF and PBDE injection volume is 2 µl, PCB – 1 µl. The minimum measured quantity of all individual compounds is presented in Figure 3.10. This figure discloses that PCDD/F has the lowest measurement quantity of which terta-dioxin and furans make 2 fg/column, penta-, hexa-, hepta- 5 – 10 fg/column, and octa- 30 fg/column. Dioxin like and non dioxin like polychlorinated biphenyls lowest measurement quantities are in the intervals from 5 to 30 fg/column.

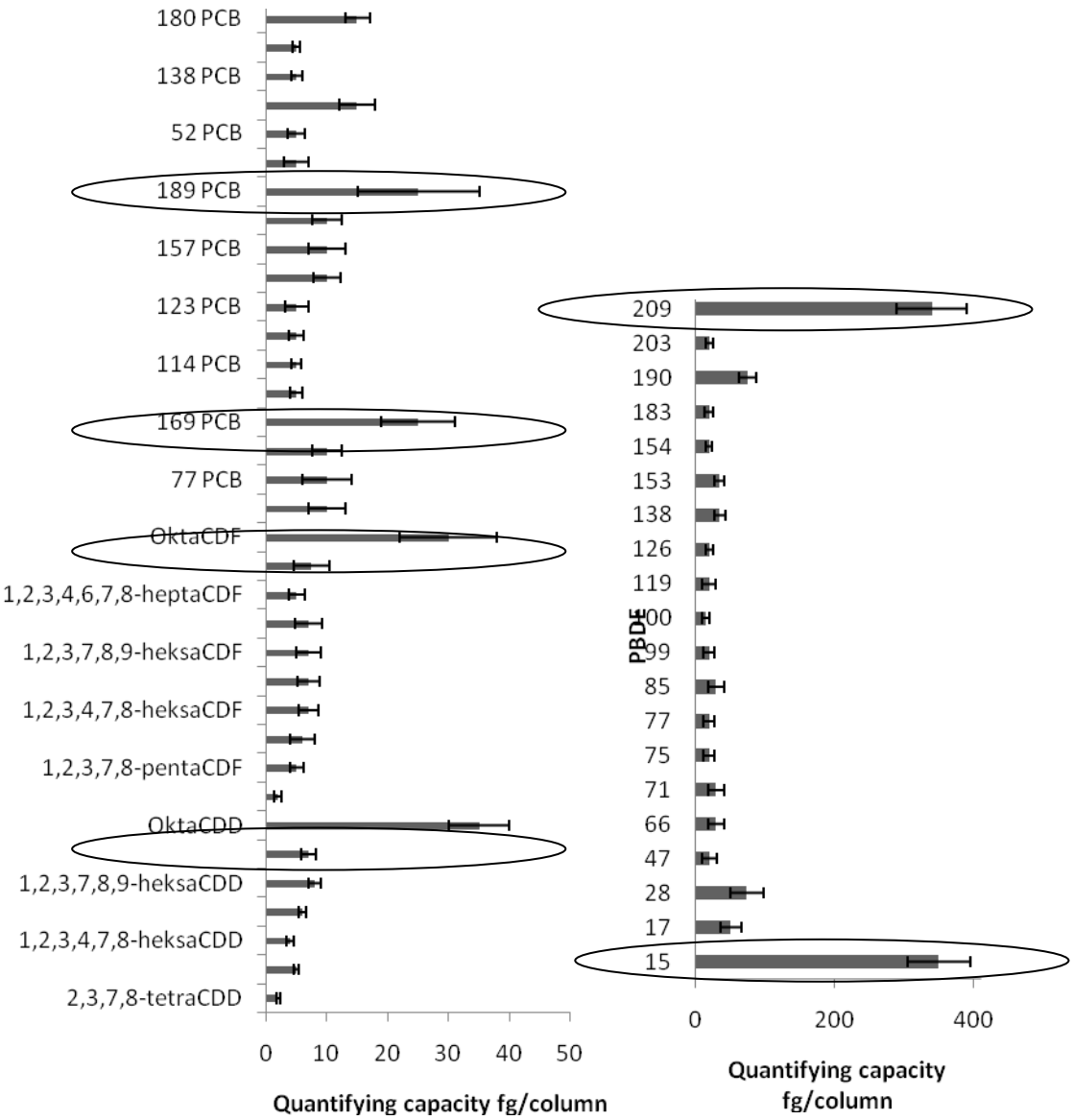

Figure 3.11. The lowest individual POP measurement quantities of PCDD/PCDF and PCB (n=10), PBDE (n=3) using the method of high resolution gas chromatography mass spectrometry.

Using the manual sample preparation method during the experiment, three matrixes have been evaluated: fish, meat, and feed. Using the automatic sample preparation procedure, detection limit has been evaluated: milk, oil, feed, and fish matrixes. The PBDE detection limit has been checked while using both methods of sample preparation has been checked only in the fish matrix.

In accordance with the data obtained, the detection limit meets the requirements of European Commission for POP using both manual and automatic sample preparation methods. The detection limits are given in Figure 3.12.

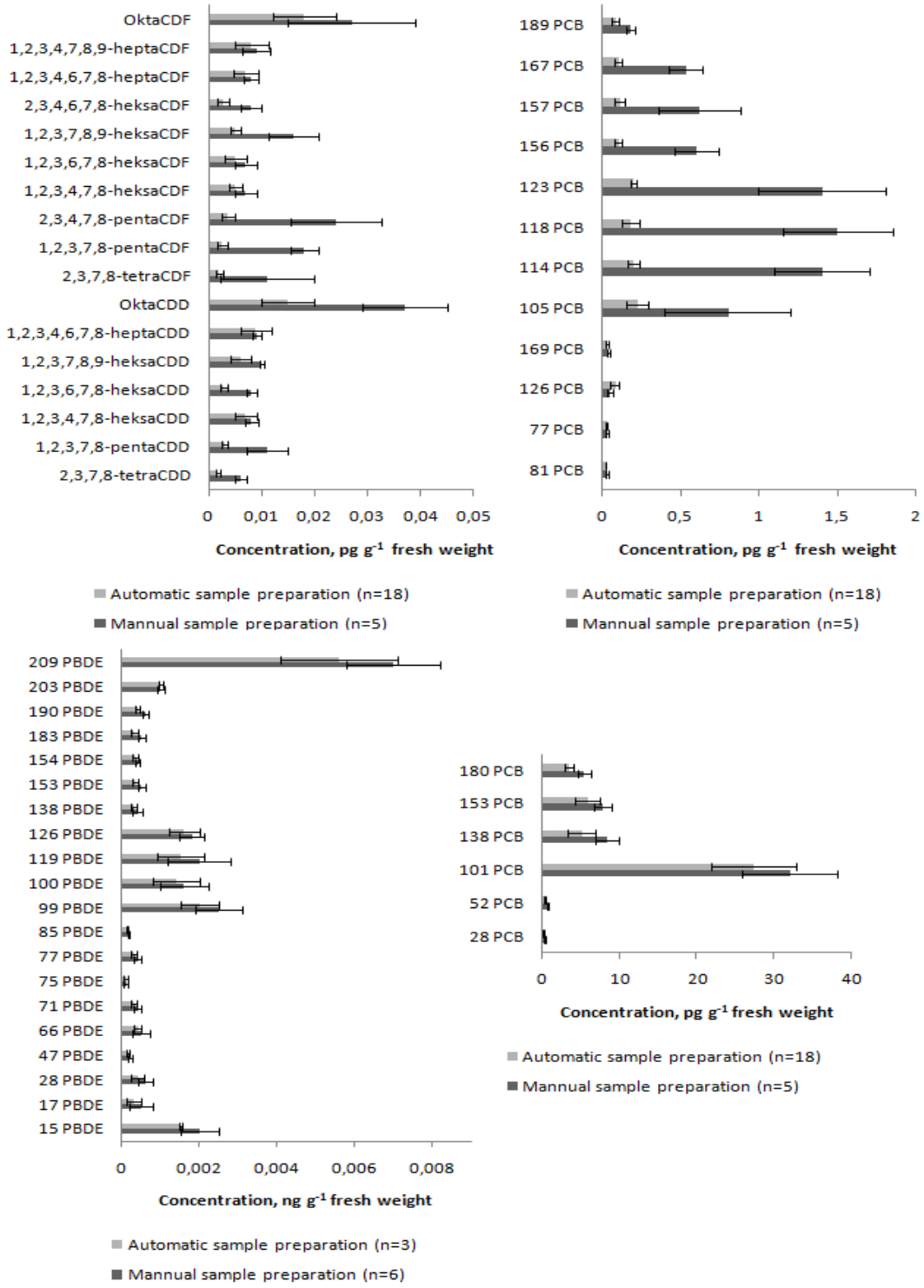

Figure 3.12. The detection limits of PCDD/PCDF, DL-PCB, non DL-PCB, and PBDE in fish using the method of high resolution gas chromatography mass spectrometry.

The detection limits meet the requirements of European Commission for the method of POP analysis, i.e. < 1/5 ML. Using the automatic sample preparation method, the detection limits are lower than using the manual sample preparation method.

3.5.2. The pollution level of blank samples

An empty sample is a sample which is analyzed without matrix effect, i.e. the preparation of the sample and qualitative and quantitative evaluation are executed without a sample. Furthermore, empty samples are analyzed for the purpose of controlling cross pollution of the dishes used in the laboratory.

Empty samples have been analyzed in respect of polychlorinated dibenzo-*p*-dioxin, dibenzofuran, and dioxin type polychlorinated biphenyls during the experiment. The results are provided in Figure 3.13. Using the manual sample preparation method n=32 while using the automatic sample preparation method n=9.

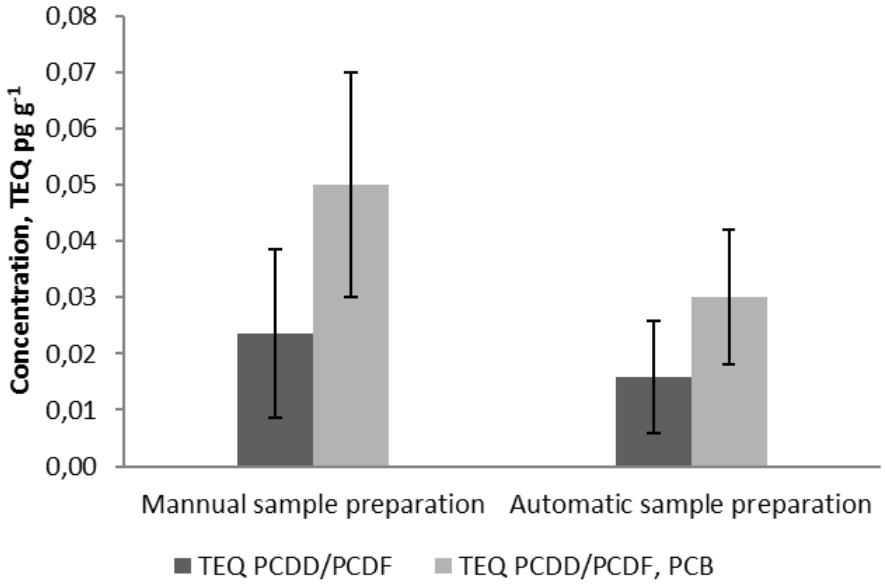

Figure 3.13. The comparison of the concentrations of blank samples using both manual and automatic sample preparation methods.

The figure shows that using the automatic sample preparation method the level of TEQ PCDD/PCDF pollution is 33% lower while TEQ PCDD/PCDF, PCB is 40% lower than using the manual sample preparation method.

3.5.3. The interval and linearity of POP analytical method calibration

The linearity of POP analytical method calibration is measured using standard solutions with relevant individual analytes and known concentrations. PCDD/PCDF ir DL-PCB calibration curves consist of 9 points, non DL-PCB consists of 7 points, PBDE consists of 5 points. The intervals of working limits for all POP are presented in Table 3.3.

Table 3.3. The intervals of polyhalogenated organic pollutants calibration curves.

Calibration point No.	**The mixture of standard analytes solution**	**Calibration point concentration, ng ml^{-1}**	**The concentration of internal standards $^{13}C_{12}$ ng ml^{-1}**	**The concentration of the recovery standard $^{13}C_{12}$, ng ml^{-1}**
1.	PCDD/F	TetraCDD/F 0,005 Penta, Hexa, HeptaCDD/F 0,025 OctaCDD/F 0,05	2,5	5
2.		TetraCDD/F 0,01 Penta, Hexa, HeptaCDD/F 0,05 OctaCDD/F 0,1		
3.		TetraCDD/F 0,05 Penta, Hexa, HeptaCDD/F 0,25 OctaCDD/F 0,5		
4.		TetraCDD/F 0,1 Penta, Hekxa, HeptaCDD/F 0,5 OctaCDD/F 1		
5.		TetraCDD/F 0,25 Penta, Hexa, HeptaCDD/F 1,25 OctaCDD/F 2,5		
6.		TetraCDD/F 0,5 Penta, Hexa, HeptaCDD/F 2,5 OctaCDD/F 5		
7.		TetraCDD/F 1 Penta, Hexa, HeptaCDD/F 5 OctaCDD/F 10		
8.		TetraCDD/F 2 Penta, Hexa, HeptaCDD/F 10 OctaCDD/F 20		
9.		TetraCDD/F 4 Penta, Hexa, HeptaCDD/F 20 OctaCDD/F 40		
1.	DL-PCB	0,1	2,5	5
2.		0,25		
3.		0,5		
4.		1		
5.		2,5		
6.		5		
7.		10		
8.		50		
9.		100		
1.	Non DL-PCB	0,1	2,5	5
2.		0,5		
3.		1		
4.		10		
5.		50		
6.		250		

7.		500		
1.	PBDE	Tetra, penta-BDE 1 Heksa, hepta, okta-BDE 2 Deka-BDE 5	10 20 150	20
2.		Tetra, penta-BDE 5 Hexa, hepta, octa-BDE 10 Deka-BDE 25	10 20 150	
3.		Tetra, penta-BDE 10 Heksa, hepta, okta-BDE 20 Deca-BDE 50	10 20 150	
4.		Tetra, penta-BDE 50 Hexa, hepta, octa-BDE 100 Deca-BDE 250	10 20 150	
5.		Tetra, penta-BDE 100 Hexa, hepta, octa-BDE 200 Deca-BDE 500	10 20 150	

The recovery standard of $^{13}C_{12}$ 1,2,3,4-TCDD is used in calibration curves of PCDD/PCDF, DL-PCB, and non DL-PCB while in PBDE $^{13}C_{12}$ 77 PBDE is used.

The linearity of calibration curves is evaluated according to both internal ($^{13}C_{12}$) and hen ($^{12}C_{12}$) relative standard deviations of standard response <20%.

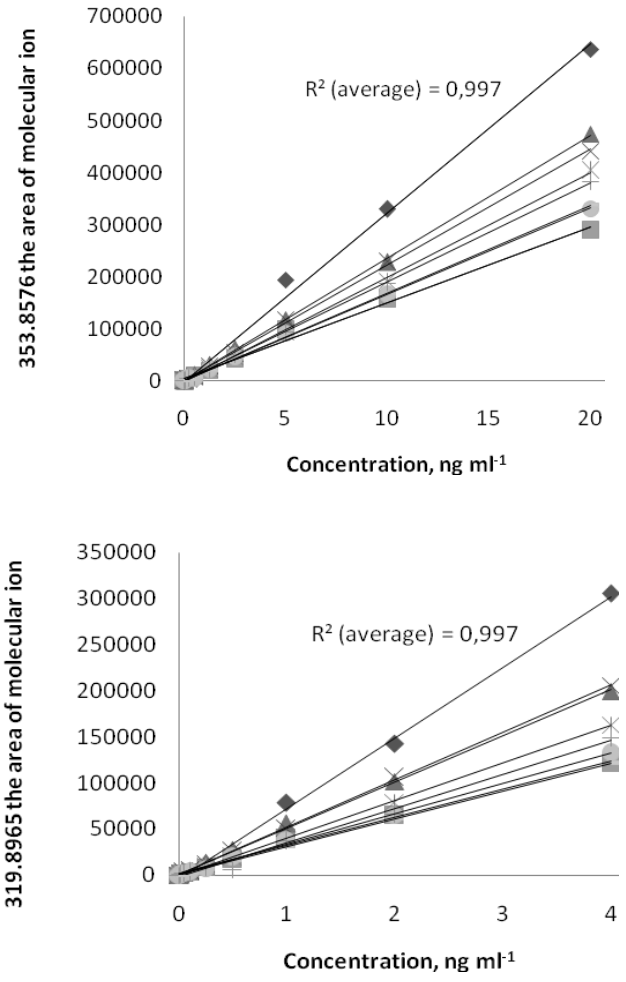

Figure 3.14. The linearity of $^{12}C_{12}$ TCDD 319.8965 and $^{12}C_{12}$ PCDD 353.8576 molecular ions in different days.

The average value of the relative standard deviation in every point of the calibration curve is in the interval from 11 to 33%. The linearity of all the other individual pollutants meets the requirements of analysis method, i.e. relative standard deviation of the response is <20%.

3.6. The comparison of the results using different sample preparation methods

In accordance with the gained results of the experiment, two sample preparation methods have been compared: manual and automatic. The results are presented in Table 3.4.

Table 3.4. The comparison of manual and automatic sample preparation methods.

Parameter	Analyte	The manual sample preparation method	The automatic sample preparation method
The duration of sample preparation	TEQ PCDD/PCDF	≈ 2 weeks	≈ 3 days
	TEQ PCDD/PCDF, PCB		
	Non DL-PCB		
	PBDE		
The level of pollution during the analysis	TEQ PCDD/PCDF	0-0.1 pg g^{-1} (avarage 0.024)	0-0.04 pg g^{-1} (avarage 0.016)
	TEQ PCDD/PCDF, PCB	0-0.22 pg g^{-1} (avarage 0.05)	0.018-0.4 pg g^{-1} (avarage 0.03)
	Non DL-PCB	-	-
	PBDE	-	-
The limit of quantification (fish)	TEQ_{1998} PCDD/PCDF	0,038 pg g^{-1}(< 1/5 ML)	0,01 pg g^{-1}(< 1/5 ML)
	TEQ_{2005} PCDD/PCDF	0,033 pg g^{-1}(< 1/5 ML)	0,02 pg g^{-1}(< 1/5 ML)
	TEQ_{1998} PCDD/PCDF, PCB	0,07 pg g^{-1}(< 1/5 ML)	0,02 pg g^{-1}(< 1/5 ML)
	TEQ_{2005} PCDD/PCDF, PCB	0,05 pg g^{-1}(< 1/5 ML)	0,05 pg g^{-1}(< 1/5 ML)
	Sum of non DL-PCB	54 pg g^{-1} (< 1/5 ML)	42 pg g^{-1} (< 1/5 ML)
	PBDE	0,02 ng g^{-1}	0,018 ng g^{-1}

The separation of individual POP	PCDD/PCDF	Yes	Yes
	PCDD/PCDF, PCB		
	Non DL-PCB		
	PBDE		
The recoveries of internal standard $^{13}C_{12}$	PCDD/PCDF	60-120%	60-120%
	PCB	60-120%	60-120%
	Non DL-PCB	60-120%	60-120%
	PBDE	50-130%	50-130%
Trueness	TEQ_{2005} PCDD/PCDF	-6.65% (1 ML)	11,81% (0.5 ML) -1.36% (1 ML) 5.54% (2 ML)
	TEQ_{2005} PCDD/PCDF, PCB	-11.92% (1 ML)	5.96% (0.5 ML) -12.1% (1 ML) -9.4% (2 ML)
	Non DL-PCB	-	-4.25% (0.5 ML) -13.37% (1 ML) -24.63% (2 ML)
	PBDE	-	-
The relative standard deviation	TEQ_{2005} PCDD/PCDF	12.4% (1 ML)	6.57% (0.5 ML) 7.48% (1 ML) 10.91% (2 ML)
	TEQ_{2005} PCDD/PCDF, PCB	2.22% (1 ML)	3.38% (0.5 ML) 3.44% (1 ML) 6.8% (2 ML)
	Non DL-PCB	-	10.2% (0.5 ML) 2.44% (1 ML) 18.73% (2 ML)
	PBDE	-	-

The results reveal that both sample preparation methods meet the requirements of European Commission for POP analysis. Nevertheless, the results using the automatic sample preparation method are superior which testifies about the ability of the method to reach lower limit of determination when the level of pollution is low. The duration of analysis is also an important factor which decreases even to 80% using the automatic sample preparation method.

3.7. The comparison of the results of POT in different food and feed samples

During the experiment in 2007-2014, 16 samples of eggs, 18 samples of milk, 32 samples of meat (9 - cattle, 9 - poultry, 14 - pork), 8 samples of vegetable origin fat (rapeseed, sunflower, coconut oil), 27 samples of animal origin fat (cattle, chicken, pork fat, fish oil), 114 samples of feed (oils, premixes, soy flour, mixed feed, feed additives, rapeseed meal, corn, fish flour, milk substitutes, guar gum, rapeseed oil, wheat flour, peas), 111 sample of fish (sprat, herring, salmon, cod, carp), 12 samples of liver were analyzed. Analysing and evaluating the results of food products and feed in terms of POP, several groups were distinguished: fish, meat, eggs, milk, feed, both fat of vegetable and animal origin.

Eggs and fat of animal origin contain OCDD which is the only predominant compound with compatible concentrations 4,2 pg g^{-1} and 1,8 pg g^{-1}. The profile of PCDD/PCDF in pigs and feed (premixes, feed of vegetable origin, combined feed, mineral supplements, vegetable oil, fish flour) samples are equal. Considering that the pigs are kept in closed cages, their nutrition mainly contain of feed. For this reason, in these matrixes OCDD, OCDF, TCDF, and 2,3,4,7,8-PCDF are prevailing compounds. OCDD concentration in pork is 0,46 pg g^{-1} fat and 0,45 pg g^{-1} 12% moisture content in feed, OCDF concentration in pork is 0,098 pg g^{-1} fat while there is 0,16 pg g^{-1} 12% moisture content in feed. More of toxic compounds such as TCDF concentration in pork is 0,23 pg g^{-1} fat and feed - 0,22 pg g^{-1} 12% moisture content, 2,3,4,7,8-PCDF concentration in pork is 0,13 pg g^{-1} in fat and feed - 0,13 pg g^{-1} 12% moisture content.

111 samples of fish were analyzed during the experiment (6 Atlantic salmon, 4 Baltic salmon, 8 salmon products, 18 Baltic sprat, 17 sprat products, 29 Baltic herring, 4 herring products, 3 cod, 22 carp, mackerel, other fish products) and 12 cod liver products in the period of 2007-2014. Since dioxins are widespread in the whole Baltic sea, this

group of products is analyzed as one of the most risky. POT profiles in fish and cod liver are similar except isolated compounds concentrations. In contradistinction to other groups, the dominant compounds in fish and cod liver are: TCDF, 1,2,3,7,8-PCDF, and 2,3,4,7,8-PCDF. TCDF concentration is 5 times higher, 1,2,3,7,8-PCDF 3 times higher, and 2,3,4,7,8-PCDF 2 times higher in cod liver comparing to fish samples.

POT profile of milk samples is the only one that goes without any similarities to other groups. The prevailing compounds are HpCDD, 1,2,3,6,7,8-HxCDD ir 2,3,4,7,8-PCDF. All 18 milk samples analyzed in the period of 2007-2014 met the requirements of European Commission for the maximum limit of concentrations in 2,5 and 5,5 pg g^{-1} fat.

The concentration of PSO TE PCDD/PCDF and PSO TE PCDD/PCDF, PCB in milk samples analyzed in Lithuania is lower than it is declared by the European Food Safety Authority (EFSA) in the protocol of 2010, respectively 1,05 pg g^{-1} and 2,42 pg g^{-1} in fat.

The dominating pollutants in feed are OCDD (0,0022-6,1 pg g^{-1} 12% moisture content), OCDF (0,0024-6,8 pg g^{-1} 12% moisture content), TCDF (0,0013-4,2 pg g^{-1} 12% moisture content), and 2,3,4,7,8-PCDF (0,00049-1,9 pg g^{-1} 12% moisture content).

The profiles of polychlorinated biphenyls in all the matrixes are similar except its concentrations. Only fat of vegetable origin demonstrates a higher 77 PCB concentration 4,8 pg g^{-1} of fat.

Several individual compounds can be distinguished which make the biggest influence on the concentration of toxic equivalent. TCDD (28% in eggs and 8,9% in chicken), pentaCDD (23% in eggs, 9,7% in chicken, 12% in milk, 18% in pork, and 33% in beef), 126 PCB (33% in eggs, 37% in chicken, 40% in milk 52% in pork, 57% in beef, 61% in fat of vegetable origin, and even 72% in fat of animal origin), heptaCDD constitutes 29% and 118 PCB 70% toxic equivalent in milk. The average toxic equivalent in milk is 0,74 +/- 0,17 pg g^{-1} of fat (from 0,45 – to 1,1 pg g^{-1} of fat).

The majority of food products and feed demonstrates lower concentrations than ML, except fat of animal origin, fish and cod liver. The concentration of fat of animal origin (1,3 pg WHO-TEQ g^{-1} of fat) exceeds the regulations 1259/2011 of the highest allowed concentration 1,25 pg WHO-TEQ g^{-1} of fat with P90 already included. Fish

samples do not meet the requirements of European Commission of the regulations 1259/2011 with P90 included (3,6 pg WHO-TEQ PCDD/PCDF g^{-1} > 3,5 pg g^{-1} and 7,3 pg WHO-TEQ PCDD/PCDF, PCB > 6,5 pg g^{-1}). Cod liver WHO-TEQ PCDD/PCDF, PCB concentrations exceed the highest allowed concentrations with P50 included (36 pg g^{-1} > 20 pg g^{-1}). The overall toxic equivalent is mainly influenced by PCDD/PCDF eggs (83%), combined feed (73%) and premixes in the matrix (79%), while DL-PCB in the cod liver matrix is even 79%.

The comparison of WHO-TEQ PCDD/PCDF and WHO-TEQ PCDD/PCDF, PCB with ML in different samples is demonstrated in Figures 3.15., 3.16. and 3.17.

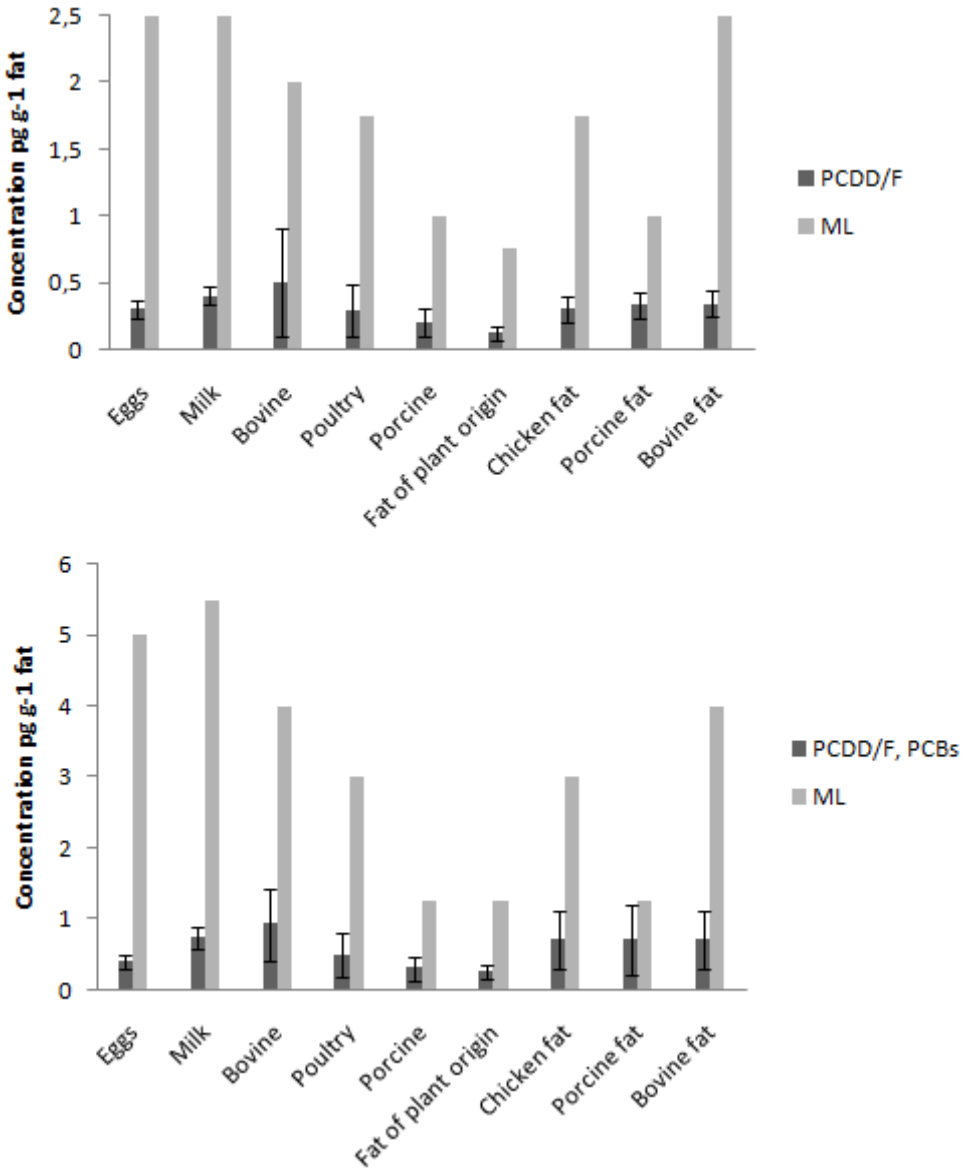

Figure 3.15. The concentrations of WHO-TEQ PCDD/PCDF and WHO-TEQ PCDD/PCDF, PCB in different food samples and its comparison with ML.

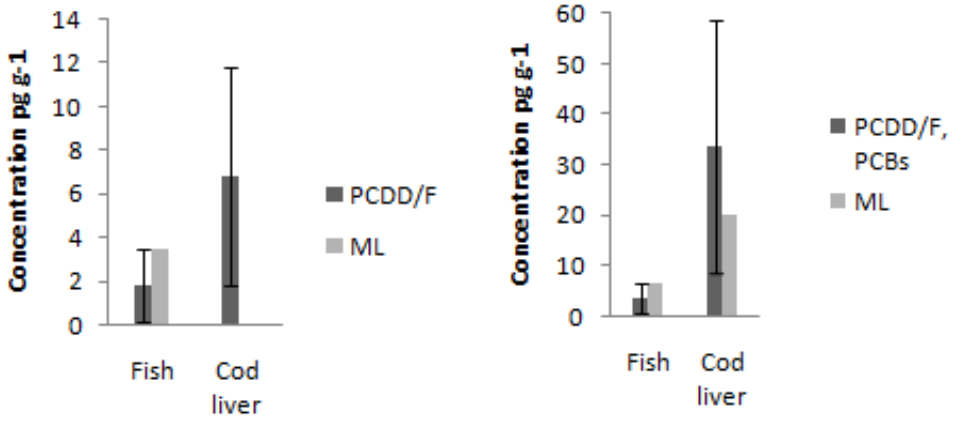

Figure 3.16. The concentrations of WHO-TEQ PCDD/PCDF and WHO-TEQ PCDD/PCDF, PCB in fish and codliver samples and its comparison with DLK.

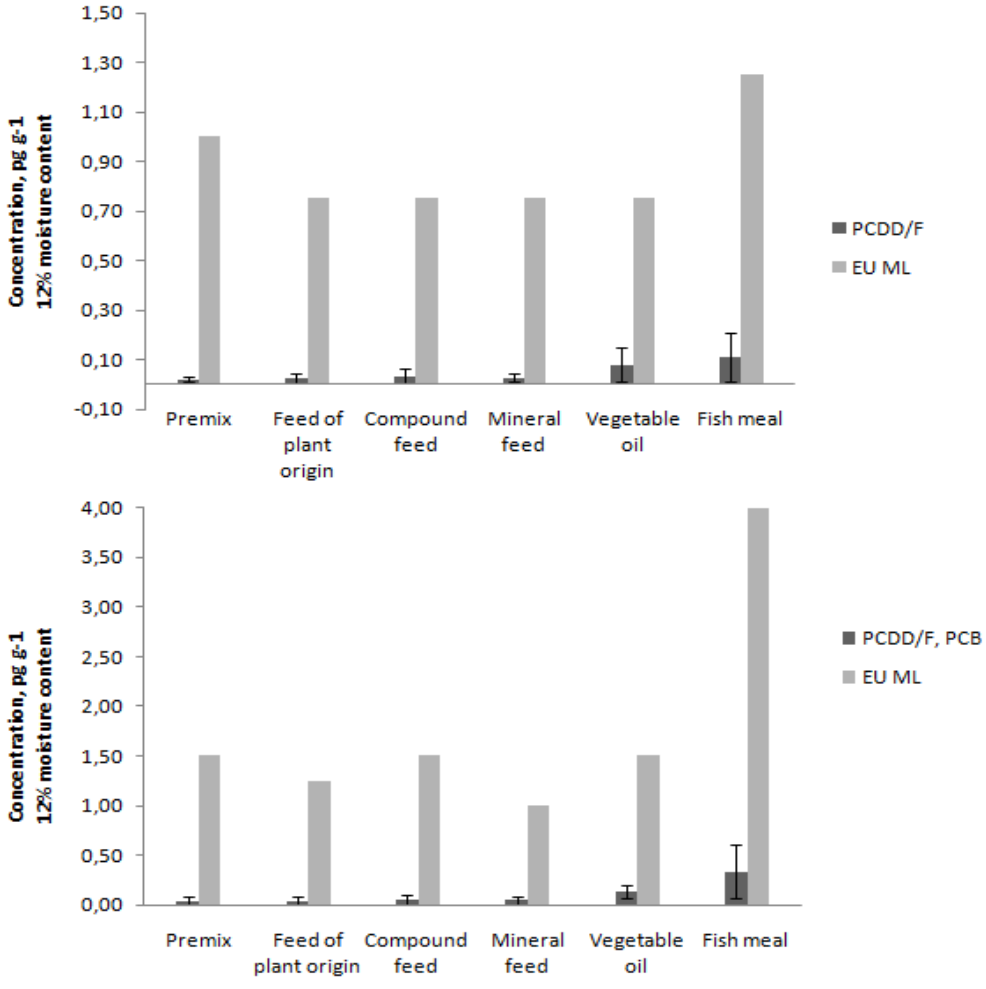

Figure 3.17. The concentrations of WHO-TEQ PCDD/PCDF and WHO-TEQ PCDD/PCDF, PCB in feed samples and its comparison with ML.

The lowest level of pollution is in fat of vegetable origin with avarage concentrations 0,12 +/- 0,048 pg g^{-1} of fat WHO-TEQ PCDD/PCDF and 0,25+/- 0,14 pg g^{-1} of fat WHO-TEQ PCDD/PCDF, PCB. The concentrations in milk, eggs, meat, and fat of animal origin are in the range of the highest allowed concentrations regulated by European Commission. Similar concentrations of pollutants are also declared by other authors.

The overall toxic equivalent in fish is in the range of 0,018 – 19 pg g^{-1}. 5,4% of fish samples did not meet the requirements of European Commission. The pollution of polychlorinated organic pollutants is also noticed by other scientists. 5 fish samples (2 samples of salmon and 3 samples of Baltic herring) and 7 cod liver samples exceeded ML regulated by EC No. 1881/2006. 1 fish sample (dried flounder) did not meet the requirements of EC No. 1259/2011. The overall toxic equivalent of the cod liver is in the range of 4,3 pg g^{-1} to 72 pg g^{-1}. The average WHO-TEQ PCDD/PCDF value of cod liver is 6,8 +/- 4,7 pg g^{-1} and the average of WHO-TEQ PCDD/PCDF, PCB is 33 +/- 25 pg g^{-1} which exceeds ML (20 pg g^{-1}). The origin of the majority of fish samples which do not meet the requirements is The Baltic sea (34% of herring, 3% of sprat, 44% of salmon). 58% of cod liver samples also did not meet the requirements of European Commission for the highest concentrations allowed in 2007-2014.

The gained results proclaim that the concentration of the persistent organic pollutant is decreasing. The decline of 76% is observed in fish samples and 80% in cod liver samples during the period of 2007-2014. After the prohibition of the use of Baltic cod in the market in 2011, no samples exceeding ML have been discovered.

3.8. Tolerable daily intake

Tolerable day intake was calculated during the period of 2005-2014. These limits were calculated for all matrixes of fresh weight. The daily intake of the cod liver was calculated by consuming 1825g per annum (5 g day^{-1}, 35 g $week^{-1}$, 150 g $month^{-1}$). Since all meat samples were analyzed with different amounts of fat, the results were recalculated with 40% of fat, and beef and chicken with 30% of fat. The results of eggs were recalculated with 10% of fat and the results of fat were submitted for a fresh

weight. The tolerable intakes were determined at the lowest, P20, P50, the average, P75, P90, P97,5 and the maximum levels of contamination.

The tolerable daily intake of meat, eggs and milk products is in the range of 0-1 pg TEQ kg body weight^{-1} , the tolerable weekly intakes do not exceed 14 pg TEQ kg body weight^{-1}, the tolerable monthly intakes do not exceed 70 pg TEQ kg body weight^{-1}, while the tolerable intakes of fish and cod liver do not meet the requirements set. Fish does not meet the tolerable weekly and monthly intakes (TWI and TMI) when there is P90 level, 18 and 78 pg WHO-TEQ PCDD/PCDF kg body weight^{-1}, respectively 18 and 76 pg WHO-TEQ PCDD/PCDF, PCB kg body weight^{-1}, when there is P50. The TWI and TMI of cod liver 19 and 83 pg WHO-TEQ PCDD/PCDF, PCB kg body weight^{-1} exceed the limits when there is P50 level and TDI 5,2 pg WHO-TEQ PCDD/PCDF, PCB kg body weight^{-1} is exceeded when there is P90 level.

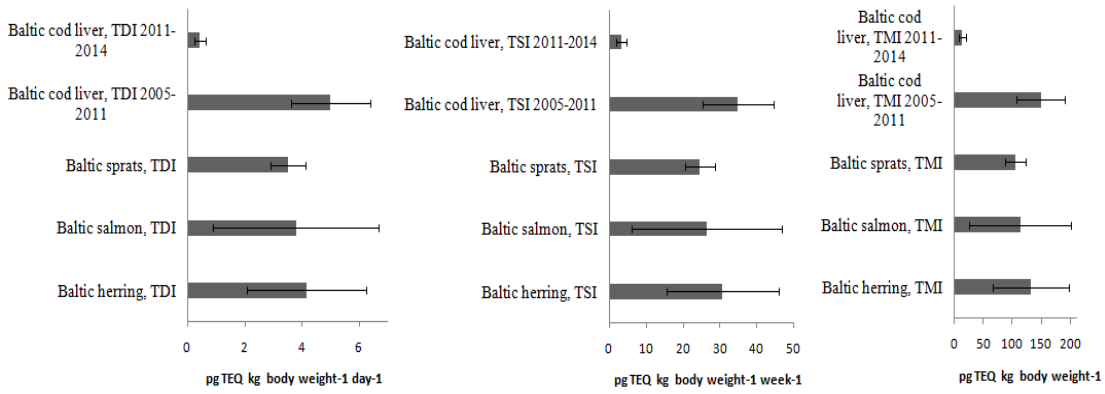

Figure 3.18. TDI, TWI and TMI in different Baltic fish.

Herring 4.2 +/- 2.1 kg body weight^{-1} day^{-1}, salmon 3.8 +/- 2.9 kg body weight^{-1} day^{1}, sprat 3.5 +/- 0.6 kg body weight^{-1} day^{-1}) and Baltic cod liver (5,0 +/- 1,4 kg body weight^{-1} day^{-1}) are in risk zone and the monitoring should be executed. TWI and TMI exceed 14 pg TEQ kg body weight^{-1} and 70 pg TEQ kg body weight^{-1} using the products every day. According to the intakes in Figure 3.19., the quantity of consumable products which does not have harmful effects for human body (origin - The Baltic Sea) should not exceed the list below:

Baltic herring ≤45 g day^{-1}, ≤150 g week^{-1}, ≤749 g year^{-1};

Baltic cod liver ≤4 g day^{-1}, ≤14 g week^{-1}, ≤70 g year^{-1};

Baltic salmon ≤47 g day^{-1}, ≤173 g week^{-1}, ≤866 g year^{-1};

Baltic sprat ≤47 g day^{-1}, ≤187 g $week^{-1}$, ≤940 g $year^{-1}$.

According to the data, the quantity of some Baltic products consumed per one week and year should be ½ of currently consumed quantities. Nine cod liver samples were analyzed during the period of 2011-2014 (origin is unknown). All the samples met the regulated requirements of European Commission. Baltic cod liver has been banned for human consumption and this prohibition is still valid because of high pollution of pollutants.

Conclusions

1. The methods of fat extraction are investigated and optimized in food products and feed. The most effective and the fastest extraction method in food products is „cold“ extraction using 250ml and 500ml of milk samples cyclohexane/dichloromethane (1/1 (v/v)) mixture, while in feed and its raw materials - Soxhlet or Twisselmann extraction, extracting with cyclohexane/toluene (1/1 (v/v)) mixture at least 6h and ethyl alcohol/toluene (7/3 (v/v)) mixture at least 6h as well.

2. The methods of extract cleaning are investigated and optimized using manual and automatic sample preparation methods. The results of the data prove that both sample preparation methods are appropriate for real sample analysis, but the automatic one is more effective and accurate.

3. The conditions of chromatographic separation and MS detection of persistent organic pollutants are optimized using the method of high-resolution gas chromatography mass spectrometry. The separation among 1,2,3,4,7,8 HexaCDF and 1,2,3,6,7,8 HexaCDF peaks reaches 13% (<25%). The most optimal injection volume of PCDD/PCDF is 2 µl (10 – 20 fg/per column), PBDE – 2 µl (15 – 350 fg/per column), DL-PCB – 1 µl (5 – 30 fg/per column) and non DL-PCB – 1 µl (5 – 20 fg/per column). The response of relative standard deviation of the calibration curve of each individual compound is <20%.

4. The analytic characteristics of the method are evaluated on the basis of European Comission requirements: the recoveries of $^{13}C_{12}$ internal standards 60-120%, the

quantification limits - <1/5 DLK (PCDD/PCDF, dioxin like and non dioxin like PCB), ≤0,01 ng g^{-1} individual PBDE, trueness +/- 15% (TEQ PCDD/PCDF and TEQ PCDD/PCDF, DL-PCB), +/- 20% (sum of non DL-PCB), the relative standard deviation <20% (TEQ PCDD/PCDF and TEQ PCDD/PCDF, DL-PCB) and <30% (sum of non DL-PCB).

5. The installed method of the analysis of persistent organic pollutants using the method of gas chromatography mass spectrometry is sufficiently sensitive and able to identify 10^{-12}g/g or even 10^{-15}g/g.

6. The effect of pollutants on human health is assessed. The tolerated daily intake of meat (pork, beef, and poultry), eggs and milk products are within the range of European Commission regulated limits 0 – 1 pg TE kg body weight-1, while Baltic fish (herring 4.2 +/- 2.1 kg body $weight^{-1}$, salmon 3.8 +/- 2.9 kg body $weight^{-1}$, sprat 3.5 +/- 0.6 kg body $weight^{-1}$) and Baltic cod liver (5,0 +/- 1,4 kg body $weight^{-1}$) are in the risk zone.

THE LIST OF SCIENTIFIC PUBLICATIONS SUMMERIZED IN DOCTORAL DISSERTATION

RESEARCH ARTICLES

1. Mašaraitė R., Petraitis J., Jarmalaitė I., Naujalis E. "Determination of dibenzo-*p*-dioxins, dibenzofurans and dioxin like PCBs in fish and meat in Lithuania." Biology, 2011: 55-62.
2. Godliauskienė R., Petraitis J., Jarmalaitė I., Naujalis E. "Analysis of dioxins, furans and DL-PCB in food and feed samples from Lithuania and estimation of human intake." Food and Chemical Toxicology, 2012: 4169-4174.
3. Godliauskienė R., Tamošiūnas V., Naujalis E. "Polychlorinated dibenzo-p-dioxins/furnas and dioxin-like polychlorinated biphenyls in food and feed in the Lithuanian market." Toxicological and Environmental Chemistry, http://dx.doi.org/10.1080/02772248.2016.1174704, 2016.

CONFERENCE MATERIALS AND ABSTRACTS

1. R. Mašaraitė, J. Petraitis, I. Jarmalaitė, E. Naujalis. Determination of dibenzo-p-dioxins, dibenzofurans and dioxin like PCBs in fish and meat in Lithuania. 5th international conference „The Vital Nature Sign 2011“, Birštonas, Kaunas.
2. R. Mašaraitė, J. Petraitis, I. Jarmalaitė, E. Naujalis. Determination of dibenzo-p-dioxins, dibenzofurans and dioxin-like PCBs in fish and meat in Lithuania. Conference „Chemija 2011“, 2011 October 4, Vilnius.
3. R. Godliauskienė, J. Petraitis, I. Jarmalaitė, E. Naujalis. The determination of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzo furans and dioxin-like polychlorinated biphenyls in Lithuania. Conference “FizTeCh 2011”, November 24-25, Vilnius.
4. R. Godliauskienė, J. Petraitis, I. Jarmalaitė, E. Naujalis. Features of analysis of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzo furans and dioxin-like polychlorinated biphenyls. Comparison of contamination of food products. Conference “FizTeCh 2012”, September 25-26 d, Vilnius.

5. R. Godliauskienė, E. Naujalis. The research of persistent halogenated organic pollutants by mass spectrometry method. Conference “FizTeCh 2014”, October 28-29 d, Vilnius.

6. R. Godliauskienė. Conference “FTMC – veikla ir ateitis”. 2012 March 8, Vilnius.

7. The participation in congresses of Reference laboratories with the topic of analysis of dibenzo-p-dioxins, dibenzo furans and dioxin-like polychlorinated biphenyls in food and feed:

- 2011 May 4-5d, York, Great Britain;
- 2011 October 25–26d, Freiburg, Germany;
- 2012 May 15–16d, Vienna, Austria;
- 2014 May 22–23d, Nantes, France;
- 2015 May 20–21d, Lisbon, Portugal;
- 2015 May 27d, CVUA-MEL, Münster, Germany.

The Curriculum Vitae

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Education

2010-2016 PhD studies at the Centre of Physical Sciences and Technology..

2004-2006 MA studies at Vytautas Magnus University, Faculty of Natural Sciences. The master of chemical analysis program.

2000-2004 BA studies at Vilnius Pedagogical University, Faculty of Natural Sciences.

Work experience

2006 – 2010 National food and veterinary risk assessment institute, chief engineer. The chemical analysis of mycotoxins, polyaromatic hydrocarbons and sudan dyes in food and feed products using high performance liquid chromatography method. The experience analyzing pesticide residues using LC-MS/MS method.

2008 – now National food and veterinary risk assessment institute, chief engineer. The chemical analysis of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzo furans, and dioxin like polychlorinated biphenyls in food and feed products using high-resolution gas chromatography mass spectrometry method.

2010 – now National food and veterinary risk assessment institute, deputy head of chemical research department. The chemical analysis of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzo furans, and dioxin like polychlorinated biphenyls in food and feed products using high-resolution gas chromatography mass spectrometry method. The organization of the work of chemical research department, monitoring, control. The installation of new analytical methods in accordance with ISO/IEC 17025 and other European Union regulations.

Internships, courses

2008-01 Further training on persistent organic pollutants chemistry issues. Laboratory staff training of polychlorinated dibenzo-p-dioxins, dibenzo furans and dioxin-like polychlorinated biphenyls determination using high-resolution gas chromatography mass spectrometry method. R. Palavinskas, NMVRVI, Vilnius, Lithuania.

2008-04 Further training on the chemical analysis of persistent organic pollutants issues. O. Paepke, NMVRVI, Vilnius, Lithuania.

2008-05 Further training on the chemical analysis of persistent organic pollutants issues. Palavinskas, NMVRVI, Vilnius, Lithuania.

2008-11 Further training on the chemical analysis of persistent organic pollutants issues. R. Malisch, Freiburg, Germany.