

JOANNA KUC\*, ADAM GROCHOWALSKI\*

## METHODS FOR THE DETERMINATION OF HEXABROMOCYCLODODECANE IN FOOD

### METODY OZNACZANIA HEKSABROMOCYKLODODEKANU W ŻYWNOSCI

#### Abstract

Methods of sample preparation with a particular emphasis on extraction and purification techniques are described. Gas and liquid chromatography techniques applied for the determination of hexabromocyclododecane are presented. Issues relating to the determination of this compound in food samples are discussed.

*Keywords: BFRs, HBCD, food analysis, GC-MS, LC-MS*

#### Streszczenie

Omówiono metody przygotowania próbek do analizy ze szczególnym uwzględnieniem technik ekstrakcji i oczyszczania próbek. Zaprezentowano techniki chromatografii gazowej i ciekłej stosowane do oznaczania heksabromocykłododekanu. Przedyskutowano problemy oznaczania tego związku w próbkach żywności.

*Słowa kluczowe: BFRs, HBCD, analiza żywności, GC-MS, LC-MS*

\* M.Sc. Eng. Joanna Kuc, Ph.D. D.Sc. Eng. Ass. Prof. Adam Grochowalski, Department of Analytical Chemistry, Department of Chemical Engineering and Technology, Cracow University of Technology.

## Abbreviations and symbols

CAS	–	Chemical Abstract Service
EINECS	–	European Inventory of Existing Commercial Chemical Substances
ESI	–	electrospray ionization
GC-MS	–	gas chromatography coupled to mass spectrometry
LC-MS	–	liquid chromatography coupled to mass spectrometry
LOD	–	limit of detection
<i>m/z</i>	–	mass-to-charge
MS	–	mass spectrometry
PBT	–	persistent, bioaccumulative and toxic
POPs	–	persistent organic pollutants
PS	–	polystyrene
REACH	–	Registration, Evaluation, Authorisation and Restriction of Chemicals
S/N	–	signal-to-noise
SRM	–	single reaction monitoring
QA/QC	–	Quality Assurance and Quality Control

## 1. Introduction

Hexabromocyclododecane (HBCD) is a cycloaliphatic bromide which has been produced through the bromination of *cis,trans,trans*-1,5,9-cyclododecatriene since the 1960s and it's the most commonly used brominated flame retardant (BFR) [1, 2]. The technical product of HBCD consists of three predominant isomers:  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD among 16 possible stereoisomers: 6 diastereomeric pairs of enantiomers and 4 meso forms [3, 4]. The structures of the  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD isomers are shown in Fig. 1. The presence of traces of two minor diastereomers,  $\delta$ - and  $\epsilon$ -HBCD in the technical HBCD product, has also been reported [5].

HBCD inhibits ignition and combustion processes by interfering with the free radical mechanism in the gas phase during the combustion process [6, 7].

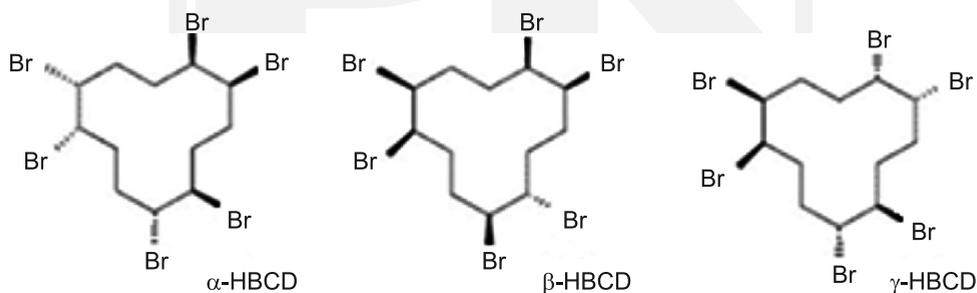


Fig. 1. Structures of  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD isomers

HBCD is a white solid substance produced in high quantities and it is commercially available under two names, corresponding to CAS and EINECS numbers [8–10].

Table 1

**Identification of a commercial product of HBCD**

<b>1,2,5,6,9,10-Hexabromocyclododecane</b>	
CAS No	3194-55-6
EINECS No	221-695-9
<b>Hexabromocyclododecane</b>	
CAS No	25637-99-4
EINECS No	247-148-4
<b>Major diastereoisomers CAS No</b>	
$\alpha$ -HBCD	134237-50-6
$\beta$ -HBCD	134237-51-7
$\gamma$ -HBCD	134237-52-8
<b>Trade names</b>	
Cyclododecane, hexabromo; HBCD; Bromkal 73-6CD; Nikkafainon CG 1; Pyroguard F 800; Pyroguard SR 103; Pyrovatex 3887; Great Lakes CD-75; Dead Sea Bromine Group Ground FR 1206 I-LM;	

HBCD is used mainly in expanded (EPS) and extruded (XPS) polystyrene foams which are applied as thermal insulation panels in the building industry [11–13]. The presence of HBCD in PS foams leads to significant improvements in the fire behavior of these materials. In case of exposure to a fire source, the foam shrinks rapidly, what results in a reduced possibility of ignition [14]. HBCD is also added to polymer matrices for cotton containing textile mixtures – this is used in the production of upholstery textiles such as furniture, wall coverings and draperies [15–17]. Some researches indicate the use of HBCD in high impact polystyrene (HIPS) applied in electrical and electronic equipment [7, 16, 18]. HBCD is not covalently bound to materials, and it can therefore be released from the product into the environment during the production, processing, and storage of waste containing this compound [15]. HBCD is considered to be persistent, bioaccumulative and toxic (PBT) and has been recently proposed for inclusion in the Protocol on POPs of the Stockholm Convention [7–9, 26, 27]. In the European Union, HBCD is identified as a Substance of Very High Concern (SVHC) under REACH [10] according to which, after 21 August 2015 only authorized applications of HBCD will continue to be allowed. Directive 67/548/EEC classifies HBCD as very toxic to aquatic organisms, causing long-term adverse effects in the aquatic environment. HBCD is also hazardous to unborn children and breastfed babies [7–9].

The wide use of HBCD has led to widespread contamination of this compound in different biotic and abiotic environmental compartments [16, 28–31] and in humans [32, 33]. The level of HBCD is studied in fish most frequently, due to their high position in the food chain and their ability to absorb high concentrations of contaminants. A relatively high

concentration of HBCD in fish is observed [24, 34-39]. At present, none of the alternative flame retardants are considered to be a suitable replacement for HBCD in PS foams [7], therefore, the monitoring of this contaminant and its determination in food is highly advisable.

Table 2

**Physicochemical properties of HBCD**

Property	Value	References
Chemical formula	$C_{12}H_{18}Br_6$	–
Molecular mass (g/mol)	641.7	–
Boiling point (°C)	Decomposes at >190°C	[20]
Melting point (°C)	175–195	[11]
	179–181°C ( $\alpha$ -HBCD)	[2]
	170–172°C ( $\beta$ -HBCD)	
	207–209°C ( $\gamma$ -HBCD)	
Density (kg/m <sup>3</sup> ) (25°C)	2403	[21]
Vapour pressure (Pa) (21°C)	$6.3 \cdot 10^{-5}$	[22]
Water solubility (mg/L) (20°C)	$4.88 \times 10^{-2}$ ( $\alpha$ -HBCD)	[23]
	$1.47 \times 10^{-2}$ ( $\beta$ -HBCD)	
	$2.08 \times 10^{-3}$ ( $\gamma$ -HBCD)	
Log Kow (octanol-water partition coefficient) (25°C)	5.81	[24]
	$5.07 \pm 0.09$ ( $\alpha$ -HBCD)	[25]
	$5.12 \pm 0.09$ ( $\beta$ -HBCD)	
	$5.47 \pm 0.10$ ( $\gamma$ -HBCD)	

## 2. Methods for the determination of HBCD in food

Methods of HBCD analysis are similar to those of POPs. These methods have been well developed over the past several years and are commonly used as a reference in research [40]. The selection of suitable sample preparation methods is an important element in HBCD determination in food samples. Whilst sample preparation is a crucial element of every analytical methodology, it is also the principal source of errors. Food sample preparation is most commonly a multi-step and time-consuming process. Particular care must be taken to avoid sample contamination. The amount of sample used for analysis depends primarily on the expected level of contamination and the sensitivity of the available detection

techniques. Every step of the analytical procedure requires attention and monitoring to ensure high reliability of results (QA/QC) [41]. The typical procedure for the determination of HBCD in food samples is shown in Fig. 2.

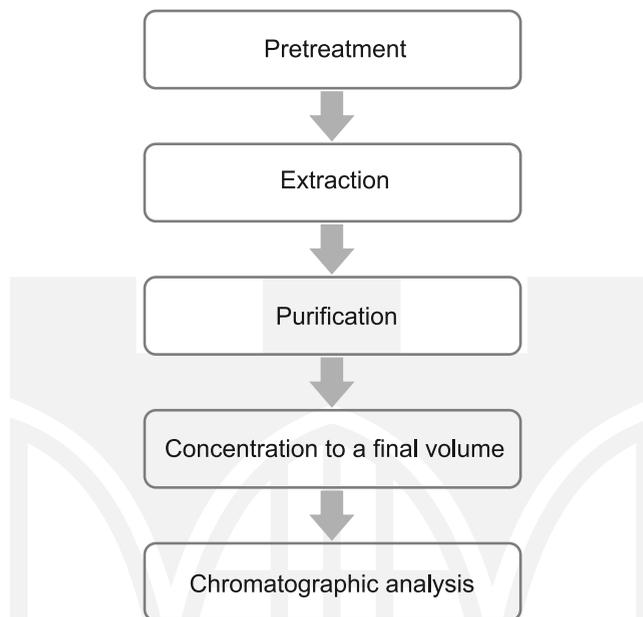


Fig. 2. Scheme of typical procedure for the determination of HBCD in food samples

Pretreatment of solid or semi-solid food samples includes washing, removal of irrelevant matter, mass reduction and drying. Mass reduction is carried out by using a mortar, homogenizer, automatic grinder or high-speed blender. Drying of the sample is most frequently carried out by vacuum methods in a freeze-dryer (lyophilizer) [42].

### 2.1. Extraction methods

For the determination of organic compounds, the essential step of sample treatment is extraction with an appropriate organic solvent or a mixture of solvents. Extraction techniques are most often used for the isolation and enrichment of analytes in food and environmental samples. Conventional Soxhlet extraction is a primary option for HBCD determination, as well as for other organic food contaminants – this has been well summarized [43–46]. The need to decrease the time of extraction and solvent consumption has resulted in the introduction of other extraction methods, e.g. accelerated solvent extraction (ASE) [47], supercritical fluid extraction (SFE) [48], microwave-assisted extraction (MAE) [49], and ultrasound-assisted extraction (UAE) [50]. The major advantage of these techniques is the possibility of extracting multiple samples simultaneously. ASE (DIONEX, Thermo Scientific) [51] is a pressurized fluid extraction (PFE) technique carried out under increased pressure and at higher temperatures, this results in a short extraction time and more favorable

kinetics of extraction [52]. In SFE, the fluid introduced into the sample exhibit solubilities similar to organic solvents [53]. MAE is typically carried out in a sealed Teflon vessel (a bomb) at high temperatures. A variety of solvents may be used [54–55]. To perform UAE, the sample is placed in a glass or metal vessel and inserted into an ultrasonic bath [56–57].

## 2.2. Clean-up methods

The co-extraction of interfering matrix components is an inherent difficulty in the extraction of food samples. It is therefore necessary to apply additional purification methods. The most commonly used clean-up methods are: gel permeation chromatography (GPC); dialysis with semipermeable membranes (SPM); multi-layer silica gel column [42]. GPC, also known as size exclusion chromatography (SEC), is an effective technique for the separation of components in a solution, based on their molecular size (hydrodynamic volume) [58]. GPC is often used for polymer analysis, however, this technique is also useful in analytical chemistry for separation of analyte from lipids. The authors [37, 45, 59] indicated that GPC does not sufficiently remove the lipids from food samples, therefore, other sample purification steps are required. For this purpose, treatment with modified silica gel [59, 60] or florisil [61] has been applied after GPC clean-up. The application of SPM as a clean-up technique for high fat food samples is based on dialysis [62, 63] and is a simple approach for analyte isolation. The future purification with silica gel is required [63].

## 2.3. Detection methods

Both gas chromatography (GC) and liquid chromatography (LC) methods were used for the determination of HBCD. However, the separation of isomers is possible only by using LC [64–66]. Determination of individual HBCD isomers by GC is not feasible due

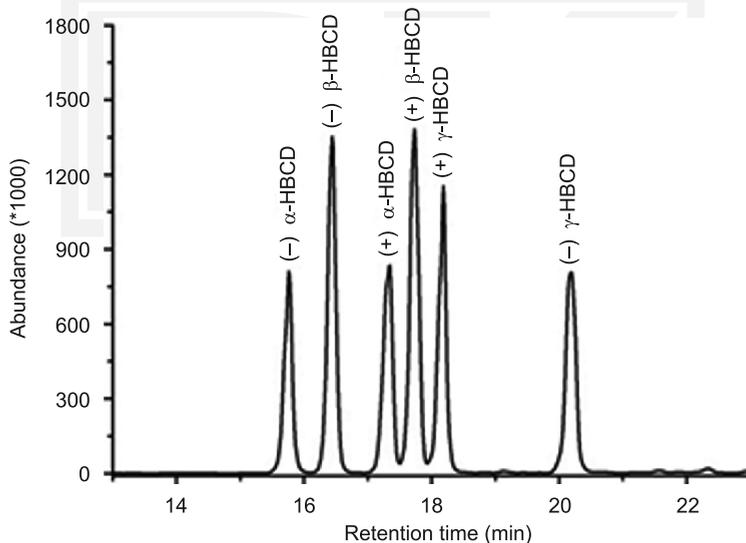


Fig. 3. LC-MS chromatogram of the (±)α-HBCD, (±)β-HBCD and (±)γ-HBCD enantiomers [60]

to the thermal inter-conversion of HBCD isomers at 160°C [20], decomposition of this compound at 240°C [67] and partial breakdown in dirty GC systems [68].

The research of two inter-laboratory studies for comparison of GC-MS and LC-MS application for HBCD determination shown no statistically significant differences between results obtained with both methods [66]. Exemplary chromatogram of the HBCD enantiomers is shown in Fig. 3.

A sensitive LC-MS method coupled with ESI ionization source with LOD of 4–6 pg for  $\gamma$ -HBCD standard solution had been developed [70] and subsequently optimized [34] resulting in LOD of 0.5 pg for  $\gamma$ -HBCD standard solution and 5 pg for fish extracts. With this method, a SRM transition of deprotonated molecular ion  $[M-H]^-$  ( $m/z$  640.6) to bromine ion  $[Br]^-$  ( $m/z$  79 and 81) was observed. However, inability of monitoring of this transition using a single quadrupole MS was noticed [71], which is due to the cut-off value of the instrument is higher than daughter ion  $m/z$  ratio. Although the decreased response at  $m/z$  676.7  $[M + Cl H]^-$  originating from a chlorine adduct was reported [71], the authors [63] applied ion monitoring based on two SRM transitions simultaneously – the quantitative  $[M + Cl-H]^- \rightarrow [M-H]^-$  and the confirmative  $[M-H]^- \rightarrow [Br]^-$ , resulting with S/N ratio  $\geq 3$  and 1 pg/g fresh weight of fish tissue for individual HBCD isomers and more than 0.997 for the correlation factors of the linear regression line for both the quantitative and confirmative SRM transitions.

### 3. Conclusions

A dramatic growth in HBCD production and usage is observed. Therefore, the levels of this compound in the environment have increased. Due to the fact that HBCD is a potential hazard to aquatic environments and humans, the monitoring of this contaminant in the environment and food chain is highly advisable.

Most of the analytical methods for food sample preparation and the determination of HBCD are similar to those of POPs. Typically, HBCD is extracted from the sample, the extract is then purified and concentrated. The final analysis is most frequently done by LC-MS techniques coupled with an ESI ion source. The GC-MS technique may also be applied, however, separation of HBCD isomers is only possible by using LC-MS.

Currently, the challenge for researchers is to reduce the time-consuming procedures and solvent consumption. This is achieved by developing new or modifying existing analytical methods.

### References

- [1] Marvin C.H., Tomy G.T., Armitage J.M., Arnot J.A., McCarty L., Covaci A., Palace V., *Hexabromocyclododecane: Current understanding of chemistry, environmental fate and toxicology and implications for global management*, Environmental Science & Technology, vol. 45, 2011, 8613-8623.
- [2] Smith K., Liu C-H., El-Hiti G.A., Kang G.S., Jonas E., Clement S.G., Checquer A.D., Howarth O.W., Hursthouse M.B., Coles S.J., *An extensive study of bromination of cis, trans-1,5,9-cyclododecatriene: product structures and conformations*, Organic & Biomolecular Chemistry, vol. 3, 2005, 1880-1892.

- [3] Heeb N.V., Schweizer W.B., Kohler M., Gerecke A.C., *Structure elucidation of hexabromocyclododecanes – a class of compounds with a complex stereochemistry*, Chemosphere, vol. 61, 2005, 65-73.
- [4] Heeb N.V., Schweizer W.B., Mattrel P., Haag R., Gerecke A.C., Kohler M., Schmid P., Zennegg M., Wolfensberger M., *Solid-state conformations and absolute configurations of (+) and (-)  $\alpha$ -,  $\beta$ -, and  $\gamma$ -hexabromocyclododecanes (HBCDs)*, Chemosphere, vol. 68, 2007, 940-950.
- [5] Arsenaault G., Konstantinov A., Marvin C.H., MacInnis G., McAlees A., McCrindle R., Riddell N., Tomy G.T., Yeo B., *Synthesis of the two minor isomers, delta- and epsilon-1,2,5,6,9,10-hexabromocyclododecane, present in commercial hexabromocyclododecane*, Chemosphere, vol. 68, 2007, 887-92.
- [6] Troitzsch J., *Overview of flame retardants*, Chimica Oggi/Chemistry Today, vol. 16, 1998, 1-19.
- [7] Priority Existing Chemical Assessment Report No. 34 Hexabromocyclododecane, Sydney 2012.
- [8] TemaNord 2008:520. Hexabromocyclododecane as a possible global POP, Copenhagen 2007.
- [9] Proposal for Harmonised Classification and Labelling Based on the CLP Regulation (EC) No 1272/2008, Annex VI, Part 2. Hexabromocyclododecane, Swedish Chemicals Agency 2009.
- [10] ECHA European Chemical Agency. Doc:ED/67/2008, *Inclusion of substances of very high concern in candidate list*, Helsinki, 28 October 2008.
- [11] ACCBFRIP American Chemistry Council Brominated Flame Retardant Industry Panel, 2005, *HPV data summary and test plan for hexabromocyclododecane (HBCD)*, CAS No. 3194556, 20 December 2001, Updated March 2005, Arlington (VA).
- [12] BSEF Bromine Science and Environmental Forum. Factsheet, Brominated Flame Retardant, Hexabromocyclododecane, October 2012.
- [13] EUMEPS Healthy building with EPS, Belgium, August 2002.
- [14] EUMEPS Behavior of EPS in case of fire, Belgium, August 2002.
- [15] de Wit C.A., *An overview of brominated flame retardants in the environment*, Chemosphere, vol. 46, 2002, 583-624.
- [16] Remberger M., Sternbeck J., Palm A., Kaj L., Strömberg K., Brorström-Lundén E., *The environmental occurrence of hexabromocyclododecane in Sweden*, Chemosphere, vol. 54, 2004, 9-21.
- [17] Kajiwara N., Sueoka M., Ohiwa T., Takigami H., *Determination of flame-retardant hexabromocyclododecane diastereomers in textiles*, Chemosphere, vol. 74, 2009, 1485-1489.
- [18] Vilaplana F., Karlsson P., Ribes-Greus A., Ivarsson P., Karlsson S., *Analysis of brominated flame retardants in styrenic polymers: Comparison of the extraction efficiency of ultrasonication, microwave-assisted extraction and pressurised liquid extraction*, Journal of Chromatography A, vol. 1196–1197, 2008, 139-146.
- [19] de Wit C.A., Herzke D., Vorkamp K., *Brominated flame retardants in the Arctic environment – trends and new candidates*, Science of the Total Environment, vol. 408, 2010, 2885-2918.
- [20] Peled M., Scharia R., Sondack D., *Thermal rearrangement of hexabromocyclododecane (HBCD)*, [in] *Advances in Organobromine Chemistry II*. Ed., Desmurs J-R., Gérard B. and Goldstein MJ. Elsevier, Amsterdam 1995, 92-99.
- [21] Great Lakes Chemical Corporation, *Material Safety Data Sheet. Great Lakes CD-75-P, CD-75PM and CD-75PC*, West Lafayette (IN): Great Lakes Chemical Corporation. MSDS Number: 00177. Effective Date: 10/14/2005.
- [22] Stenzel J.I., Nixon W.B., *Hexabromocyclododecane (HBCD): Determination of the vapor pressure using a spinning rotor gauge*, Wildlife international Ltd. 1997, Easton, Maryland. 439C-117, pp. 44.

- [23] EBFRIIP European Brominated Flame Retardant Industry Panel. 2004a, *Determination of water solubility of hexabromocyclododecane (HBCD) using a generator column method*, Easton (MD): Wildlife International, Ltd. Project No. 439C-138.
- [24] Veith G.D., DeFoe D.L., Bergstedt B.V., *Measuring and estimating the bioconcentration factor of chemicals in fish*, Journal of the Fisheries Research Board of Canada, vol. 36, 1979, 1040-1048.
- [25] Hayward S.J., Lei Y.D., Wania F., *Comparative evaluation of three HPLC-based KOW estimation methods for highly hydrophobic organic compounds: polybrominated diphenyl ethers and hexabromocyclododecane*, Environmental Toxicology and Chemistry, vol. 25, 2006, 2018-2027.
- [26] EUROPEAN CENTRE FOR ENVIRONMENT AND HEALTH BONN OFFICE *Hazard assessment: five substances proposed for POPs protocol of CLRTAP*, 13th Meeting of the Task Force on Health in Bonn, April 2010.
- [27] ECB – SUMMARY FACT SHEET PBT WORKING GROUP – PBT LIST NO. 58, *Results of the evaluation of the PBT/VPVB properties of hexabromocyclododecane*, 1st Draft, March, 2008.
- [28] Alae M., Arias P., Sjödin A., and Bergman A., *An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release*, Environment International, vol. 29, 2003, 683-689.
- [29] Covaci A., Gerecke A.C., Law R.J., Voorspoels S., Kohler M., Heeb N.V., Leslie H., Allchin C.R., and De Boer J., *Hexabromocyclododecanes (HBCDs) in the environment and humans: a review*, Environmental Science & Technology, vol. 40, 2006, 3679-3688.
- [30] de Wit C.A., Alae M., Muir D.C.C., *Levels and trends of brominated flame retardants in the Arctic*, Chemosphere, vol. 64, 2006, 209.
- [31] Kohler M., Zennegg M., Bogdal C., Gerecke A.C., Schmid P., Heeb N.V., Sturm M., Vonmont H., Kohler H.P., Giger W., *Temporal trends, congener patterns, and sources of octa-, nona-, and decabromodiphenyl ethers (PBDE) and hexabromocyclododecanes (HBCD) in Swiss lake sediments*, Environmental Science & Technology, vol. 42, 2008, 6378- 6384.
- [32] Thomsen C., Froshaug M., Broadwell S.L., Becher G., *Brominated flame retardants in breast milk from Norway*, Organohalogen Compound, vol. 67, 2005, 509-512.
- [33] Thomsen C., Stigum H., Froshaug M., Broadwell S.L., Becher G., Eggesbo M., *Determinants of brominated flame retardants in breast milk from a large scale Norwegian study*, Environment International, vol. 36, 2010, 68-74.
- [34] Janak K., Covaci A., Voorspoels S., Becher G., *Hexabromocyclododecane in marine species from the Western Scheldt Estuary: diastereoisomer- and enantiomer-specific accumulation*, Environment Science & Technology, vol. 39, 2005, 1987-1994.
- [35] Law R.J., Herzke D., Harrad S., Morris S., Bersuder P., Allchin C.R., *Levels and trends of HBCD and BDEs in the European and Asian environments with some information for other BFRs*, Chemosphere, vol. 73, 2008, 223-241.
- [36] Shaw S.D., Berger M.L., Brenner D., Kannan K., Lohmann N., Papke O., *Bioaccumulation of polybrominated diphenyl ethers and hexabromocyclododecane in the northwest Atlantic marine food web*, Science of the Total Environment, vol. 407, 2009, 3323-3329.
- [37] Nakagawa R., Murata S., Ashizuka Y., Shintani Y., Hori T., Tsutsumi T., *Hexabromocyclododecane determination in seafood samples collected from Japanese coastal areas*, Chemosphere, vol. 81, 2010, 445-452.
- [38] Rawn D.F.K., Sadler A., Quade S.C., Sun W.F., Lau B.P., Kosarac I., Hayward S., Ryan J.J., *Brominated flame retardants in Canadian chicken egg yolks*, Food Additive & Contaminants: part A, vol. 28, 2011, 807-815.

- [39] Hu D., Hu X., Li J., Wang P., Guo S., *Determinations of hexabromocyclododecane (HBCD) isomers in plastic products from china by LC-MS/MS*, Journal of Liquid Chromatography & Related Technologies, vol. 35, 2012, 558-572.
- [40] Xu W., Wang X., Cai Z., *Analytical chemistry of the persistent organic pollutants identified in the Stockholm Convention: A review*, Analytica Chimica Acta, vol. 790, 2013, 1-13.
- [41] Namieśnik J., *Jakość wyników analitycznych*, [in] *Ocena i kontrola jakości wyników pomiarów analitycznych*, Wydawnictwo Naukowo-Techniczne, Warszawa 2007.
- [42] Curren M.S.S., King J.W., *Chapter 25: Sampling and sample preparation for food analysis*, [in] *Comprehensive Analytical Chemistry XXVIZ*, J. Pawliszyn (Ed.) 2002 Elsevier Science B.V.
- [43] Luque de Castro M.D., Priego-Capote F., *Soxhlet extraction: Past and present panacea*, Journal of Chromatography A, vol. 1217, 2010, 2383-2389.
- [44] US EPA (1996), Method 3540C: soxhlet extraction.
- [45] Frederiksen M., Vorkamp K., Svensmark B., *Method development for simultaneous analysis of HBCD, TBBPA, and dimethyl-TBBPA in marine biota from Greenland and the Faroe Islands*, International Journal of Environmental Analytical Chemistry, vol. 87, 2007, 1095-1109.
- [46] Hu X., Hu D., Song Q., Li J., Wang P., *Determinations of hexabromocyclododecane (HBCD) isomers in channel catfish, crayfish, hen eggs and fish feeds from China by isotopic dilution LC-MS/MS*, Chemosphere, vol. 82, 2011, 698-707.
- [47] US EPA (2007). Method 3545A: pressurized fluid extraction (PFE).
- [48] US EPA (2007). Method 3562: supercritical fluid extraction of polychlorinated biphenyls (PCBs) and organochlorine pesticides.
- [49] US EPA (2007). Method 3546: microwave extraction.
- [50] US EPA (2007). Method 3550C: ultrasonic extraction.
- [51] Accelerated Solvent Extraction (ASE®) Sample Preparation Techniques for Food and Animal Feed Samples, Technical Note 209, DIONEX, ThermoSc.
- [52] Haukås M., E.Mariussena, Ruus A., Tollefsen K.E., *Accumulation and disposition of hexabromocyclododecane (HBCD) in juvenile rainbow trout (Oncorhynchus mykiss)*, Aquatic Toxicology, vol. 95, 2009, 144-151.
- [53] Altwaiq A., Wolf M., van Eldik R., *Extraction of brominated flame retardants from polymeric waste material using different solvents and supercritical carbon dioxide*, Analytica Chimica Acta, vol. 491, 2003, 111-123.
- [54] Eskilsson C.S., Björklund E., *Analytical-scale microwave-assisted extraction*, Journal of Chromatography A, vol. 902, 2000, 227-250.
- [55] Ganzler K., Salgo A., Valko K., *Microwave Extraction: A Novel Sample Preparation Method for Chromatography*, Journal of Chromatography, vol. 371, 1986, 299-306.
- [56] Tadeo J.L., Sánchez-Brunete C., Albero B., García-Valcárcel A.I., *Application of ultrasound-assisted extraction to the determination of contaminants in food and soil samples*, Journal of Chromatography A, vol. 1217, 2010, 2415-2440.
- [57] Luque-Garcia J.L., Luque de Castro M.D., *Ultrasound: a powerful tool for leaching*, Trends in Analytical Chemistry, vol. 22, 2003, 41-47.
- [58] Moore J.C., *Gel permeation chromatography. I. A new method for molecular weight distribution of high polymers*, Journal of Polymer Science, vol. 2, 1964, 835-843.
- [59] Kuc J., Grochowalski A., *Isolation of hexabromocyclododecane from fat matrix using size exclusion chromatography*, Czasopismo Techniczne, 2-Ch/2012.
- [60] Gómara B., Lebrón-Aguilar R., Quintanilla-López J.E., González M.J., *Development of a new method for the enantiomer specific determination of HBCD using an ion trap mass spectrometer*, Analytica Chimica Acta, vol. 605, 2007, 53-60.
- [61] Esslinger S., Becker R., Jung C., Schröter-Kermani C., Bremser W., Nehls I., Chemosphere, vol. 83, 2011, 161-167.

- [62] Bergqvist P.-A., Strandberg B., Bergek S., Rappe C., *Lipid reduction during the analyses of PCDDs, PCDFs and PCBs in environmental samples using semipermeable membrane technique*, *Organohalogen Compounds*, 11, 1993, 41-44.
- [63] Kuc J., Grochowalski A., Mach S., Placha D., *Level of hexabromocyclododecane isomers in the tissue of selected commonly consumed fish in Central European countries*, *Acta Chromatografica* DOI:10.1556/ACHrom.26.2014.4.1 online preview.
- [64] Schecter A., Szabo D.T., Miller J., Gent T.L., Malik-Bass N., Petersen M., Paepke O., Colacino J.A., Hynan L.S., Harris T.R., Malla S., Birnbaum L.S., *Hexabromocyclododecane (HBCD) Stereoisomers in U.S. Food from Dallas, Texas*, *Environ Health Perspective*, vol. 120, 2012, 1260-1264.
- [65] Frederiksen M., Vorkamp K., Bossi R., Svensmark B., *Analysis of HBCD and TBBPA by GC-MS versus LC-MS-MS – indications of systematic differences in obtained results*, 4<sup>th</sup> International Symposium On Flame Retardants BRF2007, Amsterdam, Netherlands 2007.
- [66] Haug L.S., Thomsen C., Liane V.H., and Becher G., *Comparison of GC and LC determinations of hexabromocyclododecane in biological samples – results from two interlaboratory comparison studies*, *Chemosphere*, vol. 71, 2008, 1087-1092.
- [67] Barontini F., Cozzani V., Cuzzola A., Petarca L., *Investigation of hexabromocyclododecane thermal degradation pathways by gas chromatography/mass spectrometry*, *Rapid Communication of Mass Spectrometry*, vol. 15, 2001, 690-698.
- [68] Covaci A., Voorspoels S., de Boer J., *Determination of brominated flame retardants, with emphasis on polybrominated diphenyl ethers (PBDEs) in environmental and human samples – A review*, *Environment International*, vol. 29, 2003, 735-756.
- [69] Guerra P., Eljarrat E., Barceló D., *Determination of halogenated flame retardants by liquid chromatography coupled to mass spectrometry*, *Trends in Analytical Chemistry*, vol. 30, 2011, 842-855.
- [70] Budakowski W., Tomy G., *Congener-specific analysis of hexabromocyclododecane by high-performance liquid chromatography/electrospray tandem mass spectrometry*, *Rapid Communication of Mass Spectrometry*, vol. 17, 2003, 1399-1404.
- [71] Morris S., Bersuder P., Allchin C.R., Zegers B., Boon J.P., Leonards P.E.G., de Boer J., *Determination of the brominated flame retardant, hexabromocyclododecane in sediment and biota by liquid chromatography electrospray ionisation mass spectroscopy*, *Trends in Analytical Chemistry*, vol. 25, 2006, 343-349.

