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JOANNA KUC*, ADAM GROCHOWALSKI*

METHODS FOR THE DETERMINATION OF HEXABROMOCYCLODODECANE IN FOOD

METODY OZNACZANIA HEKSABROMOCYKLODODEKANU W ŻYWNOŚCI

A b s t r a c t

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*

S t r e s z c z e n i e

Omówiono metody przygotowania próbek do analizy ze szczególnym uwzględnieniem technik ekstrakcji i oczyszczania próbek. Zaprezentowano techniki chromatografii gazowej i cieczowej stosowane do oznaczania heksabromocyklododekanu. 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
m/*z*	–	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: α-, β- and γ-HBCD among 16 possible stereoisomers: 6 diastereomeric pairs of enantiomers and 4 meso forms [3, 4]. The structures of the α-, β- and γ-HBCD isomers are shown in Fig. 1. The presence of traces of two minor diastereomers, δ- and ε-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].

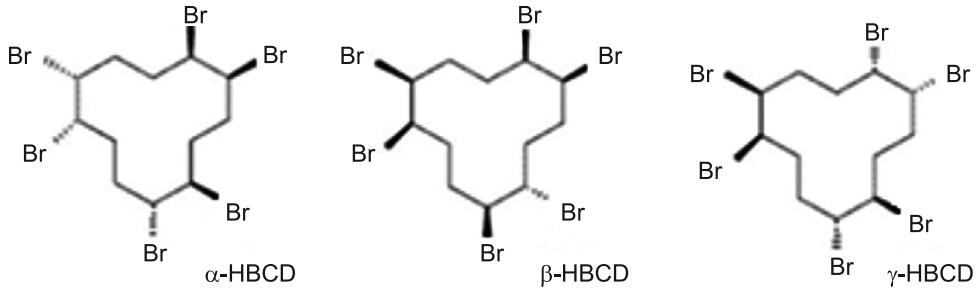

Fig. 1. Structures of α-, β- and γ-HBCD isomers

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

1,2,5,6,9,10-Hexabromocyclododecane	
CAS No	3194-55-6
EINECS No	221-695-9

Hexabromocyclododecane	
CAS No	25637-99-4
EINECS No	247-148-4

Major diastereoisomers CAS No	
α-HBCD	134237-50-6
β-HBCD	134237-51-7
γ-HBCD	134237-52-8

Trade names
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 (α-HBCD) 170–172°C (β-HBCD) 207–209°C (γ-HBCD)	[2]
Density (kg/m³) (25°C)	2403	[21]
Vapour pressure (Pa) (21°C)	$6.3 \cdot 10^{-5}$	[22]
Water solubility (mg/L) (20°C)	4.88×10^{-2} (α-HBCD) 1.47×10^{-2} (β-HBCD) 2.08×10^{-3} (γ-HBCD)	[23]
Log Kow (octanol-water partition coefficient) (25°C)	5.81	[24]
	5.07 ± 0.09 (α-HBCD) 5.12 ± 0.09 (β-HBCD) 5.47 ± 0.10 (γ-HBCD)	[25]

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.

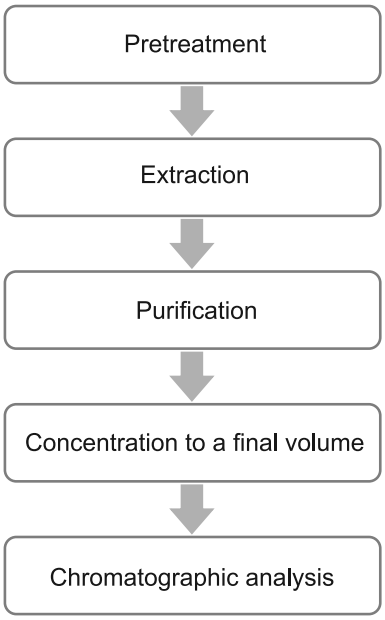

Fig. 2. Scheme of typical procedure for the determination of HBCD in food samples

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 witch 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

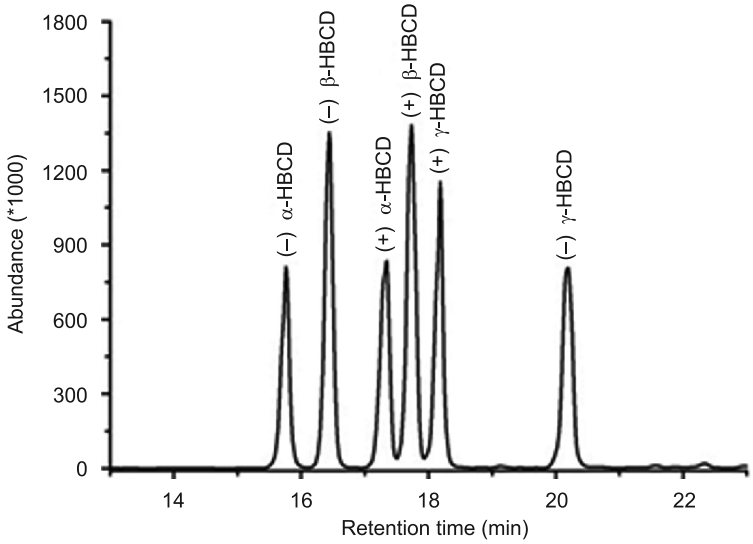

Fig. 3. LC-MS chromatogram of the (±)α-HBCD, (±)β-HBCD and (±)γ-HBCD enantiomers [60]

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 γ-HBCD standard solution had been developed [70] and subsequently optimized [34] resulting in LOD of 0.5 pg for γ-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]− → [M–H]− and the confirmative [M–H]− → [Br]−, resulting with S/N ratio ≥ 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.

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