A Review of Sample Preparation and Analysis Methods for the Hexabromocyclododecanes (HBCDs) in the Environment

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Abstract

Hexabromocyclododecane (HBCD) has gained comprehensive attention in the recent decades because of their persistent, bioaccumulative, and toxic characteristics. In order to identify existing problems in the fate and effects of HBCD on environment and human health, special attention is given to the numerous advances in the methods of analysis, toxicological effects, migration, transformation, and degradation of HBCD and its by-products, as well as distribution of HBCD in the environment and human bodies. Beside the studies of toxicity and distribution, the analysis method of HBCD did not get enough attention and the studies and reviews which related to the analysis method were very few. However, the analysis of HBCD is a continuing challenge mainly because of the limited concentrations in the environment and the large amounts of interfering substances during the measurement. The mistakes during the analysis procedure will seriously affect the study on HBCDs. According, this review paper mainly focuses on the studies of HBCD in recent years and summarizes the sample pretreatment, including sample collection, extraction, concentration and fractionation, and the instrumental analysis of HBCD.

Keywords: Hexabromocyclododecane; Sample pretreatment; Instrumental analysis; Different media

Introduction

Hexabromocyclododecane (HBCD) is a class of synthetic organobromines mainly used as a flame-retardant additive in extruded and expanded polystyrene foams. used as thermal insulations of buildings and other constructions, back coating for upholstery, wall coverings, car cushions and roller blinds, and plastic materials for electrical and electronic equipment and applicants such as wire and cable distribution boxes, audiovisual equipment cabinets, and refrigerator lining [1]. HBCD has been available on the world market since the 1960s and has been produced in notable quantities in China, Europe, Japan, and the USA. For example, an annual production of 23000 tons has been reported. In theory, HBCD possesses 16 possible stereoisomers, comprising six pairs of enantiomers and four mesoforms [2]. These isomers in technical grade HBCD mixtures were addition products of bromine to 1,5,9-cyclododecatrienes, but it is elucidated that technical HBCD mainly contains a mixture of three diastereomers: around 12% α-, 6% β-, and 82% γ-HBCD [3], while relative amounts of δ- and ε-HBCD, the minor components in the technical HBCD, are only 0.5% and 0.3%, respectively [4]. As a contaminant posing health and environmental concerns, HBCD has been studied extensively since its first identification in sediment and fish in Sweden [5]. Occurrences and levels of HBCD, especially the three major diastereomers, have been reported in various abiotic and biotic environmental media (air, soil, sediment, sewage sludge and terrestrial, freshwater and marine freshwater and marine organisms), food and human bodies in many countries [6], even in remote area of the arctic [7], which indicates the global distribution and potential for long-range environmental transport property of HBCD. As an addictive not covalently bind with the material, HBCD is likely to leach out of products in use or after disposal, as well as be released and discharged from manufacturing sites. Moreover, in vivo and in vitro animal models and human cell studies have confirmed the thyroid toxicity of HBCD, and have showed several chronic effects of HBCD at current environmental concentrations/exposures, such as the potential reproductive effects, altered thyroid and lipid metabolism, nervous system damage and oxidative stress [8]. Due to its persistent, bioaccumulative, and toxic properties, HBCD has already been on the European Chemicals Bureau PBT-list, identified as a substance of very high concern (SVHC) in EU, included in Annex XIV of European Union Chemicals Management Regulations (REACH) as a substance subject to authorisation, and has become one of the Stockholm Convention POPs since 2013. The production and use of HBCD-containing articles has been banned over the world universally. However, under specific circumstances, HBCD may still be used until 2024 because of authorisation for socio-economic benefits and enough time to switch to HBCD alternatives in EU [9]. In an environmental context, HBCD is highly resistant to degradation whether through chemical, biological, or photolytic processes. It is proposed that long-term monitoring programs of HBCD should be included in Swedish. The complex stereochemistry of HBCD, and the different toxicity characteristics, environmental behavior and fate, different degradation possibility of its isomers make chemical analysis, risk assessment for environment and human health and environmental remediation of HBCD still an unsolved problem and difficult.

In the past few years, most peer-reviewed publications about HBCDs were mostly focusing on reporting the fate, toxicological effects, and environmental concentrations of HBCD appeared. Environmental aspects of production and use of HBCD were retrieved in the review [10], in which particular concern is accumulation of HBCD in the food chain and limited toxicological information for
assessing its long-term implications for human health and the environment, such as the toxicological research of HBCD in endocrine, substance transformation, and metabolism disrupting effect, reproductive toxicity, neurotoxicity and immunotoxicity [11], the levels of HBCD in indoor dust around the world and discussed human exposure to it through dust [12].

Despite the numerous papers focusing on determination of the HBCD levels in various matrices, standard analytical procedures have not yet been devised. A variety of analytical approaches including both preconcentration and/or different instrumental analysis processes have been adopted in the previous and recently reviewed articles. Preextraction and preconcentration of HBCD from target samples, followed by purification and fractionation, if required, before final chromatographic separation and detection were routine procedures. The objects of the review articles were to summary the available data newly reported on selected analytical procedures applied to the determination of HBCD in various kinds of samples.

Sample Preparation

Natural samples preparation

**Air samples:** The whole sample preparation mainly includes sample collection, extraction, concentration and fractionation. For monitoring concentrations of HBCD in the air, indoor air, ventilation air and air samples from at the point sources were taken using a low volume air sampling configuration, while outdoor air were taken using a high volume air sampling configuration usually for monitoring concentrations of HBCD in the air. 13C-labeled HBCD was used for surrogate standard in general. It has been reported that air sampling using pre-cleaned polyurethane foam plugs (PUFs) collected HBCD in the gas phase and glass, or quartz fiber filters trapped HBCD in the particle phase respectively [13,14]. It is notable that concentrations were not corrected for losses of the sampling efficiency standard in some sampling cases and that PUF disk samplers may not be appropriate for using at low temperatures due to the possible majority reside of airborne HBCD in the particulate phase. The extraction method is mainly Soxhlet extraction with organic solution (toluene, CH2Cl2 or a mixture of n-hexane and acetone), pressurized fluid extraction with hexane/acetone (70:30, V/V) and ultrasonic assistant extraction using CH2Cl2 for 30 min [15-18]. The disadvantages of Soxhlet extraction are time (≥ 24 h) and toxic solvents consuming. During extraction, activated copper granules were added for removing elemental sulfur and further using acidic silica and elution with hexane:methylene chloride (1:1, V/V) in order to decrease matrix effects [19]. Concentration is mainly carried out using a rotary concentration method or nitrogen blowing method, using sulfuric acid and Florisil columns, gel-permeable chromatography, and then using an activated silica gel column or a deactivated silica gel column combined with an activated silica gel column [16,20]. Fractionation on a water-deactivated silica gel containing Na2SO4 or an activated carbon column was deployed to monitor α-, β-, and γ-HBCD in the air.

**Water samples:** Researchers have discovered many pretreatment methods for HBCD from ordinary water samples. As a preconcentration step, liquid-liquid extraction and Solid-phase extraction (SPE) using Oasis HLB cartridges bamboo charcoal SPE adsorbents have been successfully applied for the enrichment of HBCD in water samples [21-23]. It was reported that the losses in the SPE method were mostly due to losses in the cartridges, and that poor recovery efficiencies for HBCD were obtained for SPE compared with CH2Cl2 extraction procedure [24]. Polymer SPE columns and Bond Elut NEXUS cartridge columns with dichloromethane/methanol (1:1, V/V) were also confirmed an alternative for concentration of water samples contaminated by HBCD [24]. The recoveries obtained with spiked experiments in lake water ranged from 64.3% to 86.4%. For preconcentration, the rotary evaporation or a gentle nitrogen stream is always used to dry and concentrate the extract or eluate. Besides, some other pretreatment techniques were also applied. A temperature controlled ionic liquid dispersive liquid phase microextraction method has been developed for the enrichment of HBCD in water samples [22]. This method has been used to analyze real environmental water samples, and the recovery rate is between 77.2% and 99.3%. The main advantage of this method is that there is no toxic organic solvent. In addition, the ionic liquid/ionic liquid dispersion liquid–liquid microextraction procedure was also used to rapidly enrich HBCD in actual environmental water samples. The recovery of this method was 88.0% to 114% after some important parameters that could affect extraction efficiency were optimized [25].

More and more researchers have been working on the pretreatment of HBCD from special water samples. HBCD in river water, sewage influent and sewage effluent samples can be extracted using a solid extraction disk and a glass fiber filter, and the disk and filter can be extracted with n-hexane Soxhlet for 3 hours [26]. The recovery rates for spiked standards of α-, β-, γ-, δ-, and ε-HBCDs were 101%, 105%, 99.7%, 92.1%, and 105%, respectively. Conditioned Oasis HLB cartridges have also been developed for the determination of HBCD from 12 industrial pollutants in surface water and effluent wastewater. It showed that the single SPE procedure could extracted five phenolic compounds (nonylphenol (NP), bisphenol A (BPA) and methyl-, ethyl- and propylparabens), six perfluoroalkyl compounds (Perfluorooctanesulfonic acid (PFOS) and five perfluorooalkyl carboxylic acids), and the brominated flame retardant HBCD simultaneously and effectively [23]. A novel liquid-liquid microextraction technology based on ultrasound-assisted dispersion of floating organic solidification can simultaneously extract four hormones (17α-ethinylestradiol, 17β-estradiol, estriol and estrone), three preservatives (methylparaben, ethylparaben, and propylparaben), six perfluoralkyl compounds (PFOS and five perfluorinated Alkylcarboxylic acids) plasticizers (double Phenol A), and brominated flame retardants HBCD from surface and tap water [27]. This method is not only fast and simple, but also has less solvent consumption and high precision.

**Dust, sediment, soil samples:** Many different preparation methods for HBCD and isomers in dust, sediment and soil have been developed in recent years. Exclusion of UV-light has been designed for all of the analytical procedures, due to the potential UV-degradability of HBCD [28]. Here are some important preparation methods for HBCD and isomers from dust, sediment and soil.

A method by extraction with CH2Cl2 in an ultrasonic bath can measure isomer-specific HBCD, tri-decabrominated diphenyl ethers, decabromodiphenyl ethane (DDBPE), 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE), (2-ethylhexyl) tetrabromobenzoate (TBB), and bis (2-ethylhexyl) tetrabromophthalate (TBPB) in a same dust sample collected on surfaces 1 m above the floor [29]. This method has a high recovery rate (60-120%) and is accurate when compared with the certified values (PBDEs in NIST SRM 2585) using the 13C-labelled internal standard [30].
A novel analysis method used to the simultaneous determination of 27 brominated flame retardants (BFRs), namely PBDE, isomers of HBCD, TBBPA and several novel BFRs (NBFRs), together with 18 perfluoroalkyl substances (PFASs) in indoor dust was verified [31]. In order to achieve complete separation of analytes from the sample, a miniaturized method based on matrix solid-phase dispersion (MSPD) has been used and has been successfully applied to monitor organohalogen contaminants in 18 dust samples collected by households. The combination of ultrasonic-assisted extraction and SPE enables the separation of PBDE from HBCD or brominated FRs from organophosphate FRs, which was mainly used in household dusts. This method is easy to use and can use simpler scanning instruments.

Pressurized liquid extraction followed by cleaning the sample with a silica gel column is primarily used for the simultaneous determination of emerging flame retardants (including α-, β- and γ- HBCD, anti-and syn-isomers of dechlorane plus (DP), tetrabromobisphenol-A (TBBP-A)) and two novel flame retardants (decabromodiphenylethane (DBDPE) and 2-bis (2,4,6-tribromophenoxo) ethane (BTBPE)) in marine sediments [32].

The selective separation of HBCD, polychlorinated naphthalene (PCNs), and TBBPA in soil was also developed [33]. The recovery of PCNs, HBCDs and TBBPA in different components was almost 100% under the optimal conditions of the best sorbent type and elution solvent volume and composition. Soxhlet extraction with acetonen-hexane (1:1, V/V) for 48 h followed by a clean up using activated copper powder and multilayer silica/alumina columns was adopted for soil samples.

A selective pressurized liquid extraction (S-PLE) method which is used for rapid determination of 3 classes of halogenated organic contaminants polychlorinated biphenyls (PCBs), HBCD and polybrominated diphenyl ethers (PBDEs) in indoor dust, soil and sediment samples was studied [10]. This method has achieved remarkable results in the application of actual samples and the target analyte recovery rate is good after 3 cycles of extraction. Compared with the traditional method, this method has many advantages, including less solvent consumption, short analysis time, minimal sample contamination and high sample throughput, which is suitable for environmental monitoring with large sample volumes.

**Industrial and biological samples**

**Plastic samples:** For plastic samples processing, smashing the chunks into small particles is of the first importance. HBCDs in shells of small household electrical appliances products were lyophilized, smashed first and then extracted effectively by heating reflux extraction with toluene: methanol (10:1, V/V) [34]. Polymer was removed by counter precipitation method. Acceptable recovery of 68.0-75.2% was obtained with relative standard deviations (RSD)<5%.

A preconcentration method that was accelerated solvent extraction of methanol/toluene (1:1, V/V) has been successfully used for simultaneous determination of Bisphenol A, TBBPA, nonylphenol and HBCD in electrical and electronic products. Under optimized condition, recoveries of all the analytes satisfactorily ranged from 88.7% to 105.5% [35]. Robust Soxhlet extraction with n-hexane/isopropanol (1:1, V/V) was used for extracting HBCD from plastic products including polyethylene (PE), polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC) and acrylonitrile butadiene styrene (ABS). The extraction efficiency of this method is satisfactory by using XRF to analyze bromine before and after extraction [36].

**Biotic samples and foodstuffs:** For biotic samples, homogenization is the first processing step generally. Soxhlet apparatus with CHCl₃/n-hexane (1:1, V/V) added to dissolve the residue used for drying the extracts and GPC packed with S-X3 Bio-Beads [37-39]. The acceptable recoveries of all these compounds ranged from 75.5% to 105.0% were obtained by using d 18-α-HBCD, d 18-β-HBCD and d 18-γ-HBCD as working standards and little lipid residual indicated the lipid removal was successful. An automated method for the analysis of TBBPA and the three major HBCD stereoisomers in eel, egg, cheese and salmon samples was developed, using a specific mesh-size sodium sulphate and an acid silica column combined with a Sep-pack Plus silica cartridge in accelerated solvent extraction (ASE). The recovery of this method is between 80% and 110% [40].

A new analytical method has demonstrated high recovery rates for the simultaneous determination of HBCD, TBBPA, three brominated phenols and four hydroxylated derivatives of polybrominated diphenyl ethers (OH-PBDEs) in the muscle tissue of lean and fat fish [41]. The purification process of the combination of C18 and primary secondary amine (PSA) adsorbents was fast, simple, and had high throughput, with recoveries of between 80% and 115% and RSD <13% for all analytes.

A stimulate method for determination of PBDEs, derivatives (OH-PBDEs and MeO-PBDEs), TBBPA and HBCD in egg samples was also developed by gel permeation chromatography (GPC) and dispersive solid phase extraction (DSPE) [23]. The analytes were extracted with mixture of hexane and dichloromethane (1:1, V/V) by accelerated solvent extraction (ASE) and purified by 100 mg C-18 dispersive SPE sorbents followed with GPC. The recoveries of objects were 65.4%-97.2% and 65.6%-109.2% (except BDE85 was 54.8% and OH-BDE-137 was 47.4%) spiked at 1.0 μg/g or 5.0 μg/g in egg white and egg yolk, respectively.

**Human serum, plasma, milk and feces samples:** The simultaneous extraction of HBCD, TBBPA and PBDE in human serum using MTBE and hexane (1:1, V/V) combined with removal of lipid by sulfuric acid and purification using SPE with an LC-Si column [42]. The average recoveries were from 80.3% to 108.8% at two spiked levels of 0.5 and 5 ng/g for HBCD, 0.05 and 0.5 ng/g for TBBPA and BDE-209 with the RSDs between 1.02% and 11.42%. A method for the concurrent extraction of PBDEs, α-, β-, and γ-HBCD and TBBPA in human milk and serum was developed and validated [43]. The milk and serum samples were extracted with accelerated solvent extraction with acetone/hexane (1:1, V/V) and liquid-liquid extraction with methyl-tert-butyl ether/hexane (1:1, V/V), respectively. Co-extracted biomaterials are mainly treated by GPC followed by sulfuric acid treatment. Fractionation of HBCD, PBDEs and TBBPA was achieved using a Supelco LC-Si SPE cartridge.

Soxhlet extraction has been used for extracting HBCD from human breast milk powder [44]. The extract was dried and dissolved in cyclohexane/ethyl acetate (1:1, V/V) and then purified by GPC. The effluent was concentrated with rotary evaporation and then redissolved in hexane and sulphuric acid added to remove the fat for further. It is feasible to use feces as a non-invasive matrix to estimate serum concentrations of BFR in young children for biomonitoring purposes [45]. Due to the complex nature of the matrix, the sample
extract can be cleaned with concentrated sulfuric acid (H$_2$SO$_4$) prior to fractionation on a SiO$_2$ SPE column [45].

**Instrumental analysis**

HBCD has been traditionally analyzed using GC coupled to ECD analyzer or MS with ECNI, MS with NCI, for which Br- was monitored sensitively [16,46-48]. As standard analysis method, GC-EI-MS is used to determine ∑HBCD in electrical and electronic products in China (GB/T 29785-2013). Despite of higher sensitivity of above GC based instrumental analytical methods compared with analytical methods based on LC and MS, high temperatures can lead to misleading results and therefore limit their application [49,50]. Decrease of the injection port temperature to 230°C is favorable for the determination of ∑HBCD due to the very small residue and the negligible decomposition of HBCD at this temperature [51]. In addition, the partition of HBCD on GC column stationary phases is poor [52]. It was reported that this analytical method was applied to determine trace amounts of the APE together with lower substituted PBBS (PBB1, PBB10, PBB18 and PBB49), HBCD and TBBPA from wastewater treatment plant samples under the same GC-MS conditions. The recovery of the target compound in the simulated water sample is more than 60%, and the method has a lower detection limit and limit of quantitation. GC-ECD or GC-MS are only applicable for total HBCD quantitation.

Liquid chromatography-quadrupole linear ion trap mass spectrometry (LC-QqLT-MS) methods can analyze α-HBCD, β-HBCD and γ-HBCD together with TBBPA and related compounds and showed excellent detection limit in SRM mode, even better results in enhanced product-ion (EPI) mode [53]. LC with chiral, permethylated β-cyclodextrin columns was proved efficient for separating and determining enantiomers of the three HBCD stereomers [54]. Reverse-phase LC, coupled to ESI- or APCLI-MS, is preferred for stereoisomers (α-, β- and γ-HBCD) specific identity of HBCD compared with GC but significantly lower ion intensities disadvantaged the use of APCLI-MS. The sensitivity of the LC-MS method is 10 times less than that of GC-ECNI-MS, making it less suitable for samples with low HBCD [55]. Moreover, APCLI-MS/MS method has been used to quantify 16 BFRs including TBBPA, HBCD, PBDE, because many non-polar BFRs cannot be sensitively detected with ESI-mode [10]. A new LC/MS/MS analytical method developed using a coupled reversed-phase column has shown excellent results for ten HBCD diastereomers [56]. Compared with other previous methods, this method has good reproducibility, sensitivity and good resolution after optimization of parameter conditions.

A congeners-specific analysis method of HBCD by HPLC-ESI-MS/MS using MRM was applied to analyze fish samples (whitefish, sauger, walleye, drums) from Lake Winnipeg [57]. A linear relationship on-column produced from γ-HBCD by this method is better. A two-dimensional high-performance liquid chromatography (HPLC) approach has been developed for enantiospecific analysis of isomers of HBCD [58]. This method is mainly used to isolate the HBCD isomers in the sand oyster oil, white gull and ring seal samples.

The UPLC-ESI-MS/MS methods for evaluating the pattern of HBCD in fish and marine oil supplements was developed and LOQs for α-HBCD, β-HBCD and γ-HBCD on column were 20 pg, 20 pg and 40 pg respectively and elution time for HBCD is less than 8 min [59]. Since UPLC requires less elution time and can provide greater peak capacity, higher resolution and sensitivity than common LC, UPLC-ESI-MS/MS was developed for the determination of the three major isomers of HBCD [34]. The three isomers were separated effectively in only 3 min when ACQUITYT UPLC T3 was used. The linear ranges of the compounds were 1.6-32.4 mg/L for HBCD with correlation coefficient greater than 0.996. The LOD and the LOQ for HBCD were 0.014 and 0.068 mg/L. It has been reported that simultaneous determination of bisphenol A, TBBPA, nonylphenol, and HBCD can be performed using UHPLC-ESI-MS/MS [35]. Using the optimized parameters, the stereoisomers of HBCD can be rapidly baseline separated in a short period of time. The UHPLC-ESI-MS/MS has also been validated for simultaneous determination of HBCD isomers, TBBPA, three brominated phenols, and four hydroxylated derivatives of polybrominated diphenyl ethers (OH-PBDEs) [41]. An analytical method for determination of tetrabromomethylphenyl anhydride (TBBPA), TBBPA, HBCD, tris(2,3-dibromopropyl) phosphate (TBPB), triester organophosphate flame retardants (OPFRs), triphenyl phosphate (TPhP) and ethylhexyl diphenyl phosphate (EHDP) have been studied, mainly using reversed-phase ultra-high performance liquid chromatography (UHPLC) with a UV detector, and UHPLC coupled with high resolution (HR) orbitrap mass spectrometry featuring heated electrospray ionization (HESI-II) interface operated in negative ion mode was employed for the quantitative determination of HBCD [60]. The developed method can be used for regular diastereomer-specific monitoring of HBCD content in fish samples and represents a good alternative to existing LC-MS/MS methods for sensitivity and accuracy. An Ultra high performance liquid chromatography-time of flight high resolution mass spectrometry method was used to the analysis of HBCD diastereomers [61]. The time of flight high resolution mass spectrometry (TOF-HRMS) analyzer has a high resolution and the scan m/z range reaches the picogram limit.

Rapid resolution liquid chromatography–electrospray tandem mass spectrometry (RRLC-ESI-MS/MS) has been applied for the analysis of HBCD in actual environmental water samples [22]. The LOD and precision were in the range of 0.005-0.015 μg/L and 4.59-7.47%, respectively. A flowing atmospheric-pressure afterglow ion source for mass spectrometry (FAPA-MS) in negative-ion mode, a fast, effective alternative to GC, LC methods for HBCD detection for the chemical characterization and determination of HBCD was presented [62]. This technique is an effective method for the detection of HBCD, and it can achieve high detection efficiency and sensitivity when it is effectively ionized in the liquid phase.

A rapid method using highest strength silica gel (HSS) C18 1.8 μm particle size chromatography columns and supercritical carbon dioxide and methanol was reported to be able to isolate the three most abundant diastereomers of HBCD within three minutes [63]. Supercritical CO$_2$ plays an important role in determining the diastereoisomers of HBCD, mainly because a unique elution order of the α-, β- and γ-HBCD diastereomers using supercritical CO$_2$ was observed. This method is mainly performed using a negative ion electrospray ionization tandem quadrupole mass spectrometer, operating in multiple reaction monitoring (MRM) mode.

Determination of HBCD in polystyrene (PS) can be achieved by loop-based multiple center-cut (MHC) two-dimensional liquid chromatography (2D-LC) and has been performed several times as a method for quantifying HBCD Test [64]. MHC 2D-LC has good accuracy and repeatability for quantitative analysis of HBCD. MHC 2D-LC plays an important role in quantitative analysis of difficult-to-separate samples when traditional one-dimensional separation fails.
Conclusions

This review completely compiles and critically reviews the main research achievements regarding HBCD published recently, excluding aspects that have been mentioned in previous reviews. The article mainly summarizes sample preparation, including sample collection, extraction, concentration and fractionation, and instrumental analysis of HBCD and its by-products. Instrumental analysis methods are mainly described by GC-MS, LC-MS, high-performance liquid chromatography (HPLC), UPLC-ESI-MS/MS, Rapid Resolution liquid chromatography-electrospray tandem mass spectrometry and some other methods. Although each of these methods has its own strengths and weaknesses, we still need to work on more efficient analysis methods.

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