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Fast clean-up for polychlorinated dibenzo-*p*-dioxins, dibenzofurans and coplanar polychlorinated biphenyls analysis of high-fat-content biological samples

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Abstract

A fast clean-up procedure for the low level analysis of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls in highly fatty biological matrices using high capacity disposable multi-layer silica columns is presented. Results were compared with gel permeation chromatography for removal of lipids. Analytical criteria such as recovery rates, repeatability, reproducibility and robustness are evaluated through a broad range of biological matrices and reference materials analysis. The final proposed procedure for the complete analysis, including pressurized liquid extraction, Power-Prep system clean-up and GC–high-resolution MS analysis requires only 48 h, and allows the simultaneous preparation of up to 10 samples.

Keywords: Sample preparation; Pressurized liquid extraction; Polychlorinated dibenzodioxins; Polychlorinated dibenzofurans; Polychlorinated biphenyls

1. Introduction

Among the class of persistent organic pollutants (POPs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and associated coplanar polychlorinated biphenyls (cPCBs) represent the most toxic category [1]. These planar chlorinated hydrocarbons are anthropogenic chemicals found in the environment [2]. Even if only the most toxic member of the family, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has been classified as carcinogenic to humans by the Internal Agency for Research on Cancer (IARC) [3], they all have endocrine disrupter properties through aryl hydrocarbon receptor (AhR) mediation [4–6]. This affinity for AhR has been used to build a toxic equivalency factor (TEF) list, which is used in risk assessment of these toxic halogenated aromatics [7,8].

Since the so-called ''chick edema factor story'' that occurred in the USA in 1957, it is well known that contamination of animal feed can have dramatic consequences, not only the quality of produced foodstuffs, resulting in higher exposure risks for humans, but also on the food web economy [9,10]. Summer 1999 contamination in Belgium also demonstrated the importance of monitoring the feeding stuffs entering our food web via cattle breeding, as well as final food products in order to prevent risks

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of exposure and to trace the source of contamination [11]. Since these events, the Belgian government has adopted a norm of 5 pg I-TEQ/g fat regarding 17 PCDD/Fs present in foodstuffs containing more than 2% fat [12]. Knowing that usually more than 90% of the human exposure to PCDD/Fs is due to ingestion of food, mainly animal fat-based food, it is clear that an exposure survey has to be done through animal feed and resulting foodstuffs monitoring.

Biological matrices are often characterized by high amounts of fat and low levels of dioxin contamination (ppt or less). Due to the high lipophilicity of these compounds, the analysis requires many purification steps. A multi-step procedure consisting of sample extraction, adsorption chromatography columns clean-up and, finally, analysis using gas chromatography coupled to high-resolution mass spectrometry in the isotopic dilution mode (GC–IDHRMS) is necessary to isolate and quantify these analytes [13,14]. The aim of the extraction step is to isolate the lipid fraction containing compounds of interest.

This process is typified by such techniques as Soxhlet or liquid–liquid extraction [15–17]. They are however labor-intensive, time consuming and generally require large amount of solvents. In order to overcome these drawbacks, alternative extraction strategies have been developed, offering analysts the choice between newer techniques [18]. Pressurized liquid extraction (PLE, also called accelerated solvent extraction) is one of the most used techniques in replacement of Soxhlet for extraction of POPs [19]. It has been demonstrated to be a quantitative extraction process with respect to Soxhlet, spike levels or certified values [20,21]. Automation of this process allows the extraction of a large number of samples, either sequentially (ASE 200, Dionex, Sunnyvale, CA, USA) or in parallel (FAST-PSE, Applied Instrumentation, Allentown, PA, USA). After gravimetric determination of the lipid content, the desired amount of fat has to be processed through clean-up to allow GC–MS analysis.

From a recent compilation of data, it appears that levels of the usual background contamination occurring in animal foodstuffs are often in the sub-ppt range (down to 0.1 ppt, depending on the congeners) [22]. In addition, limits of quantification (LOQs) for classical GC–IDHRMS operating in general laboratory environments are located around the sub-ppt range and global recoveries are contained between 65 and 100%. The consequence is that more than 1 g of fat is actually needed to allow the analysis to be done in good conditions and a minimum quantity of fat is then located around 4 g. Last year, following research conducted at the Centers for Disease Control and Prevention (CDCs) [23], an automated clean-up system (the Power-Prep system from Fluid Management Systems (FMS)), using a classical set of multi-layer silica, basic alumina and carbon columns, was developed in order to increase the number of samples treated simultaneously [24]. This system has recently been evaluated for abiotic environmental samples [25,26] and is used for clean-up of low fat content samples [27]. However, in its standard mode, the clean-up system uses disposable multi-layer silica columns (4 g of acidic silica, 2 g of basic silica and 1.5 g of neutral silica) that do not allow the handling of more than 1 g of lipids in order to produce clean extracts. This means that in the case of animal foodstuffs considered here, a pre-reduction in the quantity of lipids is required before the Power-Prep clean-up.

Several possible routes such as acidic digestion [28], saponification [29,30], acidic silica columns [31,32] or gel permeation chromatography (GPC) [33,34] are possible to carry out lipid elimination. Among them, GPC separation presents several advantages: it is versatile, robust, can be used repetitively without regeneration and is quite easy to automate [35]. Although sequential injection of samples is conceptually possible to bring into play, practical application is far from easy. Lipid solutions in organic solvent are actually prone to precipitation, viscosity change, auto-injector clogging, etc. In practice, the GPC needs constant supervision and cannot be performed 100% automatically. In addition, after elution and collect of the PCDD/Fs and cPCBs fraction, the solvent has to be evaporated to allow its replacement by a more efficient solvent for the next silica-cleaning step. Total time required, including GPC column wash and solvent evaporation, is more than 3 h per sample. Then, while the extraction step produces a large number of lipid extracts automatically, the central GPC step, which operates sequentially and requires more than 15 h to produce five pre-cleaned samples, acts as a bot-

tleneck before the fast automated clean-up capable of treating up to 10 samples at a time and becomes the time limiting step of the whole process. In order to reach a higher sample throughput, slow GPC purification has been replaced by a new-high-capacity disposable silica (HCDS from FMS) column containing 28 g acidic, 16 g basic, 6 g neutral silica and which is incorporated in the classical set of PowerPrep columns. The HCDS column results from research conducted with FMS; they are also commercially available and do not require manual preparation, as previously reported [36]. Total clean-up time is reduced to less than 2 h for series samples containing up to 4 g of lipids each. This study presents the evaluation of the new HCDS columns and the resulting improved procedure for fast analysis of PCDD/Fs and cPCBs in high-fat-content biological samples.

2. Experimental

2.1. Chemicals

Water, hexane, pentane, toluene, ethyl acetate, cyclohexane and dichloromethane are Pestanal reagents (Riedel-de Haën, Seelze, Germany). Nonane puriss analytical-reagent grade standard for GC was purchased from Fluka (Steinheim, Germany). Sodium sulfate anhydrous was Baker analyzed (J.T. Baker, Deventer, Netherlands), silica gel 60 (0.063–0.200 mm) was column chromatography (Merck, Darmstadt, Germany), glass fiber thimbles (43×123 mm) were from Schleicher and Schuell (Dassel, Germany) and borosilicate solids glass beads (3 mm) were from Aldrich (Milwaukee, WI, USA). Liquid nitrogen was purchased at Air Liquide (Liege, Belgium).

The $^{13}C_{12}$ labelled internal standard solution containing PCDDs, PCDFs and cPCBs was from Cambridge Isotope Labs. (Andover, MS, USA). This EDF-4144 internal standard solution contains 2,3,7,8-TCDD (TCDD), 1,2,3,7,8-PeCDD (PeCDD), 1,2,3,4,7,8-HxCDD (HxCDD 1), 1,2,3,6,7,8-HxCDD (HxCDD 2), 1,2,3,7,8,9-HxCDD (HxCDD 3), 1,2,3,4,6,7,8-HpCDD (HpCDD), OCDD, 2,3,7,8-TCDF (TCDF), 1,2,3,7,8-PeCDF (PeCDF 1), 2,3,4,7,8-PeCDF (PeCDF 2), 1,2,3,4,7,8-HxCDF (HxCDF 1), 1,2,3,6,7,8-HxCDF (HxCDF 2), 1,2,3,7,8,9-HxCDF (HxCDF 3), 2,3,4,6,7,8-HxCDF (HxCDF 4), 1,2,3,4,6,7,8-HpCDF (HpCDF 1), 1,2,3,4,7,8,9-HpCDF (HpCDF 2), OCDF, 3,3′,4,4′-TCB (PCB 77), 3,4,4′,5-TCB (PCB 81), 3,3′,4,4′,5-PeCB (PCB 126) and 3,3′,4,4′,5,5′-HxCB (PCB 169). The recovery standard solution EDF-4145 (Cambridge Isotope Labs.) contains [$^{13}C_{12}$]3,3′,5,5′-TCB, [$^{13}C_{12}$]1,2,3,4,7,8,9-HpCDF and [$^{13}C_{6}$]1,2,3,4-TCDD. The nine calibration solutions (EDF-4143, Cambridge Isotope Labs.) contain the native and labelled congeners in different concentrations. All concentrations of the standard solutions are listed in Table 1.

2.2. Samples

Poultry, eggs and mackerel (fillet) are issued from the Belgian food market and sperm whale blubber was from animals grounded on the Belgian coast in winter 1994. Yolks were separated from raw eggs for analysis and adipose tissue samples were carved. All samples were homogenized using dissecting and/or mortar equipment and frozen under liquid nitrogen before freeze–drying. The freeze-dried products were ground in order to obtain a fine powder. A pre-filtration step on sodium sulfate was sometimes required in the case of suppurating adipose samples prior to freeze–drying. Dairy fat, extracted and isolated by industrial processes and obtained as pure fat from manufacturers, was considered as an extracted product and directly dissolved in appropriate solvents for subsequent clean-up. All results presented here represent at least triplicate analyses.

2.3. Extraction

2.3.1. Pressurized liquid extraction (PLE)

All samples except milk and dairy fat were extracted by PLE using a Dionex (Sunnyvale, CA, USA) ASE 200 extractor capable of sequentially extracting up to 24 samples. Conditions were: 33 ml extraction cells filled with freeze-dried sample and sodium sulfate (80:20), 20 ml of hexane per cycle, 5 min cycle time, two cycles per extraction, pressure of 1500 p.s.i. (1 p.s.i.=6894.76 Pa). The fat extracts

Table 1
Calibration, internal and recovery standard solutions for PCDD/Fs and cPCBs analysis

Compounds	Concentration (pg/μl). EDF-4143 (calibration)									EDF-4144 (internal)	EDF-4145 (recovery)
	1	2	3	4	5	6	7	8	9		
[$^{12}C_{12}$]2,3,7,8-TCDD	0.04	0.10	0.20	1.00	2.00	7.0	20.0	35.0	50.0	–	–
[$^{12}C_{12}$]1,2,3,7,8-PeCDD	0.04	0.10	0.20	0.50	1.00	2.00	5.0	10.0	20.0	–	–
[$^{12}C_{12}$]1,2,3,4,7,8-HxCDD	0.04	0.10	0.20	0.50	1.00	2.00	5.0	10.0	20.0	–	–
[$^{12}C_{12}$]1,2,3,6,7,8-HxCDD	0.10	0.25	0.50	1.25	2.50	5.0	12.5	25.0	50.0	–	–
[$^{12}C_{12}$]1,2,3,7,8,9-HxCDD	0.20	0.50	1.00	2.00	5.0	10.0	20.0	25.0	30.0	–	–
[$^{12}C_{12}$]1,2,3,4,6,7,8-HpCDD	1.00	2.00	5.0	10.0	20.0	25.0	50.0	100.0	200.0	–	–
[$^{12}C_{12}$]1,2,3,4,6,7,9-HpCDD	0.04	0.10	0.20	0.50	1.00	2.00	5.0	10.0	20.0	–	–
[$^{12}C_{12}$]OCDD	10.0	20.0	50.0	100.0	200.0	300.0	400.0	500.0	600.0	–	–
[$^{12}C_{12}$]2,3,7,8-TCDF	0.04	0.10	0.20	0.50	1.00	2.00	5.0	7.5	10.0	–	–
[$^{12}C_{12}$]1,2,3,7,8-PeCDF	0.04	0.10	0.20	0.50	1.00	2.00	5.0	7.5	10.0	–	–
[$^{12}C_{12}$]2,3,4,7,8-PeCDF	0.04	0.10	0.20	0.50	1.00	2.00	5.0	7.5	10.0	–	–
[$^{12}C_{12}$]1,2,3,4,7,8-HxCDF	0.04	0.10	0.20	0.50	1.00	2.00	5.0	7.5	10.0	–	–
[$^{12}C_{12}$]1,2,3,6,7,8-HxCDF	0.04	0.10	0.20	0.50	1.00	2.00	5.0	7.5	10.0	–	–
[$^{12}C_{12}$]1,2,3,7,8,9-HxCDF	0.04	0.10	0.20	0.50	1.00	2.00	5.0	7.5	10.0	–	–
[$^{12}C_{12}$]2,3,4,6,7,8-HxCDF	0.04	0.10	0.20	0.50	1.00	2.00	5.0	7.5	10.0	–	–
[$^{12}C_{12}$]1,2,3,4,6,7,8-HpCDF	0.20	0.50	1.00	2.00	5.0	10.0	20.0	25.0	30.0	–	–
[$^{12}C_{12}$]1,2,3,4,7,8,9-HpCDF	0.04	0.10	0.20	0.50	1.00	2.00	5.0	7.5	10.0	–	–
[$^{12}C_{12}$]OCDF	0.04	0.10	0.20	0.50	1.00	2.00	5.0	7.5	10.0	–	–
[$^{12}C_{12}$]3,3′,4,4′-TCB	0.80	1.60	4.0	8.0	16.0	20.0	40.0	80.0	160.0	–	–
[$^{12}C_{12}$]3,4,4′,5-TCB	0.80	1.60	4.0	8.0	16.0	20.0	40.0	80.0	160.0	–	–
[$^{12}C_{12}$]3,3′,4,4′,5-PeCB	0.80	1.60	4.0	8.0	16.0	20.0	40.0	80.0	160.0	–	–
[$^{12}C_{12}$]3,3′,4,4′,5,5′-HxCB	0.80	1.60	4.0	8.0	16.0	20.0	40.0	80.0	160.0	–	–
[$^{13}C_{12}$]2,3,7,8-TCDD	50	50	50	50	50	50	50	50	50	25	–
[$^{13}C_{6}$]1,2,3,4-TCDD	25	25	25	25	25	25	25	25	25	–	25
[$^{13}C_{12}$]1,2,3,7,8-PeCDD	50	50	50	50	50	50	50	50	50	25	–
[$^{13}C_{12}$]1,2,3,4,7,8-HxCDD	120	120	120	120	120	120	120	120	120	60	–
[$^{13}C_{12}$]1,2,3,6,7,8-HxCDD	120	120	120	120	120	120	120	120	120	60	–
[$^{13}C_{12}$]1,2,3,7,8,9-HxCDD	120	120	120	120	120	120	120	120	120	60	–
[$^{13}C_{12}$]1,2,3,4,6,7,8HpCDD	120	120	120	120	120	120	120	120	120	60	-
[$^{13}C_{12}$]OCDD	250	250	250	250	250	250	250	250	250	125	–
[$^{13}C_{12}$]2,3,7,8-TCDF	50	50	50	50	50	50	50	50	50	25	–
[$^{13}C_{12}$]1,2,3,7,8-PeCDF	50	50	50	50	50	50	50	50	50	25	
[$^{13}C_{12}$]2,3,4,7,8-PeCDF	50	50	50	50	50	50	50	50	50	25	–
[$^{13}C_{12}$]1,2,3,4,7,8-HxCDF	125	125	125	125	125	125	125	125	125	62.5	–
[$^{13}C_{12}$]1,2,3,6,7,8-HxCDF	125	125	125	125	125	125	125	125	125	62.5	–
[$^{13}C_{12}$]1,2,3,7,8,9-HxCDF	125	125	125	125	125	125	125	125	125	62.5	–
[$^{13}C_{12}$]2,3,4,6,7,8-HxCDF	125	125	125	125	125	125	125	125	125	62.5	–
[$^{13}C_{12}$]1,2,3,4,6,7,8-HpCDF	125	125	125	125	125	125	125	125	125	62.5	
[$^{13}C_{12}$]1,2,3,4,7,8,9-HpCDF	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	–	62.5
[$^{13}C_{12}$]OCDF	250	250	250	250	250	250	250	250	250	125	–
[$^{13}C_{12}$]3,3′,4,4′-TCB	48	48	48	48	48	48	48	48	48	24	–
[$^{13}C_{12}$]3,4,4′,5-TCB	48	48	48	48	48	48	48	48	48	24	–
[$^{13}C_{12}$]3,3′,5,5′-TCB	48	48	48	48	48	48	48	48	48	–	48
[$^{13}C_{12}$]3,3′,4,4′,5-PeCB	72	72	72	72	72	72	72	72	72	36	–
[$^{13}C_{12}$]3,3′,4,4′,5,5′-HxCB	96	96	96	96	96	96	96	96	96	48	–

were dried on sodium sulfate prior to lipid content determination using gravimetric analysis. Aliquots of about 4–5 g fat were used for the clean-up step.

2.3.2. Soxhlet extraction

All milk samples were Soxhlet extracted using pentane-dichloromethane (1:1) as the solvent. Glass

fiber thimbles were extracted 2 h with hexane before use. Extraction with 400 ml of solvent containing borosilicate solid glass beads was carried out overnight on 10 g of spray-dried milk slurry with 10 g of water in order to increase accessibility of the solvent during extraction and compensate the very strong conditions used during spray-drying [37]. Subsequently, 10 g of sodium sulfate as well as 10 g of silica gel were also mixed into the slurry. Resulting extracts were dried on sodium sulfate and the extraction solvent was removed using a rotary evaporator. Lipid content was determined gravimetrically. Dairy fats were directly processed using the clean-up without any extraction step, and using sample sizes of 4–5 g.

2.3.3. Spiking

Since this research was dedicated to the evaluation of the new clean-up procedure and that PLE was reported as a quantitative extraction method for many biological matrices using hexane and sufficient sodium sulfate [20,21]; recoveries were calculated by spiking the fat after the extraction step. Once the extraction solvent was evaporated, the desired amount of fat (up to 4 g) is selected and diluted in the appropriate volume of solvent (Fig. 1) and spiked with 10 μl of sonicated EDF-4144 internal standard. Fat solutions were briefly agitated on a vortex mixer before the loading on the first column of the clean-up.

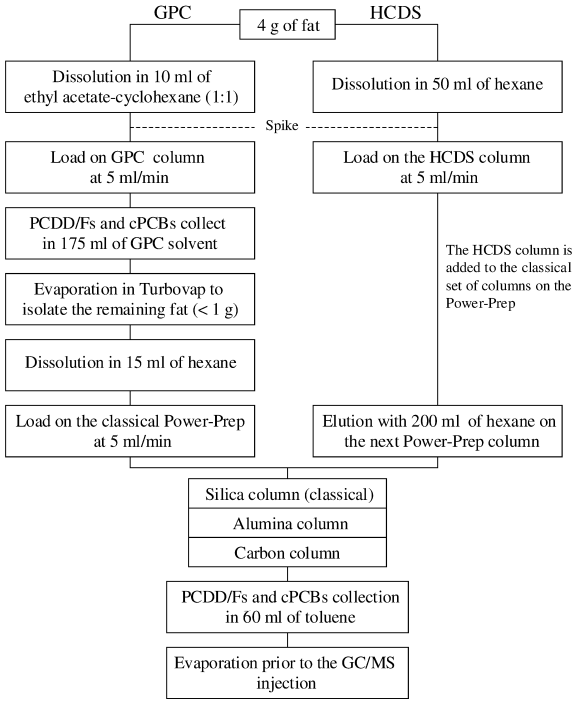

Fig. 1. Comparison of the GPC and HCDS options for the clean-up.

2.4. Clean-up

2.4.1. Gel permeation chromatography (GPC)

GPC purification was carried out on a Latek LC-12-3 column (Latek, Eppelhein, Germany) connected to a Latek P100 piston pump equipped with a Superfrac fraction collector (Amersham Pharmacia Biotech, Uppsala, Sweden). The column was packed with 70 g of S-X3 Bio-Beads (Bio-Rad, Nazareth, Belgium) using ethyl acetate–cyclohexane (1:1) as solvent. Bio-Beads were conditioned overnight in solvent at room temperature before packing. The lifetime of a column was estimated at approximately 50 samples. The fat sample (up to 4 g) was dissolved in 10 ml of ethyl acetate–cyclohexane (1:1) and loaded on the column via a 10 ml loop at 5 ml/min. The fraction corresponding to the first 25 min of elution, containing more than 80% of the fat, was discarded to waste. The fraction containing the analytes eluted between 25 and 60 min, and the resulting 175 ml were collected for the remaining clean-up steps (Fig. 1). A column wash and regeneration was then performed with 75 ml of solvent prior to the next sample.

2.4.2. Automated Power-Prep system

An automated multi-column clean-up was performed on the Power-Prep system (FMS, Waltham, MA, USA). This system is made of independent chromatography panels capable of treating up to 10 samples in parallel. The system is controlled by a personal computer via a control module that pilots the valve drive modules connected to the pump and pressure modules responsible for the solvent flow in the valve module. Internal pressure of the system is monitored by pressure gauges and never exceeds 35 p.s.i. Programming of the solvent volumes, types,

flow-rates and directions is realized in an FMS patented software operating under Windows.

The classical clean-up for PCDD/Fs and cPCBs run uses disposable multi-layer silica columns (4 g acid, 2 g base and 1.5 g neutral), basic alumina (8 g) and PX-21 (2 g) carbon columns. These columns are packed in PTFE tubes individually sealed in Mylar packaging and manufactured by FMS. Samples coming from GPC were concentrated in a Turbovap II Concentration Workstation (Zymark, Hopkinton, MA, USA) in order to remove the ethyl acetate–cyclohexane (1:1) solvent mixture and isolate the remaining lipids (less than 1 g). Lipids are then diluted in hexane prior loading on the silica column (previously conditioned with 100 ml of hexane at 10 ml/min) at 5 ml/min (Fig. 2). After a flush of 100 ml of hexane at 10 ml/min through alumina to the waste (F1), 60 ml of hexane–dichloromethane (98:2) are dispensed at 10 ml/min to alumina to eliminate low polar interferences (e.g. some PCBs) (F2). PCDD/Fs and cPCBs are eluted from alumina and fixed on carbon using 120 ml of hexane–dichloromethane (1:1) at a flow-rate of 10 ml/min, remaining interferences are discarded to the waste (F3). The carbon column is then back flushed with 60 ml of toluene at 5 ml/min to elute the PCDD/Fs and cPCBs that are collected in a 250 ml Zymark evaporation tube (F4). At the end of the process, the system is automatically decontaminated via a special solvent program.

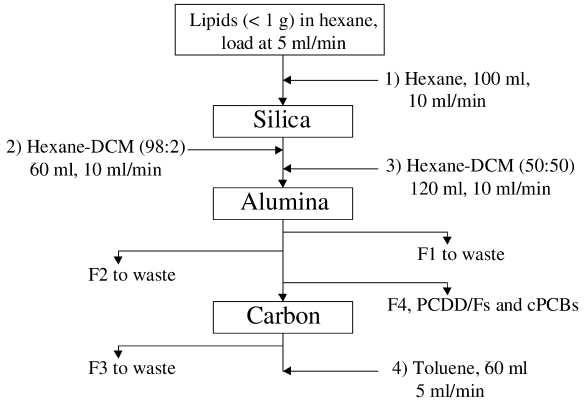

Fig. 2. Flow chart for the classical set of columns used for PCDD/Fs and cPCBs isolation using the Power-Prep automated clean-up system.

The toluene extracts are concentrated to approximately 150 μl, using the optical sensor and time options available on the Turbovap II workstation, and transferred to conical vials containing 4 μl of nonane used as keeper. The remaining toluene is slowly evaporated at room temperature by placing the vial in a dust-free evaporation box connected to the hood, prior to GC–HRMS injection.

2.4.3. HCDS columns

HCDS columns (28 g acidic, 16 g basic, 6 g neutral) are a new type of disposable silica column dedicated to removal of large amounts of fat. They are packed in PTFE tubes individually sealed in Mylar packaging and manufactured by FMS. The HCDS column is added to the classical set of columns and is the first one in contact with the sample (up to 4 g of lipids), which is diluted in 50 ml of hexane and loaded on the system at 5 ml/min, once the HCDS has been conditioned with 130 ml of hexane at 10 ml/min. The HCDS column is eluted with 200 ml of hexane (instead of the 100 ml required for the classical run, and due to the larger size of the HCDS column) to the classical silica and alumina columns as carried out in step (1) of Fig. 2. The remaining part of the PCDD/Fs and cPCBs program is the same as for the classical run (Fig. 1).

2.5. Analysis

All analyses were performed by GC–HRMS using a MAT95XL high-resolution mass spectrometer (Finnigan, Bremen, Germany) and a Hewlett-Packard (Palo Alto, CA, USA) 6890 Series gas chromatograph.

GC conditions were optimized to separate the 21 congeners present in the EDF-4144 solution. The column is a RTX-5SIL-MS (30 m×0.25 mm I.D., 0.25 μm film thickness) capillary column (Restek, Evry, France); splitless injection of 2 μl of extract at 275°C, initial oven temperature: 140°C; temperature programming: 140°C, held for 2 min, then increased at 15°C/min to 220°C, then increased to 240°C at 1.2°C/min, then increased to 270°C at 4°C/min, then increased to 300°C at 10°C/min and held at this temperature for 1 min. Pure GC grade He, 99.9999% (Air Products, Vilvoorde, Belgium) is used as carrier gas at a constant linear velocity of 25 cm/s.

The mass spectrometer is operated in the electron impact ionization mode using selected ion monitoring (SIM). Electron energy was set to 60 eV. Source temperature was 270°C. The MS system was tuned to a minimum resolution of 10 000 (10% valley) and masses issued from FC-5311 (perfluorophenanthrene) tuning compound were used as lock mass. Principal parameters such as ions monitored, dwell times and isotope ratios are listed in Table 2. In order to determine the linear response zone for each congener, nine calibration solutions are injected every 3 months. These solutions contain

Table 2
Parameters for selected ion monitoring of PCDD/Fs and cPCBS in isotopic dilution mode

Compounds		Ions monitored		Dwell times (ms)	Theoretical isotopic ratios	Acceptable range (15%)
		Quantify mass[a]	Ratio mass[b]			
Window 1	[$^{12}C_{12}$]TCB	291.9194 [M+2]	289.9224 [M]	113.32	0.77	0.65–0.88
8–12 min	[$^{13}C_{12}$]TCB	303.9597 [M+2]	301.96.26 [M]	36.86	1.77	0.65–0.88
	Lock mass	292.9824 l.m.2c[c]	316.9824 c.m.[d]	5.46		
Window 2	[$^{12}C_{12}$]PeCB	325.8804 [M+2]	327.8775 [M+4]	84.65	0.64	0.56–0.75
12–14 min	[$^{13}C_{12}$]PeCB	337.9207 [M+2]	339.9177 [M+4]	16.38	0.64	0.56–0.75
	[$^{12}C_{12}$]TCDD	321.8936 [M+2]	319.8965 [M]	84.65	0.77	0.65–0.88
	[$^{13}C_{12}$]TCDD	333.9339 [M+2]	331.9368 [M]	16.38	0.77	0.65–0.88
	[$^{13}C_{6}$]TCDD	331.9078 [M+6]		84.65		
	[$^{12}C_{12}$]TCDF	305.8987 [M+2]	303.9016 [M]	84.65	0.77	0.65–0.88
	[$^{13}C_{12}$]TCDF	319.8965 [M+2]	317.9389 [M]	16.38	0.77	0.65–0.88
	Lock mass	316.9824 l.m.[c]	366.9792 c.m.[d]	4.10		
Window 3	[$^{12}C_{12}$]HxCB	359.8415 [M+2]	361.8385 [M+4]	96.94	0.81	0.69–0.94
14–18.5 min	[$^{13}C_{12}$]HxCB	371.8817 [M+2]	373.8788 [M+4]	19.11	0.81	0.69–0.94
	[$^{12}C_{12}$]PeCDD	355.8546 [M+2]	353.8576 [M]	96.94	0.61	0.53–0.71
	[$^{13}C_{12}$]PeCDD	367.8949 [M+2]	365.8978 [M]	19.11	0.61	0.53–0.71
	[$^{12}C_{12}$]PeCDF	339.8597 [M+2]	337.8627 [M]	96.94	0.61	0.53–0.71
	[$^{13}C_{12}$]PeCDF	351.9000 [M+2]	349.9029 [M]	19.11	0.61	0.53–0.71
	Lock mass	316.9824 l.m.[c]	366.9792 c.m.[d]	4.10		
Window 4	[$^{12}C_{12}$]HxCDD	389.8157 [M+2]	391.8128 [M+4]	128.34	0.81	0.69–0.94
18.5–24.5 min	[$^{13}C_{12}$]HxCDD	401.8559 [M+2]	403.8529 [M+4]	31.40	0.81	0.69–0.94
	[$^{12}C_{12}$]HxCDF	373.8208 [M+2]	375.8179 [M+4]	128.34	0.81	0.69–0.94
	[$^{13}C_{12}$]HxCDF	385.8610 [M+2]	387.8580 [M+4]	31.40	0.81	0.83–1.3
	Lock mass	366.9792 l.m.[c]	404.9760 c.m.[d]	5.46		
Window 5	[$^{12}C_{12}$]HpCDD	423.7767 [M+2]	425.7738 [M+4]	101.03	1.04	0.88–1.20
24.5–30.5 min	[$^{13}C_{12}$]HpCDD	435.8169 [M+2]	437.8140 [M+4]	32.77	1.04	0.88–1.20
	[$^{12}C_{12}$]HpCDF	407.7818 [M+2]	409.7789 [M+4]	101.03	1.04	0.88–1.20
	[$^{13}C_{12}$]HpCDF	419.8220 [M+2]	421.8190 [M+4]	32.77	1.04	0.88–1.20
	Lock mass	404.9760 l.m.[c]	454.9728 c.m.[d]	4.10		
Window 6	[$^{12}C_{12}$]OCDD	459.7348 [M+4]	457.7377 [M+2]	101.03	0.89	0.75–1.01
30.5–35 min	[$^{13}C_{12}$]OCDD	471.7750 [M+4]	469.7779 [M+2]	32.77	0.89	0.75–1.01
	[$^{12}C_{12}$]OCDF	443.7399 [M+4]	441.7428 [M+2]	101.03	0.89	0.75–1.01
	[$^{13}C_{12}$]OCDF	455.7801 [M+4]	453.7830 [M+2]	32.77	0.89	0.75–1.01
	Lock mass	454.9728 l.m.[c]	504.9697 c.m.[d]	4.10		

[a] This mass is used for the calculation of the concentrations.
[b] This mass is used to verify the isotropic ratios.
[c] Lock mass.
[d] Calibration mass.

native and labelled congeners in the working concentration range (Table 1) and multiple injections allow determination of the mean relative response factor (RRF) for each congener.

Two daily checks were performed to ensure operation of the MS system. Sensitivity test consists of an injection of a 0.04 pg/μl solution (2 μl, 80 fg) containing [$^{12}C_{12}$]2,3,7,8-TCDD and measuring a signal-to-noise ratio of at least 10 for the less abundant of the two monitored ions (m/z: 319.8965) with a peak width larger than 3 s. The second test concerns the check of RRFs for [$^{12}C_{12}$]2,3,7,8-TCDD, [$^{12}C_{12}$]1,2,3,4,6,7,8-HpCDF and [$^{12}C_{12}$]3,3′,4,4′,5-PeCB which are representative congeners; these RRFs must be within a 95% confidence interval built on twice the standard deviation (2σ) of calibration solutions' values.

Quantification was performed using internal standards and the isotopic dilution technique. The labelled compounds were added to the fat after the extraction step. The isotopic ratio of the characteristic ions for each congener was verified and had to be within 15% of the theoretical value (Table 2). Percentages of recoveries were calculated using the recovery standard solution EDF-4145, which was added to the reconstituted nonane, extracts prior to the injection on the GC system. TEQs of all congeners were calculated using 2,3,7,8-TCDD TEFs reported by the World Health Organisation (1998) [8].

2.6. Quality control

Procedural blanks (both instrumental and method) and quality control (QC) samples were included to ensure that the analytical system was maintained under control. For samples extracted using ASE, our ''in-house'' QC samples consisted of beef fat fortified with the 17 PCDD/Fs to have a content of about 9 pg TEQ/g fat (pool 1) and 4 pg TEQ/g fat (pool 2). All real samples were analyzed in series containing five unknown samples, one method blank and one QC. Reference materials (RM 533) [38] and certified reference material (BCR 607) [37], a spray-dried milk, were Soxhlet extracted (5 g of powder) using pentane–dichloromethane (1:1), followed by the same clean-up procedure.

3. Results and discussion

3.1. Reference materials

Analysis of reference materials was carried out on milk (RM 533 and certified BCR 607) in order to demonstrate the accuracy of the method. Results obtained for RM 533 (mean PCDD/Fs 2.84 pg TEQ/g, RSD=3%) were in very good agreement with the assigned values (mean PCDD/Fs 2.86 pg TEQ/g, estimated RSD>4%) [38]. Table 3 shows results ($n=5$) using the certified milk material. Most of the measured values are inside uncertainty limits and the total value is very close to the assigned one. The usual higher congener values may be due to coeluting non-2,3,7,8-congeners since the gas chromatography column was a RTX-5SIL-MS of 30 m length. Absence of values for TCDF and PeCDF 1 is due to the combination of their very low concentration and the small amount of milk that was used (5 g).

3.2. Quality control samples

Fig. 3 represents the measured amounts for each of the 17 congeners present in the QC for both GPC and HCDS clean-ups. Results do not indicate any significant difference either for observed values or standard deviations, which have a mean value of 3 and 4% for GPC and HCDS, respectively. An example of a recovery chart is illustrated in Fig. 4. As mentioned in paragraph 2.3.3, recovery rates

Table 3
Congener-specific values in pg/g milk powder on dry mass basis for the certified reference material BCR 607

Compounds	Mean	RSD (%)	Certified value	Uncertainty (95%)	Accuracy (%)
TCDD	0.29	3	0.25	0.03	116
PeCDD	0.81	7	0.79	0.04	102
HxCDD 1	0.46	8	0.42	0.07	110
HxCDD 2	1.09	7	0.98	0.11	115
HxCDD 3	0.38	8	0.34	0.05	113
TCDF	nd.		0.05	0.03	
PeCDF 1	nd.		0.05	0.01	
PeCDF 2	1.81	5	1.81	0.13	101
HxCDF 1	0.92	8	0.94	0.04	101
HxCDF 2	1.08	3	1.01	0.09	109
HxCDF 4	1.07	8	1.07	0.05	100
Total	7.92	4	7.71		104

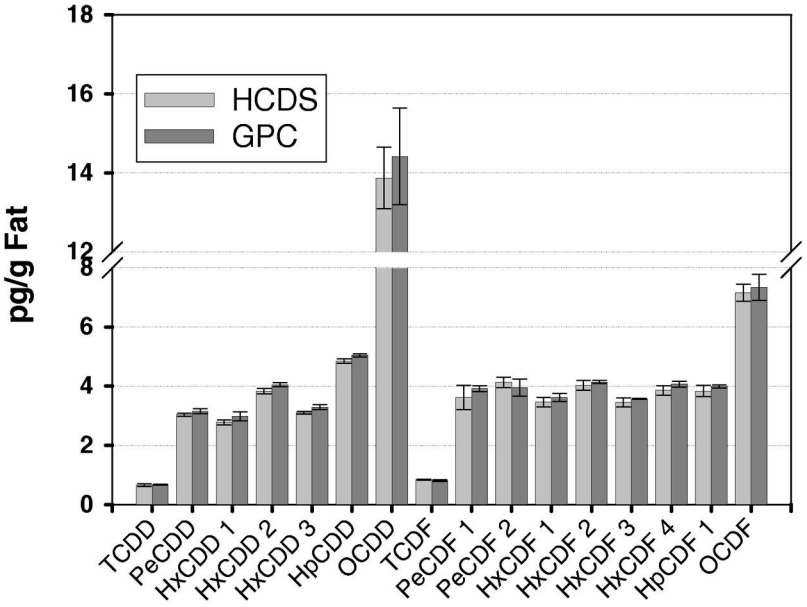

Fig. 3. HCDS vs. GPC results for quality control samples (pool 1). Results are expressed in concentration.

were calculated excluding the extraction step. Both procedures demonstrated the same profile, and were not significantly different from each other. Nonetheless, it is important to note that the isolation of cPCBs appears to be very good, which is of prime interest when one knows that the contribution of these congeners to the TEQ can reach 80% [27].

Repeatability (short-term standard deviation) and reproducibility (long-term standard deviation) of the new system were evaluated on QC samples con-

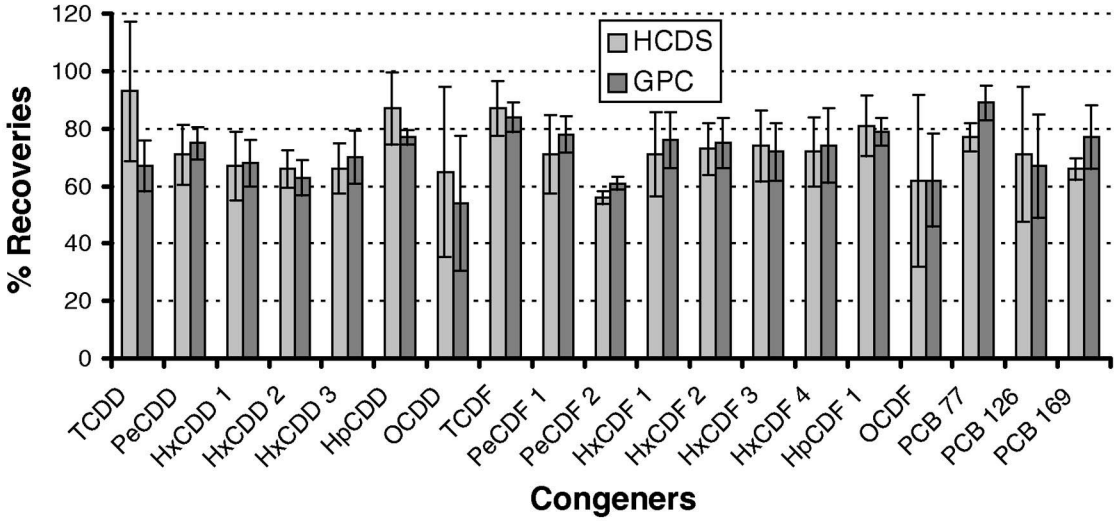

Fig. 4. Recovery rates obtained for quality control samples (pool 1) spiked after extraction.

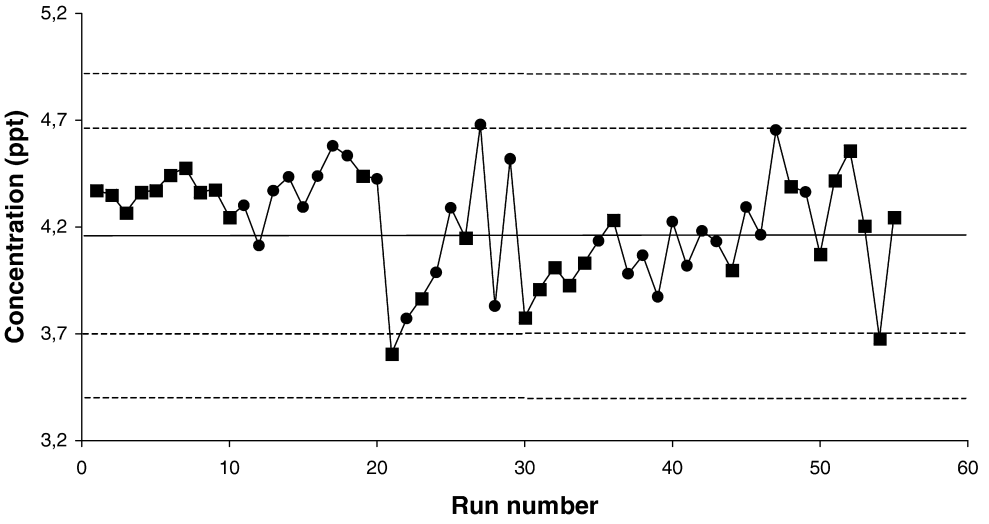

Fig. 5. Quality control chart for PCDD/Fs in fortified beef fat (pool 2); ■ represent HCDS values and ● represent GPC values.

taining both PCDD/Fs and cPCBs (pool 2). Concerning repeatability, the 10 HCDS results illustrated in the left part of Fig. 5 were obtained using QC processed on the same day by the same operator all the way to quantification. Due to the very low relative standard deviations (RSD=2–9%) observed, 95 (2σ) and 99% (3σ) control limits were set arbitrarily using a default RSD value of 20%. Since the QC pool was an ''in-house'' sample, the mean value was recalculated each time a new value was available. Concerning reproducibility, a quality control chart (Fig. 5, except the 10 HCDS repeatability values on the left) was built up over a 6-month period where both GPC and HCDS were used with different operators over time. The chart demonstrates the reproducibility of the HCDS columns against GPC. No samples were ever outside the 99% control limit.

3.3. Comparison between HCDS and GPC for real samples

Several types of food samples (poultry, eggs, mackerel fillet and dairy fat) were investigated during this study to determine the robustness and applicability of the proposed purification process. This was essential to demonstrate that, as for extraction, the same clean-up could be used independently of matrix type and fat content. All samples were extracted in order to produce 4 g of lipids, which were spiked prior to purification. Fig. 6 shows results expressed in TEQ for PCDD/Fs and cPCBs for the case of egg samples. Analyses were carried out on lipids extracted from yolk without any albumin treatment. No additional interferences were observed when the size exclusion process was replaced by the acidic treatment on silica column and a good correlation is observed between both types of lipids reduction.

If we look in more detail at congener-specific values (Table 4), it clearly appears that RSDs are generally lower for HCDS, which indicates a better reproducibility for this method. The difference in total PCDD/Fs values is a general tendency that has been observed during the evaluation study of this new type of disposable column. One reason that accounts for the generally higher GPC values for highly chlorinated congeners such as HpCDD/F and OCDD/F is the reusable character of the polymer beads constituting the column. Even if a decontamination step can be added in the procedure, the use of the same solvent as for the separation run is absolutely necessary to preserve the column properties, which prevent the use of stronger decontamination solvents. In the conditions used here, blanks were always higher for GPC (HpCDD≤1 pg, OCDD≤5 pg, OCDF≤1 pg, PCB 77≤30 pg) than for HCDS (OCDD≤1 pg and PCB 77≤5 pg/g). It is clear that

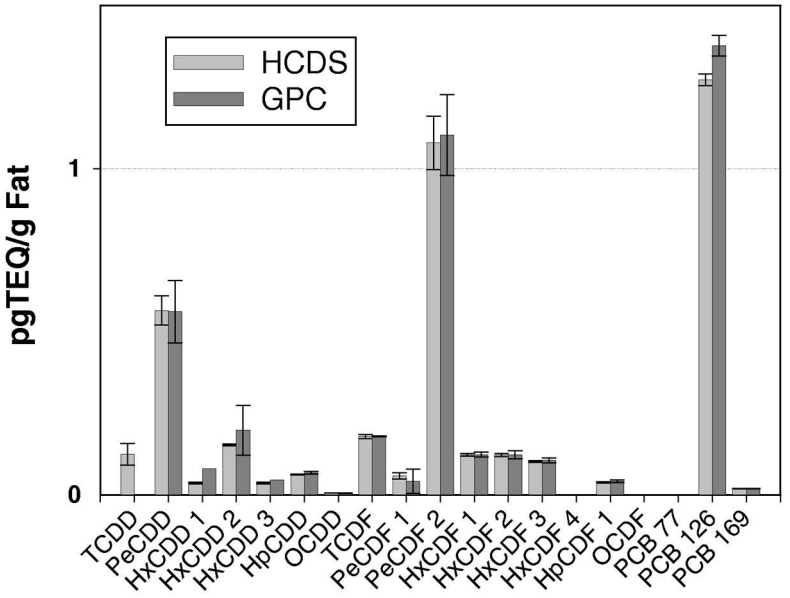

Fig. 6. HCDS vs. GPC results for real egg (yolk) samples.

Table 4
Congener-specific values for PCDD/Fs and cPCBs in poultry sample. Results obtained with different operators over the time

Method compounds	HCDS (n=3)			GPC (n=3)		
	Conc. (pg/g fat)	RSD (%)	Recovery (%)	Conc. (pg/g fat)	RSD (%)	Recovery (%)
TCDD	0.08	11.97	70	0.12	2.15	74
PeCDD	0.23	5.56	85	0.22	35.15	74
HxCDD 1	0.10	3.25	62	0.14	6.47	73
HxCDD 2	0.30	1.73	64	0.45	10.29	66
HxCDD 3	0.05	7.26	66	0.07	10.27	68
HpCDD	0.24	13.54	99	0.44	46.90	67
OCDD	0.15	27.68	66	interference	interference	59
TCDF	1.62	2.01	101	1.83	5.36	84
PeCDF 1	0.44	1.96	88	0.54	6.94	78
PeCDF 2	2.31	10.68	87	2.27	8.03	66
HxCDF 1	0.43	3.36	72	0.71	10.59	79
HxCDF 2	0.20	7.18	73	0.29	9.07	77
HxCDF 3	0.04	9.19	70	0.18	22.30	78
HxCDF 4	nd.	nd.	68	nd.	nd.	76
HpCDF 1	0.04	21.59	112	0.18	17.95	75
OCDF	0.04	86.60	69	0.25	95.54	73
Total PCDD/Fs	**6.29**			**7.68**		
PCB 77	<LOQ	<LOQ	91	<LOQ	<LOQ	87
PCB 126	21.33	0.97	83.55	21.95	22.07	76
PCB 169	2.28	1.76	72.5	2.22	40.73	72
Total cPCBs	**23.61**			**24.17**		

the risk of cross-contamination is minimized using the disposable silica columns after a simple wash of the sample preparation apparatus [27]. It is also interesting to note that the difference becomes negligible when the amounts are represented in TEQ (1.76 pg TEQ/g fat and 1.60 pg TEQ/g fat in PCDD/Fs for HCDS and GPC, respectively). This re-equilibration in favor of HCDS translates the derisory effect of the concentrations of the more chlorinated isomers on the TEQ.

During the study, sperm whale blubber was also included in the analysis scheme to measure the effect of a highly-contaminated biological matrix on the background level of the purification tool. In that case, no noticeable effect was observed and the only remaining traces of contamination were OCDD and PCB 77 at the same levels reported above. PCB 77 is always present in background, mainly due to solvent contamination and this baseline level is responsible for the high LOQ for this congener [39].

Results obtained for a variety of different matrices indicate the strong correlation existing between both types of lipid elimination procedures (Table 5). For both PCDD/Fs and cPCBs, recovery yields are usually the same or higher for HCDS, except for mackerel where rates decrease. This seems to be due to a higher reactivity of the mackerel extract towards the acidic silica, which leads to a saturation of the column. This problem is overcome by decreasing the quantity of lipids loaded on the column, which is allowed since contamination levels are higher for this type of marine matrix. Anyway, even in the case of 4 g, recovery rates are still in good agreement (>50%) with requirements of admitted procedures and permit quantification in good conditions.

Regarding analysis by GC–IDHRMS, none of the investigated matrices produced any significant differences in chromatograms. Signal-to-noise ratios remain very good and limits of detection (LODs) did not change at all. After several months and hundreds of samples analyzed using the HCDS as clean-up tool, no additional gas chromatography column degradation or liner clogging were encountered. The mass spectrometer ion source was kept in good

Table 5
Comparison of PCDD/Fs and cPCBs values using HCDS and GPC with different biological matrices

Matrices	Concentrations (pg TEQ/g fat)		Recovery (%)	
	PCDD/Fs	cPCBs	PCDD/Fs	cPCBs
Poultry (n=3)				
GPC (%RSD)	1.6 (13)	2.2 (22)	75 (12)	79 (17)
HCDS (%RSD)	1.8 (3)	2.2 (1)	80 (10)	82 (2)
% HCDS vs. GPC	113	100	107	104
Eggs (n=3)				
GPC (%RSD)	2.6 (10)	1.4 (2)	63 (13)	69 (20)
HCDS (%RSD)	2.6 (2)	1.3 (1)	62 (26)	69 (39)
% HCDS vs. GPC	100	93	96	100
Dairy fat (n=3)				
GPC (%RSD)	0.4 (6)	0.7 (3)	66 (10)	56 (7)
HCDS (%RSD)	0.5 (4)	0.6 (2)	90 (15)	86 (18)
% HCDS vs. GPC	125	86	136	154
Mackerel (n=3)				
GPC (%RSD)	49.5 (1)	250.6 (3)	77 (12)	98 (12)
HCDS (%RSD)	52.2 (7)	260.1 (7)	44 (57)	71 (60)
% HCDS vs. GPC	105	104	57	72
Sperm whale (n=3)				
GPC (%RSD)	299.7 (9)	374.8 (5)	69 (34)	45 (97)
HCDS (%RSD)	271.9 (2)	350.9 (4)	91 (22)	110 (20)
% HCDS vs. GPC	91	94	132	244

condition during the entire time without any additional cleaning-up downtime. An example of a typical chromatogram obtained using HCDS clean-up for a QC (pool 2) sample is presented in Fig. 7.

3.4. Practical aspects

From the point of view of the solvent consumption, the reduction is sensible noticeable**.** The use of around 300 ml of ethyl acetate–cyclohexane (1:1) (including column conditioning and wash) per sample for GPC is replaced by 150 ml of hexane during the automate sequence of steps. This has the effect to reduce the solvent manipulations and to avoid the evaporation step necessary to remove the GPC solvent prior to Power-Prep clean-up. This step takes more than 1 h and is critical due to the necessity of removing all traces of the ethyl acetate–cyclohexane mixture before re-dissolution of the extract in the multi-columns suitable solvent (hexane).

In addition to the reduction of personnel training and maintenance, suppression of the entire GPC step allows the operator to focus all attention to the remaining parts of the analysis. The return on investment of the Power-Prep system apparatus is then increased, even if the price per sample is roughly the same. Duration of the clean-up step for high fat content biological matrices is reduced by half and the sample capacity increased drastically. Once the time-limiting step has been removed, global analysis time can be greatly reduced and the sample throughput is then greatly improved. The proposed analysis scheme allows the analysis of up to 10 samples in 48 h when freeze drying and accelerated solvent extraction can be carried out automatically during the night (Fig. 8).

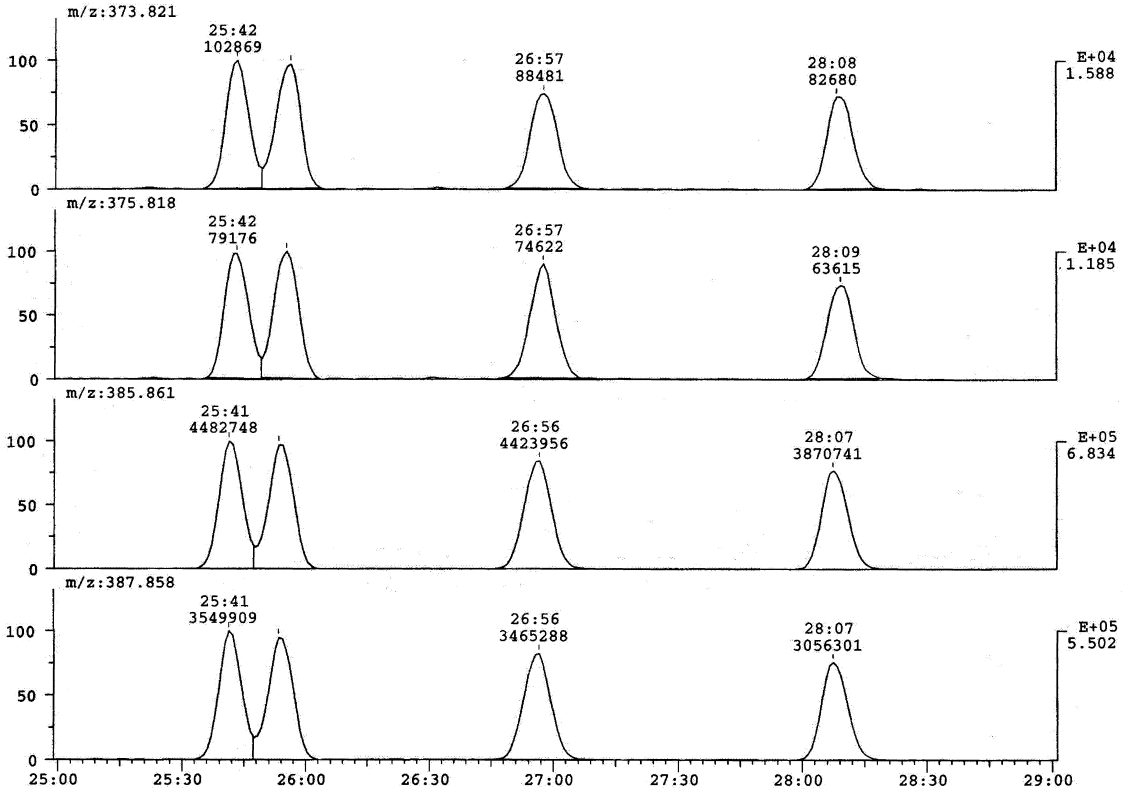

Fig. 7. Typical GC–IDHRMS chromatogram obtained for the four HxCDFs congeners after a Power-Prep clean-up of 4 g beef fat (QC pool 2) using HCDS columns. Both native (m/z: 373.821 and m/z: 375.818) and labelled (m/z: 385.861 and m/z: 387.858) compounds are represented. Time scale in min.

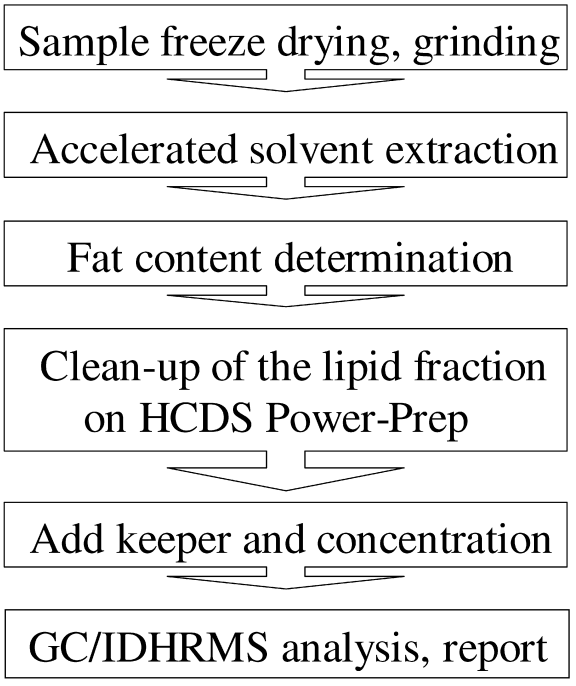

Fig. 8. Proposed analysis scheme for high-fat-content biological matrices.

4. Conclusions

A new, fast clean-up for high-fat content biological samples has been proven to be suitable for high-throughput analysis of PCDD/Fs and cPCBs. Use of HCDS columns overcomes the need for a time-limiting GPC run before the automated clean-up using the Power-Prep system. Deleting the entire GPC step results in a single, automated clean-up step between extracted lipids (up to 4 g) and the evaporation before GC–IDHRMS injection. Reasonable cost, high efficiency, rapidity, as well as the broad range of compatible matrices, present the proposed analysis scheme as a powerful tool for large numbers of sample analyses often required in monitoring programs. Moreover, total analysis time could be reduced further by optimizing the final evaporation step prior to GC–MS injection by using a large-volume injection technique [40]. Research is being conducted to include additional PCBs (seven markers as well as mono-ortho) in the analysis [39], and to replace the current GC–HRMS method by an alternative programmable temperature vaporization–large volume injection system coupled to GC–ion trap tandem in time mass spectrometry [41].

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