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New approach for removing co-extracted lipids before mass spectrometry measurement of persistent of organic pollutants (POPs) in foods

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HIGHLIGHTS

- New approaches for removing fat from food extracts for POPs.
- An automated approach and a manual approach are comparable.
- Quality criteria acceptable for spikes and proficiency samples.

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ABSTRACT

Persistent organic pollutants (POPs) methods for foods and animal feeds require sufficient sample intake followed by an extensive removal of interfering matrix components and concentration before a gas chromatographic mass spectrometry (GC-MS) method can be applied. The extraction dissolves associated lipids in animal foods or feeds. Methods must eliminate all co-extracted lipids before determination by GC-MS. A new approach for removing lipids is presented using basic silica gel or metal ion immobilized silica gel (Ag+) in a single step. Absorbent order, adsorbent amounts, and flow rates were found to be essential for consistent results. KOH/silica gel or Ag^+ ion ($AgNO_3$) silica gel were both shown to retain 75–85% of the co-extracted lipids without using sulfuric acid. KOH/silica gel method applied to butter fortified at 7.3 pg TEQ/g lipid with polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs) produced accurate results for all fortified congeners with 20% of predicted (n = 6). Ag^+ silica gel incorporated into the Miura GO-EHT automated system produced similar results fortified at 3 pg TEQ/g lipid. During PCDD/F fortifications of butter with PCDD/Fs (n = 6), labeled standard recoveries for PCDD/Fs and planar polychlorinated biphenyls (PCBs) were all acceptable (52–99%) averaging 77% using the Miura system. A reduction in the amounts of sulfuric acid silica gel needed was possible in the completion of co-extractant removal. PCDD/F spikes into butter and for a spiked sunflower oil (PCDD/Fs and coplanar PCBs) were within ± 20% of the predicted using the Miura system; suitable for current methods criteria for foods including criteria in EU legislation.

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1. Introduction

POPs are environmental pollutants produced through diverse sources, both human-made and naturally produced (Schmittle et al., 1958, Ferrairo et al., 2000, Hayward et al., 1999a). They persist in the environment and bio-accumulate in the food chain. POPs such as polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs) produce multiple toxic endpoints in animals and humans (Higginbotham et al., 1968, IARC, 1997). Animal derived foods account for >95% of the non-occupational exposure of these POPs to humans (Travis and Hattemer-Frey, 1991). Animal based foods have periodically become contaminated starting with the early discovery of PCDDs in chickens and chicken feed during a 1957 feed contamination episode in several states in the USA (Schmittle et al., 1958). Subsequent feed contamination has occurred, however infrequently (Bernard et al., 2002, Hayward et al., 1999a; Heres et al., 2010, Hoogenboom et al., 2015,

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Hoogenboom and Traag, 2003; Malisch, 2000; Rappe et al., 1998. Animal based foods and their associated feed components are a logical focus for monitoring efforts (Malisch and Kotz, 2014).

Methods developed for preparation of foods and feeds have been reviewed periodically (Firestone, 1991; Focant et al., 2004; Reiner, 2016) and compared for their effectiveness (Brumley et al., 1981) and quantitative reliability (Stephens et al., 1992). The methods often use combinations of polar and/or nonpolar solvents or acid digestion with solvent extraction followed by acid/base and neutral columns for co-extractant removal and fractionation with alumina, florisil and/or carbon (Brumley et al., 1981; Firestone, 1991; Stephens et al., 1992; Reiner, 2016). Commonly employed materials and methodological approaches were compared by Brumley et al. (1981) in a study of six laboratories each using a different method. One option not used in Brumley et al. (1981) was gel permeation chromatography (GPC) examined in detail by Focant et al. (2001) during a discussion of an automated POPs extract preparation system.

The first step in food preparation methods after extraction is the removal of the lipids/co-extractants as completely possible. US EPA methods use mineral acids and bases in two approaches for the removal of lipids/co-extractants in the human fat, fish or other environmental matrices (EPA 8290 1992; EPA 1613 1994; EPA 1668C 2010; EPA 1614 2007). One approach uses concentrated sulfuric acid which is mixed with a hexane dissolved extract to react with lipids. Spent acid is removed and fresh acid added until all lipids are removed. The second approach uses glass columns containing top to bottom neutral silica gel, acid silica gel, neutral silica, basic silica gel and neutral silica. The hexane dissolved extract is transferred to the column and eluted.

Modified silica gels described in EPA methods, the amounts and order of packing columns are identical to what is described by Langorst and Shadoff (1980) for one column described by their method. This description is identical in all EPA POPs oriented methods from 1990 onwards (8280A, 8290, 1613, 1668, 1614). The relevant section of EPA 1613 always has the sulfuric acid silica gel as the top bed with much lesser amounts of NaOH silica gel and neutral silica at the bottom. Langorst and Shadoff also describe a silver nitrate/silica column (not included in EPA methods) and an alumina column. Automated systems (Focant et al., 2001) use a similar type lipid removal column as their first stage of food extract preparation. Automated systems often offer different sized columns with increased or decreased amounts of modified silica gels intended to accommodate foods/other matrices with greater or lesser co-extractant amounts.

The EPA methods also describe additional procedures for co-extractant removal EPA 1613 revision B (1994). They include HPLC or GPC systems and are quite effective for removing lipophilic components (Firestone, 1991; Focant et al., 2001; Hayward and Pisano, 2006, Hoh et al., 2008). GPC is somewhat limited in their lipid removal capacity in a single step (Focant et al., 2001). GPC systems process extracts sequentially such that improved throughput would require the use of several systems at once; a rather cumbersome arrangement. It is, therefore, not surprising that most automated systems rely on sulfuric acid modified silica columns as the first step in purifying food or other matrix extracts.

Carbon columns are also useful in direct clean-up of high fat foods, either after GPC Hoh et al. (2008) or without prior GPC clean-up (Kedikoglou et al., 2018, Shelepchikov et al., 2019) for PCDD/Fs and PCBs. Carbon materials such as Carboxen 1000 and AX-21 have been used to eliminate co-extractants directly in dual layer carbon columns reducing the amounts of solvent and absorbents required Shelepchikov et al. (2019). In an updated method described first by Liem et al. (1990), a replacement for Carbosphere (FU 4652) was tested successfully for direct clean-up of up to 10 g fat (Kedikoglou et al., 2018). AX-21 carbon was also the basis for fat removal in older methods (Smith et al., 1984) and newer methods (Shelepchikov et al., 2019).

A fully automated system from extraction to final sample volume has long been desired for POPs preparation owing to the need for a moderately large sample intake, low detection limits, multiple solvent consuming purification steps and concentration to small volumes. Systems have been proposed and commercialized such as the FMS PowerPrep™ that has been implemented in laboratories. Automatable approaches for extraction and clean-up have been reviewed by Focant et al. (2004). The integrated and automated extraction could use SPE columns for liquids and pressurized fluid extractor (PFE) for solids coupled to a PowerPrep™ (Focant et al., 2004).

Another approach described by Focant et al. (2004), involves using a pressurized fluid extractor with acid silica gel in the extraction cell as a fat retainer. Focant et al. (2004) suggested that this process could unduly wear on the cells and frits. This approach with acid silica was successfully tested with food and feeds (Wiberg et al., 2007). PFE purified extracts were applied to a carbon column Wiberg et al., (2007). PFE extraction with an acid a fat retainer was found useful by Hayward and Pisano (2006). The PFE step was followed by GPC coupled directly to a carbon SPE (Hayward and Pisano, 2006). PFE worked well for any food or feed so along as a GPC step was used between the PFE and final SPE column. GPC was also employed with fish oils in combination with SPE carbon by Hoh et al. (2008).

In this study, the performance of a new approach for eliminating the co-extractants from high lipid foods is described that uses modified absorbents in a different order, amounts with control of column flow rate using hexane solvent in limited amounts for elution of PCDD/Fs, PCBs and PBDEs. The approach reproducibly provides final extracts that present few issues to gas chromatography and the MS measurement technique and is suitable for an automated flow path. A similar approach has been reported successfully on a smaller scale using 0.5 g amounts of pork fat, sunflower and fish oil (Shelepchikov et al., 2018).

2. Materials and Methods

Materials: PCDD/F and PCB standards were purchased from Cambridge Isotope Laboratories Inc, (CIL), Tewksbury, MA, USA. CIL mixtures were EDF-9999-1-5, EC-4187, EC-4986 (planar PCBs), CIL-4058, CIL-5179 (marker PCBs), EDF-8999 (labeled PCDD/Fs), and EDF-5999 (recovery standard), EDF-7999 (precision and recovery; PAR). Other mixtures of PCBs and polybrominated diphenyl ethers (PBDEs) were from Wellington Laboratories Inc., Guelph, Ontario were BP-MO, MBP-MO (coplanar PCBs) and MXFS (labeled BDEs), BDE-MXF (BDEs), MXFR (recovery BDE). All solvents were high purity HPLC grade or pesticide grade: hexane, toluene, dichloromethane (DCM), ethyl acetate (Fisher Scientific, Fair Lane, NJ), tert-butyl methyl ether (ACROS Chemicals Geel, Belgium). Silver nitrate silica gel, Wako Pure Chemicals Industries, Japan. KOH/silica gel was prepared as described in US EPA 8290 and 1613 for NaOH silica gel. Nonane and silica gel 60 70–230 mesh were from Sigma-Aldrich, St Louis, MO, USA and Alumina B Activity Super I MP EcoChrom™ was from MP Biomedicals, Santa Ana CA, USA. Acid silica gel was prepared as per US EPA 8290 and 1613 for 40% sulfuric acid silica gel. Reference materials used were from the National Institute of Standards and Technology (NIST) SRM 1953 human milk, salmon muscle homogenate for the Norwegian Institute of Public Health (NIPH) and PCDD/F and PCB fortified sunflower oil was prepared by Rikilt, Wageningen UR, the Netherlands.

Extraction: Butter test portions were melted at 45 °C on water bath then combined with 10–15 mL hexane and sunflower oil was

dissolved in hexane directly. If a butter aliquot (3.8 g) was fortified with PCDD/Fs, then 10 or 25 μL of a 0.4 μg/L solution in nonane (4 pg or 10 pg) was added into the melted and dissolved butter solution before column chromatography. The extracts in hexane solution were dried over sodium sulfate before applying to the Miura system (Method option 2) or were placed directly into a Kontes column 5 cm × 30 cm (Kontes Glass, Vineland NJ) which already contained sodium sulfate with 40 g KOH/silica gel for retaining >70% of the co-extractants (Method option 1). Human milk and fish were extracted as described previously Hayward et al. (1989), Hayward et al., 1999b

Manual lipid removal (option 1): Butter aliquots (3.8g) were melted at 45 °C water bath before adding 50 mL hexane and all 17 2,3,7,8-PCDD/Fs (for native spikes) followed by 15 2,3,7,8−$^{13}C_{12}$ labeled PCDD/Fs (50 pg each), 14 $^{13}C_{12}$ PCB congeners and 7 $^{13}C_{12}$ PBDE congeners (500 pg each). The butter dissolved in 50 mL of hexane was applied to a 5 cm × 30 cm Chromflex column made by Kontes Glass, (Vineland, NJ, USA) containing 40 g KOH/silica gel made by mixing 30 g 1 N KOH with 100 g silica gel. The butter extracts (50 mL hexane) were passed through the KOH/silica gel column connected directly to an AX21 carbon column Hayward et al. (1989), Smith et al. (1984) followed by 50 mL hexane. Fraction one (PCBs and PBDEs) from the carbon column (remaining lipid containing fraction) was applied to a 1.5 cm × 30 cm column packed with sodium sulfate, 2g silica gel, 12 g 40% sulfuric acid silica gel, sodium sulfate. The column was washed with 25 mL hexane, then the sample was applied in 3 x 1 mL hexane and eluted with 50 mL hexane. No effort was made to measure or control the flow rate of hexane through any column which were either gravity eluted or assisted with nitrogen gas. Both the PCDD/F and PCB fractions were submitted to alumina column as previously described (Hayward et al., 1989).

Automation development: A series of experiments were conducted to test configurations that might be used in an automated single flow path. The KOH/silica gel from option 1 was combined with an acid/silica gel layer (20−40g) in a single column. The two modified silicas were placed in a 2.5 cm × 30 cm glass column with a stop cock for controlling flow rates. The 40% sulfuric acid silica layer was increased initially to 20 g and place at the bottom while 20 g KOH/silica was placed on the top followed by sodium sulfate. The column was washed with 50 mL hexane and then the butter dissolved in 10 mL hexane was applied. Columns were eluted with 100 mL of hexane (or 150 mL for PBDEs as well) and were gravity eluted and flow rates were not controlled or measured initially. The hexane elution was then evaporated and applied in hexane to a pipet containing acid silica (check lipid removal) eluting directly into a pipet containing 18% Carbopack C on silica gel eluting into an alumina column (Hayward et al., 1989) to separate dioxins from PCBs and PBDEs. After transfer with hexane the PCDD/Fs and PCBs/PBDEs were eluted separately from the carbon and alumina columns using 20 mL toluene and 4 DCM, respectively. All experiments used 3.8 g unsalted butter (~3g lipid). Note: these columns were used to assess lipid removal qualitatively (unlike Table 1), labeled standard recoveries and the quality of the GC chromatography. The columns were not used to form the methods used with native spikes to butter (method options 1), PT samples of oil (method option 2), salmon (method options 1&2) or NIST SRM human milk (method option 1). AgNO3 silica gel was also tested in place of KOH/silica gel, because it was part of the Miura automated system which we planned to test if it became available to us.

Automated lipid removal (option 2): An automated, fully independent system under computer control using a series of four disposable columns to prepare food extracts after being exchanged into hexane. The system (GO-EHT-2) was obtained from DSP systems, XR Ede, The Netherlands. The automated system is made by the Miura Company LTD, 7 Horie, Matsuyama, Ehime 799−2696, Japan. The Miura system uses a silver nitrate silica gel (AgNO3 silica gel) column, connected to a sulfuric acid on silica gel column, which is then directly connected to a carbon column, followed by an alumina column. Test portion extracts are exchanged to 10−15 mL of hexane and loaded into the first column, silver nitrate on silica gel ($AgNO_3$ silica), then the remaining columns are connected, and all four columns are placed into the automated system. When analyzing only PCDD/Fs and PCBs, 90 mL of hexane is pumped through all four columns with the first two modified silica columns set at 60 °C while the carbon and alumina are not heated. Then, the temperature of the carbon and alumina columns is raised to 90 °C, then 1−1.5 mL of toluene elutes in the reverse direction, first the alumina and then the carbon column. Toluene elutes into a 2.2 mL autosampler vial. The Miura system was employed as directed by the manufacturer developed methods with changes for PCDD/F spiked unsalted butter, a PT sunflower oil (PCDD/F and PCBs), and PT fish sample (PCBs).

Safety: PCDD/Fs and PCBs are highly toxic, carcinogenic and should be handled to avoid skin contact and inhalation of dust or aerosols. Internal standard amounts in butter were 3x the daily allowable intake from food.

GC-Orbital Trapping Mass Spectrometry (QE-GC): Butter, human milk, salmon and vegetable oil extracts were measured using a TRACE 1300 GC interfaced to a Q-Exactive™ (Orbitrap™) mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). A 40 m × 0.18 mm ID DB-5ms column (Agilent Santa Clara, CA, USA) was used Hayward et al. (2018). The same temperature program and column was used for the determination for PCDD/Fs, PCBs and PBDEs. The GC was programmed from 120 °C for 2 min, 20 °C/min. to 200 °C, 5 °C/min to 240 °C, 240 °C for 12 min, 10 °C/min. to 280 °C and 280 °C for 10 min with a total run time of 39 min. PCDD/Fs and PCBs (all PBDEs) were injected splitless from separate fractions as would be the case with EPA methods (separate methods). PCDD/Fs were calibrated as previously described Hayward et al. (2018). In brief, all POPs were acquired in the QE-GC using full MS SIM mode operating at 120,000 resolution with automatic gain control with

Table 1
Percent lipid retention from 3.8 g unsalted butter by elution solvent, volume, absorbent type, amounts and the percent recovery range for $^{13}C_{12}$ spikes (50 pg PCDD/Fs) (500 pg PCB or 500 pg BDEs). AlOX = 5% deactivated alumina; KOH = KOH/silica; DCM = dichloromethane; ETAC = ethyl acetate; NA = not applicable. All test column configurations were performed once, except the KOH silica gel with 100 mL hexane (2 tests shown with internal standard recovery ranges).

Solvent	mL	Absorbent	amounts(g)	% retained	PCDD/F%	PCB%	BDE%
Hexane	100	AlOX/KOH	30/15	84	34−67	37−50	NA
Hexane	100	AlOX	30	30	27−65	20−30	NA
Hexane	200	AlOX/KOH	30/15	50	48−88	46−53	NA
Hexane 20% DCM	100	AlOX/KOH	30/15	47	66−86	65−80	NA
Hexane	100	Activated silica gel	40	98	4.5−28	1.1−9.1	NA
Hexane	100	KOH	40	78	63−91	45−101	51−77
Hexane	100	KOH	40	83	50−80	59−118	66−103
Hexane 0.63% ETAC	120	$AgNO_3$	15	85	66−98	120−173	74−126

default settings for tuning except a −2 V offset to the C-trap. Either 3 or 6 quadrupole mass windows were used 3 for PCBs/PBDEs and 6 for PCDD/Fs (PCDD/Fs; 285–340, 300–340, 335–354, 335–380, 369–410, 435–480 *m*/z). No lock mass was ever used. PCB and PBDE calibration curves were constructed separately with five concentrations between 10 and 1000 ng/mL and 20 ng/mL for $^{13}C_{12}$ labeled PCB and PBDE standards. The two curves were combined 1/1 to provide a final concentration of 5–500 ng/mL (1 ng/mL for coplanar PCBs) and 10 ng/mL for the labeled and then used to calibrate the QE-GC.

Quality Assurance: The Q-Exactive™ was calibrated daily using perfluorinated tributyl amine (FC-43). All food measurements were completed in duplicate on separate days. Unfortified butter amounts were subtracted from fortified butters. Blanks were processed when needed, however the unfortified butter was analyzed frequently before any spikes or reference materials were attempted. Method blanks were always not detected for any of the spiked compounds with the exception of a small amount of octachlorodibenzo-p-dioxin usually <1 pg (<5–10% of the spiked amount low or high) during the development of method option 1 (the method developed in our laboratory as oppose to option 2 a vendor supplied method). Q-Exactive™ files were processed in TraceFinder™ version 3.3.

3. Results and discussion

Option 1 development: A liquid chromatography column containing the absorbents (Table 1) aided with low pressurized nitrogen assisted elution was used testing the efficiency of selected absorbents at retaining lipids from unsalted butter dissolved in hexane. Deactivated alumina, KOH silica gel, activated silica gel or 10% $AgNO_3$ silica were combined or tested singly for lipid retention. Elution profiles were also varied (Table 1). KOH/silica was initially used in 15 g amounts in combination with 30 g deactivated alumina. The objective was to absorb as much of the lipids as possible, preferably >70%. Table 1 contains the results from the initial single tests and the range of recoveries for $^{13}C_{12}$ labeled standards for PCDD/Fs (15 congeners), PCBs (dioxin-like PCBs and marker PCBs) and PBDEs (7 congeners) when added.

Table 1 results suggest that the fat retention is affected by absorbent type and elution profile. The absorbents were placed in a 5 cm × 30 cm Kontes glass column which is part of our previous methods (Hayward et al., 1999b). Absorbent amounts and elution profile in any step are constrained to some extent by the need to remove co-extractants, but also to effectively elute analytes. Absorbent types and elution profiles which failed to produce at least 70% retention of the butter fat applied and gave adequate analyte recovery were not tested further (Table 1).

$AgNO_3$ silica was not tested initially but was tested later just before combining adsorbents into a single column. The AgNO3 was placed in the smaller 2.5 cm × 30 cm column using an amount thought to be similar to what is used in the Miura system. Lipid retention and analyte recoveries were the highest with either KOH/silica or $AgNO_3$ silica and a hexane eluting volume of 100 mL hexane (or 120 mL >0.63% ethyl acetate in hexane for AgNO3) (Table 1).

Option 1 would use 40 g KOH silica with 100 mL hexane elution (PCDD/Fs and PCBs only). The PCB/BDE fractions were further processed to remove residual lipids on a 1.5 cm × 30 cm column with 12 g 40% sulfuric acid silica gel eluting it with 50 mL hexane. Both the PCDD/F and PCB fraction clean-ups were completed by applying the extract to an alumina column. Native spikes were conducted with only the PCDD/Fs (2 pg TCDD/TCDF, 10 pg all other PCDD/Fs and 20 pg OCDD/F) into 3.8 g unsalted butter. Table 2 provides the results from these spikes. The concentrations are all well within 20%, however for two congeners (OCDD/F) mean results were lower by 20% than predicted. Fig. 1 illustrates the chromatography obtained from option 1 processing an unfortified dairy sample.

Table 2
Accuracy measurements (% of spike) for PCDD/Fs fortified in unsalted butter at 7.3 pg TEQ/g lipid using the KOH silica column for clean-up (Option 1). Means are corrected for incurred butter levels. PAR (precision and recovery standard) lot analysis results by Cambridge Isotopes Laboratories are shown for PAR lot used (200 ng/mL). CIL standard acceptance is ± 10%.

LOL butter n = 6 Congener	Means	Bkg % of spike	Bkg sub means	Sd	CIL means	sd
2,3,7,8-TCDD	103	1.5	101	5.5	99	2.9
1,2,3,7,8-PeCDD	98	2.1	96	4.1	97	4.8
1,2,3,4,7,8-HxCDD	96	2.0	94	0.6	99	5.1
1,2,3,6,7,8-HxCDD	101	7.3	93	2.7	94	5.3
1,2,3,7,8,9-HxCDD	94	2.5	92	3.3	95	3.6
1,2,3,4,6,7,8-HpCDD	110	17	93	5.3	99	5.4
OCDD	93	12	80	7.1	101	4.2
Mean PCDDs	99		93	4.1	98	
2,3,7,8-TCDF	96	1.3	94	5.3	94	4.2
1,2,3,7,8-PeCDF	89		89	3.2	96	3.5
2,3,4,7,8-PeCDF	94	1.5	92	2.5	93	5.4
1,2,3,4,7,8-HxCDF	93	1.7	91	3.2	95	3.5
1,2,3,6,7,8-HxCDF	90	1.6	89	2.3	94	3.4
2,3,4,6,7,8-HxCDF	92	2.1	90	4.3	96	4.9
1,2,3,7,8,9-HxCDF	88		88	3.9	96	3.4
1,2,3,4,6,7,8-HpCDF	87	4.4	87	4.6	100	3.5
1,2,3,4,7,8,9-HpCDF	89		89	3.9	95	4.5
OCDF	80		80	4.5	98	3.1
Mean PCDFs	90		89	3.8	96	
Mean All	94		91	3.9	96.6	
PCDD/F TEQ pg			21.5		21.6	

While option 1 works well preparing butter for GC-MS, an automated system would connect all the columns together in a single sequence for increased efficiency. As a start for developing an automated system, we combined the basic and acid silica beds into a single column. A column combining KOH/silica and acid/silica would need to remove all the co-extractants or least enough not to affect GC chromatography and MS measurement in order for it to be directly connected to the next column in the flow path. The eluting hexane from the combined column could be passed directly into a carbon or alumina column (Fig. 2) as is the case with automated systems (Focant et al., 2004; Marchand et al., 2014; Reiner, 2016).

Fig. 2 using 20 g KOH/silica (top layer) and 20 g acid silica (bottom layer) illustrates a successful removal of fat from 3.8 g butter extract. The acid silica pipet column on the right used after is free of charring (Fig. 2) indicating the acid silica has removed all lipid that could be retained from butter aliquot. Subsequent GC chromatography and internal standard responses confirmed the extract could be measured and meet all performance criteria. While these results were encouraging, they were not uniformly reproduced in that more often than not co-extractants were not completely removed.

Fig. 3 illustrates examples of this problem. The column on the left in Fig. 3 visually appear to have absorbed all the lipids while the pipet columns to the right used to test the eluate show lipids were still present in the eluted hexane. These results were obtained often such that the procedure needed to be modified. We experimented with doubling the size of the acid silica absorbent bed, but that did not eliminate the residual lipids in the hexane elution.

It was found during the use of a narrower column (2 cm) that the critical factor was the flow rate. Both KOH/silica gel and AgNO3/silica columns were used to process butter 4 times each with no control on the flow rate (stop cock open completely 2.5 cm ID column). All but three tests failed to remove the co-extractants

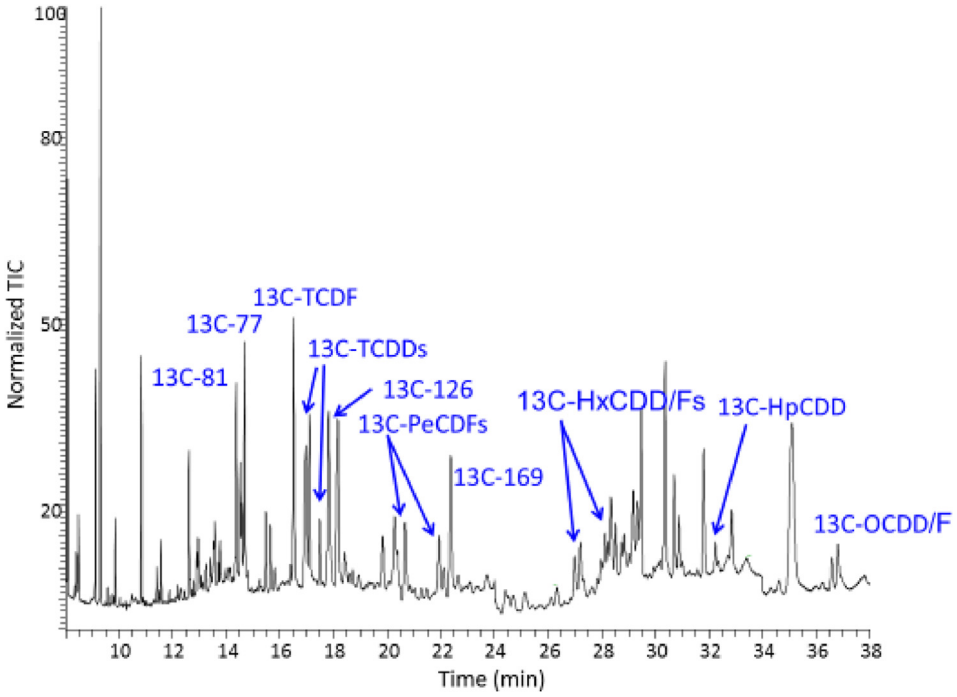

Fig. 1. Total ion current (TIC) for an unfortified dairy fat (3 g fat) processed using option 1. Responses for $^{13}C_{12}$-PCDD/F and PCB internal standards indicated by arrows. Standards fortified at 50 pg for PCDD/F and 125 pg for PCBs. Chromatograms show the response for approximately 7 or 20 pg for PCDD/F and PCBs, respectively, on column mass. All internal standards are easily observed in the TIC (sum of 285–340, 300–340, 335–354, 335–380, 369–410, 435–480 *m/z*). Note that larger TIC intensities are observed for the early eluting congeners relative to later eluting congeners owing to sharpness of the responses nevertheless good separation for all HxCDD/Fs was observed.

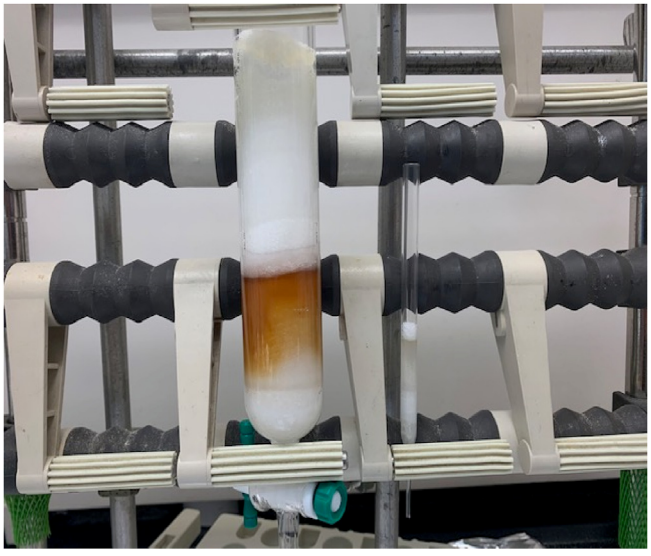

Fig. 2. A column combining 20 g KOH/silica top bed and 20 g 40% sulfuric acid silica gel bottom (left) just used for 3.8 g butter preparation eluted with 150 mL hexane in the processing of all target POPs. Note absence of charring in acid silica pipet column on the right used to process extract after the combined bed column.

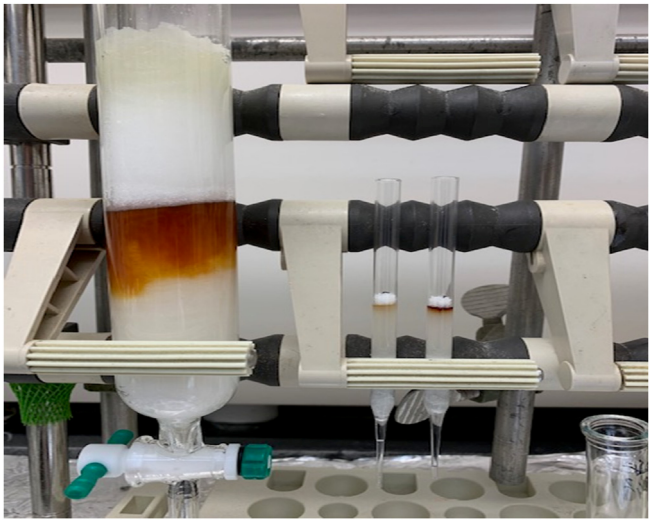

Fig. 3. A column combining two modified silica beds just used for the preparation 3.8 g butter. Top bed was 20 g KOH/silica with 40 g 40% sulfuric acid silica (bottom). The pipet column on the right shows charring in the acid layer before the final alumina column step blank pipet column is shown for comparison on left.

completely with the combined column only. If the flow rate was maintained at ≤2.5 mL/min, residuals lipid would not occur as was observed in Fig. 3. The flow rate was controlled easily with the Chemglass column by partly closing the stop-cock and measuring the flow at the start of elution. Aliquots of butter (3) and 10 g egg yolk extracts (2) showed no residual lipid after the combination column with either KOH/silica or AgNO3/silica when the flow rate was slowed to 2.5 mL/min. A stop-cock full open with a 2.5 cm × 30 cm column packed with 20 g of KOH silica and 20 g acid silica was later measured and found to run at ~5 mL/min. This problem was not observed in the initial tests with butter (4) or native spikes in butter (6) using option 1 when the two adsorbent materials were in different columns probably due to the second column with acid silica gel being of narrower diameter (1.5 cm) noticeably slowing the flow rate (Table 2).

Option 2: Recently companies have developed and marketed newly designed automated PCDD/F and PCB preparation systems

applicable for foods and other environmental samples. We acquired one such system that uses a procedure that is the closest to our new method. Based on our manual tests in development of our method this system should perform very well with foods. This system is manufactured by Miura company in Matsuyama, Japan and marketed by DSP-systems. This system uses a combination of $AgNO_3$ silica, followed by an acid silica column, then directly through a carbon column followed by an alumina column. The column bed order used by the Miura system is the same as we had been testing. While the Miura system does maintain the flow rates at or below 2.5 mL/min, the Miura also heats the lipid removal columns to 60 °C before and during elution with hexane presumably increasing the reaction rates. When reverse eluting through the carbon and alumina columns, those columns are heated to 90 °C degrees allowing use of a small solvent volume for each of the two obtained fractions (≤1.5 mL toluene) and still achieving the desired recovery.

The PCDD/Fs native spikes were repeated into unsalted butter using the Miura system except the amounts fortified were reduced to 4 pg/congener in a 3.8 g butter aliquot. Table 3 provides the results using the Miura system with PCDD/F spiked butter. Mean values measured on 6 replicate spikes for nearly all congeners were within 20% of the predicted and most <10%. The values for HpCDD and OCDD were higher and more variable due to recent laboratory background not present when spikes were done earlier with our manual method option 1. The TEQ calculated from the mean butter spike was within 2% of predicted and within 5.7% of the CIL standard calculations (Table 3). Labeled standard recoveries averaged 77% in butter spikes. The measurements of a fortified vegetable oil by the Miura system are provided in Table S1. The TEQs for PCDD/F and planar PCBs were within 4 or 12% of the consensus TEQ values. The Miura system provided results with 90% of measured congeners within 20% of reference values. The results confirm that the Miura system provides accurate results on PCDD/Fs and PCBs.

PCB and PBDE proficiency sample and SRM determinations: PCBs were determined in a salmon proficiency test sample from the Norwegian Institute of Public Health and PCBs and PBDEs in a NIST SRM 1953 (single determination for NIST 1953). Measurements were performed using a single GC-MS acquisition for PCBs/PBDEs and using the same GC column and temperature program used with the PCDD/Fs. A salmon fillet homogenate provided by Norwegian Institute of Public Health (NIPH) in 2018 was measured for PCBs. The PCB results are provided in Table 4 (measured once each with options 1 or 2). The PCB TEQs are within 4% and 14% of consensus using option 1 or option 2, respectively. Individual PCB congeners were mainly within 15% of the consensus except for marker PCB CB-180 was higher in both options. Higher values were measured on four mono-ortho dioxin-like PCBs (PCB 156, 157, 167 and 189 by options 1&2, but slightly lower on PCB 156 and 189 by option 1. All four of these PCB congeners were within 4–35% of consensus by both methods. These mono-ortho PCBs had concentrations between 10 and 100 pg/g with little impact on the PCB-TEQ (Table 4).

The NIST SRM 1953 human milk was also measured for 14 PCB congeners (Table S2) and for 9 PBDE congeners (Table S3) using option 1. PBDE congeners were within 1–9% of the certified values or within the uncertainty, except BDE-66 and 85. The 14 PCB congeners were all within 20% of the certified values and 11 were within 2–9% or within uncertainty (CB-126) (Table S2).

Conclusions: Automated sample preparation has been a goal for POP analytical methods and especially the preparation of food matrices for POPs determination owing to the many steps and large reagents amounts required. While the approach outlined here works well and will save time, solvent and reagent use, it cannot be viewed as universally comprehensive even if limited to only food matrices. Some foods such as certain oils or fish oil, certain feeds and vegetables may require an additional purification step for at least one fraction derived from the extract processing in order to maintain proper chromatography and sensitivity. Despite these concerns, this new approach will increase throughput for many important foods and feeds in a monitoring program.

Table 3
Mean concentrations and relative standard deviations for PCDD/F spikes in butter (0.8 pg TCDD/F, 4 pg PeCDD/F, HxCDD/F, HpCDD/Fs, 8 pg for OCDD/F) compared with Cambridge Isotope Laboratories (CIL) analysis of their precision and recovery standard lot used for spiking (200 ng/mL). Theoretical amounts are in the column on the far right. Miura spikes were corrected for incurred butter amounts.

	Miura		CIL		
	Means(pg)	RSD	Means	RSD	Theoretical
	n = 6				pg spiked
2,3,7,8-TCDD	0.96	5.2	0.79	2.9	0.8
1,2,3,7,8-PeCDD	4.0	4.7	3.9	4.8	4.0
1,2,3,4,7,8-HxCDD	4.0	3.1	4.0	5.1	4.0
1,2,3,6,7,8-HxCDD	4.1	2.8	3.8	5.3	4.0
1,2,3,7,8,9-HxCDD	4.0	4.7	3.8	3.6	4.0
1,2,3,4,6,7,8-HpCDD	5.9	33	4.0	5.4	4.0
OCDD	19.5	62	8.1	4.2	8.0
2,3,7,8-TCDF	0.93	4.7	0.75	4.2	0.8
1,2,3,7,8-PeCDF	3.8	4.2	3.8	3.5	4.0
2,3,4,7,8-PeCDF	3.9	3.7	3.7	5.4	4.0
1,2,3,4,7,8-HxCDF	3.9	2.3	3.8	3.5	4.0
1,2,3,6,7,8-HxCDF	4.0	4.2	3.8	3.4	4.0
2,3,4,6,7,8-HxCDF	4.0	3.9	3.8	4.9	4.0
1,2,3,7,8,9-HxCDF	3.8	4.0	3.8	3.4	4.0
1,2,3,4,6,7,8-HpCDF	4.3	5.3	4.0	3.5	4.0
1,2,3,4,7,8,9-HpCDF	3.8	2.6	3.8	4.5	4.0
OCDF	8.3	4.3	7.8	3.1	8.0
TEQ (pg spiked)	9.28		8.79		9.13

Table 4
PCBs measured in salmon proficiency sample Norwegian Institute of Public Health (NIPH) 2018. TEQs measured using option 1 or option 2 for PCBs are within 4 or 14% respectively using a single GC = MS acquisition for PCBs and PBDEs. Nearly all PCB congeners are within ±20% of the consensus values.

Congener	Option 1	Option 2	Consensus[a]	%df(1)	%df(2)
PCB-81	0.41	0.42	0.39	4	7
PCB-77	15.9	14.4	15	6	4
PCB-126	5.7	4.7	5.6	2	16
PCB-169	1.4	1.2	1.3	9	12
PCB-28	169	132	152	11	13
PCB-52	405	337	295	37	14
PCB-101	1020	984	856	20	15
PCB-105	305	298	266	15	15
PCB-114	17.3	16.5	16	8	3
PCB-118	856	831	766	12	9
PCB-123	13	9.4	9.8	33	4
PCB-138	1578	1416	1400	13	1.1
PCB-153	2104	2167	1859	13	17
PCB-156	96	125	100	4	25
PCB-157	32	27	24	33	13
PCB-167	65	60	54	21	11
PCB-180	694	1216	534	30	128
PCB-189	8	15	11	27	35
PCB-TEQ	**0.666**	**0.548**	**0.638**	**4**	**14**

[a] Median values reported to NIPH.

Disclaimer

Reference to any commercial materials, equipment, or process does not, in any way, constitute approval, endorsement, or recommendation by the US Food and Drug Administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.127023.

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