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Original Research

Determination of PCBs in Selected Components of a Food Chain by Means of SPE and GC/MS

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Abstract

The goal of this work was the isolation of PCBs from different environmental matrices by means of solid-phase (SPE) extraction and gas chromatography/mass spectrometry (GC/MS). The analyses include the determination of PCBs in fourteen samples of transformer oils and different kinds of fodders (for poultry, pigs, and cattle). The recovery rates for different congeners were on the level of 75-85%. The detection limits of five congeners of PCBs were in the range 85 to 130 μg/kg. The RSD was on the level of 2.7 – 4.4%. The Ishikawa diagram was used for evaluation of uncertainty.

Keywords: monitoring, PCBs, transformers oil, fodder, Ishikawa diagram

Introduction

Polychlorinated biphenyls (PCBs) constitute a group of 209 congeners but only fewer than 90 have been detected at significant concentrations in environmental matrices [1, 2]. Their production on an industrial scale was initiated in the late 1920s to be applied as dielectric fluid in transformers and condensers.

Despite their wide application in different branches of industry, their production was terminated and the study of their properties and influence on human organism was initiated. PCBs are very persistent chemicals due to thermal stability and resistance to biodegradation. Polychlorinated biphenyls are characterized by a large affinity to fats ($K_{ow} > 3$) as well as by great durability, which makes them an easy subject for bioaccumulation [3].These compounds were widely used in almost all branches of industry because they are characterized by good stability in various conditions, solubility in non-polar solvents, low pressure of vapours, low electric conductivity, high thermal conductivity and high resistance to chemical factors [4]. The monitoring of these pollutants in natural environment began when PCBs in environmental samples were discovered in the early 1970s. The materials containing more than 50 μg/g of total PCBs are subjected to strict regulations in the USA and other countries [5].

The main sources of emission into the environment are leakage from capacitors and transformers. Another potential source of PCBs can be uncontrolled storage altered or improper regeneration of transformer and motor oil [6, 7]. However, spontaneous chemical reaction from precursors containing chlorine, burning waste or whitening cellulose pulp by chlorine can be expected as a relatively low abundant source of PCB.The investigation of PCB properties led to their classification to group 2A, namely compounds that are probably carcinogenic, decrease immune activity systems, disrupt the development of the psychomotor system, disrupt hormone function and increase tumours, which decide negative influences on health and people's life [8]. The aforementioned compounds, due to their possible negative influence on humans, have found their place in legal regulations concerning their presence in different parts of the ecosystem.

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Waste oils containing PCBs have been short listed as hazardous substances in Poland since 1993. Available data have indicated that in national power plant installations about 1,400 t of transformer and capacitor oils are used [6]. However, well known incidents involving feed and food stuff contamination with organochlorine compounds have led to an increase in the control of PCB levels in food and feed products [9]. Legal regulations have established maximum residue levels of seven indicator isomers. These directives also call for routine analysis of large numbers of samples [10]. Therefore, authors can expect contamination of feeders by transformer oils which still contain PCBs. Plant and animal oils are added to fodders as a carrier for fat-soluble vitamins and also as one of the fundamental component of well balanced diet with proteins and carbohydrates. Other additives like vitamins, dietary fibers, and microelements include various by-products from nutritional industry. The fodder contamination illustrates the close correlation between the environment and food production.

An important part of any analytical process is sample preparation, which involves isolation and preconcentration of various analytes of interest. Guidelines of CEN (European Committee for Standardization) recommends different methodologies for extraction, clean up and analysis [11, 12]. The extraction procedures based on microwave-assisted extraction (MAE) [13, 14] supercritical fluid extraction (SFE) [15, 16], and accelerated solvent extraction (ASE) [17] are widely applied. It is well known that the clean up step is important before chromatographic analysis [18]. Additionally, many analytical protocols recommend clean up procedures. The SPE is a widely used pre-treatment method which has the property of removing interferences from a sample matrix. Therefore, this method based on dual sorbent bed: aryl sulfonic acid and silica gel (Ar-SO_3/SiOH) was used for cleaning sample extracts.

The objective of the present study was to determine the level of total PCBs in fodder and transformer oil samples collected from different regions of Poland. The determination of PCBs isolated from getting products, which are a part of human food chain state, is a very important problem for analytical chemists [19, 20].

Experimental

Materials and Reagents

The fourteen samples of transformer oils from the Factory of Transformer and Apparatus Traction in Warsaw and Meat Factory Morliny (Ostróda, Poland) were collected. The nine samples of commercially available fodder samples have been collected in the Polish market.

The n-hexane, methanol and isooctane were purchased from Fluka (Buchs, Switzerland). The chromatographic standards of PCB (2.4.4'-trichlorobifenyl, IUPAC No. 28; 2,2',5,5'-tetrachlorobifenyl IUPAC No. 52; 2,2',4,5,5'-pentachlorobifenyl IUPAC No. 101; 2,2',3,4,4',5'-heksachlorobifenyl IUPAC No. 138, 2,2',3,4,4',5,5'-heptachlorobifenyl IUPAC No. 180) were purchased from Promochem (Wessel, Germany). The technical mixtures of PCBs: Aroclor 1242, 1254 and 1260 were obtained from Supelco (Bellefonte, PA, USA). The different working standard solutions were prepared by adding the appropriate weight primary standard to hexane. The solid phase cartridges PCB-N with dual sorbents (Ar-SO_3/SiOH) 1000 mg/3ml were purchased from J.T Baker (S.Witko, Łódź, Poland). Helium of 99.999% purity was purchased from Linde (Gliwice, Poland) and Rtx – 5 (Restek, Bellefonte PA, USA) column (30 m × 0.25 mm × 0.25 μm). The ultrasonic bath UM-4 was purchased from Unimasz (Koszalin, Poland).

Apparatus and Analytical Conditions

Chromatographic Investigations

The temperature of the GC/MS Turbomass (Perkin–Elmer Co., Norwalk, CT, USA) with split-splitless injector was 250°C. Splitless time was 0.7 min, split ratio 1:25. The carrier gas was helium – linear velocity 35 cm/sec. Ionisation: EI, 70 eV, MS mode: SIM. Ion source and transfer line temperatures: 210 and 200°C, respectively. The acquisition of chromatographic data was performed by Turbomass software (Perkin–Elmer Co.). Oven temperature programme for both instruments was the following: initial 50°C hold for 1.5 min, then ramp (I) 15°C/min to 150°C (hold 2 min), then ramp (II) 5°C/min to 280°C (hold 10 min) and ramp (III) 3°C/min, 295°C (hold 6 min).

Sample Preparation

A) Fodder samples
Ultrasound-assisted solvent extraction with hexane was used for the preparation of fodder. The 10.00 g of fodder sample was homogenized with 5 g of silica-anhydrous sodium sulfate (1:1, ww.) mixture loaded in a laboratory flask containing 35 ml of hexane and extracted in an ultrasonic bath. Extraction was performed three times for 30 min. Extracts were combined, filtered and evaporated to dryness in a vacuum evaporator. Afterwards, the residue was dissolved in a few ml of hexane, transferred in 10 ml volumetric flask and filled up to their volume with hexane. A 250 μl aliquot of this solution was applied to SPE column.

B) The transformer oil samples
The 1.00 g of transformer oil was dissolved in hexane and diluted with this solvent to 10 ml in a volumetric flask. The 500 μl of this solution was loaded in an SPE column.

C) Clean – up
The solid phase extraction was used for preconcentration and clean-up of both kind of extracts. 2 ml of

hexane was added to elute PCB from SPE column. The collected extract was evaporated under gentle stream of nitrogen and then residue was dissolved in 25 µl of isooctane. The 1.5 µl of final extract were injected.

Results and Discussion

Before PCB analysis, the linear range of MS detector was determined [21]. The detector response was established for six congeners containing different number of chlorine atoms. The clean–up method had been tested with samples spiked with 25 µg/ml of each individual congener (PCB 28, 52, 101, 138, 180). The recovery was determined in the range from 75 to 85% for all tested congeners (Fig. 1). The precision, calculated as a RSD was less than 13%. Ten replicate injections of standard solutions (at least two concentration levels) were injected into GC/MS to check system performance and calibration validity. The RSD was generally less than 5%. Limit of quantitation (LOQ) of each isomer was on the level 65 ng/g.

However, data calculated according to the Ishikawa method indicate that the uncertainty in determining detection limit can change in some ranges.

Identification of PCBs in the samples was performed by comparison of chromatograms of purified extracts with those obtained from standard solutions. This method is based on simultaneous detection, at corresponding retention time, of chromatographic signals of the two selected ions for each congener (Table 1), which are chosen according to full scan mass spectrum and chromatogram of target compounds.

In addition, the isotopic ratio should be maintained within an appropriate range, i.e. 20% of calculated value for the same ions. Finally, the chromatographic signals of two selected ions of isotopic cluster should be detected at the same time as those of the corresponding standard compounds. Peaks were accepted as PCB if they fulfilled the following criteria [22]:

The retention time was within 0.1 min of the retention time of the relevant congener in the standard.

The ratio between two monitored ions was within satisfactory level (±0.2) of the ratio found for the relevant congener in the standard.

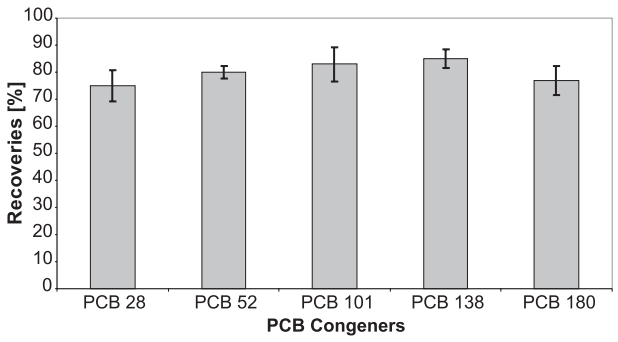

Fig. 1. Recoveries of congeners PCB 28, 52, 101, 138, 180 after SPE clean-up (n=5, spiking level 25 µg/g of each congener).

Table 1. Selected ions for qualification and quantitation.

PCB	Monitored ions
28	258, 256
52	290, 292
101	324, 326
138	360, 362
180	394, 396

The presence of selected Aroclors indicated that the pattern of peaks resembled the observed peak standard. Qualitative estimation of sample extracts was carried out with Aroclors 1242, 1254, 1260 and selected congeners. Fig. 2 shows exemplary chromatogram in SIM mode of transformer oil obtained after extraction and SPE treatment.

The results of determination demonstrated that two from fourteen transformer oils contained PCB and all fodder samples were not contaminated (Table 2). The profile of peaks on the chromatograms of sample No. 6 corresponding to the mixture of Aroclor 1254 and 1242 was observed. The second sample probably contained congeners derived from different commercial mixtures. The total concentration of PCBs was determined by visual pattern matching and summing selected peaks to obtain a total amount. The analyzed samples of transformer oils contained a PCB (as a total sum) in the concentration range 760 µg/g and 630 µg/g in samples No. 12 and No. 7, respectively. The total concentration of PCBs was more than one order of magnitude higher than the generally accepted limit according to the US EPA (50 µg/g). Therefore, these oils could be recognized as dangerous waste.

The concentration of individual congeners was shown in Table 3. The level of selected PCBs in the samples ranged from 15 to 43 µg/g. The analysis of individual congeners confirmed the presence of Aroclor in sample No. 2 because the sum of PCBs corresponded to the content of these congeners determined by other authors in the standard solution [22]. In Table 2, apart from the total concentration of PCBs in oil sample, the electric power of transformers is shown, where oil was used, year of production of transformer oil and the name of the company where transformer oil was used.

Previously, when the transformers were installed, the presence of PCBs was not taken into consideration. But now it is an important problem because the large quantities of oils containing PCBs for increasing electric resistance were applied. In general opinion, higher concentrations of PCBs in transformer oils are connected with electric power. Our data is not exhaustive but does not confirm this opinion. It is possible to expect that if a transformer device is old, the concentration of PCBs in oil is higher than in modern devices.

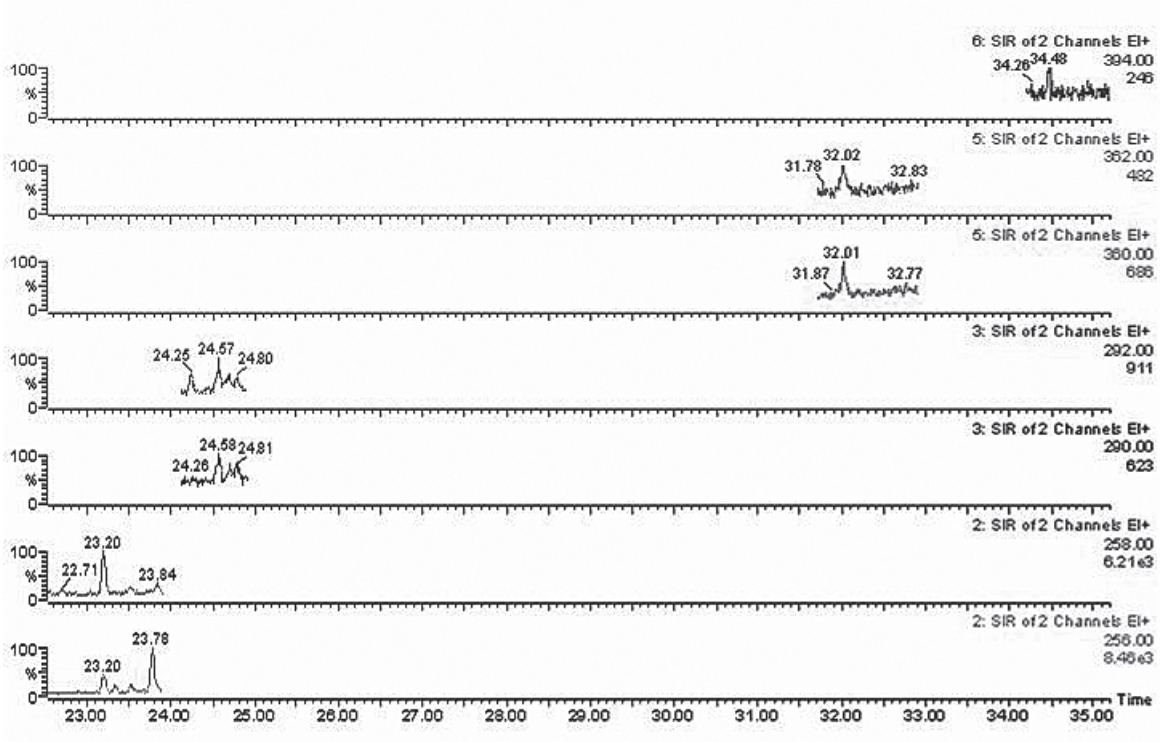

Fig. 2. Selected ion mod chromatogram of transformer oil sample after clean-up.

Table 2. The total concentration of PCB in oils [a]) I- factory of transformers and apparatus traction, II- Morliny meat factory; n.d.- not detected.

No	Power of transformer [kVA]	Year of production	Total concentration of PCB [μg/g]	Origin [a]
1	2000	1984	n.d.	I
2	1600	1973	n.d.	I
3	1000	1978	n.d.	I
4	2000	1973	n.d.	I
5	1000	1978	n.d.	I
6	1000	1978	n.d.	I
7	1000	1974	760.21 ±0.05	I
8	1600	1974	n.d.	I
9	1600	1974	n.d.	I
10	1600	1974	n.d.	I
11	1000	1974	n.d.	I
12	1000	1974	630.15 ±0.05	II
13	250	1974	n.d.	II
14	250	1974	n.d.	II

Table 3. Concentration of individual congeners in transformer oils (n=4).

PCB	Sample No. 7 Concentration [µg/g]	Sample No. 12 Concentration [µg/g]
28	20	20
52	35	15
101	59	43
138	15	20
180	30	21

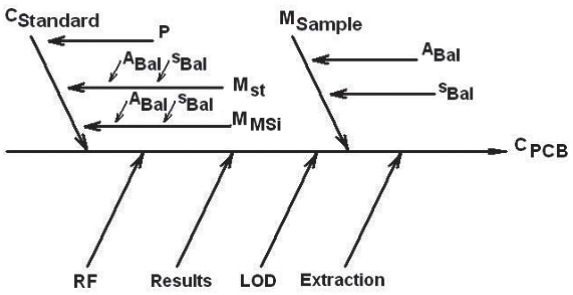

Fig. 3. Ishikawa Diagram for quantitative analysis of PCBs in transformer oil samples.

Table 4. Composition of the selected fodders.

Fodder	Application	Metabolic energy [MJ/kg]	General protein [%]	Lysine [%]	Methionine + cystine [%]	Threonine [%]	Organic calcium [%]
Starter	Pigs	13	17.1%	1	0.6	0.6	0.89
Prestarter	Pigs	12.5	21.4%	1.16	0.67	0.6	0.9
Exo-mlyn	Pigs	17	17%	0.9	0.58	0.55	0.9

Next, we take into consideration the monitoring analyses of fodders used in animal breeding. Fodder is the main component of animal food and it may contain various additives such as: vitamins, fats, carbohydrates, cellulose, and microelements. The research objective was to assert if selected fodders used in Poland are uncontaminated, and if they are not used for the 'utilization' of matrices containing dangerous xenobiotics, such as PCBs, dioxins and pesticides. The analysis was carried out on nine fodders used for feeding pigs, chickens, hens and cattle. The fodders included: Starter ®, Exo-mlyn ®, Agrocentrum ®, Agrocentrum BG2 ®, Agrocentrum AIB-1 ®, LKH ®, Prestarter ®, Finiszer ®, and Grover ®. The characteristic of selected fodders are showed in Table 4. Results of our investigations confirm the lack of contamination of fodders used. The selected fodders proved not to be a source of PCB compounds in human food chain and may be used in livestock nutrition. However, only permanent monitoring of PCB in environmental matrices helps us to prevent poisoning of human health by contaminated food.

Ishikawa Diagram for Quantitative Analysis of PCB in Transformer Oil Sample

Apart from determination of different environmental matrices by chromatographic techniques, a very important role is being attributed to the evaluation analytic procedure. For that evaluation the Ishikawa Diagram has been applied. This diagram shows all factors that influence the end result analytical process (Fig. 3). The Ishikawa diagram is used for total calculation of the uncertainty of final result – **U**.

This diagram contains the influence of the following parameters such as:

- devices used in analytic process:
- accuracy of balance – (**A_{Bal}**)
- standard deviation of balance – (**s_{Bal}**)
- accuracy of pipette – (**A_{Pip}**)
- standard deviation of pipette – (**s_{Pip}**)
- purity of standard – (**P**)
- standard deviation of results – (**Results**)
- limit of detection – **(LOD)** – standard uncertainty of extraction process – (**Extraction**)

Formulas used to calculate total uncertainty of final result (**U**) are shown in the next equation:

$$U = k * C_{AV} * [\, u(C_{STANDARD})^2 + u(M_{SAMPLE})^2 + s^2_{RF}/n_1 + s^2_{RESULTS}/n_2 + u(C_{LOD})^2 + u(C_{EXT})^2 \quad (1)$$

where:

U- total uncertainty of final result

k – factor of widening = 2

$u(C_{STANDARD})^2$ – Standard uncertainty of concentration

$u(M_{SAMPLE})^2$ – Standard uncertainty of calculate mass sample

s^2_{RF}/n_1 – Standard uncertainty of responsible factor

$s^2_{RESULTS}/n_2$ – Standard uncertainty of results

$u(C_{LOD})^2$ – Standard uncertainty of limit of detection

$u(C_{EXT})^2$ – Standard uncertainty of extraction process

In calculating the standard uncertainty of standard concentration – $\mathbf{u(C_{STANDARD})^2}$ we must take into consideration the following factors.

$$u(C_{STANDARD})^2 = u(P)^2 + u(M_{standard})^2 + \Sigma\, u(M_{MSi})^2 \quad (2)$$

where:
$u(P)^2$ – total uncertainty of purity standard =
$u(P)^2 = (u/\sqrt{3}/P)^2$
$u(M_{STANDARD})^2$ – Standard uncertainty of calculated mass standard =
$u(M_{STANDARD})^2 = [(\sqrt{s^2_{Bal} + A^2_{bal}})/M_{Standard}]^2$
$u(M_{MSi})^2$ – Standard uncertainty of calculated mixture standard=
$u(M_{MS})^2 = [(\sqrt{s^2_{MSi} + A^2_{MSi}})/M_{MSi}]^2$

Standard uncertainty of calculated mass sample – $\mathbf{u(M_{SAMPLE})^2}$ depends on standard deviation of balance s_{Bal} and accuracy of balance $A_{bal.}$ This dependence is shown in the next equation:

$$u(M_{Sample})^2 = [(\sqrt{s^2_{Bal} + A^2_{bal}})/M_{Sample}]^2 \quad (3)$$

Other formulas which are used to calculate total uncertainty of final result – **U** are:

$$s^2_{RF}/n_1 = (RSD_{RF})^2 \quad (4)$$

$$s^2_{Results}/n_1 = (RSD_{Results})^2 \quad (5)$$

$$u(C_{LOD})^2 = (LOD/C_{Av})^2 \quad (6)$$

$$u(C_{EXT})^2 = u(C_{LOD})^2 \quad (7)$$

On the basis of Ishikawa diagram and all calculations connected with this diagram we can draw the following conclusions:

Table 5. Tabulation of values used to calculate summary uncertainty of final result – **U:**

U (C standard)

				Pure standard [mg]				Basic solution [g]			
PCB	P	u	**U(P)% [%*10⁻⁴]**	S (balance)	A (balance)	M standard	**$u(C_{STANDARD})$ [%*10⁻⁴]**	S (balance)	A (balance)	M standard	**$u(C_{STANDARD1})$ [%*10⁻⁴]**
28	0.996	0.005	**8.5**	0.01	0.01	11	**1.6**	0.002	0.002	19.75	**0.02**
52	0.99	0.005	**8.5**	0.01	0.01	7.6	**3.5**	0.002	0.002	19.75	**0.02**
101	0.99	0.005	**8.5**	0.01	0.01	11.6	**1.5**	0.002	0.002	19.75	**0.02**
138	0.996	0.005	**8.5**	0.01	0.01	23.8	**0.35**	0.002	0.002	19.75	**0.02**
180	0.998	0.005	**8.4**	0.01	0.01	15.6	**0.82**	0.002	0.002	19.75	**0.02**

U (M sample)

	Sample [g]			LOD [ng/kg]	CAv	U	
PCB	S (balance)	A (balance)	Mass sample			[ng/kg]	%
28	0.002	0.002	1.000	120	740	26.12	3.53
52	0.002	0.002	1.000	85	1200	132.72	11.06
101	0.002	0.002	1.000	85	2500	108	4.32
138	0.002	0.002	1.000	97	1900	54.34	2.86
180	0.002	0.002	1.000	130	1800	50.4	2.80

PCB	U(Cstandard)	U(Msample)	RSD (RF)	RSD (Results)	∑ **U**	LOD [%]	$u(C_{EXT})^2$ [%]
28	0.001	0.00078	1.786	3.5	**5.28**	16.22	16.22
52	0.0022	0.00078	2.990	4	**6.99**	7.08	7.08
101	0.001	0.00078	2.542	2.7	**5.24**	3.40	3.40
138	0.00089	0.00078	0.667	3.2	**3.87**	5.10	5.10
180	0.0009	0.00078	1.765	4.4	**6.16**	7.22	7.22

– uncertainty of weighing standards is very low – 0.00016% (PCB 28) to 0.000084% (PCB 180) and has no influence on total uncertainty
– uncertainty of weighing samples is on the level 2.2% (PCB 180) ÷ 11.1% (PCB 52).
– uncertainty of RSD results is rather low – 2.7% (PCB 101) to 4.4% (PCB 180)
– uncertainty of LOD is in the range 3.7% (PCB 101) ÷ 16.2% (PCB 180)
– uncertainty of extraction is average, the same as uncertainty of LOD because $u(C_{EXT})^2 = u(C_{LOD})^2$ – 3.7% (PCB 101) to 16.2% (PCB 180)

From the aftermentioned data we can affirm that on uncertainty of analytical procedure the largest influence has the uncertainty connected with calculated LOD and extraction efficiency. The experimental data, calculated according to the Ishikawa diagram, indicate that it is the determination of the detection limit that exerts the greatest influence upon the uncertainty of obtained results (Table 5). The uncertainty in determining the detection limit varies from 3.7% (PCB 101) to 16.2% (PCB 180). Another factor influencing the uncertainty of obtained results is the uncertainty connected with the course of the extraction process.

Conclusions

A sensitive, rapid and robust monitoring method has been optimized for PCB in oils and fodders. The use of ultrasonic-assisted extraction allows for efficacious sample preparation. The clean-up method based on double layered SPE column provides a simplified approach for the removal of interferences from the raw sample. The GC/MS in SIM mode was used for final determination of target congeners. This method fits the purpose of rapid screening of fodder and transformer oil samples. The PCBs were found only in two transformer oils on the level of 760 and 630µg/g. However, PCB was not detected in fodders. The Ishikawa diagram was used to calculate uncertainty factors such as: RSD, LOD, extraction. These data play important roles in the uncertainty of the analytic process.

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