

**Original Article**

A Useful Method with Appropriate Recovery and High Accuracy in Simultaneous Analysis of 12 Polychlorinated Biphenyls in Cereal-Based Baby Foods Using Gas Chromatography-Electron Capture Detector

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ABSTRACT

Background and Objectives: Reliable methods are necessary to analyze polychlorinated biphenyls in baby foods, dietary supplements commonly used for children. Nowadays, contamination of food products, mostly derived from agricultural sources, with polychlorinated biphenyls seems inevitable. Of these, cereal-based baby foods are highly important due to the long-term side effects of polychlorinated biphenyls in babies.

Materials and Methods: In this study, a validated method was developed for the assessment of 12 polychlorinated biphenyls in baby foods based on the solid-phase extraction column sample preparation and gas chromatography-electron capture detector using PCB 77 and PCB 189 as internal standards. Validation of the method was assessed by the calculated and achieved parameters for linearity, mean recovery, precision, limit of quantification and limit of detection.

Results: Recoveries at three levels of 0.5, 1 and 2 µg/kg in repeatability and reproducibility studies were in ranges of 78.89–98.32 and 77.28–98.45%, respectively. Linearity was presented as R² value from 0.9980 to 0.9999, indicating good correlations between the concentrations and peak areas. Limit of quantification and limit of detection were 0.5 and 0.16 (ng/g). Analysis of 30 samples showed that six polychlorinated biphenyls were available in 7% of the samples; of which, 93% were not contaminated with polychlorinated biphenyls. None of the samples contaminated with polychlorinated biphenyls included contamination higher than the maximum residue limit.

Conclusions: Validated methodology was used in polychlorinated biphenyl analysis in various trademarks of cereal-based baby foods commercialized for the Iranian markets. Samples were screened based on the maximum residue limit by the European Union. This method is a simple method and can be carried out in a short time with high accuracy and precision.

Keywords: Analytical procedure, Cereal, GC-ECD, Polychlorinated biphenyls, PCB, Validation

Introduction

Based on the Stockholm Convention on Persistent Organic Pollutant (POP), polychlorinated biphenyls (PCBs) are described as a group of aromatic compounds, which are formed from replacing of hydrogen atoms with various numbers and positions on the biphenyl molecule, which are composed of two bound benzene rings (1, 2, 3). The PCBs include a wide range of uses, from sanitary health to agricultural and industrial uses (4). Furthermore, PCBs are hydrophobic synthetic components, which are known for their chemical stability, insulating characteristics and dielectric characteristics, and are released and diffused to the environment through anthropogenic processes such as

incineration, combustion and metal reclamation from biogenic sources (2, 5, 6, 7). The PCBs are categorized as POPs because of their lipophilic nature and persistence in the environment (8,9). They accumulate in the food chain, including meat, fish, egg and milk. Especially, they can be found in tissues and foods with high-fat contents (2, 10, 11, 12, 13, 14). The POPs, including PCBs, accumulate in the human fatty tissue and can cause health issues such as problems in nervous, reproductive and immune systems (5, 15, 16). Negative effects of the prenatal PCB exposure can lead to the neurological problems (1). The maximum residue levels for pesticides in foods are assessed by

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European legislations to establish in Regulation (EC) 396/2005, indicating necessity of a routine, sensitive, specific analytical method for the assessment of PCBs in foods (13). The maximum allowed value for sum of PCB 25, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180 in foods for infants and young children is 1 ng/g wet weight reported in Commission Regulation (EC) (15, 17). Due to the PCB toxicity, a total elimination of PCBs and other compounds is planned by 2025 under Stockholm Convention (2).

Everyday aspects of life depend on analytical measurements from food quality control to clinical assistance and drug synthesis for the research supports (18, 19). Numerous extraction methods have been used for the specific analysis of PCBs in food matrices, including Soxhlet extraction, high speed blending with solvent and matrix solid-phase dispersion (11). These methods include various procedures and limits in assessing specificity, linearity, precision, accuracy and other characteristics (18). Due to the complex nature of agricultural products, removing interferences of the effects of matrices using appropriate pre-treatment processes is necessary. These interferences can affect qualitative and quantitative measurements (20). For a wider use of the developed analytical methods, their reliability, reproducibility and repeatability need to be verified through performances in various laboratories with various instruments and analyzers (18). For the practical uses or regulatory submissions of the analytical methods, they need systematic validations by statistical analysis through assessing parameters such as specificity, linearity range, accuracy and precision (18).

Guidelines of method validation and associated topics are provided by the international organizations such as Association of Official Analytical Chemists (AOAC), American Society for Testing and Material (ASTM), Food and Agricultural Organization (FAO), United States Food and Drug Administration (FDA) and International Conference on Harmonization (ICH) (21). Quality of developed analytical methods is assessed by recovery, sensitivity, analyte stability, suitability for the purpose and the matters of time and cost (18). The WHO recommends that breast milk consumption is not sufficient to support activities in infants older than six months. Relatively, infant formulae are alternatives to breast milk that often play important roles in infant diets and may promote health situations of non-breastfed children during early development. Therefore, potential contamination of these formulae with PCB should be considered (22). In regions such as USA and European Union (EU) countries industrially processed formula milk and/or solid foods have become important sources of nutrition for infants (23). Only a few studies investigated PCB levels in infant formulae, with most of the studies focused on dioxins in human milk (22). No recorded studies are available on the analysis and measurement of PCBs in baby foods in Iran.

Hence, the aim of the present study was to develop an analytical method based on ASTM 4059-00 extraction method, which was improved for the assessment of twelve PCBs in cereal baby foods using gas chromatography-electron capture detector (GC-ECD). Procedure for the analytical assessment of the developed extraction method was followed by ICH regulation.

Materials and Methods

Samples, chemicals and Reagents

The n-hexane analytical grade (99%) and acid sulfuric (H_2SO_4 , 98%) were purchased from Merck, Germany. The PCB standards (nos. 18, 28, 31, 44, 52, 77, 101, 114, 138, 142, 153, 180, 194 and 189) were supplied by Dr. Ehrenstorfer Reference Materials, Augsburg, Germany. Solid phase extraction (Florisil column, 3 ml, 500 mg) were purchased from Chromabond, Germany. The matrix in this study included cereal baby foods. A domestic sample purchased and analyzed five times for ensuring blank samples. Then, 30 samples of cereal-based baby foods from various brands were randomly purchased from pharmacies in Tehran, Iran.

Standard solutions

Initial standards were purchased in powder form. The n-hexane was used to prepare and dilute the standards. The source standard was 200 ng/ml; then, dilution was carried out as follows:

200 (ng/ml) standard: 1 μg of standard dissolved in 5 ml of solvent (n-hexane),

10 (ng/ml) standard: 5 μL of 200 (ng/ml) standard, 75 μL n-hexane, 20 μL PCB77 (internal standard), 10 μL PCB 189 (internal standard),

20 (ng/ml) standard: 10 μL of 200 (ng/ml) standard, 70 μL n-hexane, 20 μL PCB77 (internal standard), 10 μL PCB 189 (internal standard),

50 (ng/ml) standard: 25 μL of 200 (ng/ml) standard, 55 μL n-hexane, 20 μL PCB77 (internal standard), 10 μL PCB 189 (internal standard),

and 100 (ng/ml) standard: 50 μL of 200 (ng/ml) standard, 20 μL n-hexane, 20 μL PCB77 (internal standard), 10 μL PCB 189 (internal standard).

Apparatus

The GC instrument included Agilent 7890 A, USA, equipped with ECD and autosampler with DB-S column (30 m \times 0.250 μm \times 0.25 μm). The oven temperature of GC was set as an initial temperature of 130 $^\circ\text{C}$ held for 2 min; then, increasing to 250 $^\circ\text{C}$ at a rate of 3 $^\circ\text{C min}^{-1}$ and holding the final temperature at 250 $^\circ\text{C}$ for 8 min. Injector and detector temperatures were set at 250 and 300 $^\circ\text{C}$, respectively. The purge gas was nitrogen at a rate of 3 ml min^{-1} . Helium at 0.8 ml min^{-1} rate was used as the carrier gas. The split mode with a ratio of 5:1 was used. The

makeup gas was nitrogen at a rate of 30 ml min⁻¹ for 45 min. Volume for each injection included 1 µL.

PCB extraction

Based on the selected analytes and matrices, purification and extraction methods can be various (9). Solvent for the extraction included n-hexane. Briefly, 5 g of the finely ground cereal-based baby food were mixed with sufficient quantities of PCBs at assessed concentrations and 20 µL of PCB-77 as one of the internal standards with 1 µg/ml concentration. The contaminated sample was stored at room temperature for 1 h to allow PCB complete absorption into cereals and then developed extraction procedure was used as follows: sample was mixed with 15 mL of n-hexane using shaker (Heidolph, Vibramax 100 platform shaker) at a speed of 250 rpm for 30 min. After filtration using filter papers (CHMLab 58*58, 0.17 mm), filtered liquid was stirred using vortex (Multi Reax Brand, Heidolph, Test tube shaker 5411000011 UK) at 4000 rpm for 1 min with 1 ml acid sulfuric (98%) for removing chemical backgrounds from the resulting liquid. Suspension was centrifuged (Eppendorf 5810 R, Germany) at 3000 rpm for 10 min. The remaining n-hexane in the separated liquid from the centrifugation step was passed through a Florisil column (3 ml, 500 mg, Chromabond). Technically, 1 ml of n-hexane was passed through the column, followed by passing the extracted sample from the previous step. Column was eluted in three steps, each step used 0.5 ml of n-hexane. Effluent of the column was evaporated under the flow of nitrogen gas. Then, 100 µL of n-hexane were added to the remaining solution and agitated for 3 min using vortex. The final solution was mixed with 10 µL of PCB 189 as the second internal standard with a concentration of 1 µg/ml. Then, 1 µL of this solution was injected into GC-ECD.

Method validation

Validation of the method was assessed by the calculated and achieved parameters for linearity, mean recovery, precision (repeatability and reproducibility), limit of quantification (LOQ) and limit of detection (LOD). Accuracy was assessed by calculating mean recovery experiments. For calculating the average recovery, assessed concentrations of spiked samples were compared to their target levels. For assessing LOQ, it was assessed as the lowest concentration level that could quantitatively be assessed and validated with acceptable values for recovery using five replicates and one-third of this value was considered as the LOD (European Medicines Agency, ICH) (24). For calibration analysis, spiked level calibration curve method was used to remove effects of the sample. Five-point calibration curves were prepared using standard curve over a concentration range of 10–200 ng/ml to assess linearity. In this method, each of the twelve PCBs was prepared at five concentrations of 10, 20, 50, 100 and 200

ng/ml from the spiking solution of 200 ng/ml. Solution included 20 µL of PCB 77 as one of the internal standards before the extraction step for assessing the extraction step. Blank sample was contaminated with this solution and used in the extraction step. Then, 10 µL of PCB 189 as the other internal standard were added to the final product of the extraction step for assessing quantification by GC-ECD. The prepared solution was injected into GC-ECD for quantification and qualification analyses. The used points in GC-ECD were constructed for calibration graphs.

To assess validity of the analytical method, sample was contaminated with solutions of 12 PCBs at three various levels of 0.5, 1 and 2 ng/g. Two sets of experiments were carried out. In the first set, each level of contamination was going through the extraction and assessment steps for three replicates in one day to assess repeatability and interday precision. In the second set of experiments, each pollution level at each day was assessed via extraction and assessment for nine replicates in three successive days. Results of the second set of experiments were used to assess reproducibility and intraday precision. Recovery, standard deviation and relative standard deviation for all of these experiments were calculated. The term of specificity is used to investigate the peak occurrence in this specifically developed method for recording location of the peak of each analyte in the chromatogram of GC. This word refers to differentiation between various analytes and each peak of the chromatogram after the effect of extraction from the matrices.

Results

The LOQ and LOD were 0.5 and 0.16 ng/g, respectively. Accuracy was studied by calculating recovery assays. For recovery assessments, detected values of the extracted analytes from the matrices were compared with the initial value of each analyte. At the lowest spiking level selected based on the LOQ, 0.5 ng/g, all 12 PCBs were assessed. For setting limits of quantitation (LOQs), the lowest validated spiked levels were used (24). For assessing linearity of the method, various calibration curves and coefficient of correlation (R^2) between the concentrations and the peak areas were calculated with the concentrations in the range of 0.5–10 ng/ml. As shown in Table 1, satisfactory results for the linearity with R^2 over 0.9983 was achieved within the highlighted range (10, 20, 50, 100, 200 ng/ml) for the 12 PCBs. The range of R^2 values included 0.9980–0.9999, indicating good correlations between the concentrations and areas under curve (AUC) in the linear range. The ICH, which was the selected regulation for following its validity procedure in this study, did not report the acceptable range for R^2 . However, results were in the range of the reported values (2, 13, 25, 26). Good results in linearity from the calibration curve assured applicability of the used chromatographic system GC-ECD for the twelve PCBs.

Table 1. Validation parameter analysis of the PCBs in cereal-based baby foods

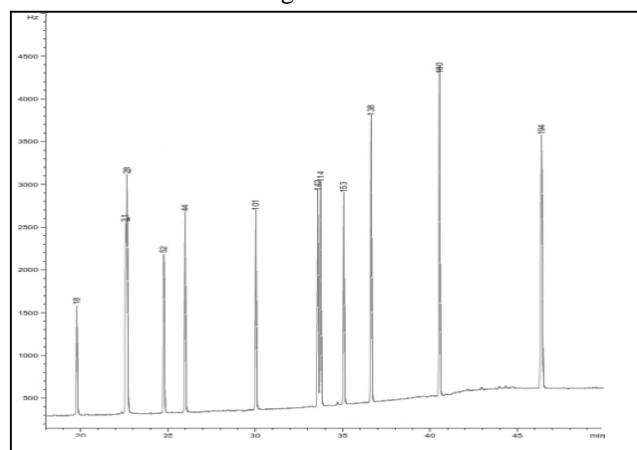
Compounds	Linear range concentration (ng/ml)	Equation	R ^{2a}	Spiking levels (ng/g)					
				0.5		1		2	
				Recovery±SD ^b	RSD _R ^c RSD _r ^d	Recovery±SD	RSD _R RSD _r	Recovery±SD	RSD _R RSD _r
PCB 18	0.5-10	y = 0.0472x + 0.0133	0.9980	89.18±9.95	11.16	87.10±8.43	9.68	89.41±11.63	13.01
				85.38±9.67	11.33	92.83±9.46	10.19	86.18±6.54	7.58
PCB 28	0.5-10	y = 0.1092x + 0.0223	0.9983	94.38±0.94	0.99	91.90±6.95	7.57	83.87±6.53	7.79
				89.90±6.07	6.75	90.84±11.18	12.31	91.81±6.55	7.13
PCB 31	0.5-10	y = 0.0673x + 0.0158	0.9987	93.57±5.44	5.82	98.45±7.64	7.76	96.35±7.41	7.69
				95.55±5.74	6.08	98.11±10.37	10.57	92.33±5.58	6.04
PCB 44	0.5-10	y = 0.0899x + 0.0187	0.9987	94.93±1.40	1.48	86.24±6.60	7.66	82.25±4.39	5.34
				88.14±8.60	9.76	84.87±9.05	10.68	89.39±6.13	6.86
PCB 52	0.5-10	y = 0.0713x + 0.012	0.9982	98.06±6.74	6.88	96.04±0.73	0.76	94.75±3.46	3.65
				94.93±4.08	4.30	98.32±7.12	7.25	94.78±5.64	5.95
PCB 101	0.5-10	y = 0.0836x + 0.0196	0.9985	89.89±5.557	6.20	89.96±4.28	4.76	82.28±7.45	9.06
				87.84±7.60	8.66	85.34±8.96	10.5	88.28±5.18	5.87
PCB 114	0.5-10	y = 0.0974x + 0.0125	0.9987	88.39±11.94	13.50	95.56±9.03	9.45	83.83±3.26	3.89
				86.90±14.59	16.79	88.84±6.98	7.86	88.73±6.22	7.01
PCB 138	0.5-10	y = 0.1301x - 0.001	0.9999	83.79±6.51	7.77	84.69±3.74	4.42	77.88±4.24	5.44
				87.89±4.59	5.82	81.73±7.72	9.45	85.52±5.33	6.24
PCB 142	0.5-10	y = 0.0962x + 0.0173	0.9991	82.27±8.29	9.95	90.13±9.50	10.54	78.57±4.70	5.98
				83.36±13.54	16.24	84.91±9.23	10.87	85.15±5.95	6.99
PCB 153	0.5-10	y = 0.0956x + 0.0042	0.9994	92.37±2.39	2.59	91.62±8.14	8.88	81.60±7.41	9.08
				89.64±9.76	10.89	83.82±9.30	11.1	91.06±5.53	6.97
PCB 180	0.5-10	y = 0.1411x - 0.0006	0.9998	86.57±7.36	8.5	84.75±8.12	9.58	77.28±5.67	7.33
				82.59±9.82	11.89	80.52±10.59	13.15	86.19±6.18	7.17
PCB 194	0.5-10	y = 0.1486x - 0.0135	0.9994	84.62±8.55	10.11	83.74±2.53	3.02	79.45±1.08	1.36
				80.65±6.20	7.69	83.83±8.17	9.75	82.88±4.47	5.39

^aRegression Coefficient, ^bStandard Deviation, ^cRelative standard deviation (Reproducibility), ^dRelative standard deviation (Repeatability)

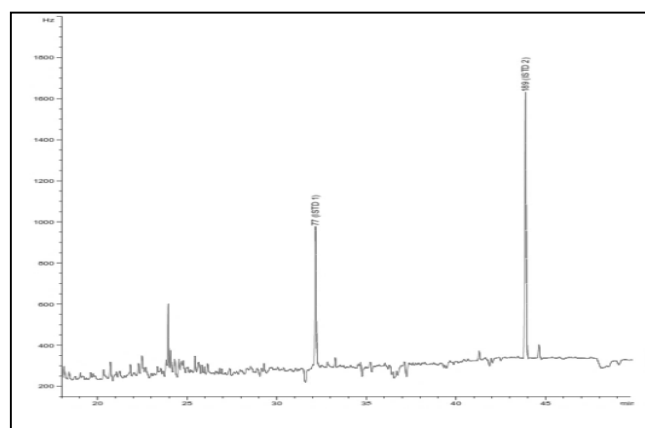
Tables 1 shows results of the precision and accuracy of the developed analytical method for the assessment of the twelve PCBs for repeatability and reproducibility. Achieved recoveries of all 12 PCBs for the spiked levels were greater than 77% and the calculated relative standard deviation was less than 17%. In the range of recoveries for the repeatability, the lowest recovery rate was 78.89% for PCB 138 at 0.5 ng/g and the highest recovery rate was 98.32% for PCB 52 at 0.5 ng/g. Results for recoveries of reproducibility varied 77.28–98.45% for PCB 180 and PCB 31, as the lowest and the highest at 2 and 1 ng/g, respectively. Thus, it can be concluded that the highest recovery for repeatability and reproducibility occurred for a greater PCB, compared to the lowest recovery. Ranges of the recovery for repeatability and reproducibility were

reported almost similarly. As another supporting argument, coefficient of repeatability of measurements and within-laboratory reproducibility, named as relative standard deviation or otherwise expressed as coefficients of variation (CV), for this method were less than 16.79 and 13.50%, respectively (Table 1). Relative standard deviations (RSD_r) associated to repeatability and reproducibility of the method in assessing recovery of the spiked levels for 12 PCBs were 0.30–16.79 and 0.76–13.50, respectively. Results achieved for precision at all concentrations included acceptable levels for precision based on the international guidelines for validation SANTE/11945/201, which was equal or less than 20% (RSD_r ≤ 20%) (26).

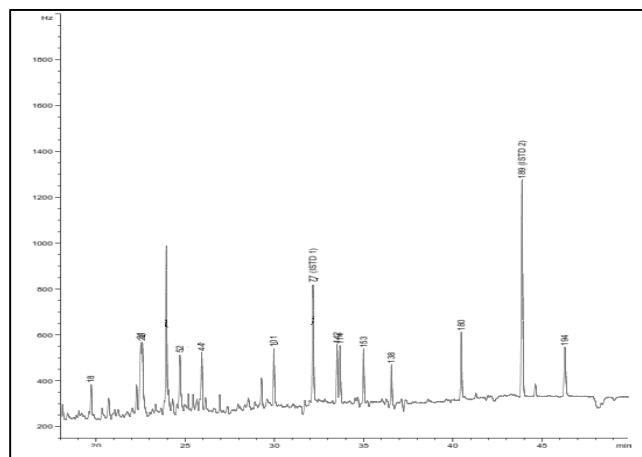
The lowest values for the CV of repeatability and reproducibility were reported for PCB 52 at 0.5 ng/g and PCB 52 at 1 ng/g, respectively. The PCB 114 showed the highest CV for repeatability and reproducibility at 0.5 ng/g. The lowest value for RSDr for repeatability experiments (4.30) was for PCB 52 at 0.5 ng/g and the highest value (16.79) was for PCB 114 at 0.5 ng/g. Based on the reported values for reproducibility, the lowest and the highest RSDr were for PCB 52 at 1 ng/g and PCB 144 at 0.5 ng/g, respectively. Results of the typical chromatograms achieved from the PCBs spiked solution, two internal standards and solution after extraction from the contaminated sample suggested clear separations of the 12 PCBs. Chromatograms indicated that developed sample preparation and analytical procedure resulted in separating each of the 12 analyzed PCBs with no interference. Chromatograms from the spiked solution of 12 PCBs, two internal standards, one extracted solution from the contaminated matrix, overlay of PCBs chromatogram with internal standards and blank chromatogram with internal standards are shown in Figure 1.



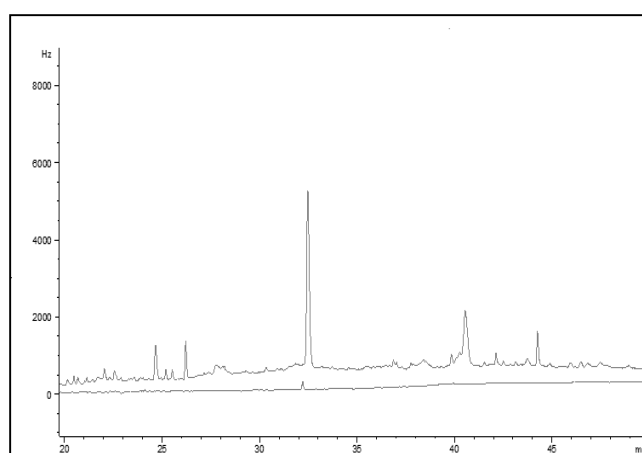
a)



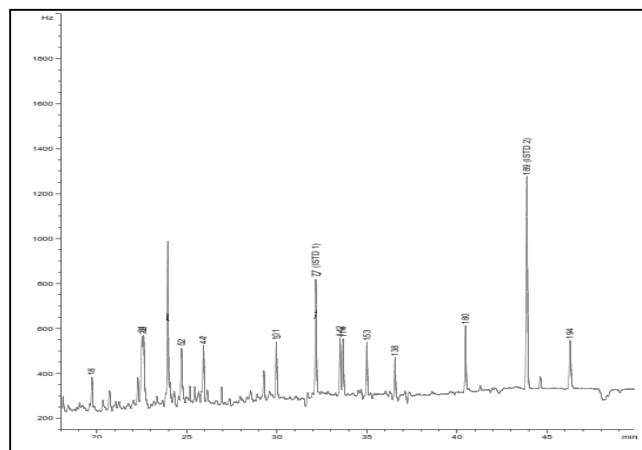
b)



c)



d)



e)

Figure 1. The GC-ECD chromatograms, a) standard solution of the 12 PCBs, b) two internal standards, c) one spiked solution of the 12 PCBs, d) overlay of the PCB standard chromatogram with internal standards, and e) overlay of the blank chromatogram with internal standards

Based on Table 2, four samples were contaminated out of a total of 30 samples, which included 7% of the total sample. In the 12 PCBs, PCBs 52, 101, 138, 153, 180 and 194 were identified in baby foods. Of these, the highest rate of contamination was linked to PCB 101. None of the

samples contaminated with PCBs included contaminations higher than the maximum residue limit. As seen in Figure 2, a total of 30 samples were analyzed; of which, the highest contamination was linked to PCB101 (1.63 ng/g), PCB180 (1.40 ng/g) and PCB194 (1.35 ng/g), respectively.

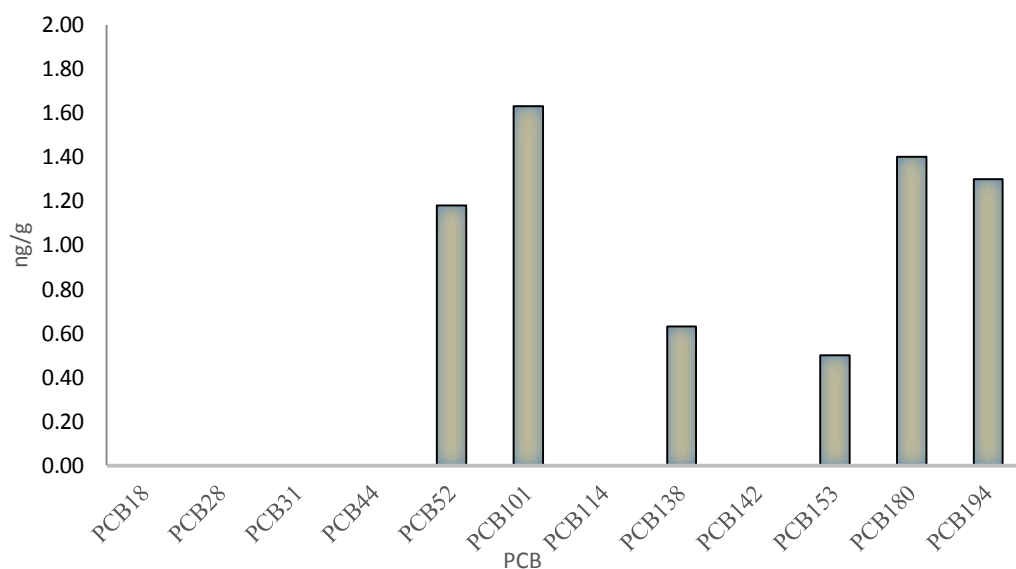


Figure 2. Total concentrations of PCBs in 30 cereal-based baby food samples

Table 2. Cereal-based baby food samples and their PCB contamination rates

Samples No.	PCB No.	ng/g	Samples No.	PCB No.	ng/g
01	-	ND	17	-	ND
02	-	ND		101	<LOQ
03	-	ND		138	<LOQ
	101	0.5	18	153	0.5
04	194	0.75		194	0.55
05	-	ND	19	-	ND
06	-	ND	20	-	ND
07	-	ND		52	0.52
08	-	ND		101	0.58
09	-	ND	21	180	0.8
10	-	ND	22	-	ND
11	-	ND	23	-	ND
12	-	ND	24	-	ND
13	-	ND	25	-	ND
14	-	ND	26	-	ND
15	-	ND	27	-	ND
	52	0.66	28	-	ND
	101	0.55	29	-	ND
16	138	0.63	30	-	ND
	180	0.6	-	-	-

Discussion

Twelve PCBs were selected for assessing selectivity and specificity of the developed method. Selected PCBs were from a wide range, light to heavy PCBs, for having a complete range from a molecular point of view. Quantitative and qualitative assessments of the selected analytes were major steps of developing and improving analytical procedures. Assessment by GC-ECD was selected for this purpose, followed by validation of the achieved results based on European Medicines Agency (EMA), ICH. Used parameters in ICH to verify that the developed method was appropriate for this use included LOD, linearity in calibration, accuracy and precision. As can be seen, the lowest RSDr and CV for repeatability and reproducibility were occurred for PCB 52, one of the lightest PCBs. The highest RSDr and CV belonged to PCB 114, in the second half of heavy PCBs. It can be concluded that while the method resulted in good validation parameters for the 12 PCBs, accuracy for the lighter PCBs could be higher.

The major reason of inaccuracy in PCB analysis by GC, especially in food matrix, was linked to the injection of interfering components from the sample or the matrix effect (27). Elimination of the matrix effect could be achieved if comprehensive sample cleanup procedures were available (28). Strategies are available with the use of alternative calibration methods such as matrix-matched calibration, standard addition, isotopically labeled internal

standards and use of analyte protectants for preventing, decreasing and compensating the matrix effect (29, 30). Use of plastic falcon tubes in analysis of PCBs interfered with phthalates. Sulfuric acid was used to methylate fatty acids (FAs) and prevent chromatogram clutter (ASTM). To increase the accuracy and precision, Florisil column was used between octadecylsilyl (C-18), graphitized non-porous carbon (Envi-Carb, Plus solid phase extraction cartridges), aminopropyl (NH₂), alumina SPE and Florisil (31). Using this method, preparation and analysis of the samples were carried out in less than 4 h with high recovery and acceptable precision for the samples as low as 0.5 ng/g for the 12 selected PCBs and two internal standards. These results suggested that the method could be used for the identification and as a quantitative method for the 12 PCBs. This method, with characteristics of simple, cost-effective and acceptable to good accuracy can be suggested for similar matrices.

Previous studies on the analysis of PCBs using gas chromatography–mass spectrometry (GC-MS) have shown similar results to the current results. For example, in a study by Nardelli in 2020 on PCB analysis in milk samples using GC-MS and GC-ECD, LOD and LOQ were reported as 0.3–0.39 and 0.44–1.30 ng/ml⁻¹, respectively. These were reported as 0.16 and 0.5 ng/g⁻¹, respectively, in the current study (32). In 2019 study carried out on the analysis of PCBs on breast milk using GC-MS, LOD and LOQ were reported as 0.22–0.58 and 0.74–1.65 ng/ml⁻¹, respectively (33). Possible PCB pollution of the agricultural-derived food products such as baby foods and potential risk of the polluted products are the reasons for this study. Developed analytical method of this study is highly important in simplicity and speed of detection of the 12 toxic PCBs in infant foods that could be sources of serious health issues. Assessment method of the 12 PCBs in baby foods was based on the extraction with n-hexane and cleaning with acid sulfuric. Moreover, eluents were analyzed using GC-ECD. The mean recoveries of the method for three spiking levels of 0.5, 1 and 2 ng/g were greater than 70%. Light PCBs showed the highest recoveries for repeatability and reproducibility. The lowest values for RSDr and CV for repeatability and reproducibility were seen for PCB 52, which was within the light PCBs, while the highest values were seen for PCB 114. From the findings, it could be concluded that the developed method was capable of determining the 12 selected PCBs in cereal matrices through a rapid, selective sensitive procedure with confident results. Results indicated that PCBs could be assessed as low as 0.5 ng/g in cereal matrices.

Conclusion

In this study, an accurate precise method was developed to determine 12 PCBs in cereals based baby

food samples, which included complex matrices. The method, including solid-phase extraction column sample preparation and GC-ECD analysis, showed a high sensitivity and confirmatory potency that was necessary for the assessment of trace levels of the PCBs. The excellent method of validation data and proficiency assessment results showed that the present quantitative method could be used for the accurate assessment of PCBs in baby food samples. Advantages of this method included use of two internal standard for error assessment in extraction and injection steps. Due to the complexity of matrices of baby foods, analysis seemed difficult. However, this study developed a simple method that could be carried out within a short time and included high accuracy and precision. Furthermore, the method could be carried out with a small volume of the samples and chemicals.

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