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# GC-MS and QuEChERS as an Analytical Tool for the Determination of Impurities in Breast Milk

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**Abstract**
Human milk is as a very significant factor contributing to proper development of children. Unfortunately milk can also carry polychlorinated biphenyls (PCBs). These compounds are highly harmful and they have negative impact on infants, whose immunological system is still developing. Thus checking milk for these compounds is of great importance. The aim of this study was to develop a suitable method of quantitative and qualitative analysis. We used gas chromatography with mass spectrometry (GC/MS). For sample preparation we used the QuEChERS technique.

## 1. Introduction

Human milk contains a lot of special nutrients, i.e. proteins, lipids, minerals, and vitamins. Unfortunately milk can also carry harmful substances [1, 2]. Polychlorinated biphenyl (PCBs) are lipophilic compounds, accumulating in fatty tissues, and in this way PCBs can be found in milk. One of the most important sources of exposure is mother's diet; in particular, diet rich in fish products. When pregnant or breastfeeding women want to get appropriate amounts of Ω-3 fatty acids (e.g., DHA), they often eat fish, whose tissues have accumulated environmental contaminants through mostly through feeding on lower organisms. Thus women may be exposed to such organic contaminats as PCBs. Long-term exposure to these substances can lead to serious health problems such as skin diseases, endocrine and reproductive disorders, and neurological problems; particularly in infants, whose immunological system is still developing and does not have defense mechanisms against the potential threat [3, 4].

The method used for determination of PCBs in most cases is gas chromatography with different detection techniques. However, in the recent years the most popular choice is gas chromatography coupled to mass spectrometry [5, 6].

The crucial step in the analytical procedure is sample preparation. Due to low concentration levels of these analytes, it is necessary to use a specific and selective method for isolating, enriching and purifying milk samples. For determining compounds in such matrices as milk, scientists use methods such as classical liquid-liquid extraction (LLE) [6, 7], solid phase extraction (SPE) [8] or accelerated solvent extraction (ASE) [9]. An interesting approach is also the QuECHERs technique [10].

This study aims at determining the PCB (No. 28, 52, 101, 138, 153, 180) content with gas chromatography coupled to mass spectrometry (GC-MS) and developing a sample preparation method used a QuEChERS techniqe.

## 2. Experimental

### *2.1 Reagents and chemicals*

The following reagents were used in this study: hexane and acetone for GC (POCH, Poland); deionized water (Mili-Q Reagent Water, Merck); magnesium sulphate anhydrous, sodium chloride (POCH, Poland), sodium citrate monobasic, sodium hydrogen citrate sesquihydrate (Sigma-Aldrich); Bondesil PSA, 40 μm (Agilent Technologies) and Bakerbond octadecyl (C18 40 μm, 60 Å). Standards for PCBs No. 28, 52, 101, 118, 138, 153 and 180 came from Dr Ehrenstorfer.

### *2.2 Instrumentation*

The method used was gas chromatography coupled to mass spectrometry (Agilent Technologies, model 6890N). Analytes were separated with Zebron ZB-5MS Guar (30.0 m × 250 mm × 0.25 mm). Flow rate was 1.1 mL/min, using helium as a carrier gas. Column temperature was programmed as follows: 60 °C (1.0 min) → 20 °C/min → 170 °C (0.30 min) →10 °C/min → 310 °C (1.20 min). The injector temperature was 265 °C. The injection volume was 1 μL. The mass ion source temperature was 300 °C.

### *2.3 Sample preparation method development*

First, we selected the method proposed by Luzardo et al. [10] to check the recovery and reproducibility of PCBs from milk. 5 ml of milk (in a 50 ml Falcon tube) was contaminated with standard solution (10 μg/L). Then 5 mL $H_2O$ was added, followed by 10 mL acetonitrile saturated in *n*-hexane. The sample was allowed to rest at room temperature for 30 min. The next step was adding the salts: 4 g $MgSO_4$, 1 g NaCl, 1 g $C_6H_6Na_2O_7.1{\cdot}5H_2O$, and 0.5 g $C_6H_7NaO_7$. The tube content was shaken (1 min) and centrifuged (5 min, 5000 rpm). The extract was transferred to a glass tube. The residue in the Falcon tube was extracted again by adding 5 mL acetonitrile saturated in *n*-hexane. Then the upper layer was added to

**Table 1**
Limit of detection and quantification and linearity of the method.

| PCB | $t_r \pm SD$ / min | $LOD$ / μg $L^{-1}$ | $LOQ$ / μg $L^{-1}$ | Range of concentration / μg $L^{-1}$ | $R^2$ |
|---|---|---|---|---|---|
| 28 | 12.112±0.002 | 0.22 | 0.74 | 2–10 | 0.997 |
| 52 | 12.733±0.002 | 0.58 | 1.93 | 2–11 | 0.999 |
| *101* | *14.339±0.002* | *0.39* | *1.31* | *2–10* | *0.996* |
| 118 | 15.487±0.003 | 0.40 | 1.34 | 2–11 | 0.998 |
| 153 | 15.884±0.003 | 0.28 | 0.93 | 2–11 | 0.998 |
| 138 | 16.395±0.002 | 0.43 | 1.45 | 2–11 | 0.998 |
| 180 | 17.588±0.003 | 0.49 | 1.65 | 2–11 | 0.998 |

the first extract. The whole extract was transferred to a Falcon tube with sorbent (500 mg PSA) and salt (900 mg $MgSO_4$). The contents were shaken (1 min) and centrifuged (5 min, 5000 rpm). The extract was evaporated under a stream of nitrogen. The residue was reconstructed with 500 ml *n*-hexane. The sample was then ready for analysis.

The QuEChERS technique was optimized by testing extraction with the use of different solvents (acetonitrile or hexane:acetone 1:1), salts (whether to use citrate salts or not) and sorbents (500 mg PSA or 500 mg PSA + 250 mg C18).

## 3. Results and discussion

### *3.1 The method of validation method of the analytical technique*

Limit of detection and quantification and linearity of the method are summarized in Table 1. It is evident, that the GC-MS method is linear in the concentration range (2–11 μg/L) with regression coefficients >0.996, and *LOQ* for the analyzed compounds is at a satisfactory level. Typical GC-MS chromatogram of standard solutions of PCBs is depicted in Fig. 1.

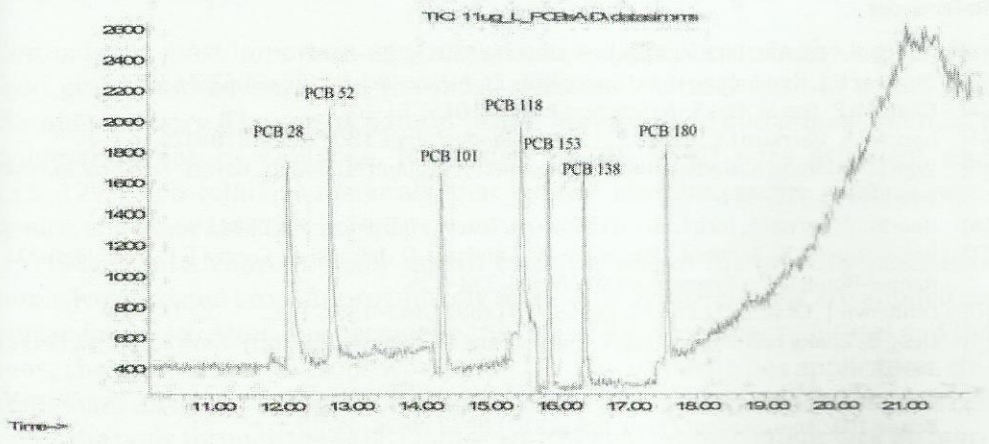


**Fig. 1** Chromatogram of standard solutions of PCBs.

**Table 2**
Efficiency of the extraction process.

| PCBs | Recovery / % | SD |
|---|---|---|
| 28 | 56.99 | 11.91 |
| 153 | 60.20 | 8.96 |
| 138 | 42.56 | 6.63 |
| 180 | 26.59 | 6.79 |

### *3.2 Sample preparation method*

The best results were obtained when we used acetonitrile saturated in *n*-hexane for extraction, the following salts: 4 g $MgSO_4$, 1 g NaCl, 1 g $C_6H_6Na_2O_7 \cdot 1.5H_2O$, and 0.5 g $C_6H_7NaO_7$. and finally the mix of the sorbents containing 500 mg PSA and 250 mg C18. When we used hexane:acetone solvent mixture, a lot of interferents were co-extracted; therefore we chose acetonitrile. The citrate salts and C18 sorbent improve the purity of the extract. The selected extraction method (QuEChERS technique) enabled us to isolate of four PCBs (Table 2).

## 4. Conclusions

The developed GC-MS method makes it possible to determine test compounds at low concentrations. Isolating analytes from milk is a challenge for the analyst because such matrix is highly heterogeneous (it contains proteins and lipids). The QuEChERS procedure allows us to isolate the four studied compounds. There are many ways to modify this method, so we can improve the efficiency of our version by making changes in the procedure (for example using different sorbents). Dispersive solid phase extraction is the crucial step in this method, because the key is to choose the type and amount of sorbents which will retain the interference, not the analyte.

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