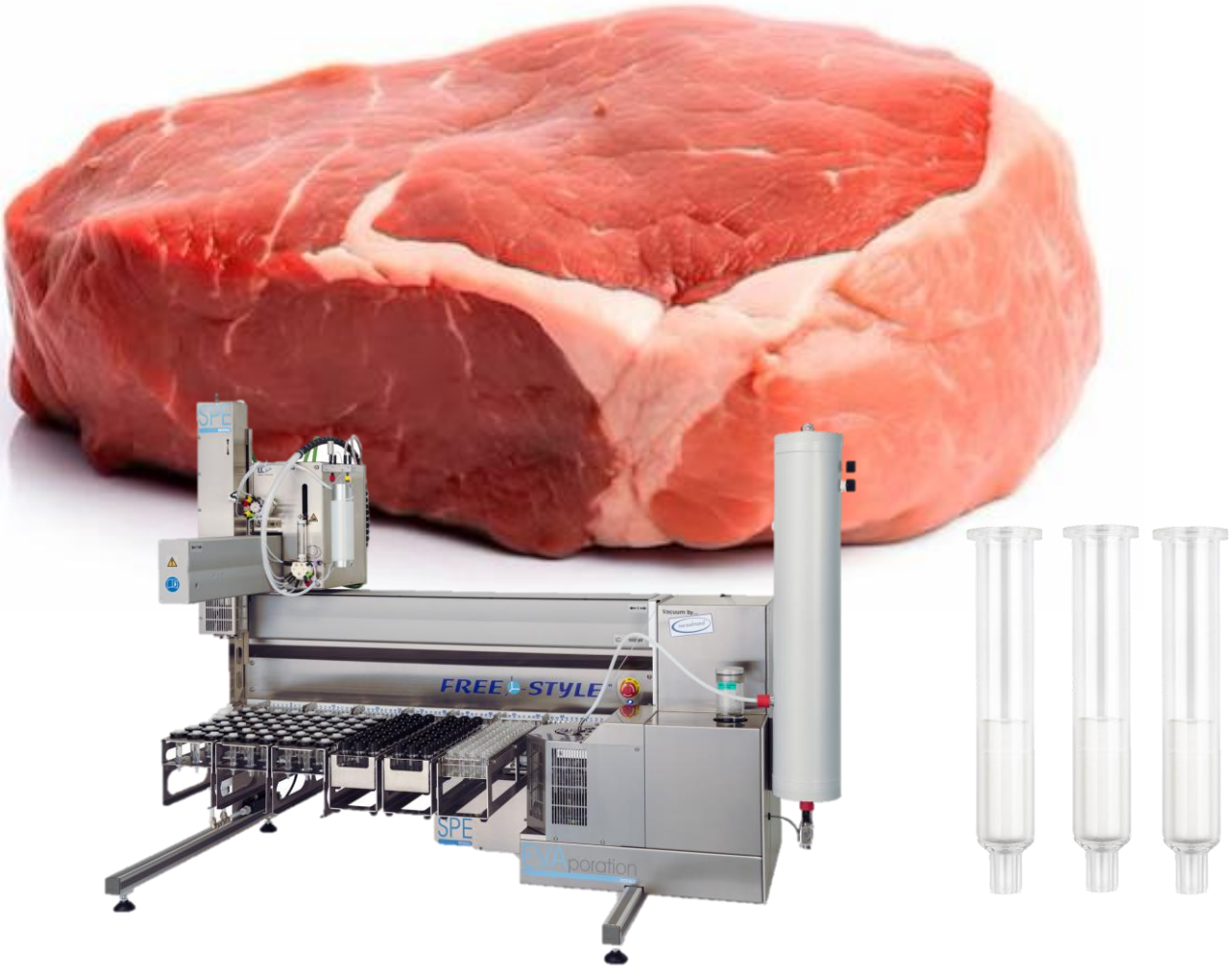
# *Fast Determination of Non-dioxin-like PCB in Meat and Fat of Animal Origin in Contaminant Control Plan Samples With Elufix Indicator PCB column Using FREESTYLE SPE-EVA System*

U. Möhlenkamp[1], L. Bahte[1], T. Bernsmann[1], T. Gersthagen (LCTech GmbH)




Application Note AN0063 | www.LCTech.de
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# Content

## Key Features

- Rapid method for non-dioxin-like PCBs (ndl-PCBs, indicator PCBs)
- Analyse samples quickly and efficiently to comply with the testing frequency set out in Regulation 2022/932 Annex I No. 1
- Meets the criteria of Regulation 2017/644
- Minimum limit of quantification of 0.2 ng/g fat
- Recovery of the internal standards of around 90 % for each indicator PCB congener
- LCTechs Elufix Indicator PCB column
- FREESTYLE SPE-EVA System for automatisation
- 45 samples unattended in one run. Approx. 45 min per sample
- Faster and cheaper than the conventional PCB/Dioxin sample prep with two or three column setup

## LCTech Products

**SPE Columns:**

Part No.: 21298

Elufix Indicator PCBs

For manual use and automated use on FREESTYLE systems

**Freestyle SPE-EVA System:**

| | |
|---|---|
| Part No.: 12663-12 | FREESTYLE BASIC, 12-port valve (for up to 6 solvents) |
| Part No.: 12668 | FREESTYLE SPE Module |
| Part No.: 13841 | FREESTYLE EVAporation Module, flexible final volume between 0.2 mL and 5 mL; automated calibration |
| Part No.: 11915 | Frame for following tray (outer width: 100 mm) |
| Part No.: 13134 | Tray for 30 pcs - 8 mL Round Bottom Vials with screw cap |
| Part No.: 13946 | Rack for Up to 18 SPE Columns; no frame needed (outer width 120 mm) |
| Part No.: 19650 | Column Adapter for Elufix Indicator PCBs columns, for usage together with caps P/N 19480 (10 pcs / pck) |
| Part No.: 19480 | Caps (PTFE) w/ O-ring for Elufix Indicator PCBs columns (10 pcs / pck) |
| Part No.: 13460 | Reusable needles, to elute Elufix Indicator PCBs columns directly into EVA |

# 1. Introduction

At the beginning of 2023, the Contaminant Control Plan was established in European legislation with the Delegated Regulation (EU) 2022/931 and the Implementing Regulation (EU) 2022/932. Annex I No. 1 of Regulation (EU) 2022/931 specifies combinations of contaminants or groups of contaminants in groups of animal products. Food of animal origin should be analysed for halogenated persistent organic pollutants (hPOPs) or metals. The group of hPOPs with maximum levels includes only the group of dioxins/PCBs or PFAS. The minimum frequency of testing is specified in Regulation 2022/932 Annex I No. 1. A disproportionate number of dioxin and/or PCB analyses have to be carried out in animal foodstuffs. In order to be able to analyse these samples quickly and without great effort, a rapid method for ndl-PCB has been developed that meets the criteria of Regulation (EU) 2017/644.

# 2. Materials and Method

## 2.1 Fat Extraction

Approx. 5 g muscle meat or adipose tissue was homogenized in a cutting mill with sodium sulfate. The homogenate was treated with n-pentane in an iodine flask and extracted in an ultrasonic bath for 15 minutes. The fat-containing solvent was filtered through a cleaned pleated filter and separated on a rotary evaporator.

## 2.2 Sample Intake

0.15 g fat was weight in a 8 mL vial and spiked with 0.5 mL of an internal standard solution (purchased from Promochem, Germany; each with a concentration of 10.000 pg/mL). The mixture was made up to 3 mL with cyclohexane and vortexed for 1 min.

## 2.3 Clean-up

The extract was processed on a FREESTYLE SPE-EVA system with Elufix Indicator PCB column. Within 20 minutes the ndl-PCB was eluted with 20 mL of cyclohexane. The extract was concentrated in the evaporation chamber to 200 µL and transferred to a 200 µL. The total processing time per sample is approx. 45 min.

FreeStyle Software Version: 14-11-08

FREE-STYLE AUTOMATISIERTE PROBENVORBEREITUNG

LCTech FreeStyle - Report on Methods: SPE -> EVA

Name: PCB_SPE_EVA.sfm — Method created: 29.01.2025 15:30:18h

SPE - Method: PCB_SPE.spe

Online ======= >

EVA - Method: PCB_EVA.evp

SPE:

| | | | |
|---|---|---|---|
| SPE Column: | LCTech_Glass.col | | |
| Extension cannula: | yes | | |
| Processing speed selection: | Standard (organic solvents) | | |
| Rinsing intensity: | Standard rinsing cycle | wo. Extra Cleaning after Load | |
| Use pressure limitation function during loading and washing: | no | | |

Step: Load — Basic type: Load - Transfer Sample-Aliquot over sample loop — Step: - ID: 621

| | | | |
|---|---|---|---|
| Volume: 3.3 ml | Suction Speed: 15 ml/min | Dispensing Speed: 2 ml/min | |
| Vial Type: Type1@8 | | | |
| | Waiting Time after Dosage: 0 sec. | Waiting Time after Step: 150 sec. | |
| | | | Dispense: direct into chamber |
| without rinsing of vial | | | |

Step: Eluting — Basic type: Eluting — Step: - ID: 622

| | | | |
|---|---|---|---|
| Volume: 20 ml | Suction Speed: 20 ml/min | Dispensing Speed: 2 ml/min | Port: 8 Cyclohexane |
| | Repetitions: 0 | | |
| | Waiting Time after Dosage: 0 sec. | Waiting Time after Step: 0 sec. | |
| | | | Dispense: direct into chamber |

| | | | |
|---|---|---|---|
| EVA: | Temperature water heating 40 °C | | Temperature bottom cone 50 °C |
| | Sample input: Online from GPC or SPE process | | |
| | Batch volume = limit from where concentration starts: 5 ml (fix) + Waiting time: 0 min. | | |
| | Vacuum during GPC online sample input: 280 mbar | | |
| | Phase 1: Concentrate to level: 1 ml | | |
| | Vacuum absolute: 180 mbar | | |
| | Rinsing volume after phase 1: 5 ml | Rinsing steps: 1 x | Solvent from Port: 8 Cyclohexane |
| | Phase 2: Concentrate to level: 0.5 ml | | |
| | Vacuum absolute: 180 mbar | | |
| | Rinsing volume after phase 2: 2 ml | Rinsing steps: 0 x | Solvent from Port: 8 Cyclohexane |
| | Time control for vacuum process: yes | | Max. Vacuum run time: 120 min |
| | to dryness: no | | |
| | Nitrogen blow-down: no | | |
| | Remove Aliquot: no | | |
| | Solvent exchange: no | | |
| | Rinsing, filling up, mixing and transfer into vials: | | |
| | Rinsing volume at the end: 0.5 ml | Rinsing steps: 1 x | Solvent from Port: 8 Cyclohexane |
| | Fill up to volume: 1 ml | | Way of mixing: with gas / air, Volume = 0 ml |
| | of Port: 8 Cyclohexane | | |
| | Concentrate: into vials | | |
| | Nr.: 1 1 [each] | Type: Type1@8 ml | Volume per vial: 3 ml |
| | Fill Quantitativ: yes | | |
| | Rinsing volume: 1 ml | Rinsing steps: 1 x | Solvent from Port: 8 Cyclohexane |
| | 1. Cleaning cycle | | |
| | Rinsing volume: 5 ml | Rinsing steps: 1 x | Solvent from Port: 7 Acetone |
| | 2. Cleaning cycle | | |
| | Rinsing volume: 5 ml | Rinsing steps: 1 x | Solvent from Port: 8 Cyclohexane |


Figure 1: FREESTYLE SPE-EVA system.


Figure 2: SPE column Elufix Indicator PCBs.

## 2.4 Measurement

The extract was dried under a gentle nitrogen stream, dissolved in 100 µl recovery standard and measured with a GC-HRMS system (DFS, Thermo Fisher, Germany) on a HT-8 column (50 m x 0.22 mm x 0.25 µm) using the average response factor of 3 calibration standards.

# 3. Results

The aim of this work was to develop a method, that complies to the criteria laid down in Regulation 2017/644 for the determination of ndl-PCB. The recovery of the internal standards has to be between 60 and 120% and the minimum limit of quantification has to be 2 ng/g fat for the individual congeners.

Only small amounts of fat can be weighed in due to the small bed volume of the sulfuric acid SPE. In general, the sample weight was in the range of 0.1 to 0.2 g and the final volume was 100 µl. With this method, it has been possible to achieve limits of quantification for the individual congeners of less than 0.2 ng/g fat. This is a factor of 10 lower than the target of the method. The gas chromatographic separation of non-dioxin-like PCB from interferences, in particular from co-eluting PCB, was achieved with the HT-8 GC column.

The performance criteria of Regulation 2017/644 could be achieved not only at the maximum level, but also in the lower ng/g range. The intermediate laboratory precision (RSD) for PCB 138, PCB 153 and PCB 180 at levels of 2 ng/g fat is 1 to 3%. The trueness is also less than 5% when tested with proficiency test material. The limit of quantification for the lower-chlorinated ndl-PCB (PCB 28, 52 and 101), which are only present at very low concentrations in food of animal origin, is 0.2 ng/g fat. Based on these limits, a minimum limit of quantification of 1.2 ng/g fat for the sum of ndl-PCB can be achieved with the method described here.

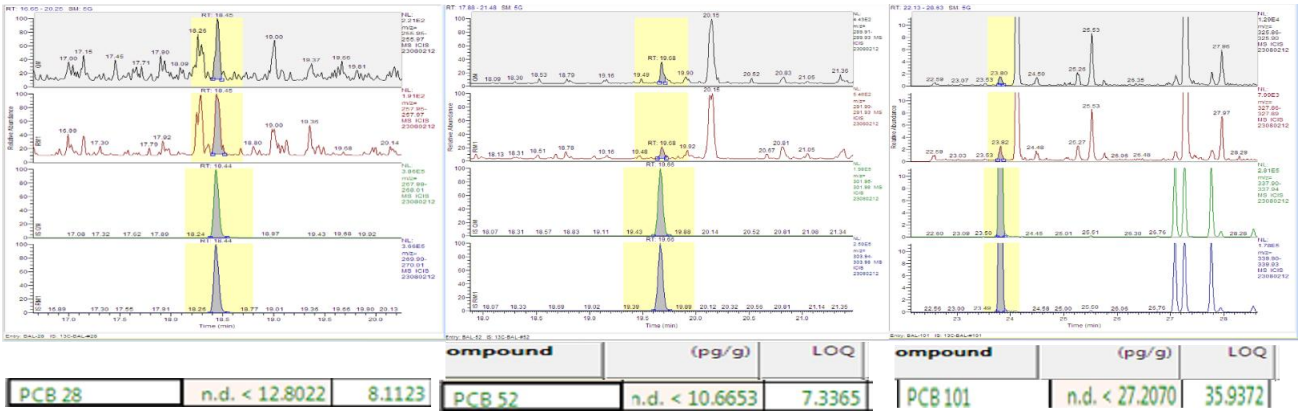


Figure 3: Chromatograms of LOQ's of PCB 28, PCB 52 and PCB 101 found in pig fat.

| | ng/g fat | ng/g fat | ng/g fat | ng/g fat | ng/g fat | ng/g fat | Av ng/g fat | SD ng/g fat | RSD % |
|---|---|---|---|---|---|---|---|---|---|
| PCB 28 | 0.026 | 0.021 | 0.026 | 0.024 | 0.021 | 0.020 | 0.023 | 0.003 | 12.4 |
| PCB 52 | 0.042 | 0.028 | 0.044 | <0.0255 | 0.033 | 0.040 | 0.037 | 0.007 | 18.1 |
| PCB 101 | 0.055 | 0.051 | 0.058 | 0.060 | 0.053 | 0.060 | 0.056 | 0.004 | 6.7 |
| PCB 138 | 3.660 | 3.671 | 3.502 | 3.452 | 3.710 | 3.633 | 3.605 | 0.103 | 2.9 |
| PCB 153 | 4.958 | 5.008 | 4.833 | 4.896 | 4.984 | 4.922 | 4.934 | 0.064 | 1.3 |
| PCB 180 | 1.944 | 1.973 | 1.919 | 1.971 | 1.949 | 1.902 | 1.943 | 0.028 | 1.5 |
| Lower Bound | 10.68 | 10.75 | 10.38 | 10.40 | 10.75 | 10.58 | 10.60 | 0.1614 | 1.6 |
| Medium Bound | 10.68 | 10.75 | 10.38 | 10.42 | 10.75 | 10.58 | 10.60 | 0.1614 | 1.4 |
| Upper Bound | 10.68 | 10.75 | 10.38 | 10.43 | 10.75 | 10.58 | 10.60 | 0.1614 | 1.5 |

Table 1: Results of 6 repetitions of a real sample pig fat.

# 4. Discussion

It can be concluded that a minimum limit of quantification of 0.2 ng/g fat is sufficient to detect the presence of ndl-PCB when comparing the performance criteria of the described method with the occurrence and levels of ndl-PCB found in food and feed in Europe (1). This method and the specially developed Elufix Indicator PCB column enables the determination of ndl-PCBs in a sufficiently sensitive, specific and robust manner. The column can be used manually or in an automated system. By using the automated FREESTYLE-SPE-EVA system, 45 samples can be processed in one run. The whole process takes per sample approx. 45 min. The system therefore enables a high throughput of samples. No supervision is necessary during sample processing and the time can be used for other work. Save labor time and increase sample numbers by using the LCTech FREESTYLE SPE-EVA system in conjunction with Elufix Indicator PCB column. Faster and cheaper than the conventional PCB/Dioxin sample prep with two or three column setup.

# 5. References

(1) Barbara Gallani, Anna Boix, Alessandro Di Domenico, Roberto Fanelli: Occurrence of NDL-PCB in food and feed in Europe Organohalogen Compounds Vol. 66 (2004) 3561- 3569

# 6. Acknowledgment

[1]The tests were carried out and the results provided by Ursula Möhlenkamp, Lothar Bathe, and Thorsten Bernsmann from Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe, Joseph-Koenig-Str. 40, 48147 Münster, Germany