SOLUTIONS BY LCTech

Feasibility Study for the Determination of Polychlorinated Naphthalenes in Food Samples Using DEXTech Plus/Pure Systems

K. Tschiggfrei, A. Schächtele (EURL for halogenated POPs in Feed and Food, Freiburg), T. Fiedler and U. Aulwurm (LCTech GmbH)

Content

1. Introduction 3
2. Experimental 4
   2.1 Analytes and Standards 4
   2.2 Samples 4
   2.3 Sample Preparation 4
   2.3.1 Sample Setup for Volatility Tests 4
   2.3.2 PCN Separation via DEXTech Plus/Pure 5
3. Results 6
   3.1 Influence of N_2 Stream and Rotary Evaporation on PCN Recoveries 6
   3.2 PCN Separation and Clean-up via the DEXTech System 7
   3.3 Long-term within-laboratory Precision and Opportunities as part of a multi-method 8
4. Summary 13
5. Acknowledgement 13
6. References 13
7. Annex 14
8. Instruments & Clean-up Columns 16

1. Introduction

Polychlorinated naphthalenes (PCNs) belong to the class of chlorinated, polycyclic, aromatic hydrocarbons. Around 150 000 t of polychlorinated naphthalenes (PCNs) have been produced as technical mixtures (e.g. Halowax in U.S., Nibren in Germany, Seekay in UK etc.) in the period of 1930-1980 (Fernandes et al. 2017). They were used as dielectrics, plasticizer, wood preservative or lubricants because of their high thermal and chemical stability, their low flammability and their good electrical isolation (Fernandes et al. 2010). Furthermore, they are a by-product at the production of technical PCB-mixtures (e.g. Arochlor, Sovol) and are formed during combustion processes (Fernandes et al. 2017). Due to their lipophilic, bio-accumulative, toxic and persistent properties, these environmental contaminants are banned in Germany since 1983 and are listed in Annex C of the Stockholm Convention since 2015 as persistent organic pollutants (POPs).

There are 75 possible PCN congeners ($C_{10}H_{8-n}Cl_n$), which can be divided in 8 homologue groups (mono- to octa-chlorination). Studies show an AhR-mediated mechanism of toxicity by PCNs because of their planar structure and similarity to 2,3,7,8-TCDD. Thereby high chlorinated PCNs show higher potencies (REPs) relative to 2,3,7,8-TCDD than low chlorinated PCNs. In total the REPs of 20% of PCN congeners vary between 0.003 and 0.000001, which is comparable with those of the mono-ortho-PCBs (WHO-TEF (2005): 0.00003) (van den Berg et al. 2006; Fernandes et al. 2017). Thus, a small contribution of the PCN-TEQ to the total dioxin-like TEQ was reported. The main rout of exposure by PCNs for humans is nutrition, whereby shellfish, fish, fats of animal origin and eggs generally showed the highest PCN levels (Fernandes et al. 2017).

The list of the Stockholm Convention of persistent organic pollutants for elimination or restricted use grows constantly. Therefore, many labs are facing the challenge to extend their scope on a regular basis in order to monitor different relevant POPs across different food groups. Automated sample preparation methods can save precious time and resources. Especially automated multi-methods are of special interest.
This application note describes a feasibility study to determine PCN congeners in food applying purification with DEXTech Plus and DEXTech Pure systems (used methods are applicable to both systems) and measurement by HRGC-HRMS (high resolution gas chromatography-high resolution mass spectrometry). The developed method can be used to determine PCDD/Fs and PCBs simultaneously with PCNs.

2. Experimental

2.1 Analytes and Standards

The following 26 congeners were included in the analysis:

PCN 27, PCN 28/36, PCN 31, **PCN 42**, PCN 46, PCN 48, PCN 49, PCN 50, **PCN 52**/60, PCN 53, PCN 59, PCN 63, **PCN 64**/68, **PCN 65**, PCN 66/**67**, PCN 69, PCN 70, PCN 71/72, **PCN 73**, PCN 74, **PCN 75**

^{13}C labelled surrogates for 8 PCN congeners (marked in bold) were used for internal standardisation. Thus for each homologue group an isotopic labelled standard was available. ^{13}C PCN 65 was used as final standard. The congeners were chosen based on the toxicological characteristics, reported levels of occurrence, congener patterns and the availability of analytical standards. Standards were obtained from LGC (Germany) and diluted to the appropriate levels in toluene.

2.2 Samples

Vegetable oil and fish oil samples were chosen for analysis. Procedural blanks and sun flower oil blank samples were expected to be free of PCNs whereas in food of animal origin, especially fish and fish products, PCN contamination was expected (Fernandes et al. 2011; Falandysz et al. 2019; Zacs et al. 2021). The chosen fish oil was contaminated with possibly interfering analytes like PCDD/Fs (WHO-PCDD/F-TEQ 1.9 pg/g fat) and PCBs (WHO-PCB-TEQ 9.2 pg/g fat).

2.3 Sample Preparation

2.3.1 Sample Setup for Volatility Tests

Before starting sample preparation with the DEXTech system, the influence of the evaporation with a nitrogen stream and rotary evaporation on PCN recoveries was tested. Isotope labelled standards (including seven congeners) and native standards were prepared in toluene in duplicate in vials and were treated in different ways as follows (test 1-3):

Table 1: Standard preparation was done in duplicate (test 1-3) or six times (test 4).

Test	Preparation
1	• 30 µl native PCNs and 30 µl ^{13}C PCNs prepared in vial • Drying by N_2 stream for 20 min to complete dryness • Re-dissolving in 30 µl ^{13}C PCN 65 standard
2	• 30 µl native PCNs and 30 µl ^{13}C PCNs prepared in vial • Drying by N_2 stream to dryness • Re-dissolving immediately in 30 µl ^{13}C PCN 65 standard

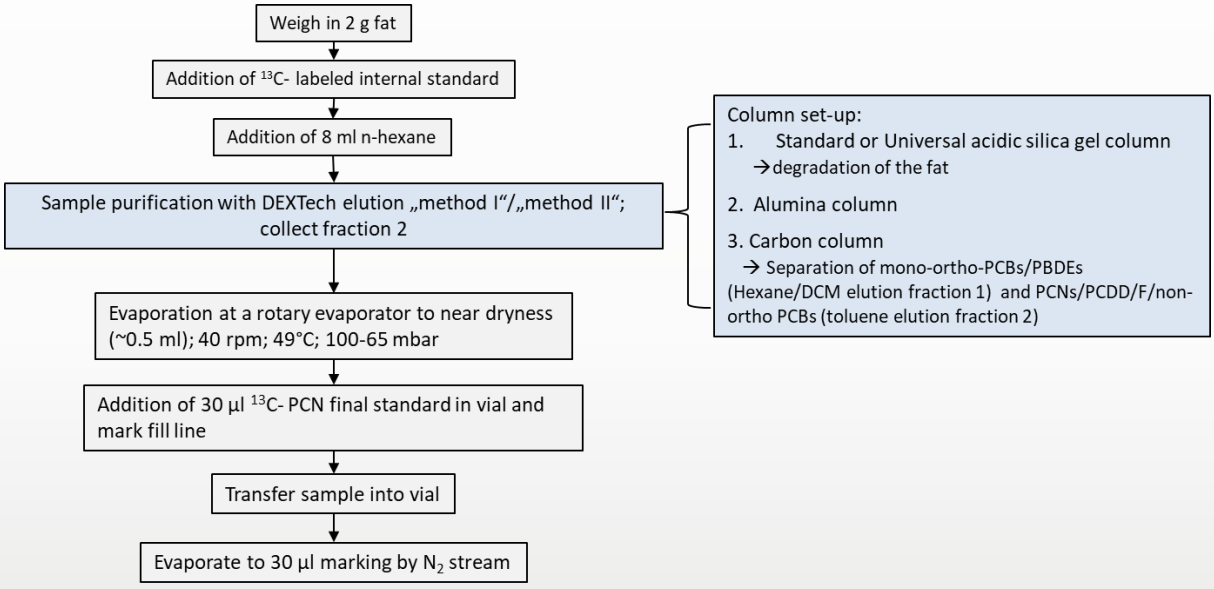
3	• 30 µl native PCNs and 30 µl ^{13}C PCNs prepared in vial • Drying by N_2 stream to less supernatant (<5 µl) • Add 30 µl ^{13}C PCN 65 standard
4	• Native PCNs and ^{13}C PCNs prepared in a peaked flask in 10 ml toluene • Evaporation in a rotary evaporator to near dryness • Transfer to vial and add 30 µl ^{13}C PCN 65 standard • Drying by N_2 stream to 30 µl supernatant (marking)

Furthermore, standards were spiked in 10 ml toluene and evaporated to near dryness by rotary evaporator (see Table 1, test 4). Remaining solvent was transferred into a GC-vial and injection standard was added before drying by N_2 stream to 30 µl marking. Altogether, the procedure of test 4 was repeated six times in different sample sequences on different days to evaluate interday and intraday precision.

Isotope labelled standard solutions and native standard solutions were prepared without any evaporation steps to compare recoveries.

2.3.2 PCN Separation via DEXTech Plus/Pure

For sample purification the methods Alox Plus Default ("method I") and Alox ("method II") were used (see annex). The tested methods are applicable to DEXTech Plus and DEXTech Pure systems. All oil/fat samples were prepared and treated as described below, if not otherwise specified:



All samples were measured by GC-HRMS at resolution 10'000 (at 5% peak height, DFS high resolution magnetic sector MS-Thermo Scientific).

3. Results

3.1 Influence of N_2 Stream and Rotary Evaporation on PCN Recoveries

Pre-tests on the volatility of PCNs were conducted. It was observed that the recoveries of especially lower chlorinated naphthalenes (tetra-/penta-) decrease significantly if PCN-standard solutions were evaporated to complete dryness (see Figure 1). Spiked solvent evaporated with rotary evaporation and N_2-stream showed lower recoveries of ^{13}C PCN congeners than pure standard solutions (without N_2 stream/rotary evaporation), too (see Figure 1).

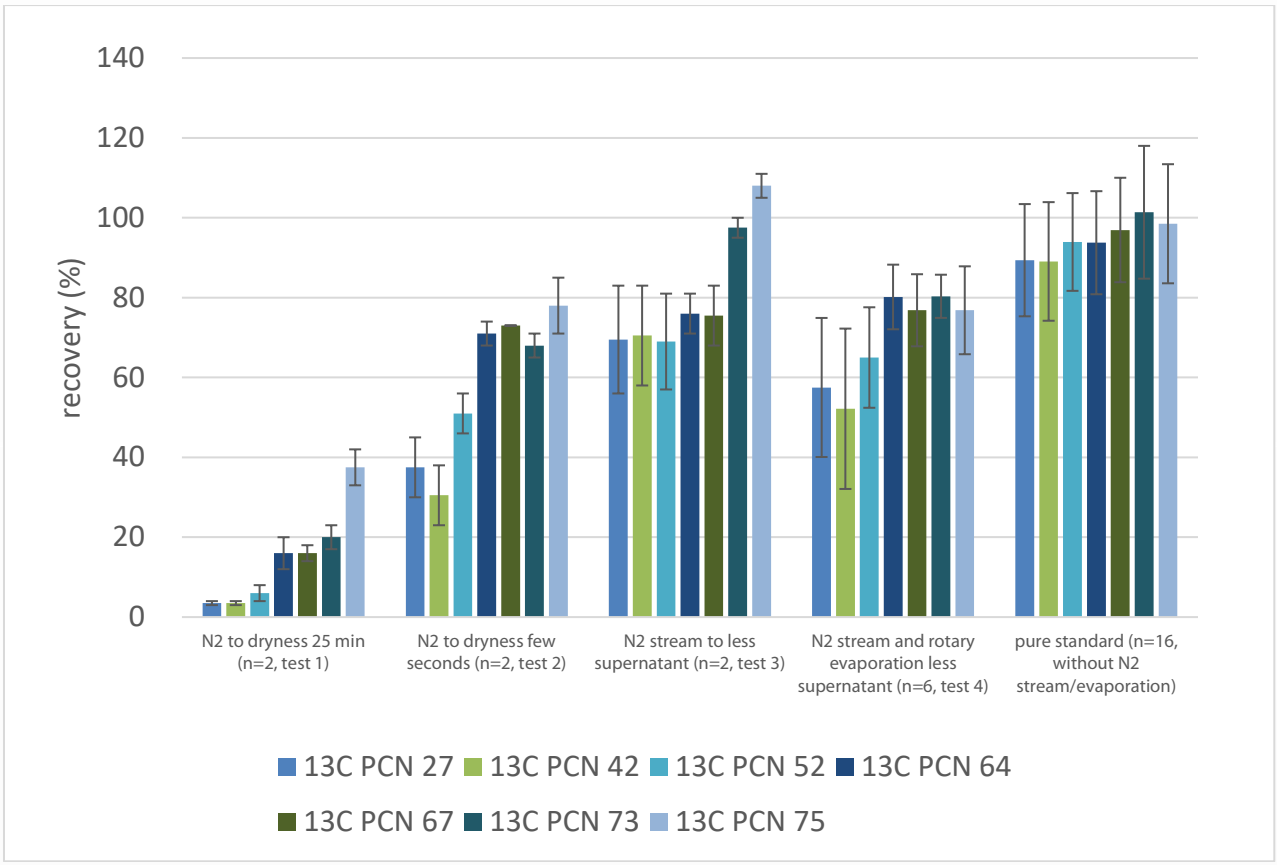


Figure 1: Influence of N_2 stream and rotary evaporation on PCN congeners. The congeners show rather high volatility.

These tests demonstrate that additionally to the clean-up, special care has to be taken for the evaporation steps during the workflow of the PCN analysis.

3.2 PCN Separation and Clean-up via the DEXTech System

In order to determine the recovery rate of ^{13}C PCNs in fraction 2 (PCDD/F, non-ortho PCB fraction), in which the major amount of the PCN-congeners monitored is expected, sun flower oil samples were spiked with 30 µl ^{13}C PCN solution and purified using method I (see annex) and a Standard column (see 2.3.2). In total 12 samples were analysed: 6 samples were analysed in parallel whereas the other 6 samples were analysed on different days. The ^{13}C PCN recoveries for all 12 samples were in the range of 34-83 %, for 6 samples analysed in parallel 44-78%. The highest standard deviation of the recoveries of 21% (n=12) or 15% (n=6) was measured for ^{13}C PCN 42 (see Figure 2).

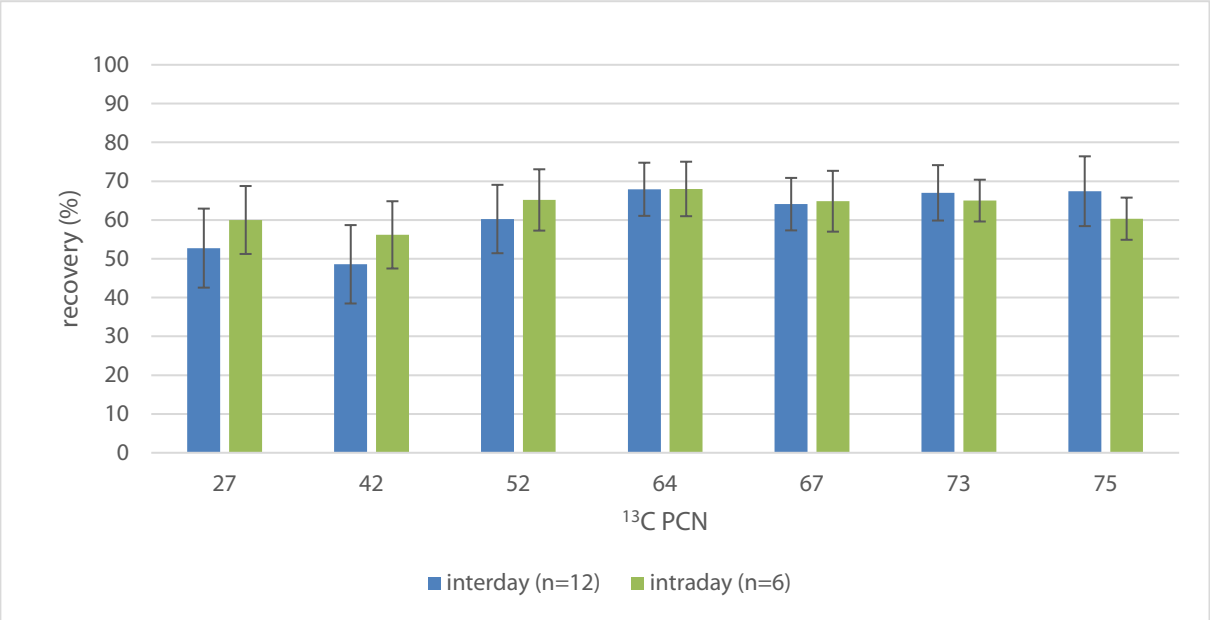


Figure 2: Recoveries of ^{13}C PCNs in fraction 2 after sample preparation of a spiked sunflower oil using method I on Standard column.

In order to check the accuracy of native PCN determination via isotope dilution, all oil samples were spiked with 30 µl native PCN mixture containing 1 pg/µl of each congener before sample preparation. The trueness of the native spiked PCNs was in a range of 73-123 % (n=12) and 78-110 % (n=6). Relative standard deviation was in a range of 5 % (PCN 64/68, PCN 73, PCN 74, PCN 75) to 11 % (PCN 31, PCN 46, PCN 53, PCN 70) (see Figure 3). The trueness of the native PCNs being close to 100 % and the low variance between the samples demonstrate that determination via isotope dilution is suitable even for low ^{13}C PCN recoveries down to 30 %.

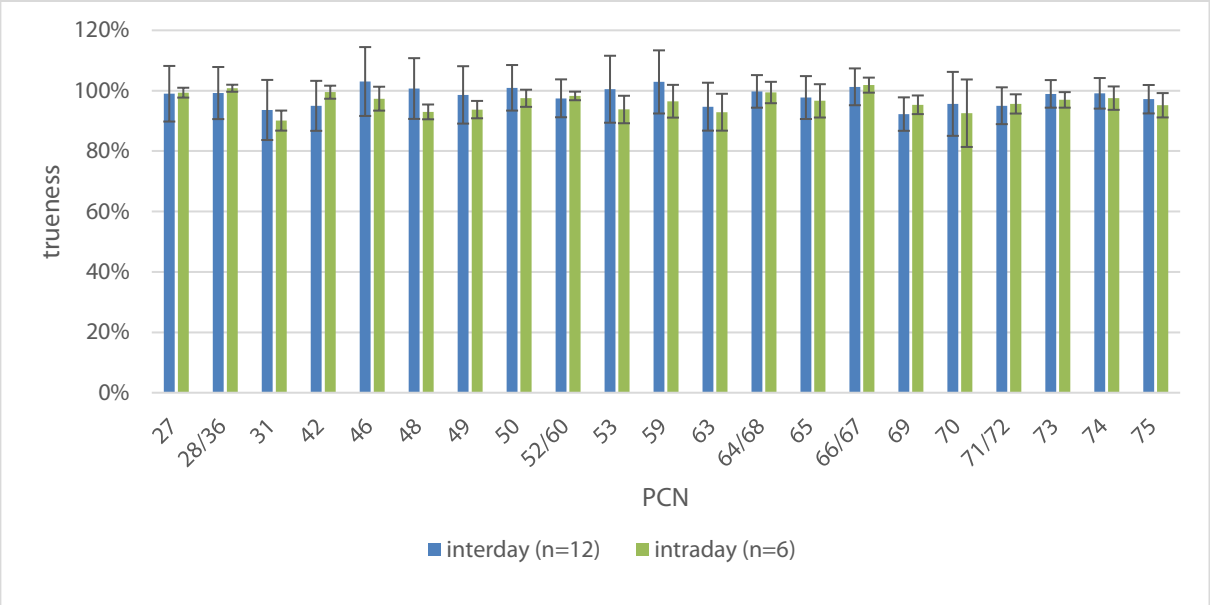


Figure 3: Accuracy (trueness and precision) of native PCN determination via isotope dilution in spiked sunflower oil at a spiking level of 15 pg/g fat for each congener.

3.3 Long-term within-laboratory Precision and Opportunities as part of a multi-method

A fish oil sample with known contamination was prepared using different DEXTech elution methods (see annex method I and method II) on different clean-up columns (Standard column or Universal column) by different operators over a time period of two years to test the long term within-laboratory precision of the method.

The recovery rates of ^{13}C PCNs in the samples (method I on Standard column n=5, method II on Standard column n=6) applying the final standard ^{13}C PCN 65 after rotary evaporation/before measurement were in an acceptable range of 38-87 % (n=11). For PCDD/F analysis the final standard is routinely added before rotary evaporation. Due to easy handling final standard ^{13}C PCN 65 was applied before rotary evaporation to 5 fish oil samples (method I on Universal column) where PCDD/Fs and PCNs were analysed in parallel. Thus, the recovery rates of ^{13}C PCNs in the samples applying final standard ^{13}C PCN 65 before rotary evaporation (Figure 4 "method I on Universal column") were higher with 41-112% (n=5) as the different congener-groups show different volatility. Especially the higher chlorinated PCNs (PCN 67, PCN 73 and PCN 75) showed under these circumstances slightly higher recoveries.

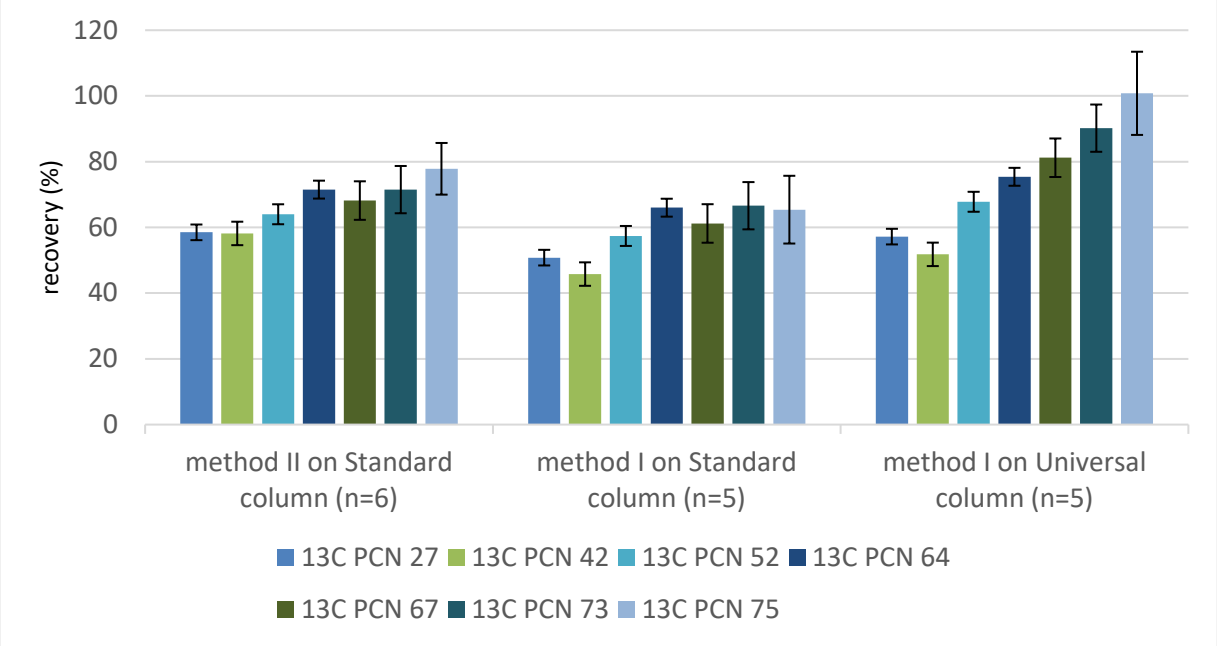


Figure 4: Recovery of ^{13}C PCNs in fish oil using different clean-up columns (Standard column or Universal column) and different clean-up methods (see annex method I/II) and addition of the final standard before (method I/Universal column) and after rotary evaporation (method I/method II/Standard column).

The tests demonstrate that a recovery of ^{13}C PCN of 40-120 % (38-112 %) and a precision of below 20 % (3-17 %) is achievable over a long period applying different clean-up columns, different DEXTech elution methods and different operators.

The sum of 26 native PCNs determined by the three different test settings was in a range of 312-320 pg/g fat (see Figure 5). PCN 52/60 (126-131 pg/g fat), PCN 42 (71-72 pg/g fat) and PCN 66/67 (40-42 pg/g fat) showed the highest levels in fish oil. The levels for PCN 31 (n.q.-2 pg/g fat) and PCN 70 (n.q.-0.5 pg/g fat) were below or at the quantification limit. No big differences between applying the method I or method II (for method II elution time of fraction 1 is extended by 4 min compared to method I), as well as using Standard column or Universal column concerning native PCN levels and their precision were determined (see Figure 5).

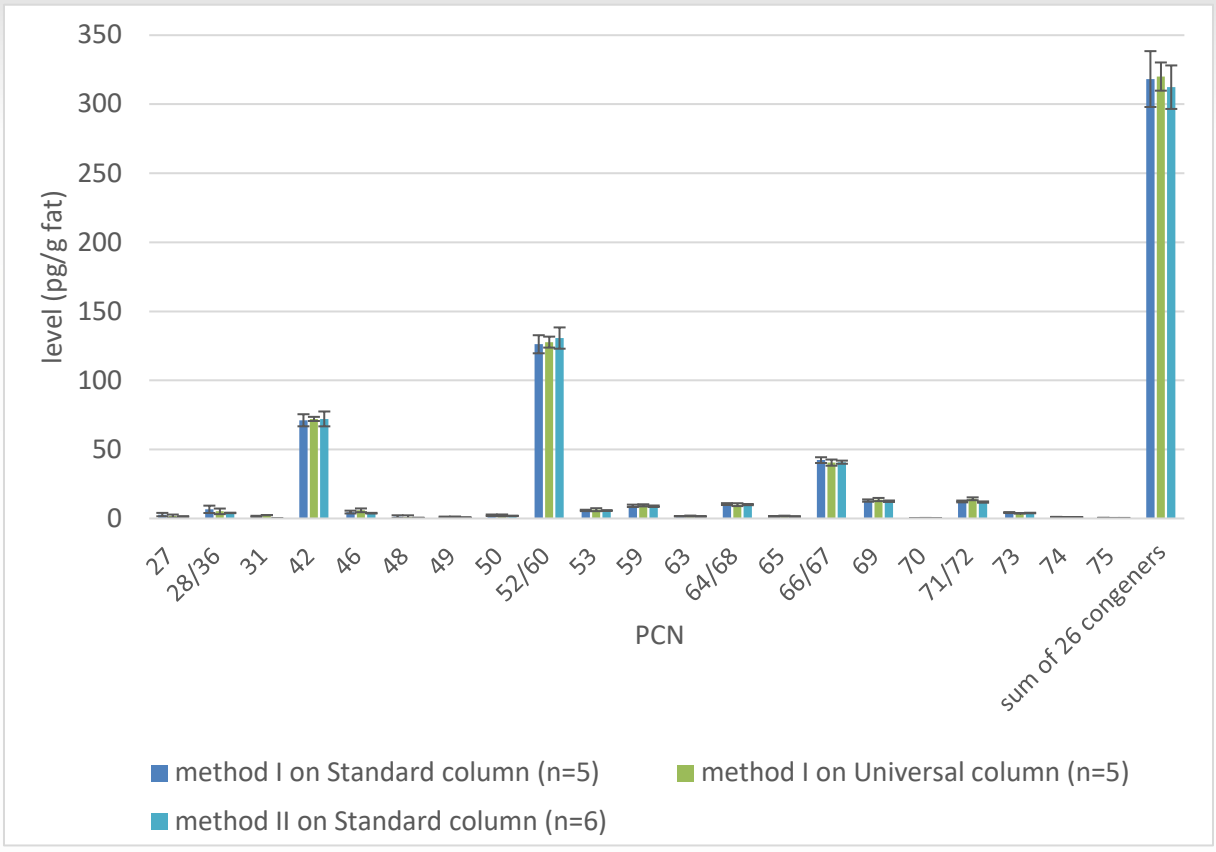


Figure 5a: Levels of native PCNs and sum of 26 congeners determined in fish oil using different sample purification settings (elution method I/II, Universal/Standard column in pg/g fat).

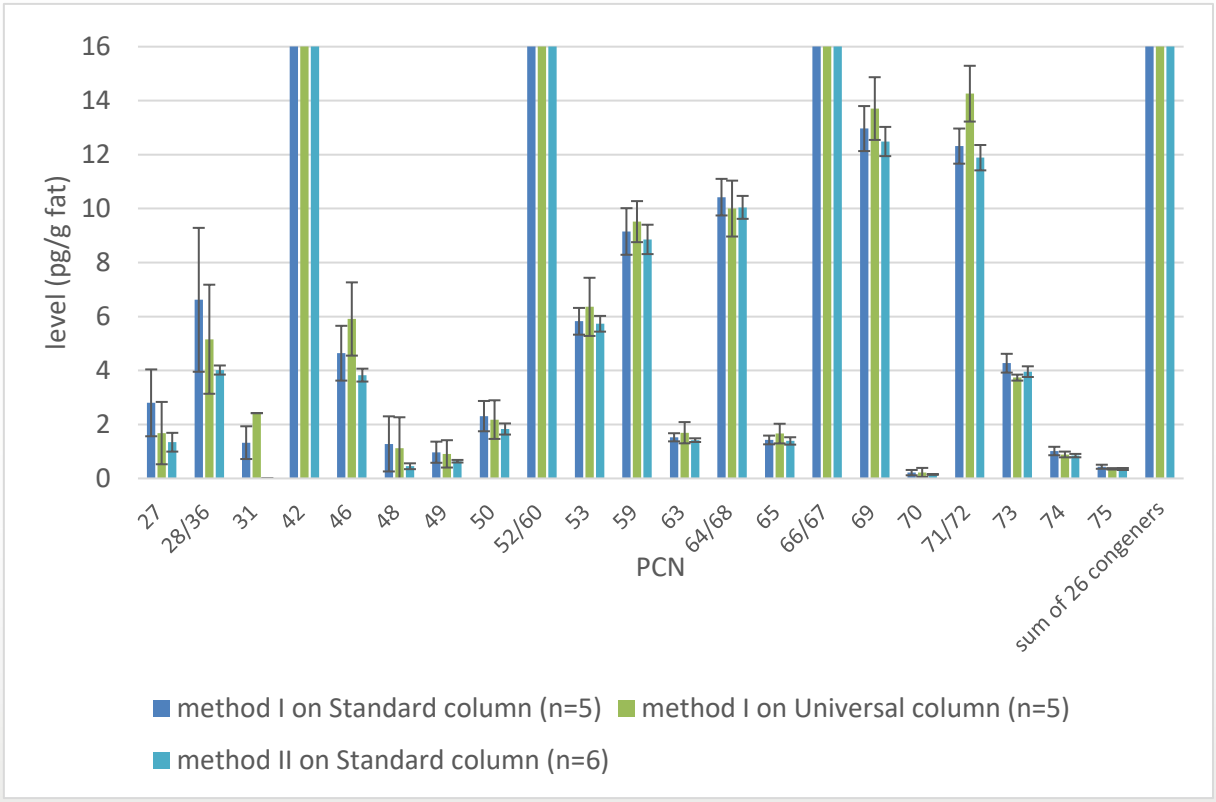


Figure 5b: Enlargement of 5a: Levels of native PCNs and sum of 26 congeners determined in fish oil using different sample purification settings (elution method I/II, Universal/Standard column).

In addition to the PCN levels, in some of the samples (n=5, method I on Universal column) the PCDD/F and PCB were also analysed. The results are shown in Figure 6a, b and c. The green dashed line represents the median of 96 measurements of the quality control sample "fish oil". The coloured dots represent the PCDD/F and PCB results of the fish oil samples where PCNs were determined within the same sample preparation. In summary, the result also show very good trueness and precision for the simultaneous analysis of PCDD/Fs and PCB together with the PCN clean-up. Results for PCDD/Fs and PCBs were well within +/- 2 times the standard deviation of the median value of 96 analyses of this routine quality control sample.

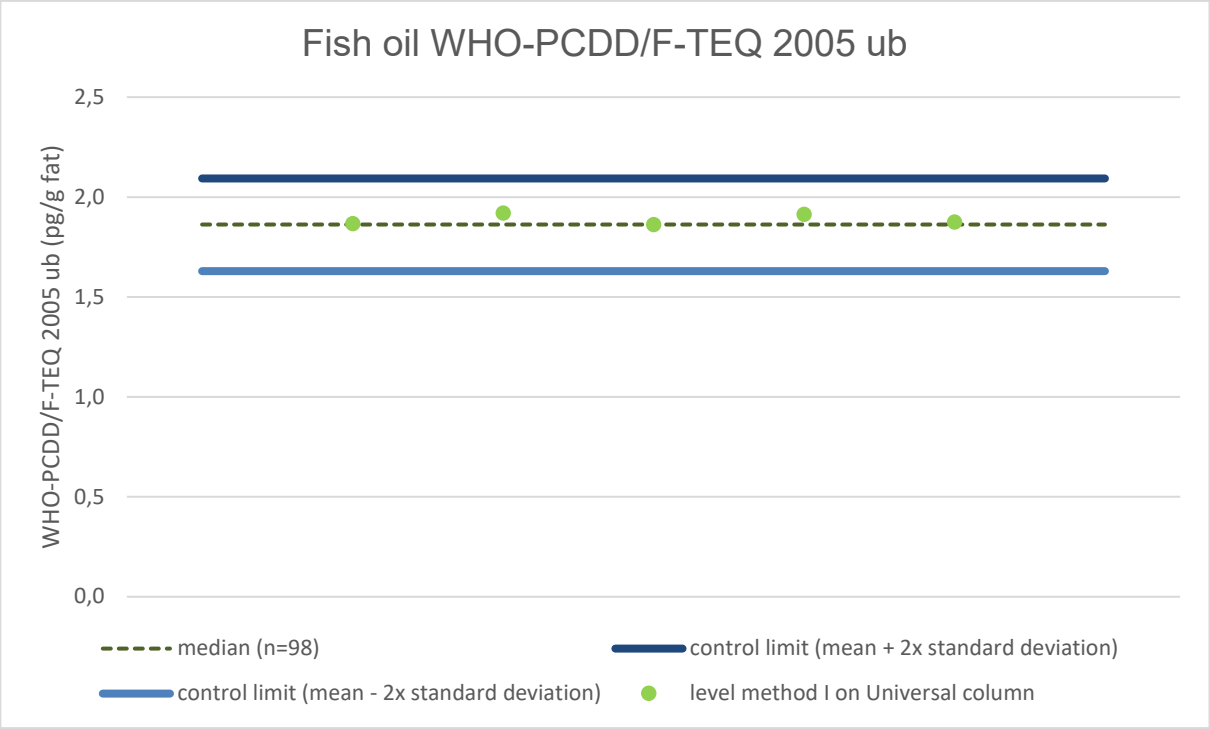


Figure 6a: PCCD/F – TEQ result for the 5 samples (green dots) simultaneously cleaned-up and tested for PCDD/Fs, PCBs and PCNs. The green dashed line represents the median PCDD/F-TEQ of 98 tests for the fish oil quality control sample.

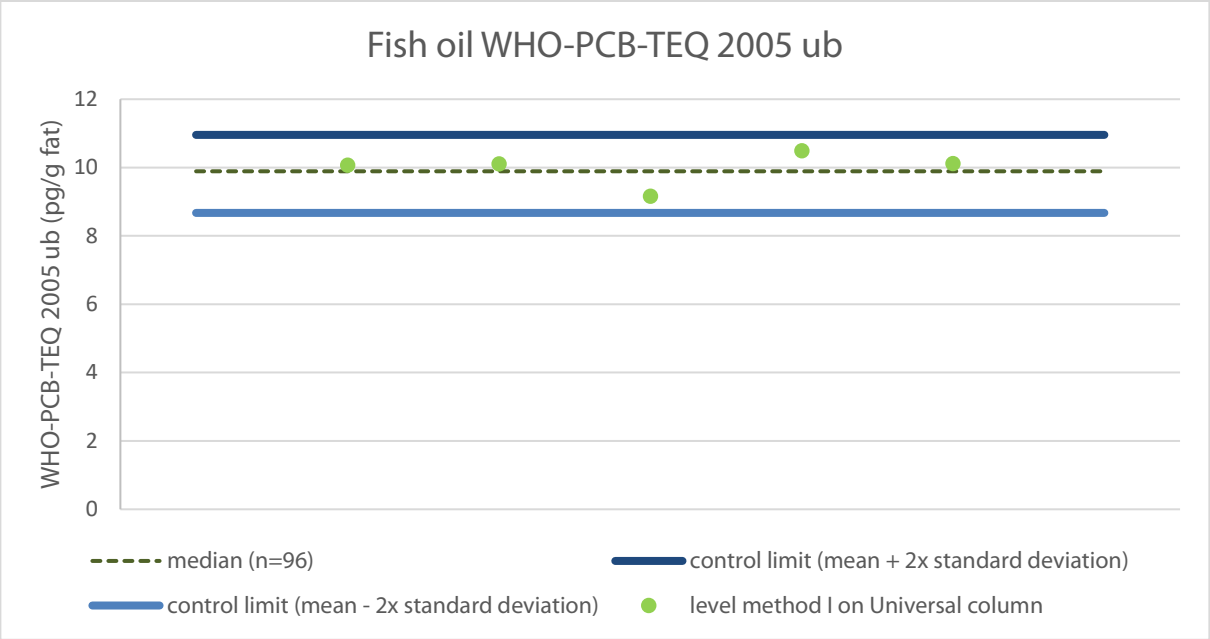


Figure 6b: WHO-PCB–TEQ result for the 5 samples (green dots) simultaneously cleaned-up and tested for PCDD/Fs, PCBs and PCNs. The green dashed line represents the median PCB-TEQ of 96 tests for the fish oil quality control sample.

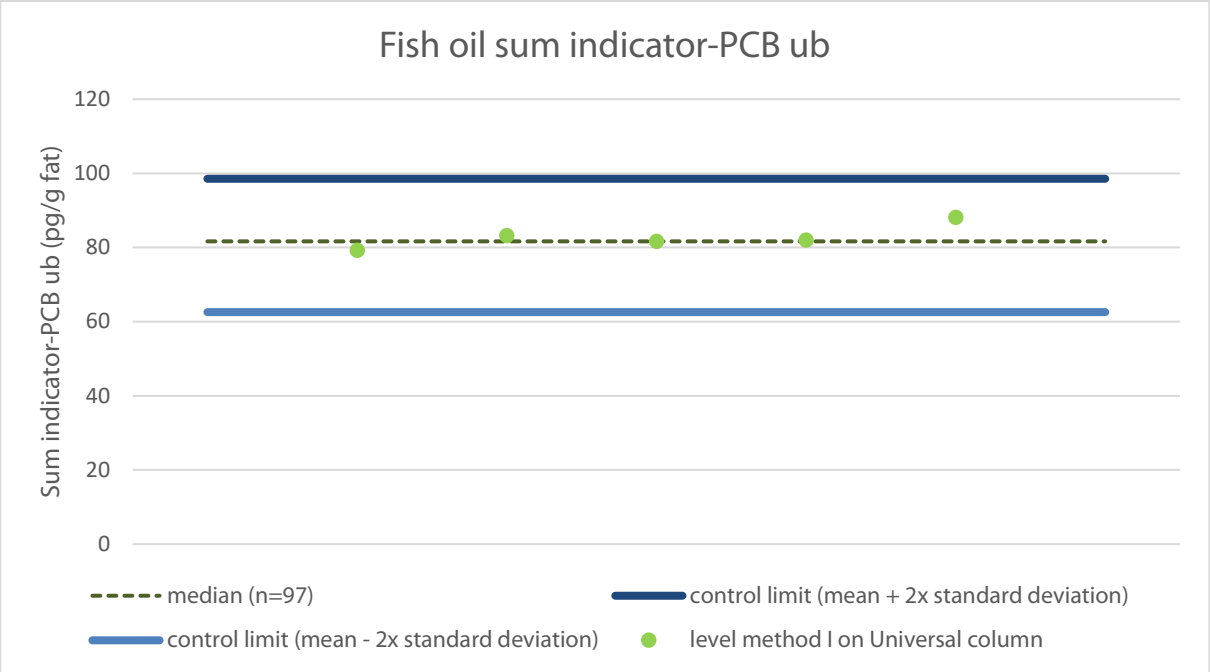


Figure 6c: Sum indicator-PCB results for the 5 samples (green dots) simultaneously cleaned-up and tested for PCDD/Fs, PCBs and PCNs. The green dashed line represents the median sum indicator-PCB of 97 tests for the fish oil quality control sample.

4. Summary

The data of this feasibility study demonstrate that the DEXTech Plus or DEXTech Pure system and methods can be used for the clean-up of PCNs before the analysis by GC-HRMS. With the Default Alox Plus method and the Standard or Universal column, the tested isotope-labelled and native PCNs were eluted in fraction 2 together with the PCDD/Fs. The recoveries of the ^{13}C labelled PCNs are in an acceptable range of 40 to 120 % and can be used for the quantification of native PCNs via isotope dilution method. Additionally, the results for the native PCNs show good precision, trueness and accuracy using the DEXTech Plus/Pure system. Therefore, these clean-up systems are suitable for the fast and simultaneous clean-up of PCBs, PCDD/Fs and PCNs.

5. Acknowledgement

All the tests were done at the European Union Reference Laboratory for halogenated POPs in Feed and Food in Freiburg. We thank Karin Tschiggfrei and Dr. Alexander Schächtele for generously providing the data for this study.

6. References

Falandysz, Jerzy; Smith, Frankie; Panton, Sean; Fernandes, Alwyn R. (2019): A retrospective investigation into the occurrence and human exposure to polychlorinated naphthalenes (PCNs), dibenzo-p-dioxins and furans (PCDD/Fs) and PCBs through cod liver products (1972-2017). In: *Chemosphere* 231, S. 240–248. DOI: 10.1016/j.chemosphere.2019.05.073.

Fernandes, A. R.; Tlustos, C.; Rose, M.; Smith, F.; Carr, M.; Panton, S. (2011): Polychlorinated naphthalenes (PCNs) in Irish foods: Occurrence and human dietary exposure. In: *Chemosphere* 85 (3), S. 322–328. DOI: 10.1016/j.chemosphere.2011.06.093.

Fernandes, Alwyn; Mortimer, David; Gem, Martin; Smith, Frankie; Rose, Martin; Panton, Sean; Carr, Melanie (2010): Polychlorinated naphthalenes (PCNs): congener specific analysis, occurrence in food, and dietary exposure in the UK. In: *Environmental science & technology* 44 (9), S. 3533–3538. DOI: 10.1021/es903502g.

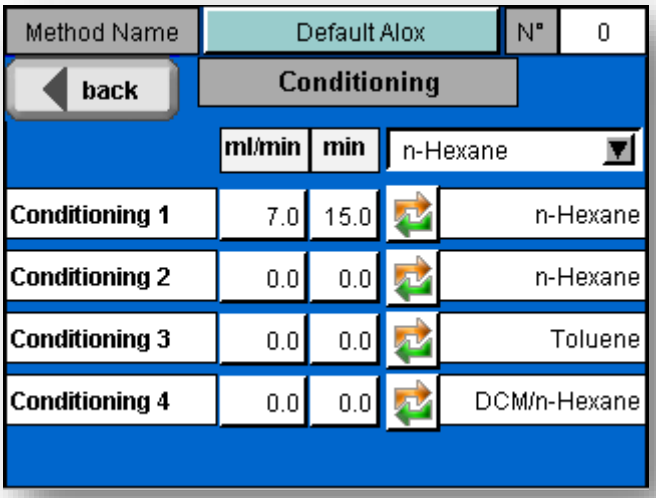
Fernandes, Alwyn; Rose, Martin; Falandysz, Jerzy (2017): Polychlorinated naphthalenes (PCNs) in food and humans. In: *Environment international* 104, S. 1–13. DOI: 10.1016/j.envint.2017.02.015.

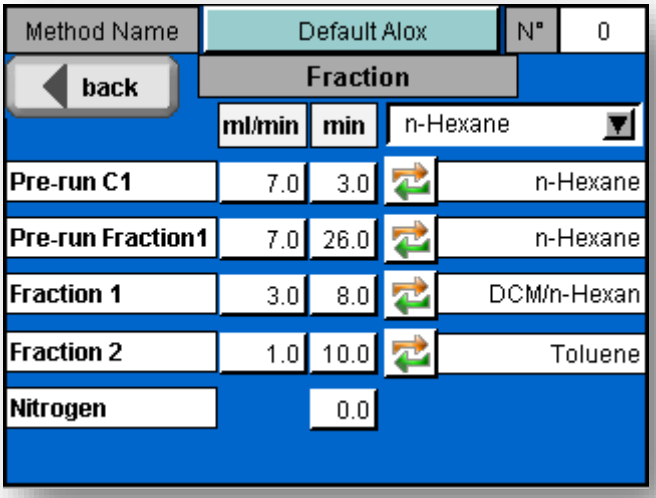
van den Berg, Martin; Birnbaum, Linda S.; Denison, Michael; Vito, Mike de; Farland, William; Feeley, Mark et al. (2006): The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. In: *Toxicological sciences : an official journal of the Society of Toxicology* 93 (2), S. 223–241. DOI: 10.1093/toxsci/kfl055.

Zacs, D.; Perkons, I.; Pasecnaja, E.; Bartkevics, V. (2021): Polychlorinated naphthalenes (PCNs) in food products in Latvia: Congener-specific analysis, occurrence, and dietary exposure of the general population. In: *Chemosphere* 264, S. 128460. DOI: 10.1016/j.chemosphere.2020.128460.

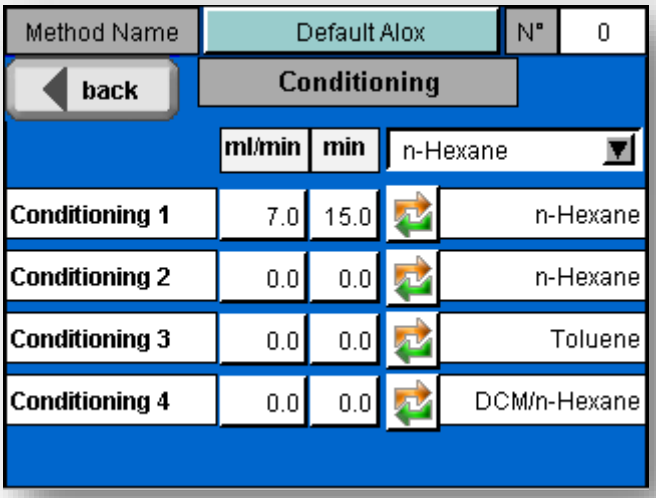
7. Annex

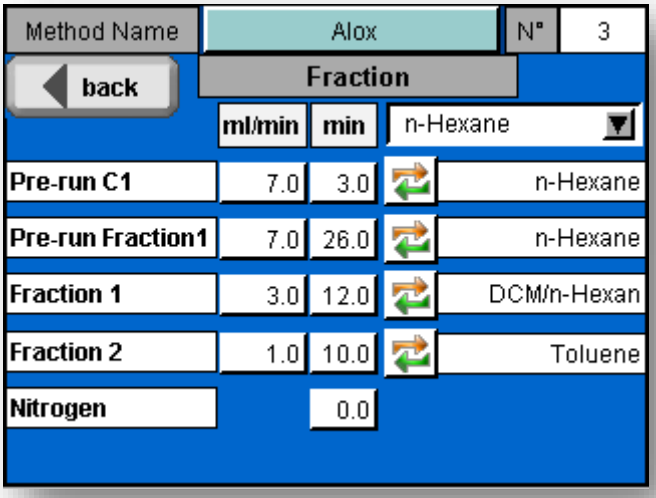
- Alox Plus Default (“Method I“)





- Alox („Method II“)





8. Instruments & Clean-up Columns

Instruments

1.	DEXTech Plus	P/N	15011
2.	DEXTech Pure	P/N	15969
3.	DEXTech Heat	P/N	16800
4.	DEXTech 16	P/N	15430

Clean-up columns

1.	Universal column	P/N	19511
2.	Smart columns	P/N	19513
3.	Alox column	P/N	15433
4.	Carbon column	P/N	15424

Contact

LCTech GmbH
Daimlerstraße 4
84419 Obertaufkirchen
Germany

Tel.: +49 8082 2717-0
E-mail: info@LCTech.de

www.LCTech.de
www.LCTech-online.com

SOLUTIONS BY LCTech