# Feasibility study for the Determination of Polychlorinated Naphthalenes in Food and Feed samples

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## Goal

1 To determine in which fraction PCNs elute on the automated "GO-xHT" purification device

2 To determine the performance of the analysis of PCNs in real samples at relevant levels using a GO-xHT and GC-HRMS

3 To determine the recovery of PCNs in Centrivap solvent evaporation

## Abstract

Selective and automated purification for the analysis of dioxins and PCBs is common nowadays. However, the use of these purification devices for the analysis of other classes of compounds are studied to lesser extent. This application note demonstrates that the automated purification device GO-xHT (Miura), is suitable to purify samples for sensitive detection of polyhlorinated naphthalenes (PCNs) in addition to dioxins, PCBs and PBDE (1). The purified extracts can be concentrated to such small volumes that pg/g detection limits in samples are possible using a GC-HRMS with good accuracy and precision.

Polychlorinated naphthalenes (PCNs) were produced early 1900 by treatment of naphthalene with chlorine. The reaction product is an oil with varying viscosity depending on the degree of chlorination. Initially use was for insulating coatings for electrical devices, as well as for impregnation of wood against insects, molds and fungi. PCNs are widespread in the environment and are listed by the Stockholm convention in annex C. Data on food shows widespread occurrence in most commonly consumed foods from different parts of the world. Toxicological studies have given a greater insight into the potencies of some congeners which are known to be toxic. (2, 3)

For the analysis of PCNs methodologies have been developed based on experience gained from the analysis of similar contaminants, such as dioxins and PCBs. In the last decade, especially sample preparation methodologies have progressed, from manual operations to automated solutions, such as the Miura GO-xHT. In this communication the feasibility of PCN analysis using a Miura GO-xHT is reported.

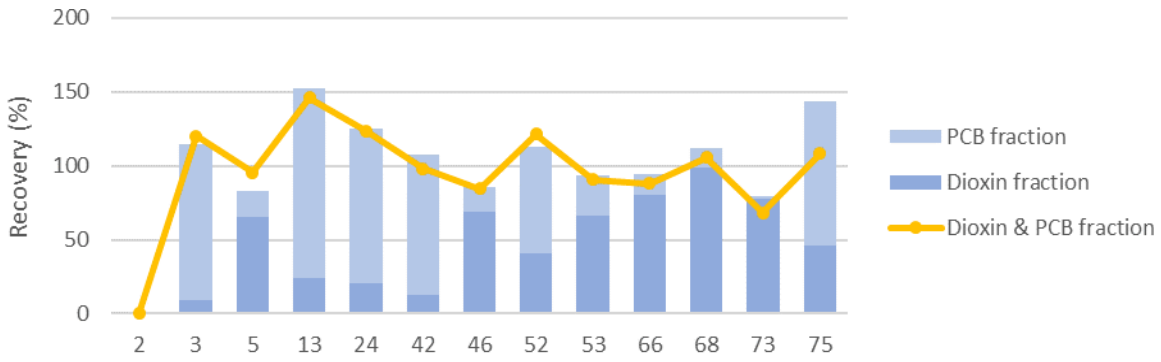


Figure 1 Recovery of PCN in a spiked blank by fraction and combined (measured) on a GO-xHT

## Introduction

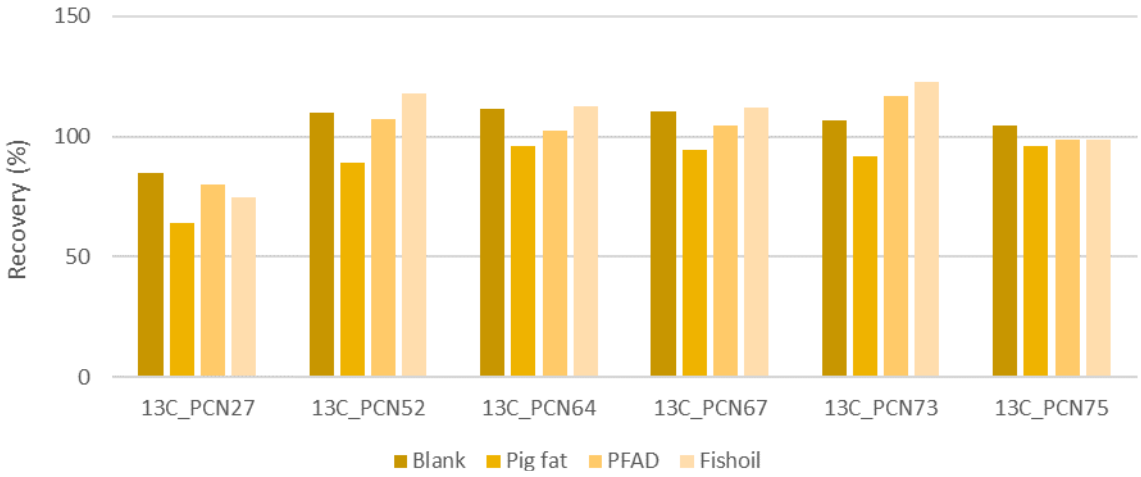


Figure 2 Recovery of internal standards in several types of samples purified using a GO-xHT (n=2, the sd is 1 – 12)

## Samples and standards

Samples pig fat, palm free fatty acid distillate (PFAD) and fish oil were chosen for analysis. These samples were specifically selected as they generally contain more interfering elements in dioxin and PCB analysis compared to other type of samples. Each sample was analysed once as such and once with addition of native PCNs.

Standard mixtures of PCNs (ECN-5558-1.2, S.I. 1) and $^{13}$C10 PCNs (ECN-5490-200X-1.2, S.I. 2) and a standard $^{13}$C12 PCB159 (EC-5336-1.2) were obtained from Cambridge Isotope Laboratories (Andover, MA) and diluted to the appropriate levels.

## Purification of samples

Samples and blanks were purified using a GO-xHT (Miura). The technology of this instrument has proven to be able to purify samples for selective and sensitive analysis of dioxins and PCBs (1,4). Due to the similarity in molecular structure of PCNs and dioxins the system was expected to be suitable for the purification of samples for the analysis of this class of compounds.

For each sample a set of four columns in-line columns is used (S.I. 3): silica gel impregnated with silver nitrate (1th); silica gel impregnated with sulfuric acid (2th); activated carbon (3th) and alumina (4th). Each extract is transferred on top of the first column and after complete adsorption which takes in general less than five minutes the set of columns is placed in the GO-xHT system.

The samples fat and oil were liquified by heating the samples at 100°C for 10 minutes allowing direct application of 2.5 gram on the column-set. On top of each sample 50 pg $^{13}$C PCNs was added and to spiked samples 200 pg native PCNs was added.

The column set was eluted with 90 ml hexane at a flowrate of 2.5 ml/min. During this step the temperature of the two purification columns is maintained at 60°C. The elevated temperature weakens the adsorption with silica gel and as a result the elution speed of PCNs, dioxins and PCBs is enhanced. Also the chemical reaction rates (amongst others, oxidation with sulfuric acid) with sample matrices is accelerated. PCDD/Fs, the four NO-PCBs and some of the PCNs are trapped on the activated carbon column while the MO, NDL-PCBs and other PCNs are trapped on the alumina column.

Both the alumina and the carbon column are eluted using a small amount of toluene resulting in two fractions, each of 1.5 ml. During these elution steps the temperature of the carbon and alumina column is set at 90°C. At first only the alumina column is eluted with 1.5 ml toluene and the collected fraction contains the MO-PCBs, NDL-PCBs and a part of the PCNs. After that the carbon column is also eluted with 1.5 ml toluene and this fraction contains all PCDD/Fs, NO-PCBs and the other PCNs.

Except for one blank with spike both fractions were combined and evaporated till just dryness using a CentriVap (Labconco). For this one blank with spike both fractions were evaporated till near dryness. Consequently, all samples were reconstituted in 20 µl dodecane containing 20 µg/ml $^{13}$C12 PCB159. Additionally, the two separate fractions of the blank with spike were combined after analysis and once again measured using a GC-HRMS.

Finally, two diluted native standards were concentrated under the same conditions and reconstituted in 20 µl $^{13}$C12 PCB159 (20 µg/ml) and $^{13}$C10 PCN (12 µg/ml) in nonane.

## GC-HRMS analysis

A GC-HRMS (DFS High Resolution Magnetic Sector MS - Thermo Scientific) was used for the analysis of PCNs. The GC-HRMS was equipped with a PTV injector (Best P.T.V. injector) using a sintered glass liner (SGE pn 092155) and a VF-5ms 60m x 0,25mm x 0.25µm + 5m EZ-guard (Varian). From each sample 5µl was injected. Relative retention times on a similar column have been reported (6) and were used for identification of constitutional isomers.

The mass spectrometer was operated in the electron ionization mode at a resolution of 10.000. The selected monitored ions are given in supplementary information 4. The linear range was from 2.5 – 50 pg on-column, but should be extendable to 0.5 pg on-column.

The lowest standard was used as reporting limit (LOR), whereas actual LOQ's were 0.25 – 1.8 pg/g based on the sd of the peak area of a 0.1 ng.ml$^{-1}$ standard (n=4).

## Results

### Experiment 1

The first goal was to determine in which fraction PCNs elutes. In both fractions, e.g. dioxin and NO-PCB fraction and MO and NDL-

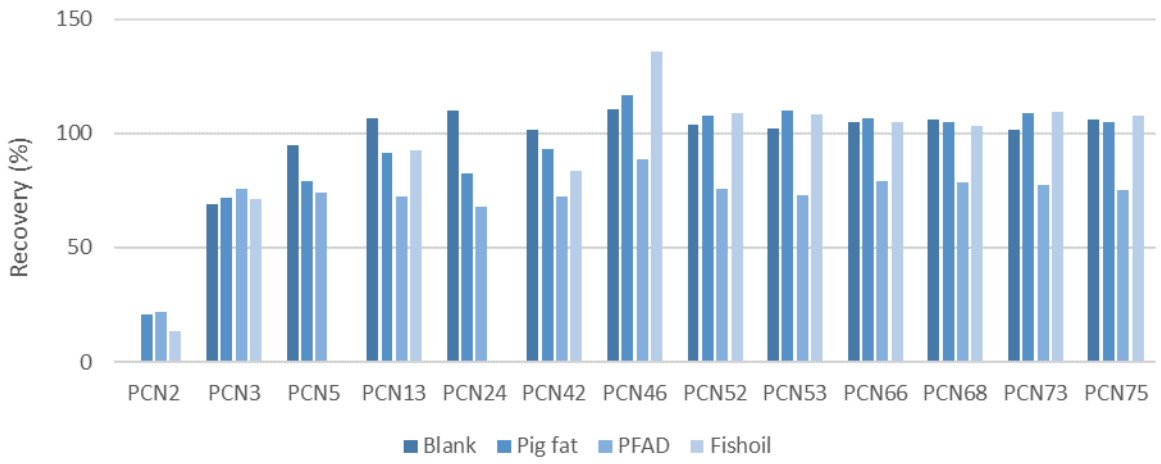


Figure 3 Recovery of spiked PCN in several types of samples (n=1), the recovery of PCN5 and PCN24 could not be determined in fish oil due to the high amount found in the sample

PCB fraction, PCNs were eluted. The ratio over the two fractions varied by congener (fig. 1).

The overall recovery of all compounds was 110 ± 25% except for PCN2. The recovery of PCN2 was 0% since PCN2 is probably not desorbed from the silica columns due to stronger absorption onto the silica.

Since the congeners elute in two separate fractions and internal standards are not available for every specific congener, it is of utmost mathematical importance to use equivalent fractions of the dioxin & NO-PCB and MO&NDL-PCB eluates for further analysis of PCN. For instance, if PCN-A elutes 100% in fraction 1 and its internal standard $^{13}$C PCN-B elutes for 100% in fraction 2, the amounts in an equivalent combined solution would be 100% and 100%, resulting in a ratio of 1:1. But if the fractions were not equivalently combined, but e.g. 10% of fraction 1 and 100% of fraction 2, the concentration ratio would 1:10. Using isotope dilution quantification this would result in a difference of a factor 10 in the final result of PCN-A.

## Experiment 2

In the samples the recovery of the internal standards varied between 64 and 123% (fig. 2) and besides PCN2 accuracies were between 68 and 136% for the native PCNs (fig. 3). PCN2 was once again not detected in the blank, yet in low amounts (13 – 22%) in samples. This implies a lower absorption strength of PCN2 on the silica columns in the presence of fat.

The accuracy of native PCNs could not be determined for PCN5 and PCN24 in fish oil due to the high amounts found in the sample (S.I. 5). In all samples at least one target PCN was found above the LOR. In addition, other peaks with the same ion ratio were observed in the chromatogram (fig. 4 and S.I. 6).

As the recoveries and accuracies do suspect, no suppressions were observed in the reference mass in either of the samples. In addition, except for two results (-25% and 25%) all ion ratios were within ± 15%.

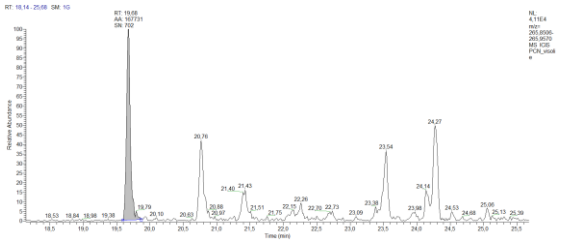

Figure 4 Chromatograms of tetra-CN in fish oil. PCN42 (39pg/g) at 19.68 minutes and PCN46 (<LOR) at 25.06 minutes

## Experiment 3

The recovery after CentriVap solvent evaporation of PCN2 ( 81 ± 6%) was lower than the other PCNs (99 ± 7%). This difference was ascribed to evaporation of PCN2 due to its low molecular mass.

## Summary

Samples pig fat, PFAD and fish oil were successfully purified on a GO-xHT for sensitive measurement on a GC-HRMS. The sample extracts were sufficiently purified to allow a large part of the sample to be injected on a GC-HRMS without interference on the GC separation or suppression in the mass spectrometer.

The PCN congeners do not all elute in the same fraction, but in both the dioxin & NO-PCB and MO-PCB & NDL-PCB fraction. All investigated congeners were recovered within acceptable range (60-140%) with the use of $^{13}$C10 PCNs internal standards except for PCN2.

The resulting detection limits were sufficiently low to monitor the relevant level in food (2).

## Literature

1 DSP-Systems, https://dspsystems.eu/wp-content/uploads/2016/08/16071844DSP-Application-note.pdf

2 Fernandes et al., Polychlorinated naphthalenes (PCNs) in food and humans, Environmental International 104, (2017) 1-13

3 Fernandes et al., Polychlorinated Naphthalenes (PCNs): Congener Specific Analysis, Occurrence in Food, and Dietary Exposure in the UK, Environemntal science & technology Vol. 44, no. 9, 2010

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5 Wiedmann et al., Quantification of chlorinated naphtha|enes with GC-MS using the molar response of electron impact ionization, J Anal Chem (1993) 346: 800

6 K. Kannan et al., Isomer-Specific Analysis and Toxic Evaluation of Polychlorinated Naphthalenes in Soil, Sediment, and Biota Collected near the Site of a Former Chlor-Alkali Plant, Environ. Sci. Technol. 1998, 32, 2507-2514

## Supplementary information

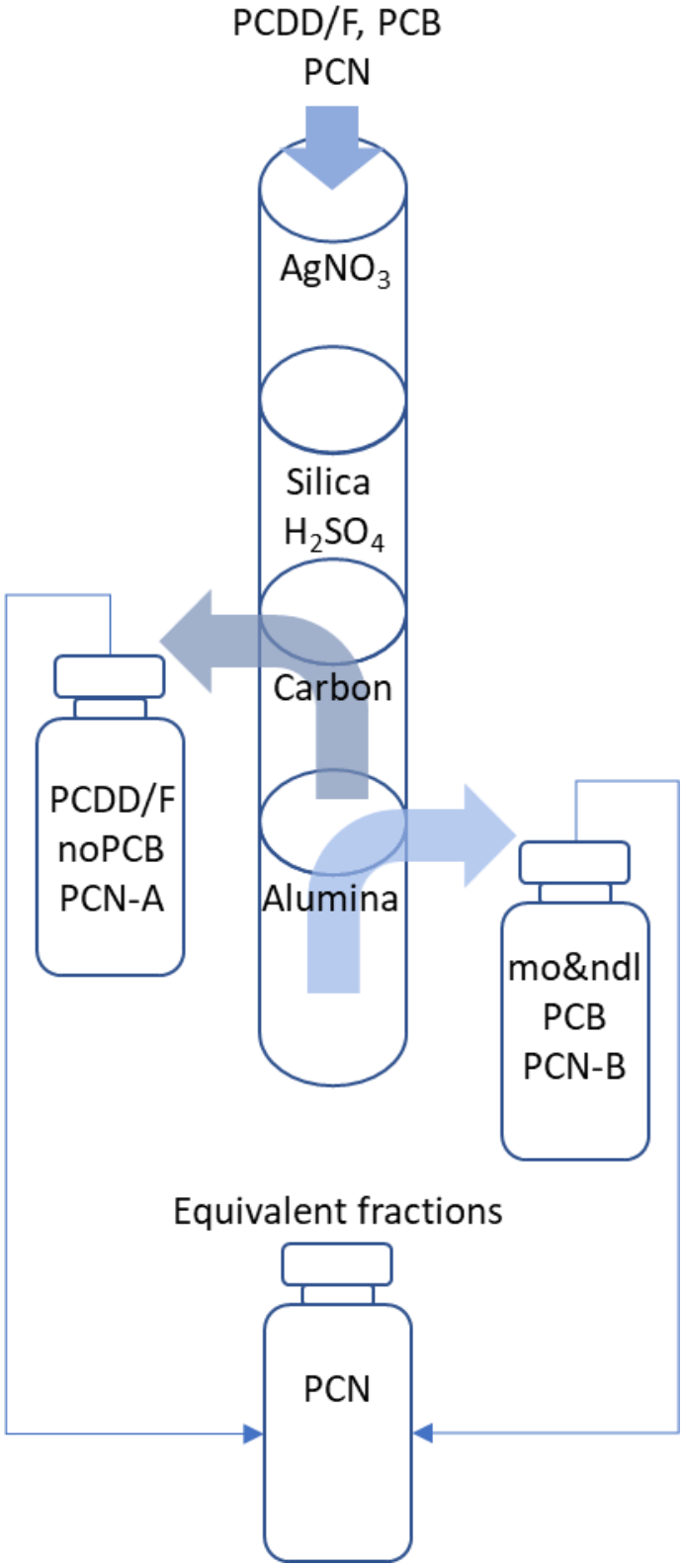
S.I. 1 PCN in standard solution ECN-5558-1.2 (CIL), 1000ng/mL in nonane

| PCN number[5] | IUPAC |
|---|---|
| 2 | 2-MonoCN |
| 3 | 1,2-DiCN |
| 5 | 1,4-DiCN |
| 13 | 1,2,3-TriCN |
| 24 | 1,4,6-TriCN |
| 42 | 1,3,5,7-TetraCN |
| 46 | 1,4,5,8-TetraCN |
| 52 | 1,2,3,5,7-PentaCN |
| 53 | 1,2,3,5,8-PentaCN |
| 66 | 1,2,3,4,6,7-HexaCN |
| 68 | 1,2,3,5,6,8-HexaCN |
| 73 | 1,2,3,4,5,6,7-HeptaCN |
| 75 | OctaCN |

S.I. 2 $^{13}C_{10}$ PCN in standard solution ECN-5490-200X-1.2 (CIL), 100ng/mL in iso-octane

| PCN number[5] | IUPAC |
|---|---|
| 27 | $^{13}C_{10}$ (99%) 1,2,3,4-TetraCN |
| 52 | $^{13}C_{10}$ (99%) 1,2,3,5,7-PentaCN |
| 64 | $^{13}C_{10}$ (99%) 1,2,3,4,5,7-HexaCN |
| 67 | $^{13}C_{10}$ (99%) 1,2,3,5,6,7-HexaCN |
| 73 | $^{13}C_{10}$ (99%) 1,2,3,4,5,6,7-HeptaCN |
| 75 | $^{13}C_{10}$ (99%) OctaCN |

S.I. 3 Schematic display of the purification for PCN analysis using a GO-HT



S.I. 4 Selected monitored ions and times

| Segment | Cycle time ms | Quantification ions (m/z) first | second | compound | Intensity au |
|---|---|---|---|---|---|
| 1 | 600 | 162.0236 | 164.0207 | Mono-CNs | 1 |
| | | 195.9847 | 197.9817 | Di-CNs | 1 |
| | | 229.9457 | 231.9427 | Tri-CNs | 1 |
| | | 265.9038 | 263.9067 | Tetra-CNs | 1 |
| | | 275.9373 | 273.9403 | 13C10 Tetra-CNs | 1 |
| | | 168.9883 | 263.9866 | lock and cali FC-43 | 5 |
| 2 | 600 | 299.8648 | 297.8677 | Penta-CNs | 1 |
| | | 309.8983 | 307.9013 | 13C10 Penta-CNs | 1 |
| | | 333.8258 | 335.8229 | Hexa-CNs, | 1 |
| | | 343.8594 | 345.8564 | 13C10 Hexa-CNs | 1 |
| | | 371.8817 | | 13C HxCB | 1 |
| | | 313.9834 | 363.9802 | lock and cali FC-43 | 5 |
| 3 | 600 | 367.7868 | 369.7839 | Hepta-CNs | 1 |
| | | 377.8204 | 379.8174 | 13C10 Hepta-CNs | 1 |
| | | 403.7449 | 401.7479 | Octa-CNs, | 1 |
| | | 413.7785 | 411.7814 | 13C10 OctaCNs | 1 |
| | | 375.9802 | 413.9770 | lock and cali FC-43 | 5 |

S.I. 5 Results of the analysis of PCNs in samples in $pg.g^{-1}$ (* deviation ion ratio > 15%)

| Compound | Blank | Pig fat | PFAD | Fishoil |
|---|---|---|---|---|
| PCN2 | NTB | NTB | NTB | NTB |
| PCN3 | <4 | <4 | 73 | 12 |
| PCN5 | 14 | 22 | 51* | 507 |
| PCN13 | <4 | <4 | 12* | <4 |
| PCN24 | <4 | 5.0 | 7.7 | 276 |
| PCN42 | <4 | <4 | <4 | 39 |
| PCN46 | <4 | <4 | <4 | <4 |
| PCN52 | <4 | <4 | <4 | 23 |
| PCN53 | <4 | <4 | <4 | <4 |
| PCN66 | <4 | <4 | <4 | <4 |
| PCN68 | <4 | <4 | <4 | <4 |
| PCN73 | <4 | <4 | <4 | <4 |
| PCN75 | <4 | <4 | <4 | <4 |

S.I. 6 Chromatograms of mono-CN to octa-CN in fish oil. PCN42 (39pg/g) at 19.68 minutes and PCN46 (<LOR) at 25.06 minutes