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HIGH SAMPLE THROUGHPUT IN A MODERN DIOXIN LABORATORY USING AUTOMATED CLEAN-UP AND DUAL ACQUISITION GC-HRMS

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Introduction

Oil or derived products intended for the use in animal feed, must have been analyzed by accredited laboratories for the sum of dioxins and dioxin-like PCBs. The operator placing the feed on the market is responsible for sampling and correct analyses. Only if the analysis result is below the maximum EU level (ML) these products can be used by the feed manufacturer. These requirements are based on EU-legislation, as laid down in Reg. (EU) No. 225/2012 and Commission Regulation (EC) No 152/2009 (ref 1,2). This "positive release" requirements puts great pressure on laboratories. Reports of analysis should not only be based on state of the art technology and fully accredited methods but more over they should be available, due to high cost before the ship can be unloaded (demurrage). In order to be able to report results of analysis within 24 hours, automation of the whole procedure is a must. At Nofalab on request by the client a number of samples can be analyzed and reported within 8 hours. This has been achieved by introducing a new sample preparation system in combination with dual acquisition GC-HRMS. This new approach is not only applicable to animal feed and ingredient but also to food and environmental samples.

Materials and methods

Extraction

In principle all samples, with exception of oil and fat, are extracted using an accelerated solvent extraction system (Speed Extractor E-916 Buchi) or recently via a new fully automated extraction system based on soxhlet according to the Randall technology (SER 158, Velp scientifica). Prior to extraction or in case of oil/fat dilution sixteen 13C labelled dioxins, four 13C labelled non-ortho PCBs, eight 13C labelled mono-ortho PCBs and six 13C labelled NDL-PCBs added to the samples. All solid samples, are extracted with a mixture of ethanol/toluene.. Extracts are concentrated down to < 0.1 ml and after solvent exchange from toluene to hexane 37Cl-2,3,7,8-TCDD (clean-up standard) is added and extracts are made up with hexane to around 10 ml.

Clean-up

Clean-up is performed using an in 2015 in Europeintridcued new technology (GO-xHT, Miura), figure 1A. For each extract a set of four in-line columns is required: Silver Nitrate Silica gel (1); Sulfuric Acid Silica gel (2); Activated carbon (3); Alumina (4). Column 1 and 2 are used for purification of the extracts while the other two columns are used for trapping compounds of interest. Extracts are transferred via a funnel to the first column. Thereafter the set of columns and tubing is assembled and placed in the GO-xHT system,figure 1B. Column set is eluted with 90 ml of hexane witha flowrate of 2.5 ml minutes. During this step the temperature of the two purification columns is maintained at 60°C. As the adsorption force with silica gel is weakened by heat the elution speed of dioxins and PCBs is enhanced, using only small volume of hexane. Also the chemical reactions rate (oxidation with sulfuric acid or nitric acid) with sample matrices and Silica columns is accelerated. PCDD/Fs and the four NO-PCBs are trapped on the activated carbon column while the MO and NDL-PCBs are trapped on the alumina column.

Figure 1: **A** Set up of the GO-xHT system capable of simultaneous purifying 2, 4 or 6 samples within 90 minutes. **B** Schematic diagram of the column flow channel of the GO-xHT system#

Finally in backflush 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 steps the temperature of the carbon and alumina column is set at 90°C. To both fraction the recovery/syringe standards 13C-1,2,3,4-TCDD and 13C

2,3,4,6,7,8 HxCDF in 20 ul nonane is added and both fraction are concentrated to a final volume of 20 ul using an evaporator (Centrivap, Labconco).

GC-HRMS

Both fractions are analysed using GC-HRMS (DFS High Resolution Magnetic Sector MS - Thermo Scientific). Each MS is equipped with two GCs each with a PTV injector (Best P.T.V. injector) using a sintered glass liner (SGE pn 092155). The GC column is a VF-5ms 60mx0,25mmx0.25µm + 5m EZ-guard (Varian). The mass spectrometer is operated in electron impact ionization mode, using selected-ion monitoring. From both fractions 4 µl is used to introduce the sample onto the GC. After data reduction, results are directly transferred to a Laboratory Information Management System (LIMS) and after approval reported to the customers. In order to achieve high sample throughput all three DFS High Resolution Magnetic Sector instruments are operated in dual acquisition mode.

Using GC-1, 4 ul of a standard solution or from a purified extract is injected and during the wait time of approximately 20 minutes, while the solvent peak as well as other volatile compounds beyond the scope of the method elute from the column, all GC eluate is diverted to waste using a waver (figure 2A). After 20 minutes, the GC eluate is directed to the ion source of the MS and MS data acquisition is started. Also at this time, a second standard or sample is injected onto GC-2, following the same procedure as used for GC-1 (i.e. during the first 20 minutes no GC-2 eluate is directed towards the MS). Once GC-1 is ready (OCDD is eluted from the column) another injection was performed onto GC-2 while cooling down the oven of GC-1 . This results in two GCs running simultaneously with staggered sample injections. In this way only part of the chromatogram containing compounds of interest is directed to the MS for data acquisition. This Dual Acquisition technique results in an increase of capacity , using one mass spectrometer with two GCs, by more than 80%. In figure 2B sequence files from both of the GCs providing consecutive data files is shown.

Figure 2: GC-HRMS with dual data acquisition.
A Micro Channel Device "Waver" used for controlling the direction of the GC eluate
B Dual Data Acquisition sequence, only at the relevant retention time compounds eluting from GC 1 or GC 2 are monitored resulting in >80% capacity improvement

Results and discussion

Advantages of the new approach are obvious. Using accelerated solvent extraction or the new automated soxhlet method, based on Randall principle, benefits in speed of extraction using less solvent and less bench space. Time needed for extraction has been reduced significantly to 1 hour in case of the speed extractor or to less than 3 hours for the automated soxhlet extractor.

Previously classical sample purification was time consuming and/or used quite a lot of organic solvent including toxic DCM and with high risk of cross contamination. The new technology developed by MIURA has many advantages amongst others; low solvent consumption of less than 100 ml per sample and as there is no contact of the sample extract with parts of the hardware the risk of cross contamination is eliminated. Even in the case of a high contaminated sediment sample followed by a low-contaminated sunflower oil sample, difference between the two samples factor 1000, no cross contamination could be observed. Another important advantage is the small volume of the two obtained fractions each of 1.5 ml, while with the classical clean-up procedure the fraction volumes are around 50 – 80 times higher. The fractions of 1.5 ml can be reduced to a final volume of 10 or 20 ul using the Centrivap within a relatively short time.

Figure 3: Chromatogram of PeCDD in a sample tocopherol; Chromatogram left obtained after classical clean-up and right after clean-up using the MIURA GO-xHT system

By using small particles in the purification columns and elevated temperature MIURA has achieved high performance clean-up resulting in better chromatography with less interferences. In figure 3 two chromatograms of a food additive are given one obtained after classical clean-up and the other after clean-up using the MIURA system.

Initially a problem was observed with extracts obtained with toluene or with samples spiked with 13C labels dissolved in toluene. In case of a small amount (e.g. 100 ul) of toluene in the hexane extract a breakthrough of approximately 30% of NO-PCBs from the activated carbon column onto the Alumina

column could occur. Since the spring of 2016 carbon columns are improved having smaller particle size than before and, therefore, enhanced adsorbing capacity which makes the column sets less sensitive to toluene traces present in the sample extract. This significantly improves the recoveries for NO-PCB's in samples which have been extracted with toluene or toluene mixtures. Initial tests showed that samples containing up to 200ul of toluene still gave very good recoveries for all PCBs, including for the four NO-PCBs (well above 80%). Still, it is advised to remove as much toluene as possible. Toluene extracts have to be evaporated until a volume of less or around 1-2 ml then, after adding 10 ml methanol, a minimum azeotrope is formed with a boiling point of 65°C. Final evaporation to 100µl results in a reduction of the toluene amount to almost zero. Addition of a keeper e.g. 100µl of dodecane, especially in the absence of fat is highly advised. The method is validated and accredited by Nofalab according to ISO 17025 for food and feed samples.

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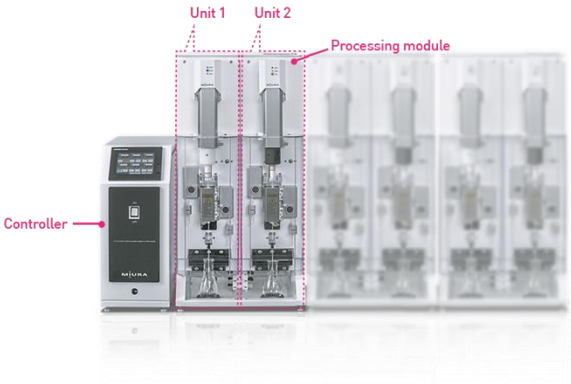
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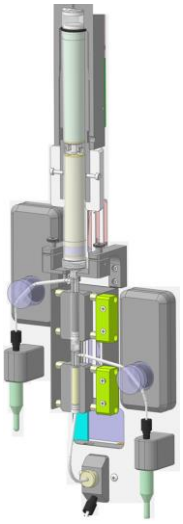

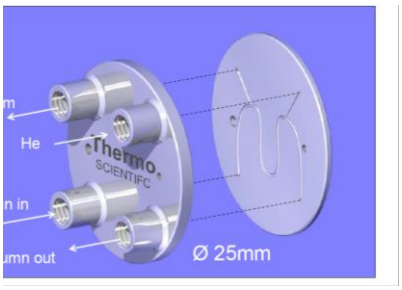
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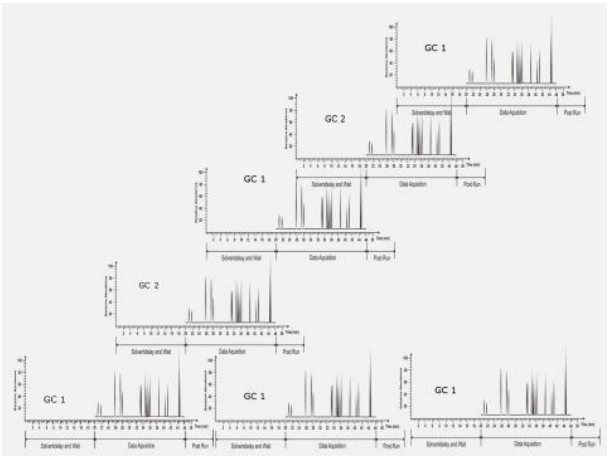
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