AXELSEMRAU

# Determination of PAHs in Foodstuff
with the CHRONECT Workstation PAH


## Application note 1802

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## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of substances from which numerous representatives are regarded as potentially carcinogenic [1]. For this reason, the control and regulation of these substances is of particular importance. The European Union (EU) has issued strict limits for the most important representatives of this analyte class, which must be strictly adhered to. The list of these so-called priority EU PAHs comprises 15 substances, which is usually extended by a 16th substance that is also suspected of being carcinogenic (so-called 15+1 EU PAHs) [2]. Of these 16 substances, four have limit values. Depending on the food and consumer group, these values range between < 1 µg/kg and 50 µg/kg [3, 4].

PAHs typically enter the foodstuff through processing. During frying, deep-frying, smoking or roasting, PAHs can be formed by incomplete combustion or pyrolysis and subsequently detected in the food. However, diesel exhaust fumes or environmental sources can also be held responsible for the contamination of individual products.

## Method and device setup

While the determination of PAHs in many foods is unproblematic, it can be extremely difficult in some products. Especially the existence of PAH-like biogenic substances or large amounts of emulsifiers can make the analysis difficult.

The detection of PAHs is generally carried out using chromatographic separation techniques on liquid (HPLC) or gas chromatography (GC). However, coupling GC with mass spectrometric detection (GC-MS) is considered the method of choice. Special attention should be paid to sample preparation. Due to the necessary sensitivity, a high quantity of food sample must be used, from which the PAHs are extracted. However, care must be taken not to extract other substances in addition to PAHs, which could falsify the analysis or, in the worst case, damage the expensive analytical equipment.

The CHRONECT Workstation PAH is based on the imPAHct method (innovative multidimensional PAH cleanup technology), which combines rapid manual sample extraction with a multidimensional chromatographic purification technique that is fully automated [5]. The obtained extract is then analyzed online by GC-MS(/MS), whereby the final sample result is available after 45 min after placing the sample into the system.

The workstation is based on Axel Semrau's LC-GC technology, which has been tried and tested for years. The LC part is used for sample preparation. The downstream transfer of the sample extract into the GC dimension is done by a so-called Large-Volume-On-Column-Transfer. The CHRONECT LC-GC interface is used for this purpose.

The actual sample purification takes place by a two-stage LC process. After injection of the sample by the XYZ robot, the sample is freed from all substances that are more polar than PAHs. A bare silica gel column in normal phase HPLC mode is used for this purpose.

However, the purified PAH fraction still contains numerous interfering substances that must be separated by means of a second LC dimension. For this purpose, a so-called π donor-acceptor chromatography is used, which can selectively enrich π electron-rich substances. The resulting PAH-rich sample extract is then transferred to the gas chromatograph as described above and separated, detected and quantified using classical GC-MS(/MS) analysis.

For the HPLC part of the system, classical components from Agilent, Knauer and Shimadzu have already been successfully used. The use of common GC-MS systems, such as those from Bruker, is also possible. The control software CHRONOS is the core element for controlling and combining the individual modules. In combination with the LC-GC interface, CHRONOS coordinates all subsystems so that they perform the right action at the right time, independent of the data system of the individual equipment manufacturers.

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**System components:**

- CHRONECT LC-GC interface
- CHRONECT Robotic Autosampler with 85 cm axis
- Software CHRONOS
- CHRONECT LC-GC interface
- Agilent 1260 Infinity II HPLC (Pump and VWD)
- Bruker EVOQ GC-TQ or Agilent MSD 5977B
- Alternatively, a system can be built from components of the manufacturer Shimadzu (LC-40 and GCMS-QP2020 NX)

## Sample preparation

All samples must be prepared before the actual measurement. Depending on the sample matrix, this can be easier or more complex.

Manual preparatory work on the sample can be reduced to a minimum by using the imPAHct method. Figure 1 shows the sample preparation procedure as a function of the food group. Edible oils can usually be injected directly after dilution, while other food products are prepared in 10-15 minutes.

## Results

To establish a two-step LC sample purification, the development of a special valve circuit was necessary. Figure 2 shows an example of the result. With only four HPLC valves, all necessary switching can be performed. Table 1 summarizes all relevant actions.

To demonstrate the performance of the system, a calibration line for benzo[a]pyrene in the range between 0.04 - 4 µg/kg was established (see Fig. 3). Furthermore, a cocoa butter previously treated with activated charcoal was spiked with 0.05 µg/kg PAHs. All 16 PAHs could be clearly detected with a recovery of more than 85 %. Thus, all important requirements of the EU directive 836/2011 are fulfilled [4].
Figure 4 shows a section of the extracted ion chromatogram of the cocoa butter. Even more demanding foodstuff such as spices can be analyzed with the described method. Pepper and amaranth oil were prepared and measured. While pepper is rich in essential oils, amaranth oil contains about 10 % of olefinic hydrocarbons, which are known to interfere with PAHs by chromatography.

Figure 5 shows chromatogram sections for both samples in which matrix interferences are usually to be expected. However, it can be clearly shown that only the desired analytes are visible.

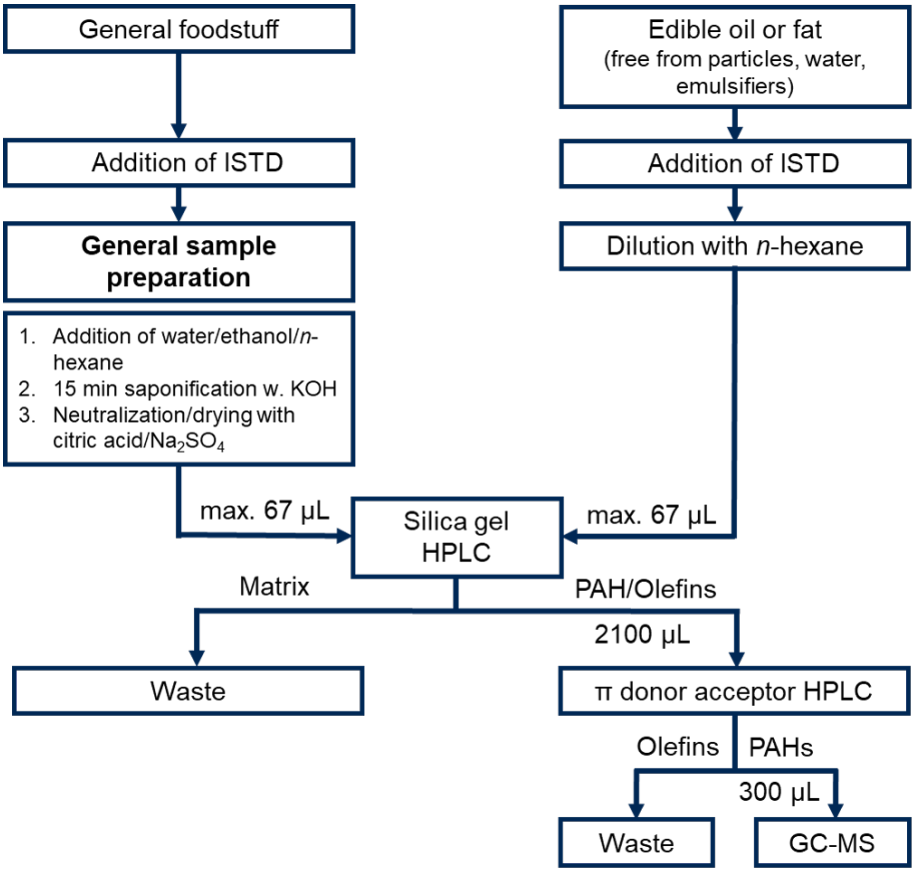


**Figure 1:** Flow chart of manual sample preparation [5].

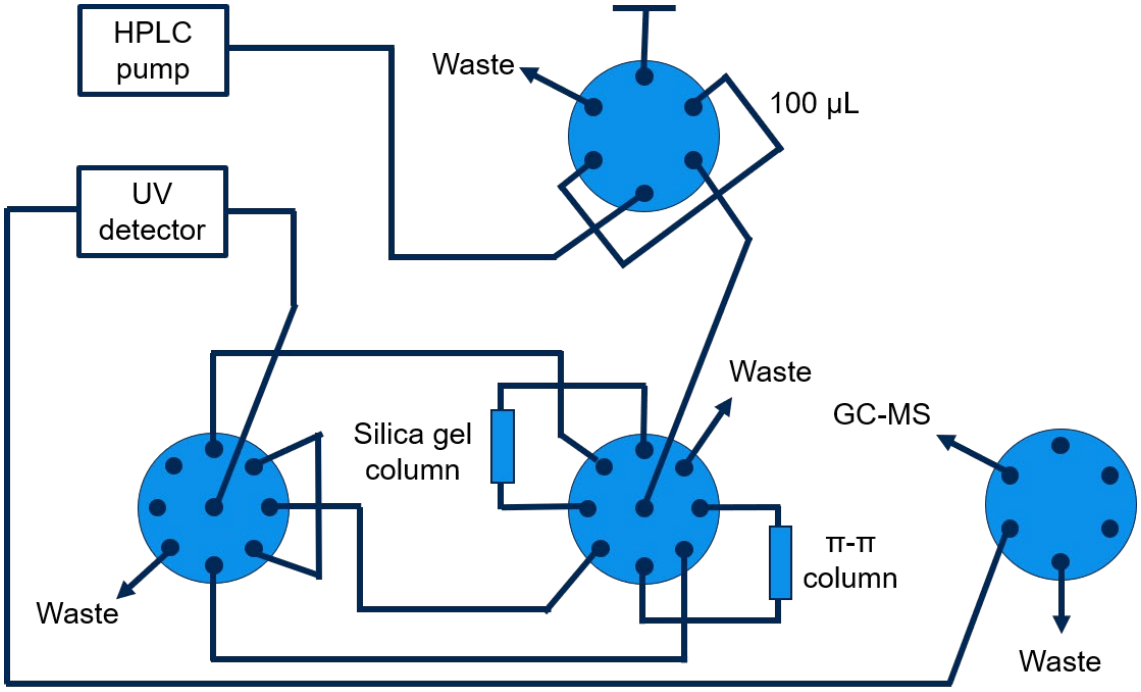


**Figure 2:** HPLC valve switching [5].

**Table 1:** Valve switching processes during a PAH analysis [5].

| Time [min] | Action |
|---|---|
| 0.00 | Injection on the silica gel column |
| 0.00 – 2.00 | Separation of PAHs & olefins from the rest of the matrix |
| 2.00 – 5.00 | Transfer of the PAH-containing fraction from the silica gel to the π-π column |
| 5.00 – 10.00 | Separation of PAHs from olefins |
| from 10.00 | Start of the backflush of the π-π column |
| 13.10 – 14.10 | Elution of the PAHs fraction from the π-π column into GC-MS |
| until 20.00 | Backflush of the π-π column for matrix removal |
| 20.00 – 29.00 | Backflush of the silica gel column for matrix removal |
| 29.00 – 34.00 | Re-equilibration of the silica gel column in forward direction |
| 34.00 – 40.00 | Re-equilibration of the π-π column in forward direction |

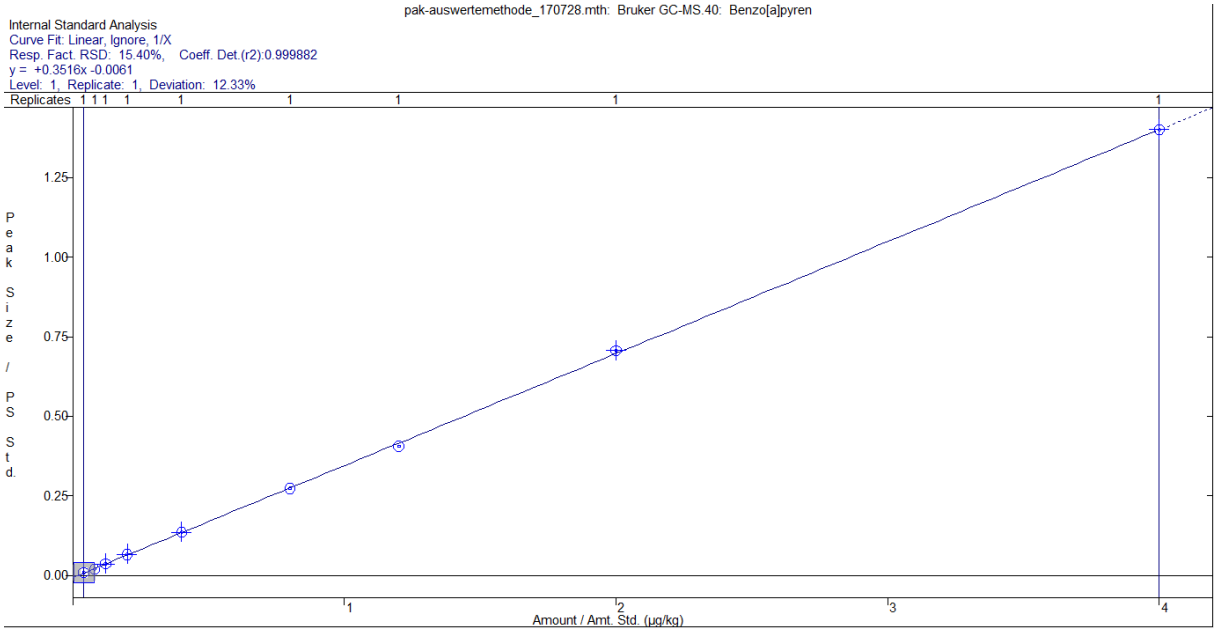


**Figure 3:** Calibration line for benzo[a]pyrene in the concentration range 0.04 - 4 µg/kg.

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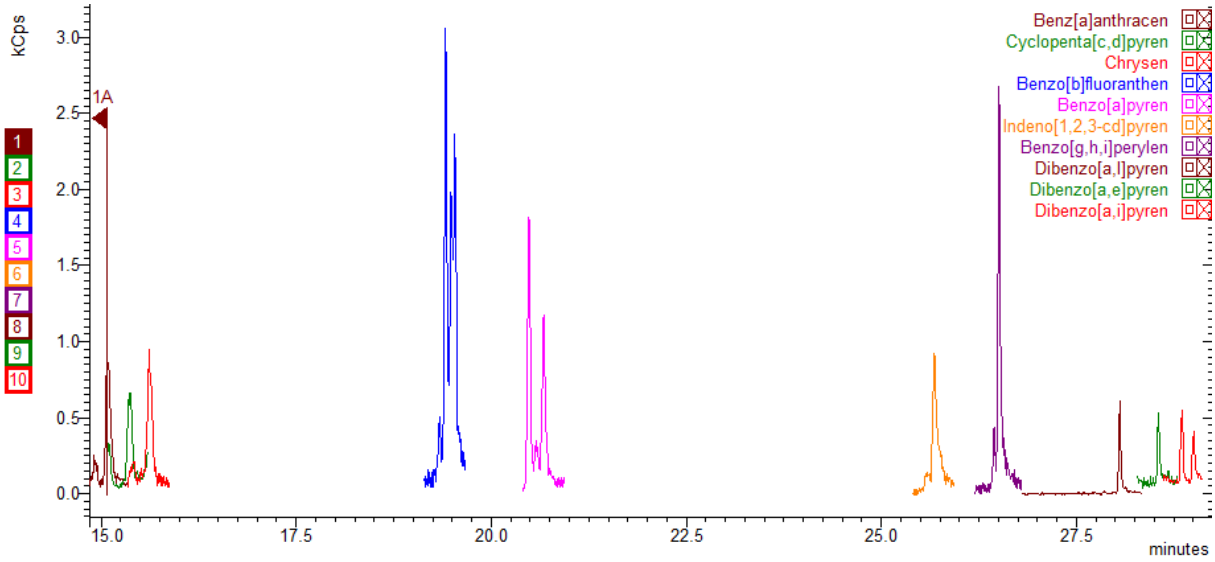


**Figure 4:** Extracted MS ion chromatogram of a cocoa butter spiked to 0.05 µg/kg. All doped PAHs are free of interference and clearly identifiable.

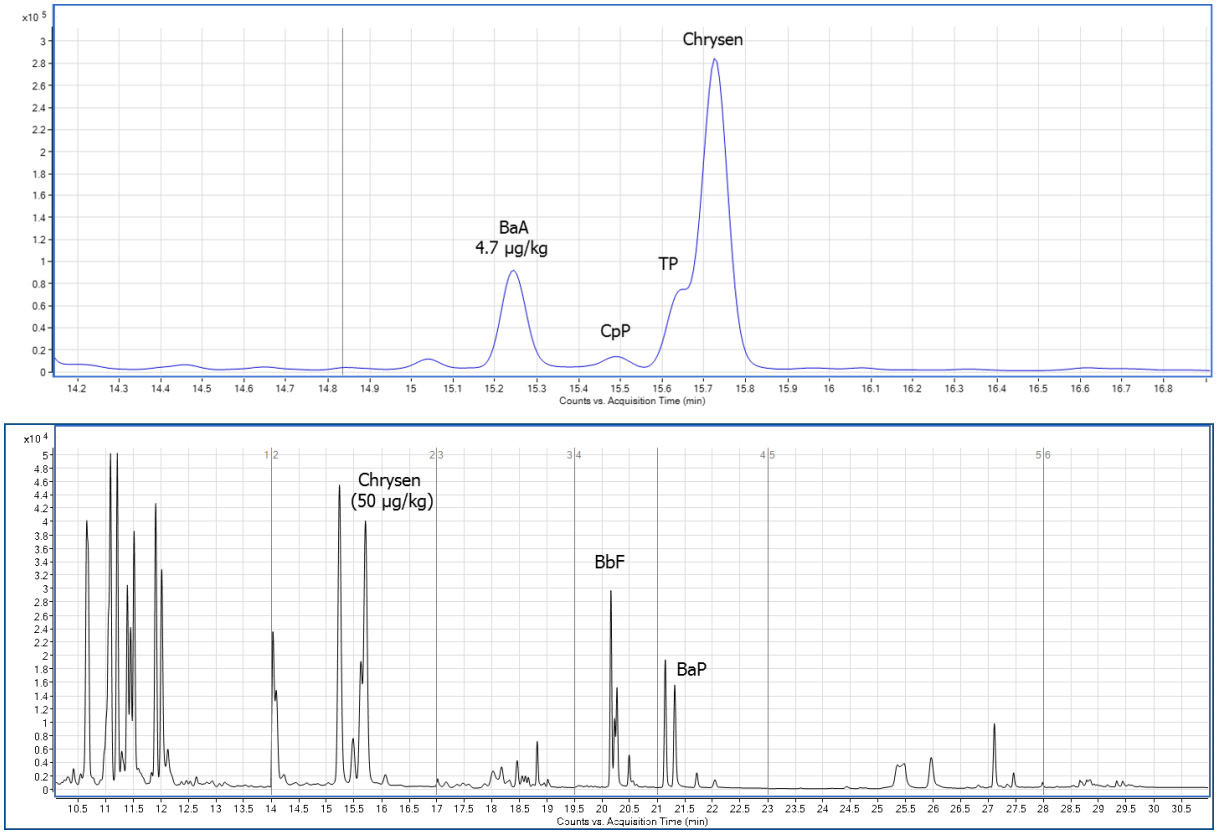


**Figure 5:** MS chromatogram section of a pepper (top) and amaranth oil sample (bottom). Especially in the range of benz[a]anthracene (BaA), cyclopenta[c,d]pyrene (CpP), triphenylene (TP), chrysene as well as benzo[b]fluoranthene (BbF) and benzo[a]pyrene (BaP) strong matrix interferences can occur.

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## Summary

The CHRONECT Workstation PAH is able to cope with the strict limits of PAH analysis for the 16 regulated EU PAHs. Depending on the GC-MS detection system, detection limits are achievable that are lower by a factor of 100 than the European limits for PAHs in infant food (1.0 µg/kg) [3]. This is made possible by imPAHct, a fast manual extraction combined with an efficient multidimensional chromatographic sample purification.

## Literature

[1] Scientific Committee on Food, Opinion of the Scientific Committee on Food on the Risks to Human Health of Polycyclic Aromatic Hydrocarbons in Food. 4 December 2002. European Commission (EC), Brussels, 2002.
[2] European Food Safety Authority (EFSA). EFSA J. 2008, 724, 1–114.
[3] The European Commission. Off. J. Eur. Unions 2011, 215, 4–8.
[4] The European Commission. Off. J. Eur. Unions 2011, 215, 9–16.
[5] Nestola, M.; Friedrich, R.; Bluhme, P.; Schmidt, T. C. *Anal. Chem.* 2015, 87(12), 6195–6203.

imPAHct and the CHRONECT Workstation PAH is a development by Axel Semrau.

**Subject to technical changes**

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