Cutting-Edge determination of PAHs in edible oils

Changes to EU Regulations concerning maximum levels for contaminants in foodstuffs came into effect on September 1, 2012: Four specific PAH compounds must now be monitored as markers for the presence of polycyclic aromatic hydrocarbons (PAHs) in food. According to an expert assessment from the European Food Safety Authority (EFSA), benzo[a]pyrene alone is not a sufficiently reliable marker for PAH contamination. Early on, staff at Eurofins WEJ Contaminants GmbH in Hamburg, Germany started developing concepts for optimizing and automating the determination of PAHs in edible oils. The key to their success lay in automated solid phase extraction (SPE).

As Aristotle taught us, one swallow doesn't make a summer. The opposite has been found to be true when it comes to PAHs in food: If benzo[a]-pyrene, probably the best-known PAH compound, is discovered in food, it is most likely not alone, but rather in the company of other PAHs. It has previously been reported that, "PAHs are always found as a mixture of several hundred individual compounds".

Food can become contaminated with PAHs via contact with air, soil, or water. These contaminants can also be formed or introduced when food is processed or prepared using techniques such as smoking, broiling, roasting, frying , baking or drying, in short, whenever food comes into direct contact with heat or smoke [1]. Why should we care about this? After all, PAHs exhibit only a low level of acute toxicity to humans. However, the long-term effects of some PAHs (see box: PAHs at a glance) could not be more dramatic: PAHs affect fertility, are mutagenic, and can trigger cancer. The International Agency for Research on Cancer (IARC) classifies PAH mixtures as Hazard Group 1 (carcinogenic to humans) while individual PAHs are classified as Hazard Group 2A (probably carcinogenic to humans) or lower.

Succes achieved through foresight and planning (from the right): Claudia Schulz, Ph.D., Business Unit Manager, Ansgar Ruthenschrör, Research & Developement, and Holger Fritz, Laboratory Manager.

In 2008 the Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority (EFSA) published a Scientific Opinion on PAHs in food, which went against conventional wisdom at the time. The conclusion: Benzo[a]-pyrene is not a reliable marker for the presence of polycyclic aromatic hydrocarbons in food. A system comprising four specific PAH marker compounds was instead proposed. As a consequence, the EU regulation, which specifies the acceptable Maximum Levels (MLs) of PAHs in foodstuffs [(EC) No. 1881/2006] was changed and amended with COMMISSION REGULATION (EU) No 835/2011 of 19 August 2011, described as "amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs".

As of September 2012, in addition to an individual ML for benzo[a]pyrene there is a combined ML for the following PAHs: Benzo[a]pyrene, benz[a]anthracene, benzo[b] fluoranthene, and chrysene. These four are collectively referred to as PAH4, which is the official marker for PAH contamination in food [1] and they must all be monitored. As stated in the regulation text: "The separate maximum level for benzo(a)pyrene is maintained to ensure comparability of previous and future data."

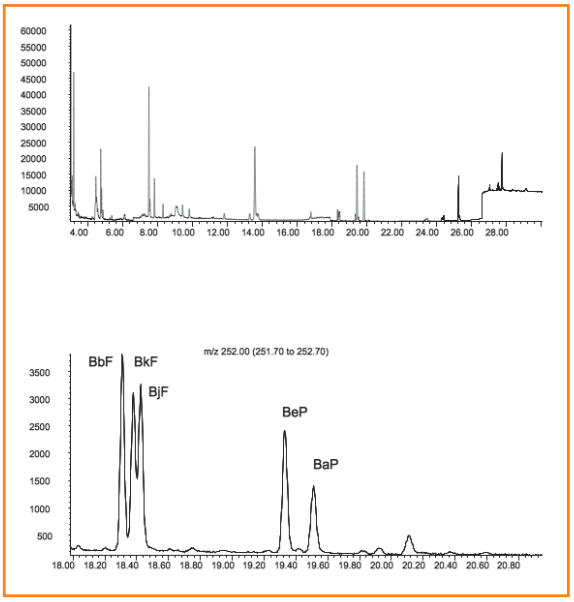
PAHs at a glance

"Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds with two or more aromatic rings. Several hundred chemical compounds belong to this group. PAHs are formed during incomplete combustion of organic materials. PAHs are found in fossil fuels such as coal and mineral oil, and are introduced to the environment via vehicle emissions or waste gas from furnaces and central heating systems." There are two classification systems for PAHs. The United States Environmental Protection Agency (EPA) recognizes 16 PAHs and the EFSA classifies 15+1 priority PAHs as relevant.

Why automate and change the laboratory process?

Even if someone is not concerned with the environmental impact of laboratory operations, supply bottlenecks and ever increasing prices should be a powerful driver towards more careful use of resources. The recent acetonitrile shortage and current helium crunch are dramatic examples. The emerging trend is a recognition that environmental and business considerations are not mutually exclusive, but often go hand in hand. Generating unnecessary waste is simply not good business practice. If you minimize and automate

Reference sample containing „food matrix" spiked with approximately 0.8 µg/kg of each analyte. The upper trace is the TIC (Total Ion Chromatogram) the lower trace is the m/z 252 Extracted Ion Chromatogram (EIC) showing the benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[j]fluoranthene (BjF), Benzo[e] pyrene (BeP), Benzo[a]pyrene (BaP), and perylene peaks.



analysis processes, you not only use less solvent, you save money and reduce the environmental impact. If at the same time you increase sample throughput and employee productivity while getting better results – why wait? Practice has shown that automation and downscaling of the analysis process provides a competitive edge.

Searching for the best solution

Eurofins WEJ Contaminants needed to find the optimal solution for their PAH analysis needs. "Until 2009, we used gel permeation chromatography (GPC) for sample clean-up in combination with GC/MS determination of PAHs in oils and other food matrices", says Claudia Schulz, Ph.D., Business Unit Manager, Eurofins WEJ Contaminants. This well tested solution delivered satisfactory results, but the process was very labor-intensive and required large quantities of solvent. "We sought to eliminate manual sample preparation steps and automate as much of the analysis as possible," adds Ansgar Ruthenschrör, Chemical Engineer in the Research & Development Unit. Holger Fritz, head of Organic Contaminants, points out that several ideas were considered. One such proposal entailed a miniaturized GPC column, which could reduce the significant amount of solvent used and associated cost. Some tests were conducted, but then a far more compelling alternative presented itself: Solid phase extraction, or SPE. "SPE offered us complete automation of the process, and provided significant added value," said Claudia Schulz. According to Ansgar Ruthenschrör, the idea of developing and implementing an SPE-GC/MS method to determine PAHs in oils and other food matrices came after reading a Journal of Chromatography A article [5]. In it, the use of a polystyrene divinylbenzene phase was reported for SPE clean-up of samples after pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE) with cyclohexane as the extraction agent. This was used as starting point for the SPE automation project. An additional SPE phase, silica gel, was tested since it is recommended in the EU regulation for detecting smoke flavorings [(EC) No. 2065/2003]. Ultimately this approach was not further pursued. After initial tests using a polystyrene divinylbenzene phase had shown encouraging results at the research laboratory of Eurofins WEJ Contaminants GmbH, the scientists simply had to find the right instrument to automate the manual SPE method.

Teamwork: Combining the know-how of all members

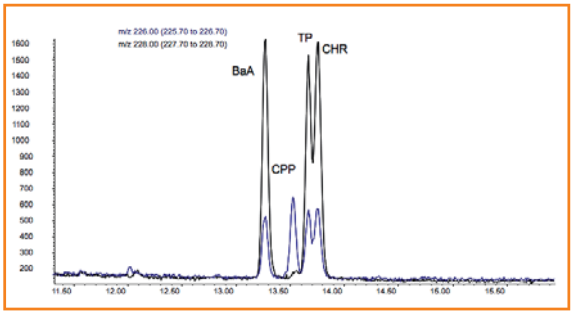
„GERSTEL was invited to present a proposal to provide instrumentation to automate the SPE method. The first priority was to clearly define the requirements of the application. This was done through a close collaboration between the GERSTEL application team and the Eurofins scientists. The Eurofins requirements included

Eurofins Scientific

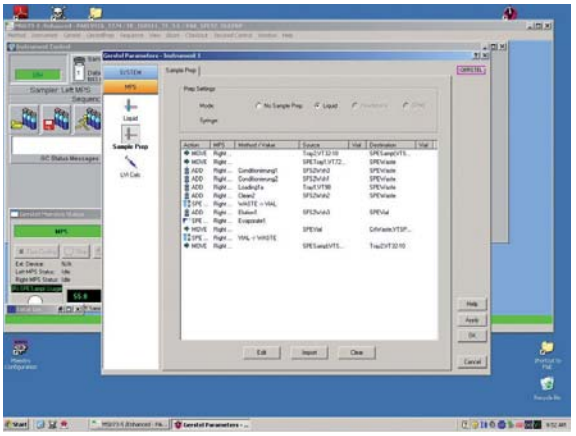
With over 13,000 staff in more than 170 laboratories across 35 countries, Eurofins Scientific is the world leader in food and bio/ pharma products testing. It is also number one in the world in the field of environmental laboratory services and one of the global market leaders in agroscience, genomics, discovery pharmacology and central laboratory services.

Eurofins WEJ Contaminants GmbH

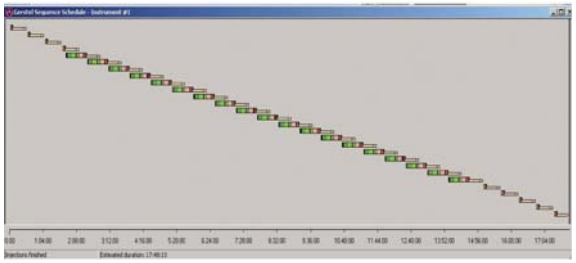
There are 40 Eurofins sites in Germany alone. One of these is conveniently located in Harburg, close to ports and highways of Hamburg. Harburg is home to Eurofins WEJ Contaminants, center of excellence for organic and inorganic contaminants, mycotoxins, and veterinary drug residues. [4]



The m/z 226 and 228 EICs showing the benzo[a]anthracene (BaA), Cyclopenta[c,d]pyrene (CPP), Triphenylene (TP), and Chrysene (CHR) peaks.



Prep method attached to the GC method. The MAESTRO software has been extended to enable the addition of priority samples including sample preparation into the running sequence.

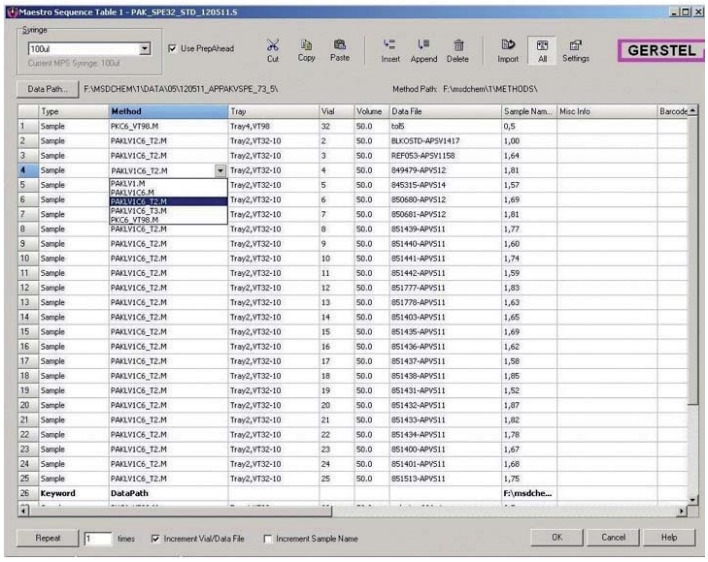


Scheduler overview showing the overlapping sample preparation and GC/MS analysis including the total analysis time for the ongoing sequence.

Partner and communication link between the user and GERSTEL: Sales manager for the German speaking countries and Key Account Manager Michael Gröger (2nd from right) visiting the GC Laboratory of Eurofins WEJ Contaminants GmbH, Hamburg, Germany.

Compound	Edible oil			Fish		
	LOQ [µg/kg]	RSD [%]	Accuracy [%]	LOQ [µg/kg]	RSD [%]	Accuracy [%]
Phenanthrene	0.3	25	90	7.3	9.4	110
Fluoranthene	0.5	31	106	3.8	11	100
Pyrene	0.6	27	91	4.9	13	97
Benzo[a]anthracene	0.4	5.1	102	4.3	11	110
Chrysene	0.4	4.2	95	3.5	9.1	102
Benzo[b]fluoranthene	0.4	5.2	115	3.7	11	104
Benzo[k]fluoranthene	0.3	5.5	113	1.3	19	99
Benzo[a]pyrene	0.4	7.7	93	2.0	11	109
Indeno[1,2,3-c,d]pyrene	0.3	8.0	87	1.4	8.9	92
Dibenzo[a,h]anthracene	0.3	11	120	1.4	10	109
Benzo[g,h,i]perylene	0.4	8.5	100	1.5	9.3	94
Benzo[e]pyrene	0.7	12	106	1.9	4.4	96
Perylene	0.8	18	96	2.0	4.0	99
Anthanthrene	0.5	4.0	83	2.1	5.3	106
Dibenzo[a,l]pyrene	0.7	8.8	99	2.3	4.7	109
Cyclopenta[c,d]pyrene	0.9	3.3	124	2.6	9.5	85
Methylchrysene	0.8	11	101	2.3	3.3	105
Benzo[c]fluorene	0.8	12	99	2.5	21	108

LOQs and quality data for the PAH determination using Eurofins' new SPE-MPS-GC/MS analysis method.



Sequence table for the combined sample preparation and GC/MS method.

automated extraction and clean-up of relatively large samples of oil and food combined with automated introduction of the resulting clean extract to the GC/MS system for analysis. This meant that the Dual-Rail version of the GERSTEL MultiPurpose Sampler (MPS PrepStation) was the ideal platform. Initial testing performed at GERSTEL by Ansgar Ruthenschrör and GERSTEL scientist Oliver Lerch, Ph.D. went remarkably well. The method was fine tuned in the GERSTEL application laboratory until a proof of concept for automation had been developed. Ansgar Ruthenschrör then pushed ahead with further optimization and implementation into the Eurofins routine laboratory workflow. The seasoned chemical engineer meticulously reviewed and optimized SPE parameters for the polystyrene divinylbenzene phase. The final method consisted of a seven step procedure (see box), which

Sample preparation

Condition the SPE phase with cyclohexane

Load the sample onto the cartridge

Elute the sample matrix

Elute the PAHs

Concentrate the PAH eluate

Introduce an aliquot to the GC/MS

GC/MS analysis

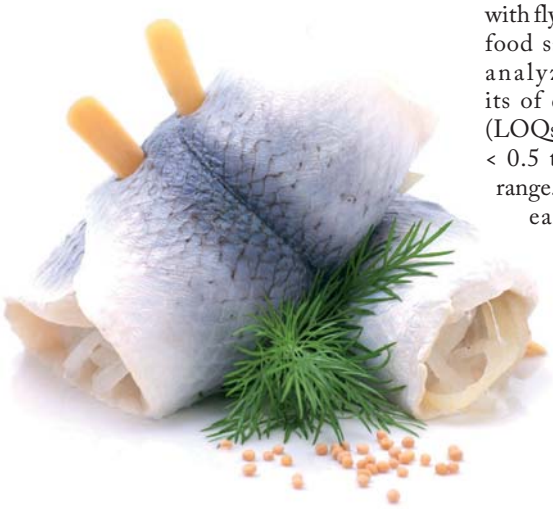
was transferred to the Dual-Rail MPS PrepStation for testing under routine laboratory conditions.

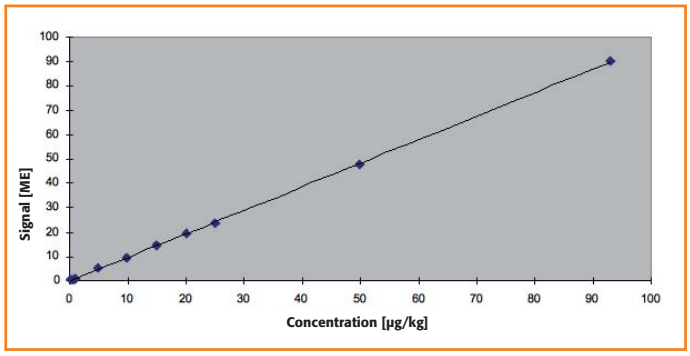
The optimized method parameters were transferred to a Prep Sequence in the MAESTRO software. This is an easy and uncomplicated process since the MPS sample preparation steps are simply selected by mouse-click from a drop down menu and automatically added to the sample preparation method. To ensure highest possible throughput, the MAESTRO PrepAhead feature was activated, enabling parallel sample preparation and GC/MS analysis. PrepAhead helps to ensure that the next sample in the

sequence table is always prepared and ready whenever the GC/MS system concludes a run. Using this „just-in-time" strategy means that the GC/MS is constantly analyzing samples and producing data, as opposed to having to wait for each sample to be prepared prior to injection. Claudia Schulz points out that this increases system throughput, a key requirement for most users, and especially for a contract laboratory.

Successful Participation in Round-Robin Tests

Comparative studies were performed in order to ensure that the automated MPS-GC/MS system fulfilled the Eurofins requirements for use in the contract laboratory. Ansgar Ruthenschrör: "The comparison resulted in convincingly similar results from the GPC-based analysis and the new automated SPE-GC/MS system. The system passed the tests with flying colors, even when real life oils and food samples were analyzed." Limits of quantitation (LOQ_S) were in the < 0.5 to 1.0 µg/kg range. Method linearity, repeatability, and comparison precision were excellent and the results accurate. "The results produced using this method are certainly correct as proven by our successful participation in round-robin tests and by cross-checking the method by analyzing spiked samples," reports Claudia Schulz. Everyone involved at Eurofins and GERSTEL emphasized how remarkably effective the collaboration had been, especially the combination of the customer's application expertise and the instrumentation know-how of the GERSTEL experts. The automated SPE-GC/MS method for determining PAHs in oils and foods had been devised, manually tested, and approved by Eurofins experts. And the GERSTEL experts took care of implementing the method on the instruments. Last but not least, the partnership had a wider impact. "Among other things," emphasizes Oliver Lerch, "Eurofins' need for an option to add priority samples during a complex SPE-GC/MS sequence triggered the development of Preplets—now a standard part of our MAESTRO software (see page 19)."





Linearity for benzo[a]pyrene of the complete method - including sample preparation on the MPS.

The collaboration between GERSTEL and Eurofins WEJ Contaminants GmbH is ongoing. The Hamburg-based laboratories now have a total of three GERSTEL SPE-GC/MS systems for PAH analysis.

Sources

[1] German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety: EU-wide Consumer Protection against Environmental Contaminants in Foodstuffs: Polycyclic Aromatic Hydrocarbons (www.bmu.de/en/topics/health-chemical-safety/gesundheit-und-umwelt/lebensmittelsicherheit/consumer-protection-eu/eu-wide-consumer-protection-against-environmental-contaminants-in-food-polycyclic-aromatic-hydrocarbons/)

[2] Oliver Lerch, Ph.D.: Simplifying PAH analysis, GERSTEL Solutions Worldwide Magazine 8 (2008) 10-13 (www.gerstel.de/pdf/sw_8_page_10_13.pdf)

[3] Eurofins WEJ Contaminants (www.eurofins.de/food-analysis/laboratories/eurofins-wej-contaminants/about-eurofins-wej-contaminants/history.aspx)

[4] Eurofins: Overview of the analytical services provided by Eurofins (www.eurofins.com/en.aspx)

[5] Veyrand, B., Brosseaud, A., Sarcher, L., Varlet, V., Monteau, F., Marchand, P., Andre, F., Le Bizec, B.: Innovative method for determination of 19 polycyclic aromatic hydrocarbons in food and oil samples using gas chromatography coupled to tandem mass spectrometry based on an isotope dilution approach. Journal of Chromatography A, 1149 (2007) 333-344