Accelerated solvent extraction is a powerful technique that can be used to reliably extract polycyclic aromatic hydrocarbons (PAHs) from environmental and food samples. It uses common organic solvents at elevated temperatures and pressure to deliver extractions equivalent to traditional techniques like Soxhlet and sonication, but with faster extraction times, reduced solvent use, and walk away capability.

PAHs are environmental pollutants containing two or more fused aromatic rings. These have been identified in a wide range of food matrices worldwide originating with a variety of anthropogenic and natural sources. A recent review has summarized analytical methods and trends in food and beverages [1]. Due to the complexity of foodstuff matrices, traditional sample preparation procedures have involved a three-step process including saponification, liquid-liquid extraction, and cleanup by chromatography or solid-phase extraction. These purification procedures can also take time and copious amounts of solvents to complete, adding to the overall cost and time of the total analytical workflow. The addition of adsorbent material to the extraction cell along with the sample can instead produce extracts essentially free from interferences [2]. Flow-through extraction allows absorbents to be added directly to the extraction cell to remove interferences, for one-step extraction and cleanup (Fig. 1). Additionally, PAHs are known to decompose by photoirradiation and oxidation, thus, light exposure during sample processing must be carefully controlled [3,4]. Regardless of the analysis pathway, the time-consuming step is the sample preparation. The use of the accelerated solvent extraction has proved to be effective in reducing the cycle time from days to hours.

Fig. 1: Schematics of flow-through, one-step extraction and cleanup

Polycyclic Aromatic Hydrocarbons in Mussel Tissue
A Novel Method with In-line Sample Cleanup for PAH-Extraction from Mussels

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Standard Methods

The German Method DIN-EN 12393-3:2009 (extended and revised version of the DFG Method S19) recognizes the accelerated solvent extraction for the extraction of pesticides residues from food of plant origin. On a more global perspective, the U.S. EPA Method 3545A, includes the accelerated solvent extraction as an official extraction method for semi-volatile organic compounds, organophosphorus pesticides, organochlorine pesticides, chlorinated herbicides, and polychlorinated biphenyls (PCBs). Further methods are the NOAA Method NWFS-NWFSC-59 (hydrocarbon and chlorinated hydrocarbon contamination in marine animal tissues, soils and sediments), the Chinese GB/T 19649-2005 (pesticides in grains and grain products) and the Mexican National Standard NMX-AA-146-SCFI-2008 (PAHs in soils and sediments).

Extraction of PAHs

PAHs are lipophilic and generally have a very poor aqueous solubility, and tend to accumulate in lipid tissue of plants and animals. Some PAHs are semi-volatile but most of them tend to adsorb on organic particulate matter. When particulates fall out into surface water, they are transported in suspension and surface adsorbed PAHs finally end up in fresh water or marine sediments. On the contrary of similar persistent compounds like PCB, PCDD/F or organochloride pesticides, most organisms have a high bio-transformation potential for PAH resulting in no significant bio-magnification in the aquatic food chain [4,5]. However filter-feeding bivalves (e.g. mussels and oysters) filter large volumes of water and have a low metabolic capacity for PAH and accumulate PAHs. They are therefore perfect bioindicators for long-term tendencies of element pollution in marine ecosystems.

Investigating Mussel Samples

An investigation of mussel samples, originating from Chile, towards there PAH content was carried out. The samples were

Fig. 2: Chromatogram of the spiked mussel sample

Fig. 3: Chromatogram of the reference sample

Fig. 4: Analysis of the a PAH-free mussel sample
and stored in a fridge until analysis. The total successively dried under a stream of nitrogen larger volumes using Soxhlet. The extracts were formed in 4 static cycles of 5 minutes each at 100 degrees centigrade and 100 bar using di-alumina (Brockmann Activity 1) was placed behind two glass fiber membrane filters. The mussel tissue was mixed with 10 g of Thermo Scientific Dionex ASE Prep DE (diatomaceous earth) dispersant and added to a 66 ml stainless steel extraction cell. The extraction was performed in 4 static cycles of 5 minutes each at 100 degrees centigrade and 100 bar using dichloromethane as a solvent, yielding a ready-to-use 12 pg on column for benzo(a)pyrene.

Table 1: LOD /LOQ for method validation, determination of linearity and recovery results LC/MS MRM, spike at 50 ppb

<table>
<thead>
<tr>
<th>Peak Component</th>
<th>LOD (pg on column)</th>
<th>LOQ (pg on column)</th>
<th>R²</th>
<th>ppb</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Naphthalene</td>
<td>10</td>
<td>34</td>
<td>0.9936</td>
<td>55</td>
<td>110</td>
</tr>
<tr>
<td>2 Acenaphthylene</td>
<td>20</td>
<td>28</td>
<td>0.9999</td>
<td>47</td>
<td>94</td>
</tr>
<tr>
<td>3 Acenaphthene</td>
<td>8</td>
<td>28</td>
<td>0.9996</td>
<td>53</td>
<td>106</td>
</tr>
<tr>
<td>4 Fluorene</td>
<td>5</td>
<td>17</td>
<td>0.9981</td>
<td>53</td>
<td>106</td>
</tr>
<tr>
<td>5 Phenanthrene</td>
<td>5</td>
<td>16</td>
<td>0.9996</td>
<td>46</td>
<td>92</td>
</tr>
<tr>
<td>6 Anthracene</td>
<td>5</td>
<td>17</td>
<td>0.9999</td>
<td>57</td>
<td>114</td>
</tr>
<tr>
<td>7 Fluoranthene</td>
<td>18</td>
<td>60</td>
<td>0.9994</td>
<td>53</td>
<td>106</td>
</tr>
<tr>
<td>8 Pyrene</td>
<td>6</td>
<td>18</td>
<td>0.9996</td>
<td>45</td>
<td>89</td>
</tr>
<tr>
<td>9 Chrysene</td>
<td>3</td>
<td>11</td>
<td>0.9956</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>10 Benzo[a]anthracene</td>
<td>4</td>
<td>15</td>
<td>0.9997</td>
<td>25</td>
<td>49</td>
</tr>
<tr>
<td>11 Benzo[b]fluoranthen</td>
<td>2</td>
<td>8</td>
<td>0.9970</td>
<td>29</td>
<td>58</td>
</tr>
<tr>
<td>12 Benzo[k]fluoranthen</td>
<td>1</td>
<td>3</td>
<td>0.9959</td>
<td>31</td>
<td>62</td>
</tr>
<tr>
<td>13 Benzo[a]pyrene</td>
<td>12</td>
<td>38</td>
<td>0.9985</td>
<td>49</td>
<td>98</td>
</tr>
<tr>
<td>14 Dibenzo[a,h]anthracene</td>
<td>2</td>
<td>8</td>
<td>0.9938</td>
<td>29</td>
<td>57</td>
</tr>
<tr>
<td>15 Benzo[g,h,i]perylen</td>
<td>2</td>
<td>7</td>
<td>0.9985</td>
<td>22</td>
<td>44</td>
</tr>
<tr>
<td>16 Indeno[1,2,3-c,d]pyrene</td>
<td>3</td>
<td>10</td>
<td>0.9988</td>
<td>20</td>
<td>41</td>
</tr>
</tbody>
</table>

Table 1: LOD /LOQ for method validation, determination of linearity and recovery results LC/MS MRM, spike at 50 ppb

The mussel tissue was spiked with equal levels of each of the 16 analytes at concentrations of 50 ppb, 1 ppm, and 10 ppm. The quantified results for each component and % recovery are listed in the table.

The results show recoveries between 114 % and 89 % for the first eight eluted PAH materials. The recovery for Benzo(a)pyrene is 98 % utilizing the internal standard. The chromatograms for the spiked mussel tissue and reference sample are shown in Figures 2 and 3. The chromatogram of the mussel sample is presented in Figure 4.

Conclusion

With accelerated solvent extraction up to 24 samples can be extracted sequentially and unattended and require only 25 minutes per sample. The in-cell cleanup using a combination of diatomaceous earth and acidic alumina as dispersing and sorbing agent respectively allow the removal of small particles and lipids from the sample matrix providing a clean extract for analysis and eliminates the need for post-extraction cleanup steps such as gel permeation chromatography (GPC). Benzo(a)pyrene with the use of an internal standard shows recovery close to 100 %.

References


Complete overview on Solutions for Food and Beverage Analysis on www.thermofisher.com/foodtesting

Find out more details on Dionex Sample Preparation Solutions including accelerated solvent extraction on www.thermofisher.com/samplepreparation

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purchased from a local grocery. 10 g of acidic alumina (Brockmann Activity 1) was placed between two glass fiber membrane filters. The mussel tissue was mixed with 10 g of Thermo Scientific Dionex ASE Prep DE (diatomaceous earth) dispersant and added to a 66 ml stainless steel extraction cell. The extraction was performed in 4 static cycles of 5 minutes each at 100 degrees centigrade and 100 bar using dichloromethane as a solvent, yielding a ready-to-inject extract without the need for additional cleanup steps. The extraction of each sample was completed in 25 minutes and the total extract volume was of only 100 ml compared to larger volumes using Soxhlet. The extracts were successively dried under a stream of nitrogen and stored in a fridge until analysis. The total analysis time – from sample receipt to final results – was less than one hour.

Determination of PAHs

Gas chromatography coupled to mass spectrometry or liquid chromatography with fluorescence detection has been the principle means of separation and quantification. The use of ultra high pressure liquid chromatography (UHPLC) greatly reduces the chromatography time. The UHPLC instrumentation allows for higher flow rates than a traditional HPLC system. The use of a 1.8 μm particle size column results in better resolution of components. Atmospheric pressure photoionization (APPI)-MS/MS provides selective ionization and detection to complement the selective extraction method. The UV gas-phase ionization enhances response for the PAHs and limits chemical noise from indigenous tissue chemistry. Additional chemical noise reduction is achieved in detection using MRM transitions characteristic of the individual PAHs monitored. This combination of accelerated solvent extraction, UHPLC, and APPI-MS/MS results in a selective streamlined method with limit of detection (LOD) of 12 pg on column for benzo(a)pyrene.

Results

The limit of detection (LOD) and limit of quantitation (LOQ) were determined for method validation using the 16 PAHs reference sample with the Thermo Scientific Dionex ASE 350 Accelerated Solvent Extractor system. The analytes were spiked at concentrations of 50 ppb, 1 ppm, and 10 ppm to bracket the levels of detection and FDA levels of concern for each analyte. The LOD and LOQ were calculated from the reference sample as the concentration that provided a baseline standard deviation ratio of 1:3 and 1:10 respectively (Table 1).

Linearity was initially determined using a standard mix at five concentrations ranging from 40 pg to 20,000 pg on column (Table 1).

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