Rapid Determination of Four Polycyclic Aromatic Hydrocarbons by HPTLC using Nano Plates

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There are well-established analytical methods to evaluate a large number of PAH like gas chromatography (GC) and high performance liquid chromatography (HPLC). Both these methods need a preceding purification (liquid-liquid partition, multiple column chromatography), which is undoubtedly a time-consuming factor. To overcome this drawback, the time consuming factor, Romanowski et al. recently used a very promising procedure, wherein the preseparated PAH fraction, effected by HPLC from the total extract, is utilized in determining PAH by GC [1].

Thin-layer chromatography (TLC) attracted attention as a highly sensitive, precise and extremely rapid technique with potential application in nearly every area of analytical interest; but the rapid increase in performance over that obtained in the usual practice of TLC, has led to the application of high-performance thin-layer chromatography (HPTLC). It must be pointed here that by using the usual TLC methods, a satisfactory separation of PAH on the TLC plates is considerably limited when original extracts are applied. Certainly, the HPTLC (nano plates) is superior to the normal TLC plates as far as application volume and the developing time for the plate is concerned, but due to the short way a spot has to run on nano plates, the most desirable separation effects cannot be easily predicted. Hence, the separation expected on a nano plate of a complex mixture sample must be considered more critically.

By application of an extract of suspended matter on reversed phase nano plates, it was found, that just benzo(a)pyrene (BaP) and benzo(e)pyrene (BeP) remained unseparated despite all efforts to optimize the chromatography. A quantitative determination of BaP in the presence of BeP remained unseparated despite all efforts to optimize the chromatography. A quantitative determination of BaP in the presence of BeP remained unseparated. Keeping in view to reduce the time-consuming factor involved in preceding purification for the BaP estimation methods, mentioned above, our main object here has to determine some other PAH beside BaP, which are regularly present in the ambient air.

Experimental

Materials. PAH reference compounds were obtained from Aldrich-Europe and were used without purification. Acetonitrile, dichloromethane were purchased from Merck, Darmstadt, and purified by distillation. Bidistilled water was prepared in quartz vessels. Silica gel fine, 100 – 200 mesh, and nano plates, C18-100 were products of Macherey/Nagel, Düren, West Germany.
Apparatus. Standard solutions and sample extracts were applied as a streak (6 mm) by means of the Linomat III (CAMAG, Muttenz, Switzerland). The plates were developed in a CAMAG HPDC Linear Chamber. The measurement of the fluorescence intensity was performed by scanning the streaks with a CAMAG TLC scanner. Spectra Physics Integrator 4270 was used for the evaluation of the areas and for the calculation of the concentrations.

Sample Collection and Preparation. For the collection of suspended particles every apparatus can be used, which operates at a flow rate of about 10 m³/h for 24 h. The air passing the filter should have a velocity not exceeding the runway distance for each component is identically marked.

PAH were eluted within a volume of 100 ml of cyclohexane. The eluate is concentrated under reduced pressure to 1 ml again.

By using a 100 μl CAMAG Linomat syringe, samples were spotted in streak form on the nano plates by means of Linomat techniques. The first four narrow bands were of the four reference substances, benzophenone, benzophenol, benzo(b)fluoranthene and benzo(a)anthracene. Each streak or narrow band is 6 mm in length and spotted 10 mm above the edge of the nano plate. The last streak was a mixture of the four reference substances.

HPTLC. The plate placed in a CAMAG linear chamber was developed with a ternary solvent system, acetonitrile/dichloromethane/water (9:1:1) for about 7 min. The plate was removed, dried and developed once again with the same selected solvent system to achieve a complete distinct separation of components.

Evaluation. Evaluation of the narrow bands was performed with a CAMAG scanner by measuring the fluorescence of the bands. The fluorescence intensity is registered as a peak by means of an integrator. The exciting wavelength was 366 nm (mercury lamp); on the emission side a secondary filter was installed, which is transmittant above 400 nm wavelength.

The concentrations of the 4PAH were calculated by means of the usual external standard method. As shown in Fig. 1 and Fig. 2 the 4 compounds are distinctly separated, both in reference components and in the extract samples. The relative standard error %

Results

Accuracy of the Method. Standard errors were calculated for standard solutions as well as for samples. A volume of 2 μl of each standard solution containing 1 ng/μl of the substance was sprayed 7 times in a row on the nano plate. The plates were developed, dried and run under identical conditions as described before for evaluation.

A similar investigation was carried out for the sample solution. Peak areas were measured by an integrator. The following standard errors were found:

<table>
<thead>
<tr>
<th>PAH</th>
<th>Relative standard error %</th>
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<tbody>
<tr>
<td>BghiP</td>
<td>0.4</td>
</tr>
<tr>
<td>BaP</td>
<td>0.4</td>
</tr>
<tr>
<td>BbF</td>
<td>0.7</td>
</tr>
<tr>
<td>BaA</td>
<td>3.5</td>
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</tbody>
</table>

1. Comparison between reference and sample components. Example of a complete evaluation with Spectra Physics Integrator No 4270. The expressions used are adapted to HPLC or GC, but they can be altered for the usual external standard method. As shown in Fig. 1 and Fig. 2 the 4 compounds are distinctly separated, both in reference components and in the extract samples. The relative standard errors were found.