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# QUANTITATIVE TRACE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND LOW-TEMPERATURE FLUORIMETRY IN SHPOLSKII MATRICES AT 4.2°K

## APPLICATION TO MEDICINAL WHITE OILS

J. M. COLIN* and G. VION

*Total–Compagnie Française de Raffinage, Centre de Recherches, Boite Postale 27, 76700 Harfleur (France)*

and

M. LAMOTTE and J. JOUSSOT-DUBIEN

*Université de Bordeaux I, Laboratoire de Chimie Physique A, ERA 167, 351 Cours de la Libération, 33405 Talence (France)*

## SUMMARY

A scheme of analysis for polycyclic aromatic hydrocarbons (PAHs) in medicinal white oils was developed; this was made possible by carrying out three initial chromatographic stages to isolate the PAHs and following this by a combination of high-performance liquid chromatography and spectrofluorimetry at room temperature. The identification of the PAHs was confirmed by trapping the chromatographic peaks and analyzing them by Shpolskii fluorimetry at 4.2°K.

## INTRODUCTION

Although high-performance liquid chromatography (HPLC) has largely been developed for the analysis of polycyclic aromatic hydrocarbons (PAHs) in, *e.g.*, air, water and automobile exhaust, this technique has seldom been applied for PAH analysis in white oils. The very low amounts of PAHs in such oils (of the order of ppt*) makes such analysis particularly difficult and requires unusually good selectivity as well as a high level of sensitivity for detection and identification.

Because of their high sensitivity, fluorescence methods appear to fulfil such requirements and to be particularly convenient for the detection of very small amounts of PAHs (which are known to be efficient fluorescent compounds). In this work, HPLC separation and quantitative analysis of selected aromatic compounds in white oils has been achieved by using a Jobin–Yvon fluorimeter (JY 3) as a detector; this instrument allows wavelength selection for both excitation and emission[1]. Moreover,

* Throughout this article, the American trillion ($10^{12}$) is meant.

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an individual PAH can be characterized by its fluorescence spectrum even at very low levels of concentration.

Spectral resolution, however, depends considerably on the experimental conditions. When in solution, at room temperature, fluorescence spectra are usually broad, the linewidth for the observed vibronic bands being typically of the order of 100 to 500 $cm^{-1}$ (Fig. 1). In fact, the specific vibrational structure that contains most of the characteristic information on a particular PAH is lost unless good purification or complete separation is achieved (which is never the case with the HPLC separation of complex mixtures). Identification of PAHs from fluorescence spectra at room temperature is difficult to establish conclusively.

A notable improvement in the specificity of PAH fluorescence spectra can be obtained by application of the so-called Shpolskii effect[2]. This effect is observed when

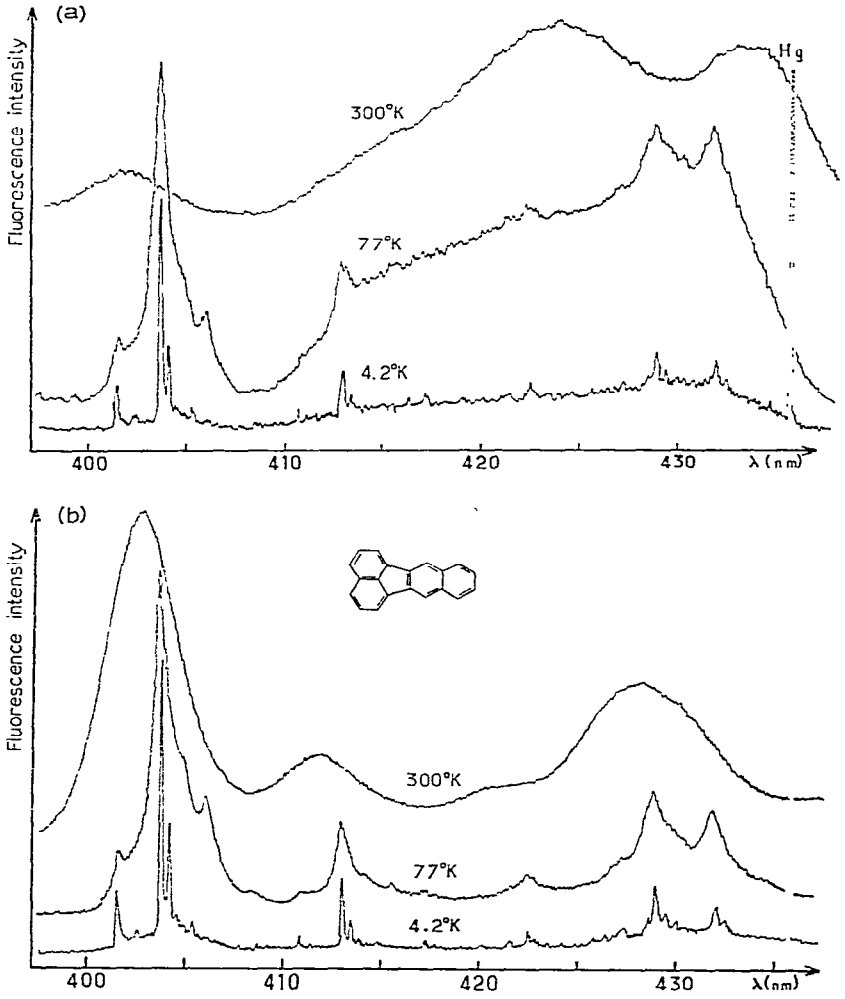


Fig. 1. Effect of temperature on fluorescence spectra. (a) Spectra from the fraction in which benzo[*k*]-fluoranthene has been identified ($\lambda_{ex.}$ = 310 nm). (b) Fluorescence spectra of benzo[*k*]fluoranthene.

aromatic compounds are dissolved in a suitable *n*-alkane matrix at low temperature. The resulting spectra exhibit well-resolved vibrational structure, and at 77°K the linewidths are reduced to *ca.* 10 to 50 $cm^{-1}$, which usually permits PAH identification with good confidence[3,4]. Even so, at this temperature the lines are still broadened by a temperature-dependent phonon interaction with the alkane lattice, but a further decrease in the linewidth to a few wavenumbers can be gained by lowering the temperature to 4.2°K (Fig. 1). At this temperature, the spectra are perfectly resolved and practically all the discrete vibronic transitions are observed. Further, the lines are split into multiplets due to various insertion sites in the crystalline matrices. The structure of the multiplet is very sensitive to the molecular shape, and hence reliable identification of a given PAH can be made even when the solution contains impurities, other aromatic compounds or isomers[5].

We have used this technique in order to confirm peak identification based on retention times and room-temperature fluorescence of fractions collected from HPLC separations of white oils. Moreover, we have taken advantage of the fact that the fluorescence due to aromatic compounds present in such a fraction can easily be separated from the diffuse emission due to other compounds to estimate the "purity" of the fraction and subsequently to correct quantitative estimations based on HPLC peak intensity.

## EXPERIMENTAL

### *Analysis by liquid chromatography*

The stages necessary for the isolation and determination of the amount of PAHs have already been described in detail[1]. Let us reiterate the main points:

(1) Frontal elution on silica gel gives a separation of all the aromatic compounds[6].

(2) By using adsorption chromatography (alumina as adsorbent), these aromatic compounds are separated into two fractions, one of which contains the PAHs.

(3) Partition chromatography on Sephadex LH-20 impregnated with dimethylformamide and water[7,8] separates this polyaromatic fraction into five sub-fractions, of which only three contain PAHs.

(4) By HPLC (Varian 5020 chromatograph) with an octadecyl-grafted silica support, coupled directly with a spectrofluorimetric detector operated at room temperature, isolation and determination of the amount of PAH in each of the three sub-fractions can be achieved. The spectrofluorimeter (Jobin–Yvon JY 3) is equipped with two concave holographic gratings (excitation and emission) and not with only filters (as is generally used in liquid chromatographic detection).

The wavelengths used for the detection of the PAHs are shown in Table I.

For the measurement of the individual PAHs, a standard solution containing the PAH to be measured is injected in the chromatograph just before the HPLC analysis. This procedure is the only one possible, because of the specificity of the detector, which does not permit use of the internal-standard procedure.

### *Identification by low-temperature fluorimetry*

*Sample preparation.* The PAHs were collected from the HPLC column as solutions (25 ml) in a mixture of water and methanol. In order to obtain high-

TABLE I

WAVELENGTHS USED FOR DETERMINING PAHs

| *Compound* | *Excitation wavelength (nm)* | *Emission wavelength (nm)* |
|---|---|---|
| Fluoranthene | 365 | 500 |
| Pyrene | 338 | 394 |
| Chrysene | 268 | 410 |
| Benz[*a*]anthracene | 290 | 410 |
| Benzo[*a*]pyrene | 390<br>365 | 405 |
| Benzo[*k*]fluoranthene | 297<br>390 | 405 |
| Benzo[*b*]fluoranthene | 365 | 500 |
| Perylene | 403 | 440 |
| Anthanthrene | 403 | 440 |
| Benzo[*ghi*]perylene | 297 | 405 |
| Indeno[1,2,3,*cd*]pyrene | 365 | 500 |

resolution Shpolskii fluorescence spectra, it was necessary to transfer the solutes into *n*-alkane solutions. This was accomplished by evaporating the first solvent under vacuum at reduced temperature in an ice bath. We verified that this process gave a recovery of better than 80% for most aromatic compounds. The fractions were then taken up in *ca.* 2 ml of *n*-heptane, which was used in all experiments at 4.2°K.

*Fluorescence spectrometry at 4.2°K.* Fluorescence spectra at 4.2°K have been obtained with use of a laboratory-made fluorimeter. Excitation was provided by a xenon lamp (Osram 450 W), the light from which was filtered through a Jobin–Yvon HRS grating monochromator (1200 lines/mm). For most measurements, bandwidths for excitation were set at *ca.* 3 nm.

The fluorescent radiation was observed at 90° through a Jobin–Yvon HRP monochromator equipped with the same grating as for excitation. The bandwidth for analysis of the fluorescence was set so as to give a resolution of between 0.1 and 0.06 nm.

An EMI photomultiplier 9789 CXB was used as detector, and the spectra were recorded on a Servotrace recorder at a rate of 5 nm/min.

The sample cell (a 4-mm O.D. silica tube) containing the solution was introduced, after a preliminary rapid cooling to liquid-nitrogen temperature, into a liquid helium Meric cryostat.

The *n*-heptane (Fluka, Buchs, Switzerland; purissimum grade) used was dried and kept over molecular sieves (5 and 10 Å); it was verified to be free of fluorescence with use of a MPF 3 Perkin-Elmer fluorimeter.

## RESULTS AND COMMENTS

The method outlined above was applied to the analysis of two medicinal white oils. The results are shown in Table II.

The chromatogram (Fig. 2) obtained by the direct coupling of HPLC and spectrofluorimetry at room temperature show that, by choosing the appropriate excitation and emission wavelengths, it is possible to detect the PAHs specifically

TABLE II

ANALYSES OF TWO WHITE OILS

| *Compound* | *Oil 1 (ppt)* | *Oil 2 (ppt)* |
|---|---|---|
| Fluoranthene | 645 | 830 |
| Pyrene | 610 | 734 |
| Chrysene | 235 | 265 |
| Benz[*a*]anthracene | 130 | 103 |
| Benzo[*a*]pyrene | 72 | 55 |
| Benzo[*k*]fluoranthene | 65 | 62 |
| Benzo[*b*]fluoranthene | 146 | 142 |
| Perylene | 17 | 13 |
| Anthanthrene | 22 | 12 |
| Benzo[*ghi*]perylene | 224 | 180 |
| Indeno[1,2,3,*cd*]pyrene | 72 | 80 |

and hence measure their concentrations. The results presented are for eleven PAHs, but the method could be applied to many more compounds, provided that reference specimens were obtainable.

Because of the high specificity of fluorescence spectra in Shpolskii matrices at 4.2°K, analysis of the PAHs trapped at the exit of the HPLC column by low-temperature fluorimetry enables identification to be made with very good confidence. It con-

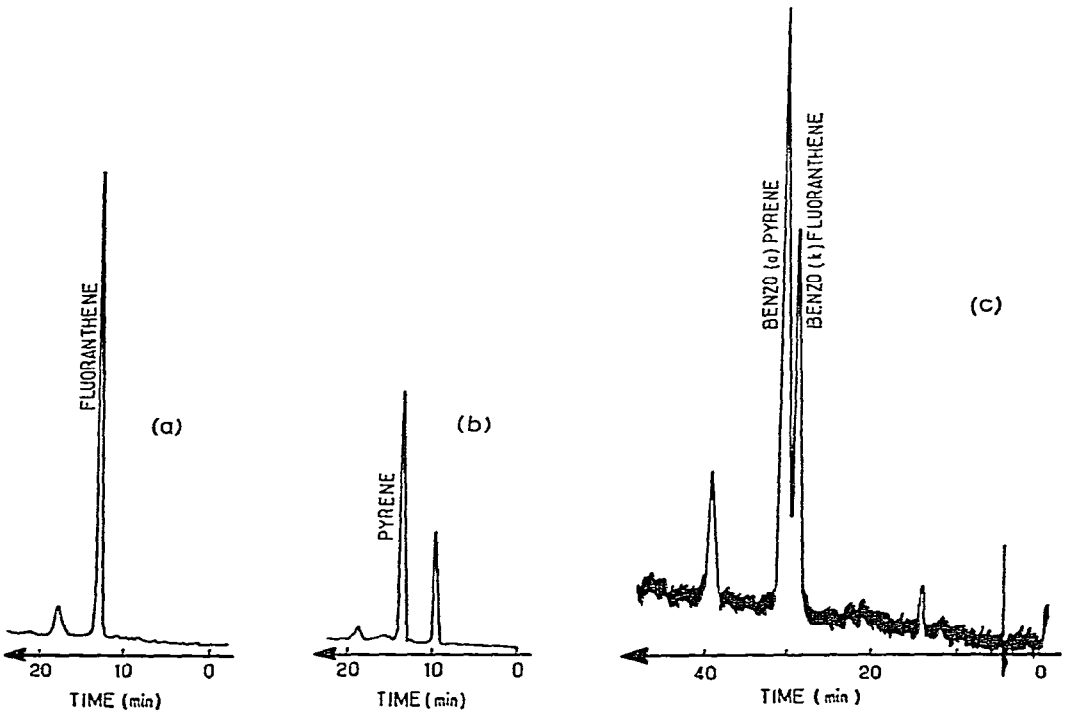


Fig. 2.

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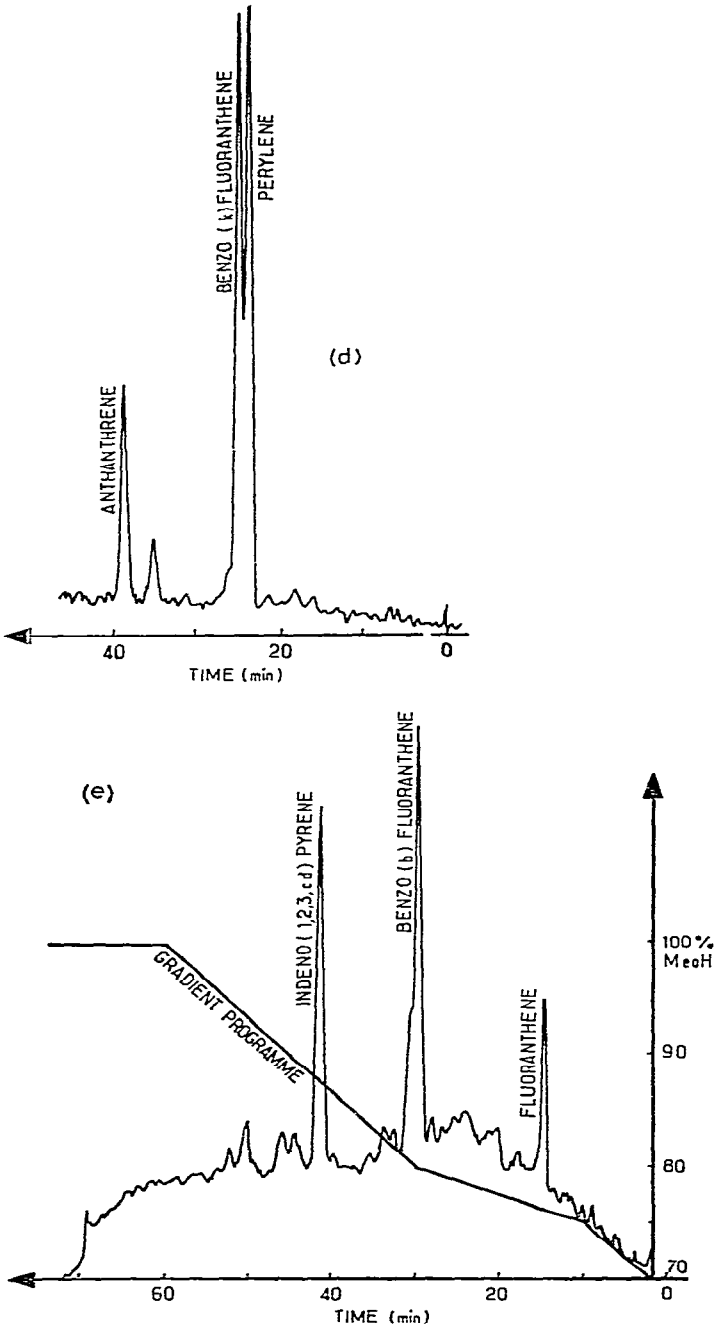


Fig. 2. HPLC separations using the Varian 5020 chromatograph with a column (30 cm × 4 mm I.D.) of Varian MicroPak MCH 10. Mobile phase, water–methanol; flow-rate, 2 ml/min; gradient programme used in all experiments shown in Fig. 2e. (a), $\lambda_{ex.} = 365$ nm, $\lambda_{em.} = 500$ nm; (b), $\lambda_{ex.} = 338$ nm, $\lambda_{em.} = 394$ nm; (c), $\lambda_{ex.} = 390$ nm, $\lambda_{em.} = 405$ nm; (d), $\lambda_{ex.} = 403$ nm, $\lambda_{em.} = 440$ nm; (e), $\lambda_{ex.} = 365$ nm, $\lambda_{em.} = 500$ nm.

firms the sometimes ambiguous identification made by traditional methods (*e.g.* retention times and fluorescence spectra at room temperature). Two examples are shown in Figs. 1 and 3.

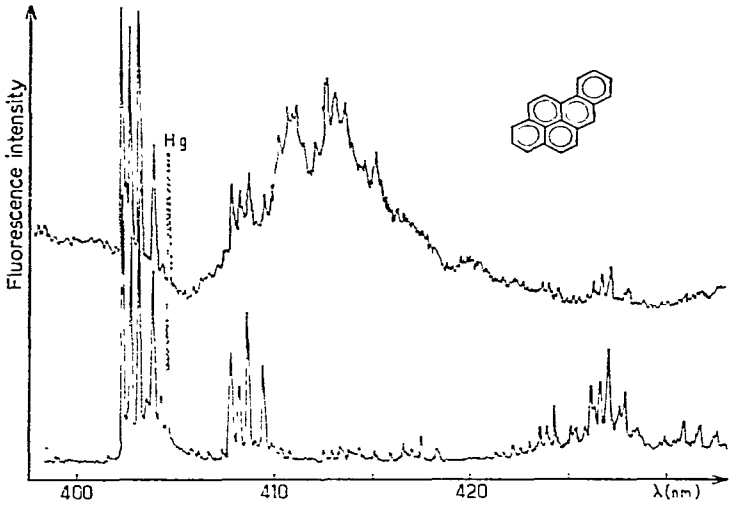


Fig. 3. Top: Spectrum from the fraction in which benzo[*a*]pyrene has been identified. Bottom: Fluorescence spectrum of benzo[*a*]pyrene.

The comparison of the spectra shows conclusively that the trapped peaks contain benzo[*k*]fluoranthene (Fig. 1) and benzo[*a*]pyrene (Fig. 3), respectively. However, in both instances, the presence of background fluorescence indicates the presence of other species, which is normal considering the complexity of the original mixture. This is particularly clear with benzo[*a*]pyrene, since the important part of the spectrum between 410 and 420 nm cannot be attributed solely to benzo[*a*]pyrene. Measurement must therefore be made between 400 and 410 nm, and great care must be taken in the region 410 to 420 nm, where other species fluoresce. In other words, one is unable to obtain precise quantitative measurements by direct coupling of HPLC and fluorimetry unless the detector used is equipped with an emission monochromator having a sufficiently narrow bandwidth ($\leqslant$10 nm).

Although Fig. 2 shows only two examples of low-temperature fluorimetry, the technique was used to confirm the identification of all the PAHs analyzed by direct HPLC–fluorimetry coupling.

The analysis being very long (*ca.* 1 month each run), we have not measured the standard deviation of the data until now, but each oil was analysed twice, the results shown in Table II being the average of two measurements. For each PAH, the difference between the two measurements is less than 20%, which seems satisfactory at such a level of concentration (10 ppt).

The lower limit of detection is between 2 ppt (for perylene or anthanthrene) and 50 ppt (for fluoranthene, pyrene or chrysene).

## CONCLUSION

The coupling of HPLC with room-temperature fluorimetry permits the measurement of traces of PAHs in medicinal white oils. Analysis by low-temperature fluorimetry of the chromatographic peaks collected from the column confirms their identification and verifies the absence of interference in the measurement.

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