Laser-induced fluorescence detection at 266 nm in capillary electrophoresis
Polycyclic aromatic hydrocarbon metabolites in biota

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Abstract

The separation of five phenolic polycyclic aromatic hydrocarbon metabolites (hydroxy-PAHs) has been performed by cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) using a 30 mM borate buffer (pH 9.0) containing 60 mM sodium dodecyl sulfate and varying concentrations of γ-cyclodextrin (γ-CD). A concentration of 12.5 mM γ-CD was found to provide a baseline separation of the five hydroxy-PAHs. We applied conventional fluorescence and laser-induced fluorescence (LIF) detection, using a new, small-size, quadrupled Nd–YAG laser emitting at 266 nm. The best limits of detection, in the low ng/ml range, were achieved using LIF detection. For all analytes, linearity was observed up to ca. 100 ng/ml. As an application, conjugated pyrene metabolites in hepatopancreas samples from the terrestrial isopods *Oniscus asellus* and *Porcellio scaber* were separated and detected. Finally, flatfish bile samples from individuals exposed to polluted sediment or crude oil, which were part of an interlaboratory study, were analyzed by CD-MEKC with conventional fluorescence and LIF detection to determine the 1-hydroxypyrene concentrations. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

At present, laser-induced fluorescence (LIF) detection for analytes exhibiting native fluorescence, following excitation in the deep UV range, has been hardly exploited for detection purposes in capillary electrophoresis (CE), mainly because LIF detection using excitation in the deep UV range is not straightforward. Until now, such excitation required the use of large frame argon ion lasers or excimer laser-pumped dye laser systems [1,2]. Unfortunately, these laser systems are expensive and impractical in analytical applications because of their large dimensions.

In this study, the performance in LIF detection of

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a new, small-size, quadrupled Nd–YAG laser emitting 266-nm radiation at a high pulse frequency was investigated. Such a laser system will be of special interest in bioanalytical studies since the 266-nm output is fully compatible with the excitation of natively-fluorescent amino acids (tyrosine, tryptophan and phenylalanine). In the present study, however, its appropriateness for detection of polynuclear aromatic hydrocarbon (PAH) metabolites is tested.

Because the metabolism of PAHs after PAH exposure is very fast for most organisms, the concentrations of PAH metabolites in biotic matrices are generally much higher than those of the parent PAHs. It is therefore advantageous to focus attention on the determination of PAH metabolites, instead of on their parents. In the last decade the quantification of PAH metabolites in excreta (urine, bile and feces) has become an important method for the biomonitoring of PAH exposure in various terrestrial and aqueous ecosystems [3–7], as well as for monitoring human PAH exposure [8–13].

Specifically, 1-hydroxypyrene has extensively been used as a biomarker for PAH exposure. The determination of 1-hydroxypyrene and other hydroxy-PAHs is performed after enzymatic hydrolysis of phase II conjugates such as 1-hydroxypyrene sulfate and 1-hydroxypyrene glucuronide. Although standards are not yet commercially available, the potential of direct monitoring of phase II metabolites has also been explored [5,9,10,13].

Phenolic and conjugated PAH metabolites are usually analyzed by liquid chromatography (LC) combined with fluorescence detection [3–13]. Few studies report on the separation of hydroxy-PAHs by CE [14–16], but until now CE has not been applied to the detection of these compounds in real (biotic) samples. In the present study the separation and detection of five hydroxy-PAHs, and of pyrene and its metabolites, in biological matrices (fish bile and isopod hepatopancreas) is studied.

2. Materials and methods

2.1. Chemicals and materials

Water demineralized and distilled in the laboratory was used to prepare buffers and analyte standard solutions. Buffers were adjusted to the required pH using 1 *M* NaOH. Boric acid and ascorbic acid were obtained from J.T. Baker (Deventer, The Netherlands), absolute ethanol (analytical-reagent grade) from Riedel-de Haën (Seelze, Germany) and sodium dodecyl sulfate (SDS) from Fluka (Buchs, Switzerland). 1,5-Naphthalenedisulfonic acid and γ-cyclodextrin hydrate were purchased from Aldrich (St. Louis, MO, USA). β-Glucuronidase/aryl sulfatase (from *Helix pomatia*, EC 3.2.1.31 and EC 3.1.6.1, 30 and 60 U/ml, respectively) was obtained from Merck (Darmstadt, Germany). Buffer solutions were filtered over 0.45-μm syringe filters (Schleicher & Schuell, Dassel, Germany) before use.

2.2. Standard solutions and samples

The hydroxy-PAHs used in this study are 1-hydroxypyrene, 3-hydroxybenzo[*a*]pyrene, 1-hydroxyphenanthrene, 1-hydroxychrysene and 2-hydroxynaphthalene. Stock solutions containing these five hydroxy-PAHs (10 μg/ml) as well as the fish bile samples were distributed as part of a European Union (EU)-funded interlaboratory study (SMT 4-CT 98-2250) [17]. The stock solutions had been prepared with ethanol–water (80:20, v/v), containing 5 mg/ml ascorbic acid to avoid oxidation. From the stock solutions, 1 μg/ml solutions were prepared using ethanol–water (80:20, v/v) with 5 mg/ml ascorbic acid. In order to prepare calibrant solutions, further dilutions were made by mixing with ethanol–water (80:20, v/v) without ascorbic acid.

Hepatopancreas samples from the isopods *Porcellio scaber* and *Oniscus asellus*, which contained conjugated pyrene metabolites, were obtained as described by Stroomberg et al. [5]. Briefly, the isopods were starved for 3 days and individually placed in scintillation vials for 48 h, together with 100 mg of ground leaves spiked with pyrene at a level of 10% (w/w) dry mass. After dissection, hepatopancreases of 10 animals were pooled in 100 μl of Tris buffer (pH 9.0) and 20 μl of protease K was added. Proteolysis was performed for 18 h at 37°C. Next, an equal volume of ethanol was added and the remaining debris was precipitated by 3-min centrifugation at 5000 rpm. Individual metabolite solutions were obtained by fractionation of the

samples by means of reversed-phase LC, with a gradient of acetonitrile and ammonium acetate buffer (10 m*M*, pH 5.0) [5]. For prolonged use, all standards and samples were stored in the refrigerator at $-20°C$. Before use, the solutions were sonicated for 5 min.

2.3. Instrumental set-up

An LS-50B fluorescence spectrometer (Perkin-Elmer, Beaconsfield, UK) was used to record fluorescence excitation and emission spectra. Electropherograms were obtained using a Prince high-voltage/injection system (Lauerlabs, Emmen, The Netherlands) in combination with a conventional FP-920 fluorescence detector (Jasco, Tokyo, Japan) or a laboratory-built set-up for LIF detection. CE separations using the conventional fluorescence detector were performed at a voltage of 20 kV with a 90 cm×75 μm I.D. uncoated fused-silica capillary (BGB Analytik, Anwil, Switzerland) with an effective length of 60.5 cm. For the LIF experiments a 104 cm capillary with an identical effective length was used; the separation was run at 23.3 kV to maintain the same field strength (221 V/cm). The injection pressure was 25 mbar and the injection time 0.1 min. For data collection a computer equipped with CLASS-VP data-acquisition software was used.

The LIF set-up is shown in Fig. 1. The light from a quadrupled Nd–YAG NanoUV laser (Uniphase,
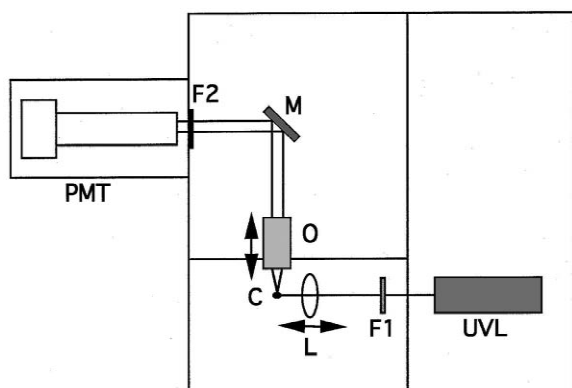

Fig. 1. Detection system for LIF detection. UVL: 266-nm laser; F1: neutral density filter; L: focusing lens; C: capillary (focusing point); O: reflecting objective; M: mirror; F2: bandpass or cut-off filter; PMT: photomultiplier tube.

Meylan, France), emitting at 266 nm with an average output power of 5.4 mW, a pulse width of 0.34 ns and a repetition rate as high as 7.8 kHz, was focused into the liquid core of the capillary by a quartz lens with a focal distance of 3 cm. A 25×, N.A. 0.4 reflective objective (Ealing, Holliston, MA, USA) was used to collect the fluorescence emission. The 9558 QA photomultiplier tube (EMI, Middlesex, UK) used for detection was connected to a 456H power supply (Ortec, Bracknell, UK) operated at 1000 V. Neutral density filters were used in order to decrease the irradiance of the capillary detection window. A UG-1 bandpass filter and BG-14 or GG-13 cut-off filters (Schott, Mainz, Germany) were used to collect all emission and discriminate against scattered excitation light.

3. Results and discussion

This section first discusses the CE separation and fluorescence detection of five hydroxy-PAHs which would be the target analytes in the fish bile samples. The parent PAHs are usually not observed in such samples. Therefore, it is not necessary to involve the parent compounds in the separation. Emphasis is on the performances of the new 266-nm LIF method compared with conventional fluorescence detection. Subsequently, the determination of pyrene metabolites in isopods and fish bile samples will be discussed. For such complex matrices the LIF method will, no doubt, suffer from the disadvantage that it offers no selectivity in excitation settings: only one, single, wavelength is available, i.e., 266 nm. Comparison with conventional fluorescence detection is, therefore, of distinct interest.

3.1. CE of hydroxy-PAHs

To perform conventional fluorescence detection under optimum conditions, the excitation and emission fluorescence spectra of the hydroxy-PAHs were recorded in the selected buffer system (see below). If more than one maximum was present, as is the case for the excitation spectra, the maximum giving the highest signal-to-noise ratio (S/N) in the electropherograms was chosen. The maxima used for detection in CE are given in Table 1. The conven-

Table 1
Limits of detection (ng/ml) for five hydroxy-PAHs using CE with LIF and conventional fluorescence (CF) detection[a]

Compound	CF	LIF, UG-1	LIF, BG-14
3-Hydroxybenzo[a]pyrene	2 (383/435)	25	2
2-Hydroxynaphthalene	40 (275/344)	3	4
1-Hydroxyphenanthrene	7 (306/361)	3	2
1-Hydroxychrysene	35 (307/370)	10	6
1-Hydroxypyrene	1 (347/387)	4	2

[a] In parentheses (CF): excitation/emission wavelengths used for detection.

tional fluorescence detector has a fixed excitation slit of 10 nm, but the emission slit can be varied (10, 18 or 40 nm bandwidth). An emission bandwidth of 40 nm gave the best results in terms of S/N.

As far as the CE separation is concerned, micellar electrokinetic chromatography (MEKC) as such does not suffice: using a 30 mM borate, 60 mM SDS buffer (pH 9.0), all analytes were found to migrate simultaneously. This is not surprising because all target compounds are rather hydrophobic and therefore have similar affinity for the micelles [18]. In order to generate selectivity, γ-cyclodextrin (γ-CD) was added to the buffer, an approach proven to be valuable in the separation of a different set of hydroxy-PAHs [14]. The selectivity that can be achieved with a γ-CD–SDS buffer system is based on the different partitioning of the analytes between the micellar, aqueous and γ-CD phases. The affinity of a particular analyte for a CD molecule strongly depends on its fit in the CD cavity. Although β-CDs are generally considered to be more efficient complexing agents than γ-CDs, the latter are preferred in CD-MEKC because the β-CD molecules are not able to bind analytes strongly in micellar buffer systems. It has been suggested that this can be ascribed to the co-inclusion of individual SDS molecules which reduces the actual cavity size, especially in the case of β-CDs [19,20].

In this work we used a 30 mM borate, 60 mM SDS, pH 9.0 buffer with varying concentrations of γ-CD (10, 12.5, 15 and 20 mM) to achieve the separation of the five hydroxy-PAHs. A concentration of 12.5 mM was found adequate to separate all hydroxy-PAHs.

The limits of detection (LODs) of the hydroxy-PAHs obtained with conventional fluorescence detection were determined from three replicate in-

jections close to the detection limit. As shown in Table 1, the LODs were in the low ng/ml range for three of the test analytes, but about 10-fold higher for 2-hydroxynaphthalene and 1-hydroxychrysene.

Using peak areas, linear ranges from 10 to 500 ng/ml were obtained for 3-hydroxybenzo[a]pyrene and 1-hydroxypyrene (with R^2 values of 0.9997 and 0.9993, respectively; five data points in duplicate for both). For peak heights, linearity was observed from 10 to 100 ng/ml with R^2 values of 0.9992 and 0.9969 (four data points in duplicate) for 3-hydroxybenzo[a]pyrene and 1-hydroxypyrene, respectively. For 1-hydroxyphenanthrene, linearity was observed from 25 to 200 ng/ml, using either peak areas or peak heights ($R^2 = 0.9940$; four data points in duplicate, and $R^2 = 0.9952$; five data points in duplicate, respectively). Because the LODs for 2-hydroxynaphthalene and 1-hydroxychrysene were rather high, no linear ranges were determined for these compounds.

In general, the linear ranges are shorter when using peak heights, which can be readily explained by the peak broadening occurring at levels above ca. 100 ng/ml. This peak broadening is presumably caused by overloading of the micellar and γ-CD phases. The co-inclusion of individual SDS molecules into CD cavities, as mentioned above, implies that the effective micellar concentrations are lower than expected from the total SDS concentration and the critical micellar concentration. Also, the concentration of γ-CD molecules with a cavity large enough for strong inclusion of hydroxy-PAHs will be reduced. These assumptions are supported by the observation that the peak of 3-hydroxybenzo[a]pyrene, which shows the highest affinity for the γ-CD molecules, shows *tailing* at high concentrations, which indicates overloading of the γ-CD

phase. On the other hand, the *fronting* observed for the 1-hydroxypyrene peak at higher concentrations suggests overloading of the micellar phase.

With the conventional fluorescence detector optimum detection sensitivity for all compounds requires programmed adjustment of the excitation and emission wavelengths. This proved to be quite difficult, because the migration times were found to show slight run-to-run variations and the compounds are migrating closely together. Moreover, the LODs for 2-hydroxynaphthalene and 1-hydroxychrysene will often be inadequate for the determination of these compounds in real samples. Therefore, it is certainly worthwhile to explore the possibilities of LIF detection.

The LIF set-up (Fig. 1) was optimized under flow-driven conditions (pressure=100 mbar), using a solution of $5 \cdot 10^{-6}$ *M* 1,5-naphthalenedisulfonic acid. The background level was checked after every adjustment to avoid collection of stray light from the capillary core and wall. This was done by adjusting the pressure to zero: then, in the absence of flow, the fluorescence signal almost instantaneously collapses due to photodecomposition.

Firstly, the capillary holder position was optimized so that the focal point on the capillary was exactly in the center of the reflective objective. Then, the focusing into the capillary liquid core was optimized by adjusting the position of the focusing lens. Subsequently, the distance of the reflective objective to the illuminated point of the capillary was optimized to achieve optimum fluorescence collection and scattered light rejection. Finally, the position and angles of the mirror projecting the fluorescence image on the photomultiplier tube were varied for optimum fluorescence intensity. Proper alignment of the LIF set-up could easily be maintained over several months, without any re-alignment. Also, excellent long-term output power stability was observed, as was apparent from the stable and flat baselines obtained.

It should be realized that the peak power of the laser used here is still as high as 1.8 kW, despite its modest average power of 5.4 mW. It was observed that such a high peak power damages the capillary within a few hours. Also, air bubbles were formed within the capillary, probably due to heating of the buffer. Fortunately, when using a neutral density

filter with a transmission (*T*) of 6.3%, the capillaries could be used for at least several weeks, while maintaining adequate detection sensitivity. A typical LIF-detected electropherogram of a standard mixture of the five hydroxy-PAHs is shown in Fig. 2. High efficiency peaks were obtained with plate numbers of 128 000, 199 000, 241 000, 191 000 and 214 000 (peak numbers 2–6, respectively).

The fluorescence excitation spectra (not shown) of the hydroxy-PAHs suggest that only 2-hydroxynaphthalene and 1-hydroxypyrene can be excited efficiently at 266 nm. For the other metabolites the extinction coefficients at 266 nm are rather low. Interestingly, these differences are not reflected in the LIF results: LODs in the low ng/ml range were achieved for all compounds studied (Table 1). Probably, measurements were performed under saturation conditions: despite the low molar absorptivities at 266 nm, maximum excitation is reached due to the high peak power. When using the BG-14 cut-off filter, the LODs for 1-hydroxypyrene and 3-hydroxybenzo[*a*]pyrene were better than with the UG-1 bandpass filter because the UG-1 filter, while conveniently excluding ambient light, is hardly transparent above 400 nm. Compared with the results obtained with conventional fluorescence detection, the LODs were significantly better for 2-hydroxynaphthalene and 1-hydroxychrysene.
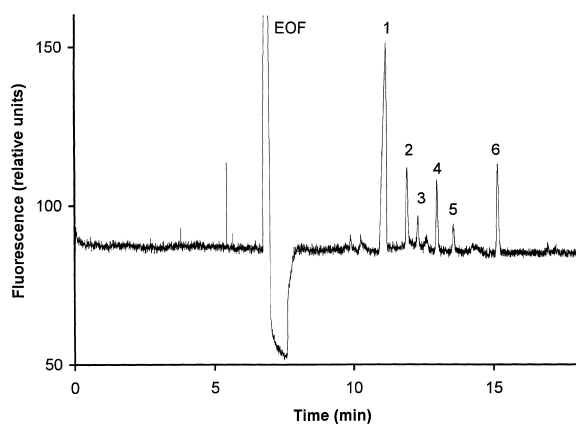

Fig. 2. Electropherogram of a mixture of five hydroxy-PAHs (20 ng/ml) using 266-nm LIF detection. Emission filter: BG-14. Other conditions: see text. EOF: electroosmotic flow; 1=ascorbic acid; 2=3-hydroxybenzo[*a*]pyrene; 3=2-hydroxynaphthalene; 4=1-hydroxyphenanthrene; 5=1-hydroxychrysene; 6=1-hydroxypyrene.

To summarize this section on standard solutions, it can be concluded that the 266-nm LIF detection method investigated here is generally applicable for analytes exhibiting native fluorescence, despite the fixed excitation wavelength. The detection limits are slightly better than those obtained with conventional fluorescence detection. A quantitative comparison with other LIF detection methods is difficult to make since differences in detection configuration play a role. In an MEKC separation of hydroxy-PAHs, Smith et al. [14] achieved LODs in the 0.08–3 μM range, values distinctly less favorable than the data in Table 1. However, they used a capillary with an inner diameter of 50 μm and made use of fiber optics. In general it can be stated that continuous wave argon ion lasers perform better in terms of achievable detection limits. In preliminary experiments on naphthalene sulfonates, using 266-nm LIF detection, LODs in the low nM range were obtained [21], which is about one order of magnitude higher than obtainable using excitation by a continuous wave argon ion laser [1,2]. Such a difference in performance should be expected in view of the pulsed nature of the present laser (duty cycle: $3 \cdot 10^{-6}$).

3.2. Pyrene metabolites in isopods

In a previous study it was concluded that the pyrene metabolite concentrations in isopods, specifically, *Oniscus asellus* and *Porcellio scaber*, can be used as a biomarker for PAH exposure in the terrestrial environment [5]. Monitoring the conjugated pyrene metabolites directly, instead of 1-hydroxypyrene after enzymatic hydrolysis, can be advantageous because the analyte detectability is 3–5-times higher in that case [9,10,13]. Moreover, detection of the conjugated PAH metabolites instead of the hydroxy-PAHs can give important information regarding phase II metabolic pathways. Here, the applicability of CE–LIF was tested for the detection of pyrene, 1-hydroxypyrene and the three conjugated pyrene metabolites, 1-hydroxypyrene sulfate, 1-hydroxypyrene glucoside and an unknown phase II pyrene metabolite. Since the conjugates are not commercially available, quantification was not possible.
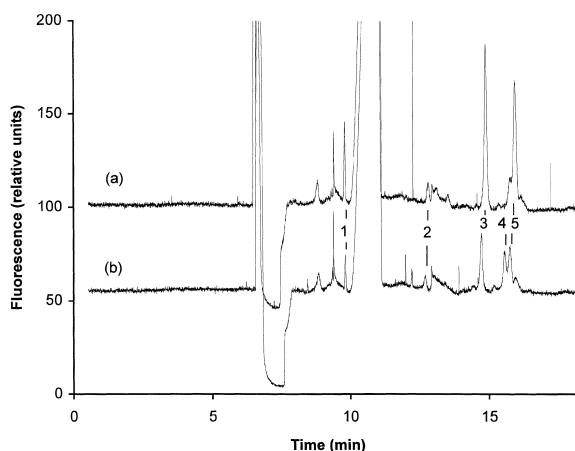
Electropherograms of the two isopod samples

Fig. 3. Electropherograms of pyrene metabolites isolated from the hepatopancreas of (a) *Oniscus asellus* and (b) *Porcellio scaber* using 266-nm LIF detection. Emission filter: BG-14. Buffer: 30 mM borate, 60 mM SDS, 10 mM γ-CD, pH 9.0. 1=1-Hydroxypyrene sulfate; 2=unknown pyrene metabolite; 3=1-hydroxypyrene; 4=pyrene; 5=1-hydroxypyrene glucoside.

mentioned above, recorded with LIF detection using the BG-14 cut-off filter, are shown in Fig. 3. Theoretical plate numbers are typically 241 000, 93 000, 137 000, 97 000 and 91 000 for peak numbers 1–5. The conjugated PAH metabolites could be well separated by CE using a 30 mM borate, 60 mM SDS, 10 mM γ-CD, pH 9.0 buffer. However, pyrene and 1-hydroxypyrene glucoside showed partial overlap (R_s: 0.40) with this buffer composition. Their resolution required 12.5 mM γ-CD in the buffer. Unfortunately, under these conditions the 1-hydroxypyrene sulfate peak overlapped with a compound present in the sample matrix. Nevertheless, when using both buffers systems all target analytes can be monitored.

3.3. 1-Hydroxypyrene in fish bile

As a second application, two fish bile samples were analyzed, bile from plaice (*Pleuronectes platessa*) exposed to crude oil, and from flounder (*Platichthys flesus*) exposed to contaminated sediment. These samples had been distributed as part of an interlaboratory study [17]. The methods used in this study by other laboratories were LC with conventional fluorescence detection and gas chromatography–mass spectrometry (GC–MS).

In this application the quantitative aspects of CE with conventional fluorescence and LIF detection, were tested, the target analyte being 1-hydroxy-pyrene. In view of the data assembled in Table 1, for this particular metabolite conventional fluorescence detection will probably be the method of choice: compared to 266-nm LIF it performs equally in terms of LODs but it provides additional excitation selectivity. The fish bile samples (50 μl) were weighed and hydrolysed enzymatically for 2 h at 37°C after the addition of 20 μl of enzyme (β-glucuronidase/aryl sulfatase) and 180 μl of water. After incubation, 1000 μl of absolute ethanol was added and the sample was centrifuged for 5 min at 7000 rpm; next, the supernatant was taken for analysis.

As was already indicated above, it was not surprising that analyzing the fish bile samples by CE with 266-nm LIF detection and using the BG-14 cut-off filter, gave rise to a large number of peaks in the electropherograms, especially with the oil-exposed fish bile. The interfering peaks inhibited reliable recognition of most of the hydroxy-PAHs discussed in Section 3.1 by means of spiking. Only 1-hydroxy-pyrene, which was found to be present at a much higher concentration than the other hydroxy-PAHs, could be unambiguously identified. In order to improve the selectivity of LIF detection, a GG-13 cut-off filter, which removes all radiation below ca. 395 nm, was used instead. This caused a substantial reduction of the number of peaks for the sediment-exposed fish bile, but not for the oil-exposed fish bile (Fig. 4).

It was expected that the use of conventional fluorescence detection would further improve the selectivity, since the excitation and emission maxima of 1-hydroxypyrene are at rather high wavelengths (cf. Table 1). Indeed, the electropherograms now became much cleaner (Fig. 5), with the electropherogram of the flounder bile exhibiting only one large peak.

Quantification was performed by using the external standard method with calibration curves in the range from 16 to 100 ng/ml (four data points in triplicate). Before injection the oil- and sediment-exposed fish bile samples were diluted with ethanol–water (80:20, v/v) to give a total dilution of 50× and 100×, respectively. Relevant results are pre-
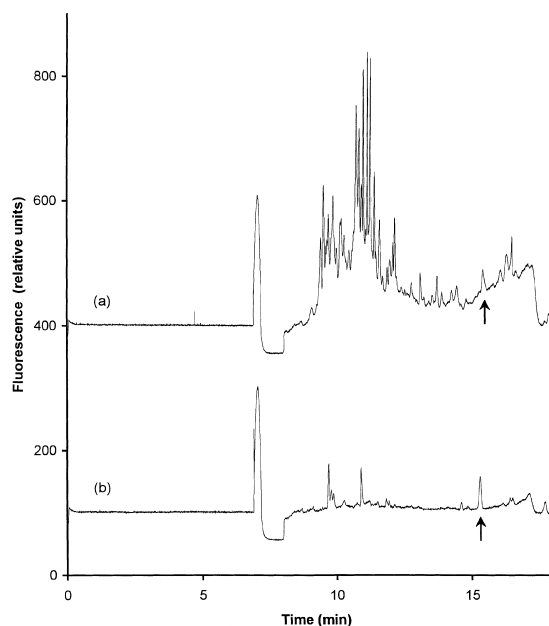

Fig. 4. Electropherograms of fish bile samples after enzymatic hydrolysis using 266-nm LIF detection. Emission filter: GG-13. Other conditions: see text. (a) Oil-exposed plaice bile, (b) sediment-exposed flounder bile. The 1-hydroxypyrene peaks are indicated by arrows.

sented in Table 2. It is interesting to compare the results with those obtained in the interlaboratory study [17]. For the sediment-exposed flounder bile the concentrations as determined independently by seven laboratories were found to be in the 3.0±1.9 μg/g range ($n=7$, 95% confidence interval), while for the oil-exposed plaice bile the range was 1.2±0.4 ($n=6$). The results presented in Table 2 are fully in line with these data. The small differences between the LIF and CF data might be systematic, but the range of 1-hydroxypyrene concentrations determined by the other laboratories is to broad for a definite conclusion.

4. Conclusions

Compared to other available deep UV laser systems the quadrupled Nd–YAG laser used in this study has several advantages. It is compact, inexpensive and rugged (expected operating lifetime: 10 000 h). Next, the alignment of the LIF set-up is easily
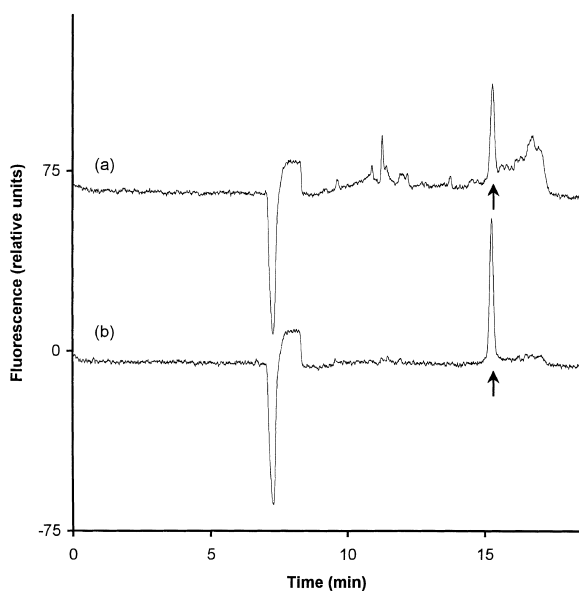
Fig. 5. Electropherograms of fish bile samples after enzymatic hydrolysis using conventional fluorescence monitoring with λ_{ex} 347 nm and λ_{em} 387 nm. Other conditions: see text. (a) Oil-exposed plaice bile, (b) sediment-exposed flounder bile. The 1-hydroxypyrene peaks are indicated by arrows.

maintained over several months and the laser shows excellent long-term output power stability providing stable baselines and reliable detection. Finally, its output power is high enough to saturate the absorption transitions even when a 6.3% T neutral density

Table 2
1-Hydroxypyrene concentrations in PAH-exposed fish bile and calibration plot characteristics, using CE with LIF and conventional fluorescence (CF) detection[a]

	Concentration 1-hydroxypyrene (μg/g)	
	LIF	CF
Plaice bile (oil-exposed)	1.8 (0.07)	1.5 (0.04)
	1.7 ($n=1$)	1.3 ($n=2$)
Flounder bile (sediment-exposed)	4.5 (0.07)	4.0 (0.28)
	4.1 ($n=2$)	3.8 ($n=2$)
R^{2}[b]	0.997	0.993

[a] For each sample hydrolysis and further sample treatment was carried out in duplicate; unless stated otherwise, subsequent quantification was based on the average of three injections. Values in parentheses: standard deviation (repeatability of three injections).

[b] Four data points in triplicate.

filter is used to reduce the incident power. The last characteristic implies that the 266-nm CE–LIF detector can be generally used for analytes exhibiting native fluorescence; even when the molar absorptivity at 266 nm is poor. This is illustrated by the LODs obtained for the five hydroxy-PAHs studied: similar LODs (2–6 ng/ml) are obtained for all of them although three of them have low absorptivities at 266 nm.

Of course, if real samples have to be analyzed, the inherent lack in excitation selectivity can be disadvantageous. This is clearly illustrated by the electropherograms of the oil-exposed fish bile. Nevertheless, the 1-hydroxypyrene concentrations determined in both bile samples using 266-nm LIF detection are comparable to those obtained with conventional fluorescence, and within the range of concentrations found by other laboratories using LC–fluorescence detection.

Finally, it is expected that 266-nm LIF will have perspective for detection in CE, especially when attention is focused on molecules like tyrosine, tryptophan and phenylalanine, because the 266-nm laser line is close to the fluorescence excitation maxima of these amino acids.

Acknowledgements

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