Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1011 (2003) 193–201
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Capillary electrophoresis with laser induced-fluorescence detection of profens derivatized with the water-soluble fluorogenic reagent 4-*N*-(4-*N*′-aminoethyl)piperazino-7-nitro-2,1,3-benzoxadiazole

Cheng Zhi Huang[a,b], Tomofumi Santa[a,*], Kohki Okabe[a], Kazuhiro Imai[a]

[a]*Graduate School of Pharmaceutical Sciences, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[b]*College of Chemistry and Chemical Engineering, Southwest Normal University, Chongqing 400715, China*

Received 4 February 2003; received in revised form 28 April 2003; accepted 11 June 2003

Abstract

Profens, including pranoprofen, fenoprofen, flurbiprofen, ketoprofen and ibuprofen (Ib), were derivatized by a water-soluble benzofurazan fluorescent reagent, 4-*N*-(4-*N*′-aminoethyl)piperazino-7-nitro-2,1,3-benzoxadiazole and then were run on capillary electrophoresis in a NH_4Ac–HAc buffer of pH 3.1 containing 2.4 m*M* β-cyclodextrin. At room temperature, the derivatization reaction was catalyzed by triphenyl phosphine and diphenyl disulfide in acetonitrile medium, and the derivatives fluoresce around 530 nm when excited at 488 nm. With the CE running on a 50 cm×50 μm I.D. length fused-silica capillary of by using Ar^+ laser induced-fluorescence detection, the detection limits attained were in the range of 0.16 to 0.3 fmol.

Keywords: Derivatization, electrophoresis; Pharmaceutical analysis; Profens; Aminoethylpiperazinonitribenzoxadiazole

1. Introduction

Profens form an important class of nonsteroidal anti-inflammatory drugs, and the separation and detection of a mixture of such a complex family is very important [1]. Particularly, the separation of newly synthesized drugs of these families is a big challenge for new drug discovery [2–4]. Although capillary zone electrophoresis and high-performance liquid chromatography (HPLC) methods have been reported [1,5], and prototype multiplex hyphenated system has been applied to the analysis of a complex mixture of this drug family by reversed-phase HPLC [6], these methods, however, are generally based on UV detection [1] or an expensive combination of spectrometry with nuclear magnetic resonance (NMR), infra-red (IR), and mass spectrometry [6].

To enhance the sensitivity and selectivity, chemical derivatization of the carboxyl moiety of this drug family is a good way of introducing strong absorption or luminescence to the molecules [7,8]. Thus, an ideal fluorescence derivatzation reagent is expected to possess the following desirable properties: (1) high molar absorptivity and fluorescence quantum yield; (2) selective reaction with the functional group

*Corresponding author. Tel.: +81-358-414-761; fax: +81-358-023-339.
E-mail address: santa@mol.f.u-tokyo.ac.jp (T. Santa).

0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)01069-0

of analytes and minimal side reactions during the derivatization procedures; (3) easy performance of the reaction in either aqueous or non-aqueous solvents; and (4) good water-solubility. In order to meet these criteria, we have developed fluorescent tagging reagents with benzofurazan (2,1,3-benzoxadiazole) skeleton with the advantages of high fluorescence quantum yield, high reactivity to the analytes [9–13]. Of these benzofurazan reagents, 4-*N*,*N*-dimethylamino-sulfonyl-7-*N*-piperazino-2,1,3-benzoxadiazole (DBD-PZ) is the best in terms of the sensitivity and reactivity [14], but it is hydrophobic and the adsorption in column or capillary is unavoidable. Therefore, the development of water-soluble reagents is a challenging strategy, and 7-(*N*,*N*-dimethylaminosulphonyl) - 4-*N*-(4-*N*-aminoethyl)piperazino-2,1,3-benzoxadiazole (DBD-PZ-NH_2) as a derivatization reagent has been synthesized for saturated carboxyl acids with high detection sensitivity for biological samples including Intralipos 20% and rat plasma [15]. The problem is that the excitation wavelength of DBD-PZ-NH_2 is in the area of 415–435 nm depending on solvents, which is not compatible or easily match laser induced-fluorescence detection.

Recently, we designed and synthesized a water-soluble benzofurazan reagent, 4-*N*-(4-*N*′-aminoethyl)piperazino-7-nitro-2,1,3-benzoxadiazole (NBD-PZ-NH_2, the molecular structure of which is displayed in Fig. 1) [16], in the hope to establish a sensitive and reproducible quantification method for carboxylic acids and their substituted derivatives including α-aryl carboxylic acids and α-aryl amino acids such as profen family drugs, α-aryl amino acid and acetic building blocks. Herein we apply NBD-PZ-NH_2 as a fluorogenic regent to the separation and detection of profens with capillary electrophoresis equipped with an argon ion laser-induced fluorescence (LIF) detector. Pre-column derivatization was completed at room temperature. Since the tertiary amino group, which is not reactive in the derivatization reaction and can be protonated in acidic medium, NBD-PZ-NH_2 is highly water-soluble and can migrate faster than other components in normal voltage controlled capillary zone electrophoresis. With this reagent, quantification of profens below

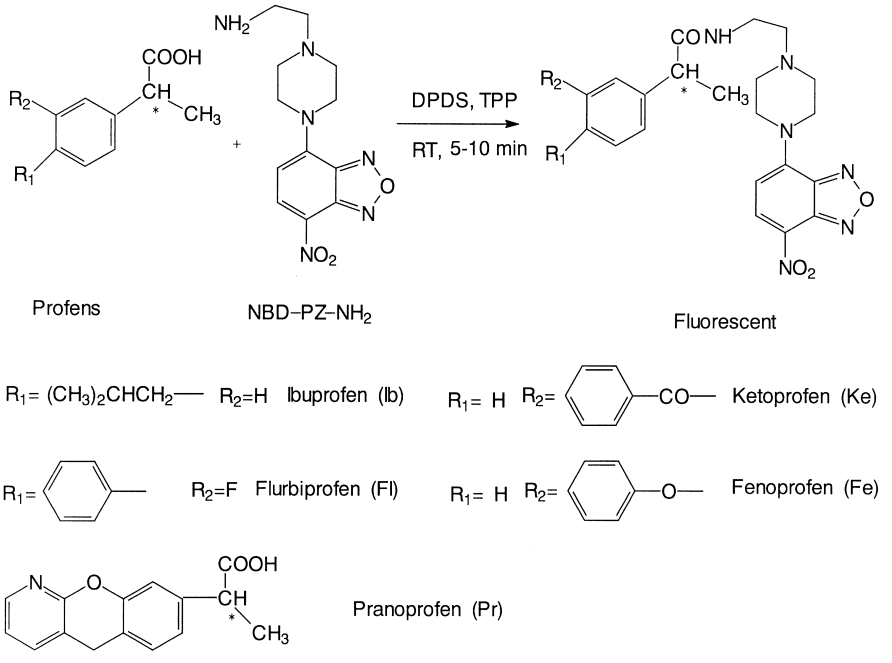

Fig. 1. The derivatization reaction of profens with NBD-PZ-NH_2.

45 fmol can be achieved with the detection limits in the range of 0.16 to 0.3 fmol.

2. Experimental

2.1. Chemicals and reagents

Profens studied herein include pranoprofen (Pr; Yoshitomi Pharmaceutical Industries, Osaka, Japan), fenoprofen (Fe; Sigma, St. Louis, MO, USA), flurbiprofen (Fl; Nagase, Kobe, Japan), ketoprofen and ibuprofen (Ke and Ib; both from Wako, Osaka, Japan). 4-Nitro-7-chloro-2,1,3-benzoxadiazole (NBD-Cl), *N*-(2-aminoethyl)piperazine, triphenyl phosphine (TPP), β-cyclodextrin (β-CD), and diphenyl disulfide (DPDS) were purchased from Wako (Tokyo, Japan), while sodium 1-hexanesulfonate (C_6–SO_3^-) and urea were from Toyo Kasei Kohyo (Tokyo, Japan). Acetonitrile (ACN) and methanol were of HPLC grade (all from Kanto Chemicals, Tokyo, Japan). Inorganic compounds including ammonium acetate, and acetic acid (both from Kanto Chemicals) were used to prepare running buffer for CE. Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents are of analytical or guaranteed grade and used without further purification.

2.2. Equipments and materials

Instruments used in this work involve a P/ACE MDQ Capillary Electrophoresis System coupled with a 488 nm laser module and a LIF detector module (Beckman Coulter, CA, USA), a SPE-200 centrifugal evaporator (Shimadzu, Kyoto, Japan), and Hitachi equipment including a M-1200H mass spectrometer with atmospheric pressure chemical ionization (APCI) system, a F-4500 spectrofluorometer, and a HPLC system consisting of an L-6200 intelligent pump, an F-7480 fluorescence detector, and a D-2500 Chromato-integrator (Hitachi, Tokyo, Japan). HPLC preparation of the pure derivatives were made by using an analytical column, TSK gel ODS-80Ts (150×4.6 mm I.D., 5 μm) (Tosoh, Tokyo, Japan). A HM-30 V pH meter (TOA Electronics Ltd., Tokyo, Japan) was used for pH measurements, and fused-silica capillary tubing of 0.050 mm I.D.×0.375 mm O.D. (GL Sciences, Tokyo, Japan) was used for the electrophoresis.

2.3. Synthesis of reagent and preparation of pure profen derivatives

NBD-PZ-NH_2 was first synthesized starting from NBD-Cl according to our previous report [16], and then 2 mg/ml NBD-PZ-NH_2 working solution was prepared by dissolving the pale brown oil product into ACN.

In order to characterize the fluorescence properties, pure derivatives of profens with NBD-PZ-NH_2 were prepared and separated by HPLC. Generally, 12 μl of each profen solutions in ACN (about 20 m*M*) was at first added to a 1.5-ml vial, then 1.2 μl of freshly prepared 180 m*M* TPP and 180 m*M* DPDS in ACN were respectively added. After vortexing, 8 μl of 2.0 mg/ml NBD-PZ-NH_2 solution was added. Additional ACN was added to keep the initial volume of 24 μl. The vial was then capped and allowed to stand for 10 min at room temperature. Then, the mixture was diluted with 0.3% trifluoroacetic acid (TFA) to 80 μl, and stored at 4 °C or subjected to HPLC separation. The HPLC purified derivatives in water–ACN mixed solvent was collected and centrifugally evaporated to dryness at room temperature with a reduced pressure. The products were used for mass spectrometric (MS) and spectrofluorometric measurements. MS measurements identified these derivatives as expected molecular mass: m/z 530 $(M+H)^+$ for NBD-PZ-NH_2-Pr, m/z 517 $(M+H)^+$ for NBD-PZ-NH_2-Fe, m/z 518 $(M-H)^-$ for NBD-PZ-NH_2-Fl, m/z 528 $(M\text{-}H)^-$ for NBD-PZ-NH_2-Ke, and m/z 481 $(M+H)^+$ for NBD-PZ-NH_2-Ib.

Using ACN–water mixtures containing 0.05% TFA and 1.0 m*M* C_6–SO_3^- for the mobile phase of HPLC preparation: eluent A, ACN–water (30:70, v/v); eluent B, ACN–water (80:20, v/v). Gradient program was 0–5 min, (eluent B composition, 0–5%), 5–35 min (B, 5–20%). The flow-rate was kept at 1.0 ml/min. Column temperature was maintained at 25 °C. Fluorescence detection was made at 530 nm with the excitation at 488 nm and the slit width of 15 nm.

2.4. Precolumn derivatization for capillary electrophoresis and HPLC detection

Generally, 5.0 μl of a mixture of the above five profens with each drug of 0.1 m*M* was at first added to a 1.5-ml vial, followed by 2.5 μl of freshly prepared 180 m*M* TPP and 180 m*M* DPDS in acetonitrile. After vortexing, 2.0 μl of 2.0 mg/ml NBD-PZ-NH_2 solution and additional ACN was added to keep the initial volume of the mixture to 50.0 μl. The vial was then capped and allowed to stand for 10 min at room temperature. Then, 50 μl of 0.3% TFA solution was added, and vortexed. The mixture was stored at 4 °C or subjected to CE analysis. For HPLC, all the procedures, including the components of the mobile phase, and the gradient programs with A and B eluents were the same as preparative separations stated in Section 2.3, except 12.5 μl of the mixture of five profens of 0.1 m*M*, and 2.0 μl of the TFA-diluted mixture was injected to HPLC for detection.

2.5. Laser induced fluorescence detection for CE

All CE experiments were performed on a P/ACE MDQ capillary electrophoresis system (Beckman, CA, USA) monitored by an IBM personal computer using P/ACE MDQ System software compatible for Windows 98. Detection was performed using 520 nm emission bandpass filter with Ar^+ laser (8 mW, 488 nm) excitation beam. A fused-silica capillary (GL Science, Tokyo, Japan) of 60 cm (50 cm to the detection window)×50 μm I.D.×375 μm O.D. was used. Detection windows were prepared by removing a section of the polyimide coating with a low temperature flame. New capillaries were flushed sequentially with distilled water for 30 min, 1.0 *M* NaOH for 30 min, and distilled water for 10 min before use. All solutions used for CE were previously filtered through a syringe driven filter unit of 0.45 μm PTFE (Millipore, Bedford, MA, USA). Operation voltage was 25 kV. The temperature of capillary was kept at 25 °C. Sample injection was performed by applying a 3.4 kPa pressure at the capillary inlet for 4 s.

To estimate injected volume of the sample by applying the pressure at the inlet of the capillary for a specified period, a 0.05% methyl red solution, prepared by dissolving the crystal in a mixture of 0.6 ml ACN and 0.4 ml 0.3% TFA, was introduced to the inlet end of the capillary which had been made transparent by removing the polyimide coating with a low temperature flame. The lengths of the red zones were measured using a scale against white background.

3. Results and discussion

3.1. Fluorescent properties

Since we make use of laser induced-fluorescence detection of the profen derivatives, it is necessary to investigate the fluorescence properties of the reagent and its derivatives at first. Fig. 2 displays the excitation and emission spectra of NBD-PZ-NH_2 and NBD-PZ-NH_2-Pr. It was found that NBD-PZ-NH_2 and its profen derivatives in an optimized medium for the CE buffer (composed by 1.7 *M* HAc, 0.06 *M* NH_4Ac, 2.4 m*M* β-CD, and 0.6 *M* urea, see below) and different ACN–water solutions for HPLC have similar fluorescence emissions. As shown in Table 1, they fluoresce at 530 nm when excited in the region of 460–490 nm, and their fluorescence quantum yield are dependent on the molecular structures of these drugs, and the components of the medium.

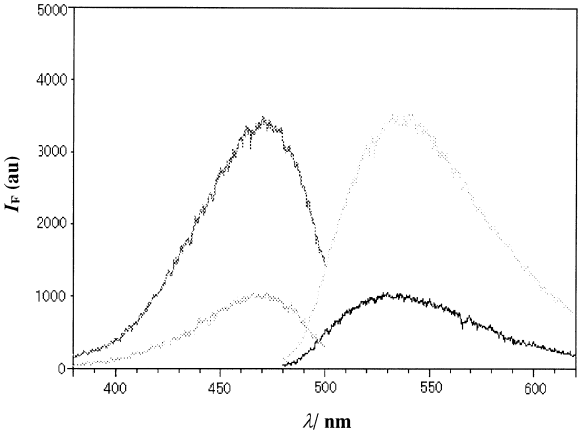

Fig. 2. The excitation and emission of NBD-PZ-NH_2 (lower curves) and NBD-PZ-NH-Pr (upper curves). The solvent was water–ACN (8:2) containing 1.0 m*M* C_6–SO_3^- and 0.05% TFA.

Table 1
Fluorescence emissions of profen derivatives with NBD-PZ-NH_2 in the running CE buffer and in ACN–water solution

Medium	pH	NBD-PZ-NH_2	Pr	Ke	Fe	Fl	Ib
CE buffer	3.10	475/534[a]	470/528	470/529	468/530	469/529	468/534
		0.0841[b]	0.2701	0.4199	0.396	0.3950	0.3945
Water[c]	2.04	474/534	469/535	477/540	477/540	477/540	456/536
		0.0102	0.0119	0.0163	0.0146	0.0158	0.0269
Water–ACN (9:1)	1.84	472/534	469/537	478/537	470/540	478/535	471/540
		0.0152	0.0223	0.0084	0.0076	0.0082	0.0111
Water–ACN (8:2)	1.76	470/536	469/532	474/532	474/537	477/534	473/539
		0.0235	0.1026	0.0086	0.0075	0.0090	0.0177
Water–ACN (7:3)	1.70	469/531	469/531	468/529	466/529	468/527	468/531
		0.0401	0.1020	0.1132	0.1019	0.0895	0.0225
Water–ACN (6:4)	1.64	469/530	465/533	474/533	468/535	471/536	466/535
		0.0622	0.1156	0.2195	0.0867	0.1790	0.1513
Water–ACN (5:5)	1.61	468/529	464/530	470/534	470/530	471/531	464/528
		0.0832	0.1137	0.2289	0.0973	0.1868	0.1436

Wavelength error, ±2 nm.

[a] Excitation and emission wavelengths.

[b] Fluorescence quantum (ϕ), determined according to following equation [23], $\frac{\varphi_x}{\varphi_{QS}} = \frac{F_x(n_x)^2 A_{QS}}{F_{QS}(n_{QS})^2 A_x}$, wherein ϕ is the fluorescence quantum yield, n is the refractive index of the solvents, F and A are integrated fluorescence intensity in 10 nm range, and the peak absorbance, respectively. The subscripts, x and QS, stand for the sample and reference (quinine sulfate), respectively. The reference value of $\phi_{QS} = 0.55$ in 0.05 mol/l H_2SO_4 solution was used when excited at 355 nm.

[c] Running buffer of pH 3.1 was prepared by mixing 3.1 ml of 1.0 *M* NH_4Ac solution, 5.0 ml of 17.4 *M* HAc solution, 5.0 ml of 24 m*M* β-CD solution and 25.0 ml of 1.0 *M* urine solution with 12.0 ml of water, and the final concentrations of the components are 1.7 *M* HAc, 0.06 *M* NH_4Ac, 2.4 m*M* β-CD, and 0.6 *M* urea. In water–ACN mixture, 1.0 m*M* C_6–SO_3^- and 0.05% TFA were contained, and the pH values are decreased increasing ACN content.

With increase in ACN content, the fluorescence quantum yields of these derivatives increase. In a same medium, the sequence of the fluorescence quantum yields for these derivatives have the order of Ke>Fl>Ib>Pr>Fe. In addition, it is obvious that the derivatives have higher fluorescence yields than that NBD-PZ-NH_2 has in the medium of CE buffer or in ACN–water solutions. Thus, this reagent is in fact a fluoregenic reagent, and could be used for the sensitive detection of these drugs.

As our former report shows, NBD-PZ-NH_2 is pH-dependent since the nitrogen atom at piperazine ring of the reagent was deprotonated in neutral or basic medium (at pH 4 and above), and quenching of the fluorescence occurred by an electron transfer from the nitrogen atom to the excited state of the fluorophore (so-called photoinduced electron transfer, PET) [17]. As Table 1 shows, the pH values of CE buffer and the mobile phase mixture for HPLC is lower than 3.5, thus no PET occurs in the media either in electrophoretic running or HPLC separation since piperazine ring is protonated.

3.2. Optimal conditions for the precolumn derivatization

Fig. 1 shows that the derivatization reaction of profens with NBD-PZ-NH_2 should be catalyzed by TPP and DPDS in ACN medium. Thus, the derivatization is obviously dependent on the concentration of the two catalysts. As shown in Fig. 3, the optimal concentration of both TPP and DPDS is 9.0 m*M* in the initial derivatization period. It was also proved that the TPP/DPDS catalyzed derivatization procedures are dependent on the reaction period. Fig. 4 shows that the derivatization could be completed within 5 to 10 min at 25 and 37 °C. Further test at 50 °C shows that this derivatization reaction is not strongly dependent on the reaction temperature in the range 25–50 °C, and the fluorescence intensities did not display significant fluctuations at these different temperatures. Thus, this derivatization reaction is easier concerning the reaction temperature and period that is much shorter than that of carboxylic group with 5-bromomethylfluorescein and poly-

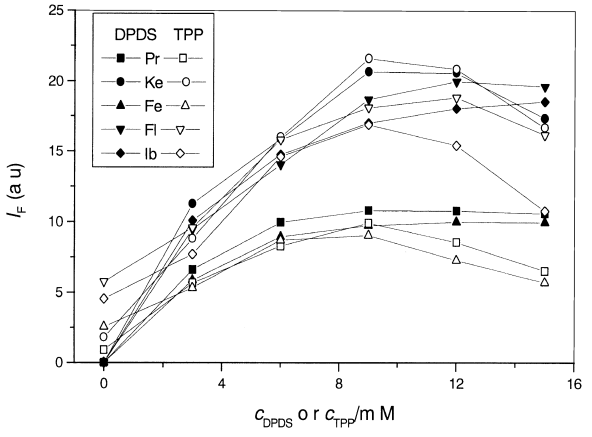

Fig. 3. Dependence of the derivatization procedure on DSPS (solid signals) and TPP (open signals) concentrations. Then 7.5 fmol of drugs were injected to CE. When testing optimal c_{TPP}, c_{DPDS} was kept at 9.0 m*M* at the initial derivatization period; on the contrary, when testing optimal c_{DPDS}, c_{TPP} was kept at 9.0 m*M*. The derivatization was made at room temperature for 10 min.

methine cyanine where the derivatization needs 3 and 24 h, respectively [18–20].

3.3. Optimization for CE conditions

The separation of these derivatization products of NBD-PZ-NH_2 depends on the composition of the running buffer for CE. Fig. 5 is a typical electropherogram, showing that good separation of these drug derivatization products with the DBD-PZ-NH_2

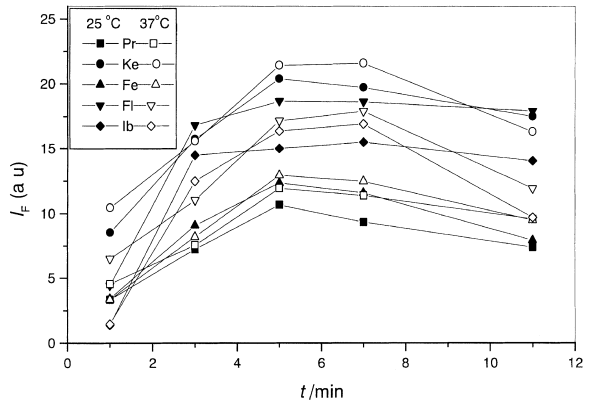

Fig. 4. Time courses of the derivatization procedure at 25 °C (solid signals) and 37 °C (open signals). Then 7.5 fmol of drugs were injected to CE. Both c_{TPP} and c_{DPDS} were kept at 9.0 m*M* at the initial derivatization period.

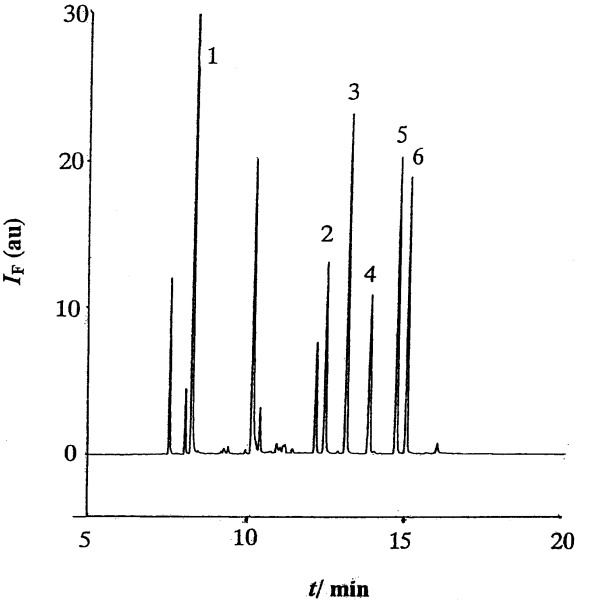

Fig. 5. A typical electropherogram showing good separation of the derivatization products of profens with the DBD-PZ-NH_2 by using CE–LIF. Then 7.5 fmol of drugs were injected to CE. Both c_{TPP} and c_{DPDS} were kept at 9.0 m*M* at the initial derivatization period during which 10 min was taken at room temperature. Peaks: 1, NBD-PZ-NH_2; 2, NBD-PZ-NH_2-Pr; 3, NBD-PZ-NH_2-Ke; 4, NBD-PZ-NH_2-Fe; 5, NBD-PZ-NH_2-Fl; 6, NBD-PZ-NH_2-Ib.

can be made by using a running buffer of pH 3.1 that contains 1.7 *M* HAc, 0.06 *M* NH_4Ac, 2.4 m*M* β-CD, and 0.6 *M* urea, and the elution order is Pr>Ke>Fe>Fl>Ib. The optimal composition of the running buffer was tested under the performed voltage of 25 kV, and the controlled temperature of capillary at 25 °C, injected samples by applying a small pressure (3.4 kPa) at the inlet of the capillary for 4 s. Experiments showed the appropriate buffer is NH_4Ac–HAc with the pH of 2.8 to 3.5, a rang that does not display significant deprotonation indicating the occurrence of positron emission tomography (PET) process.

It was found that addition of β-CD could improve separation efficiency. Table 2 shows the dependence of separation efficiency on β-CD concentration in the running buffer. It can be seen that good separation is available when β-CD in the running buffer is higher than 1.92 m*M*. In our experiment, β-CD was controlled as 2.4 m*M*. In order to increase the solubility

Table 2
Effect of β-CD concentration on the separation efficiency of profen derivatives with NBD-PZ-NH_2

β-CD/m*M*	Pr ($N\times10^{-5}$)	Ke[a] ($N\times10^{-5}/R_s$)	Fe ($N\times10^{-5}/R_s$)	Fl ($N\times10^{-5}/R_s$)	Ib ($N\times10^{-5}/R_s$)
0.24	2.65	Four peaks are merged into two overlaid peaks			
0.96	2.24	3.80/6.46	0.96/0.44	1.80/2.88	1.05/1.26
1.92	1.82	1.54/4.56	1.60/1.98	3.04/5.77	1.15/1.86
2.40	2.89	3.18/6.56	2.75/3.85	3.72/7.54	1.74/2.70
2.88	2.35	1.95/6.04	1.26/3.52	1.40/4.99	0.38/1.73
4.80	1.50	1.41/5.40	1.34/5.04	1.75/5.60	1.26/1.96

[a] Separation efficiencies are expressed between Pr–Ke, Ke–Fe, Fe–Fl, and Fl–Ib, and are calculated with $R_s = \frac{\Delta\mu}{4\mu_{avg}} N^{1/2}$, where N is plate number, $\Delta\mu$ is the mobility difference of two neighboring peaks, and μ_{avg} is average of mobility.

of β-CD in the running buffer, 1.0 *M* of urea were used [21]. The elution order of Pr>Ke>Fe>Fl>Ib shown in Fig. 5 is not related to the molecular mass of these derivatives (for these profens of Pr, Ke, Fe, Fl, and Fl, the molecular masses are 255.3, 254.3, 242.3, 244.3 and 206.3 in Da, respectively). Since these derivatives have the same positive charge (+1) in the acidic medium due to all of them having one protonated nitrogen atom at piperazine ring, the elution order is possibly related to the compatibility between the inner diameter of β-CD and the molecular size of these derivatives, which depends on the stability of complex of β-CD and the derivatives [22].

In order to display the role of β-CD in CE running, we could make a comparison between the detection using CE and HPLC. Although the derivatives have the excitation wavelength around 470 nm (Table 1 and Fig. 2), we still set the fluorescence detector excited at 488 nm in order to compare the results with HPLC and CE methods. Fig. 6 is a typical chromatogram by using reversed-phase HPLC detection. The elution time has the order of Pr>Ke>Fe>Fl>Ib, totally identical to that of CE running (Fig. 5). In order to improve the separation efficiency, C_6–SO_3^- and TFA were added to mobile phase of the ACN–water. Repeated retention behavior and fluorescence detection could be obtained.

From the chromatogram (Fig. 6), the elution order of Pr>Ke>Fe>Fl>Ib in HPLC shows that the hydrophobicity of these derivatives have the order of Pr<Ke<Fe<Fl<Ib. Since the inner environments of β-CD is hydrophobic, the hydrophobic interaction between β-CD and the profen derivatives should have the order as Pr>Ke>Fe>Fl>Ib, Thus the

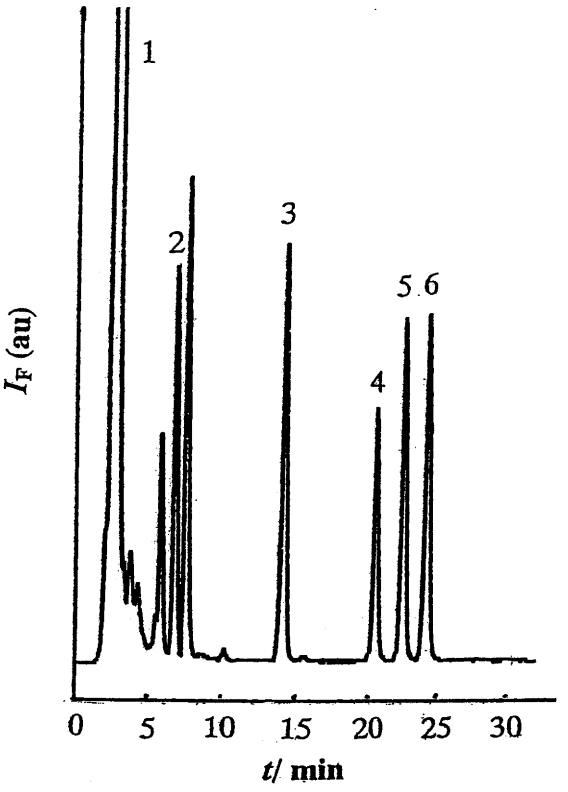

Fig. 6. A typical chromatograph showing good separation of the derivatization products of profens with the DBD-PZ-NH_2 by using reversed-phase HPLC method. Then 25.0 pmol of drugs were injected to CE. Both c_{TPP} and c_{DPDS} were kept at 9.0 m*M* at the initial derivatization period during which 10 min was taken at room temperature. Peaks: 1, NBD-PZ-NH_2; 2, NBD-PZ-NH_2-Pr; 3, NBD-PZ-NH_2-Ke; 4, NBD-PZ-NH_2-Fe; 5, NBD-PZ-NH_2-Fl; 6, NBD-PZ-NH_2-Ib. In above separation, the plate number for the five derivatives is ($\times10^{-4}$): 0.11, 0.65, 1.37, 1.18 and 1.35, respectively.

elution order in CE is strongly dependent on the hydrophobicity, and the molecular mass is not the main factor. Therefore, the role of β-CD, which improves the separation efficiency in CE running, is to supply the hydrophobic environments, making the CE and HPLC have the same elution order.

3.4. Linearity, detection limits and repeatability

Calibration curves were made between the fluorescence intensity and the content of the injected drugs. The injected volume of profen derivatives was measured by comparing the injection periods of the profen derivative solutions of drugs with that of methyl red under same pressure. It was confirmed that there was a linear relationship ($y = 0.2145x - 0.0975$, $r = 0.9998$) between the injected period (x, in seconds) and the plug length (y, in mm) of methyl red solution when applying a pressure of 3.4 kPa to estimate the volume of injection. Since methyl red was dissolved in a same composed solvent mixture as the injected solution of drugs, the factors such as viscosity, which can lead to injected volume errors because of the difference of the injected solution can be minimized. Thus, we can calculate the volume of the injected derivative solutions. Under the pressure of 3.4 kPa, a 4-s injection can result in a 0.76 mm plug length. Namely, 1.5 nl of the drug solutions was injected when employing a capillary of 0.050 mm in I.D. at the pressure of 3.4 kPa.

Table 3 lists the analytical parameters for the detection of the five drugs. It can be seen that the present method can be used for the detection of profens at femtomole level in CE–LIF detection. Importantly, the sensitivity displayed by the slopes of these regression equations is identical to the fluorescence quantum yields (Table 1). That is, the sensitivity almost has the same order as the fluorescence quantum yields: Ke > Fl > Ib > Pr ≈ Fe. In addition, it is obvious from Table 3 that CE is far more sensitive than HPLC in terms of mass detection limits. Even with the different content of ACN at different elution time in HPLC detection, the detection sensitivity also has the order of Ke > Fl > Ib > Pr > Fe, identical to the fluorescence quantum yields. In terms of the concentration, however, the detection limits of both CE and HPLC are at the same range of 10^{-7} M. That displays the priority of the CE method with small amounts of sample consumptions since it takes the advantage of by laser-induced fluorescence that focuses capabilities of laser radiation.

4. Conclusion

As stated above, the fluorogenic reagent, NBD-PZ-NH_2, can be employed for sensitive detection of

Table 3
Analytical parameters for the determinations of five profens by using CE–LIF and reversed-phase HPLC methods, respectively

	Profens	Linear range (m, fmol/pmol)[a]	Regression equations ($n = 9$, m, fmol/pmol)[a]	Correlation coefficient (r)	Detection limit (3σ)	
					m, (fmol/pmol)[a]	c (10^{-7} M)[b]
CE	Pr	0–45.0	$I = -0.67 + 1.28m$	0.9969	0.30	2.0
	Ke	0–45.0	$I = -1.74 + 2.44m$	0.9903	0.18	1.2
	Fe	2.6–45.0	$I = -2.02 + 1.48m$	0.9928	0.26	1.7
	Fl	1.6–45.0	$I = -3.25 + 2.40m$	0.9912	0.16	1.1
	Ib	1.7–45.0	$I = -3.26 + 2.32m$	0.9906	0.17	1.1
HPLC	Pr	3.6–50.0	$I = 1.29 + 0.76m$	0.9991	0.36	1.8
	Ke	2.6–50.0	$I = 1.08 + 1.06m$	0.9994	0.26	1.3
	Fe	3.8–50.0	$I = 0.70 + 0.73m$	0.9993	0.38	1.9
	Fl	2.9–50.0	$I = 0.64 + 1.04m$	0.9992	0.29	1.5
	Ib	2.9–50.0	$I = 1.32 + 0.97m$	0.9994	0.29	1.5

[a] m stands for mass quantity expressed with fmol for CE, pmol for HPLC, respectively.
[b] c stands for the concentration expressed with mol l^{-1}. The injected volumes were 1.5 nl for CE, and 2.0 μl for HPLC, respectively. Both c_{TPP} and c_{DPDS} were kept at 9 mM at the initial derivatization period during which 10 min was taken at room temperature.

profens through both CE and reversed-phase HPLC methods based on precolumn derivatization. The measurements of fluorescence quantum yields showed that NBD-PZ-NH_2 is a good fluoregenic reagent. The derivatization reaction could be finished at room temperature in 10 min, much shorter than reported methods for the derivatization of carboxylic groups [18–20]. Since the reagent is water-soluble, its derivatives can migrate fast in HPLC, and we can get that CE has shorter retention time. Due to the hydrophobic interaction between β-CD and profens derivatives, both CE and HPLC have the same elution order. The former one could be employed for detection of profens in the femtomole range, while the latter one only could be in picomole range. Thus, we believe that NBD-PZ-NH_2 is a good fluorescent derivatization reagent for CE–LIF detection, and could find further applications in the detection of sample containing carboxylic moiety in molecular structure.

Acknowledgements

We are thankful for the support from the Tokyo Biochemical Research Foundation (TBRF) for the Postdoctoral Fellowship Grant for Asian Researchers (HCZ, No: tbrf/il-19/01) and gratefully acknowledge Dr C.K. Lim of the University of London, for his kind review of the manuscript.

References

[1] F. Lelievre, P. Gareil, J. Chromatogr. A 735 (1996) 311.
[2] S. Lee, N.A. Beare, J.F. Hartwig, J. Am. Chem. Soc. 123 (2001) 8410.
[3] C.Y. Chen, Y.S. Chang, S.A. Lin, H.I. Wen, Y.C. Cheng, S.W. Tsai, J. Org. Chem. 67 (2002) 3323.
[4] Y. Chikusa, T. Fujimoto, M. Ikunaka, T. Inoue, S. Kamiyama, K. Maruo, J. Matsumoto, K. Matsuyama, M. Moriwaki, H. Nohira, S. Saijo, M. Yamanishi, K. Yoshida, Org. Proc. Res. Develop. 6 (2002) 291.
[5] F. Okeeffe, S.A. Shamsi, R. Darcy, P. Schwinte, I.M. Warner, Anal. Chem. 69 (1997) 4773.
[6] D. Louden, A. Handley, S. Taylor, E. Lenz, S. Miller, I.D. Wilson, Anal. Chem. 72 (2000) 3922.
[7] Y. Ohkura, M. Kai, H. Nohta, J. Chromatogr. B 659 (1994) 85.
[8] T. Toyo'oka, J. Chromatogr. B 671 (1995) 91.
[9] S. Uchiyama, T. Santa, N. Okiyama, T. Fukushima Uzu, K. Imai, Anal. Chem. 73 (2001) 2165.
[10] P. Prados, T. Fukushima, T. Santa, H. Homma, M. Tsunoda, S. Al-Kindy, S. Mori, H. Yokosu, K. Imai, Anal. Chim. Acta 344 (1997) 227.
[11] T. Santa, K. Kimoto, H. Homma, K. Imai, Biomed. Chromatogr. 10 (1996) 183.
[12] T. Fukushima, T. Santa, H. Homma, S. Al-Kindy, K. Imai, Anal. Chem. 69 (1997) 1793.
[13] M. Vogel, U. Karst, Anal. Chem. 74 (2002) 6418.
[14] S. Uchiyama, T. Santa, N. Okiyama, T. Fukushima, K. Imai, Biomed. Chromatogr. 15 (2001) 295.
[15] C.Z. Huang, T. Santa, K. Imai, Analyst 127 (2002) 741.
[16] T. Santa, D. Matsumura, C.Z. Huang, C. Kitada, K. Imai, Biomed. Chromatogr. 16 (2002) 523.
[17] S. Uchiyama, T. Santa, K. Imai, Analyst 125 (2000) 1839.
[18] V. Zuriguel, E. Causse, J.D. Bounery, G. Nouadje, N. Simeon, M. Nertz, R. Slavayre, F. Couderc, J. Chromatogr. A 781 (1997) 233.
[19] D.L. Gallerher Jr., M.E. Johnson, Analyst 124 (1999) 1541.
[20] D.L. Gallerher Jr., M.E. Johnson, Anal. Chem. 72 (2000) 2080.
[21] A.M. Rizzi, P. Briza, M. Breitenbach, J. Chromatogr. 582 (1992) 35.
[22] Z.D. Hu, High Performance Capillary Electrophoresis, Lanzhou University Press, Lanzhou, 1997, p. 31, 90.
[23] G.Z. Chen, X.Z. Huang, Z.Z. Zheng, J.G. Xu, Z.B. Wang, Spectrofluorometry, Science Press, Beijing, 1990, p. 16.