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Monitoring of drugs and metabolites in body fluids by capillary electrophoresis with XeHg lamp-based and laser-induced fluorescence detection

Commercial capillary electrophoresis instrumentation with XeHg lamp-based and laser induced fluorescence (LIF) detection is employed for analysis of urinary 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and its major metabolites, urinary metabolites of acetylsalicylic acid, urinary benzoylecgonine in an immunoassay format, and albendazole sulfoxide and albendazole sulfone in plasma. For the examples studied, the data suggest that the lamp-based detector can be employed for the monitoring of pharmacological and toxicological relevant solute concentrations, and thus represents an attractive alternative to LIF detection.

Keywords: Albendazole / Capillary electrophoresis / Cocaine / Ecstasy / Lamp-based fluorescence / Laser-induced fluorescence / Salicylate DOI 10.1002/elps.200305821

1 Introduction

For the past 15 years, our laboratory has specialized in applying capillary electrophoresis (CE) to the determination of drugs and metabolites in body fluids [1–3]. Selected drugs and metabolites fluoresce naturally and thus can be monitored by fluorescence detection. Fluorescence detection in CE of low-molecular-mass compounds in body fluids has been shown to provide improved selectivity and often also sensitivity when compared to absorption detection. In most of these assays solutes are being detected by laser-induced fluorescence (LIF) [4–10]. Laser-based fluorescence has produced spectacular results for solute detection in CE [10–12]. Laser suffer from a number of inherent limitations, including the limiting availability of excitation wavelengths and high cost. The nonlaser or lamp-based fluorescence detectors constructed for CE in various research laboratories, such as those (in order of decreasing performance) using xenon arc [13], mercury-xenon arc [14], pulsed xenon [15], deuterium [4, 16] and tungsten [16] lamps, typically provide poorer sensitivity but allow for wavelength selection in a wide range. Using such incoherent excitation sources, solute monitoring by simultaneous absorption and fluorescence detection has also been described [4, 15]. Recently, a XeHg lamp-based tunablewavelength CE fluorescence detector in which the fluorescent light is totally reflected within the capillary and collected with a glass ellipsoid at the downstream end of the detection cell (Fig. 1, [17]), became commercially available. This detector can be used with CE instruments of various manufacturers and, in our laboratory, is applied to the monitoring of drugs in body fluids.

Thus far, no comprehensive study dealing with the determination of drugs and metabolites in body fluids using CE with lamp-based fluorescence detection has been undertaken. In this paper, we wish to report our results that were obtained by CE with XeHg lamp fluorescence detection for the analysis of (i) urinary 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and its major metabolites, (ii) urinary metabolites of acetylsalicylic acid, (iii) benzoylecgonine in urine *via* a CE-based immunoassay, and (iv) albendazole sulfoxide (ABZSO) enantiomers and albendazole sulfone ($ABZSO_2$) in plasma extracts. For each system, data obtained with LIF detection on commercial instrumentation featuring a pulsed 266 nm diode-pumped solid-state laser, a continuous-wave 325 nm HeCd laser or a continuous-wave 488 nm Ar-ion laser are also presented. Samples analyzed comprise patient and quality control samples.

2 Materials and methods

2.1 Chemicals, reagents, and origin of samples

All chemicals used were of analytical or research grade. The standard compounds employed for the MDMA, salicylate, ABZSO, and benzoylecgonine projects were the

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Abbreviations: ABZSO, albendazole sulfoxide; **$ABZSO_2$**, albendazole sulfone; **FPIA**, fluorescence polarization immunoassay; **HMA**, 4-hydroxy-3-methoxyamphetamine; **HMMA**, 4-hydroxy-3-methoxymethamphetamine; **MDA**, methylenedioxyamphetamine; **MDMA**, 3,4-methylenedioxymethamphetamine; **OxBZ**, oxfenbendazole

same as those used previously in [18], [19], [20], and [2, 21], respectively. Stock solutions were made with bidistilled water. TDxFLx fluorescence polarization immunoassay (FPIA) reagents for cocaine metabolite (No. 9670-60) were obtained from Abbott Laboratories (Baar, Switzerland). The reagents comprise separate vials for antibody containing solution (< 1% sheep antiserum) and benzoylecgonine-fluorescein tracer solution (tracer concentration < 0.01%) whose concentrations are not exactly disclosed. All capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA) and were sequentially conditioned with 1 M NaOH, 0.1 M NaOH, water, and buffer. Prior to a set of experiments, the capillary was rinsed with 0.1 M NaOH and water (about 5 min each). Patient samples were collected in the departmental drug assay laboratory. External quality control urines were purchased from Cardiff Bioanalytical Services (UKNEQAS for drugs of abuse, Cardiff, UK). Our own urine and plasma were used as blank matrices.

2.2 Instrumentation with lamp-based fluorescence detection

Measurements were performed on a SpectraPHORESIS 100 capillary electrophoresis system (Thermo Separation Products, Fremont, CA, USA) that was equipped with an untreated, 50 μm ID fused-silica capillary (Polymicro Technologies) of 71 cm (48 cm to the detector) total length. Injection of sample was effected by aspiration during 1.0 s. The temperature of the capillary surroundings was about 25°C. Detection was effected with an Argos 250B tunable-wavelength fluorescence detector (Flux Instruments, Basel, Switzerland) that features a XeHg lamp, fiber optics to and from the detection cell, and a detection cell in which the fluorescent light is totally reflected within the capillary and collected with a glass ellipsoid at the downstream end of the cell prior to being guided into the optical fiber and to the photomultiplier (Fig. 1). Excitation was obtained with a slit-based monochromator (Optometrics DMC Model-02; Optometrics, Ayer, MA, USA) set at 256, 280 or 480 nm (1.0 mm slit providing a light source of ± ~ 4 nm of the chosen wavelength) and emission was measured with a 295, 320, 495, or 520 nm notch filter (Flux Instruments). A PC with a PC Integration Pack (Version 3.0, Kontron Instruments, Basel, Switzerland) was employed for data registration and storage. For data presentation, the conversion software Geminyx (Version 1.90; Flowspek, Basel, Switzerland) was employed and final figures were made in Sigmaplot Scientific Graphing Software windows Versions 2.01 (Jandel Scientific, Corte Madera, CA, USA) and 8.0 (SPSS, Chicago, IL, USA).

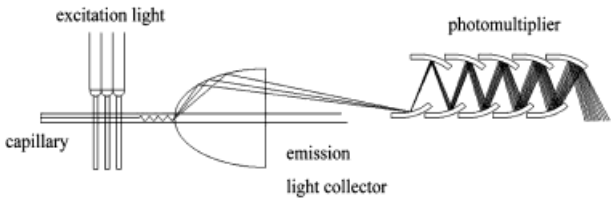

Figure 1. Schematic representation of the Argos 250B detection cell in which the fluorescent light is totally reflected within the capillary and collected by a glass ellipsoid that is sleeved over the capillary at the downstream end of the optical window.

2.3 Instrumentation with pulsed UV LIF detection

Measurements were performed on a Prime Vision capillary electrophoresis system (Europhor, Toulouse, France) that features an almost identical sampler as that of the SpectraPHORESIS 100. An untreated, 50 μm ID fused-silica capillary (Polymicro Technologies) of 83 cm (39 cm to the detector) total length was employed. Injection of sample was effected by aspiration during a time interval of 1.0 s. The temperature of the capillary surroundings was about 25°C. Detection was effected with a ZetaLIF 266 detector (Picometrics, Ramonville, France) that features a pulsed 266 nm UV laser (2 mW diode-pumped solid-state laser Nanolase/JDS Uniphase type NU-10210-100 with a repetition rate of 6 – 10 kHz; Nanolase, Meylan, France) and comprises a collinear optical arrangement with a ball lens [22]. The emission light was measured with a 290 nm notch filter (80% transmission). The photomultiplier tube (PMT) gain, the range and the rise time were set to 700, 2 and 1.0 s, respectively. The data acquisition system was the same as described in Section 2.2.

2.4 Instrumentation with continuous-wave LIF detection

Determinations using a 488 nm air-cooled Ar-ion laser (Ion Laser Technology, Salt Lake City, UT, USA) and a 325 nm HeCd laser (Model 4230NB; LiCONiX, Santa Clara, CA, USA) were made with the P/ACE 5510 CE system together with its LIF detection cell that comprises an ellipsoidal mirror for collection of the fluorescence light (Beckman Instruments, Fullerton, CA, USA). A 75 μm ID fused-silica capillary (Polymicro Technologies) of 40/47 cm effective/total length was employed and fluorescence signals were measured with a 488 nm notch filter and a 520 nm band-pass filter (Ar-ion laser) and band-pass filters between 366 and 479 nm or a 385 nm notch filter (HeCd laser).

Data were evaluated using the P/ACE Station Software (Beckman). The capillary temperature was kept at 20°C and the sample carousel was at room temperature.

3 Results and discussion

3.1 Monitoring of MDMA and its metabolites in urine

In our investigations dealing with the analysis of MDMA and its major metabolites in urine by CE with UV detection [2, 18, 23] and CE-MS [24], MDMA, 3,4-methylenedioxyamphetamine (MDA) and, after hydrolysis, 4-hydroxy-3-methoxymethamphetamine (HMMA) could be detected. The minor metabolite 4-hydroxy-3-methoxyamphetamine (HMA), however, was expected but not detected [2, 18]. All these four compounds are naturally fluorescent. Thus, it was of interest to us to find out whether fluorescence detection would provide improved sensitivity and thus the detection of urinary HMA. The feasibility of monitoring urinary MDMA by nonaqueous CE with fluorescence detection was recently demonstrated by Lin *et al*. [25, 26] using a home-made instrument with a Xe lamp and solute excitation and emission at 280 and 320 nm, respectively. Our experiments were performed with an aqueous 75 mM KH_2PO_4 buffer that was adjusted to pH 2.5 using phosphoric acid [2] and data obtained with the ZetaLIF and Argos detectors are presented in Figs. 2 and 3. Analysis of a standard solution containing 100 ng/mL of each compound revealed that HMA and HMMA are less fluorescing compared to MDMA and MDA and that the ZetaLIF detector with the 266 nm excitation is providing a higher sensitivity compared to the Argos operated at 256 nm (1 mm slit) with a notch filter of 295 nm (Figs. 2A and B, respectively). With the ZetaLIF under the employed conditions, detection limits for MDMA and MDA were noted to be about 15 ng/mL (for HMA and HMMA about 30 ng/mL), detection limits that are better than those observed by UV-absorption detection [2].

Data obtained with direct analysis of 10-fold diluted patient urines are presented in panels C and D of Fig. 2. The two urines stemmed from a patient who received 1.5 mg racemic MDMA/kg body mass and was collected before and 12 h (MDMA, MDA and HMMA contents of 22.5, 1.8 and 2.6 μg/mL, respectively [18]) after drug administration. With injection of 10-fold diluted urine, MDMA and MDA could be determined with both detectors. Furthermore, MDMA was found to comigrate with one or several fluorescing endogenous compounds. After solid-phase extraction as described in [18], MDMA and MDA could be detected without interferences (Figs. 3A and B) and all four compounds could be monitored after enzymatic hydrolysis prior to solid-phase extraction (Figs. 3C and D).

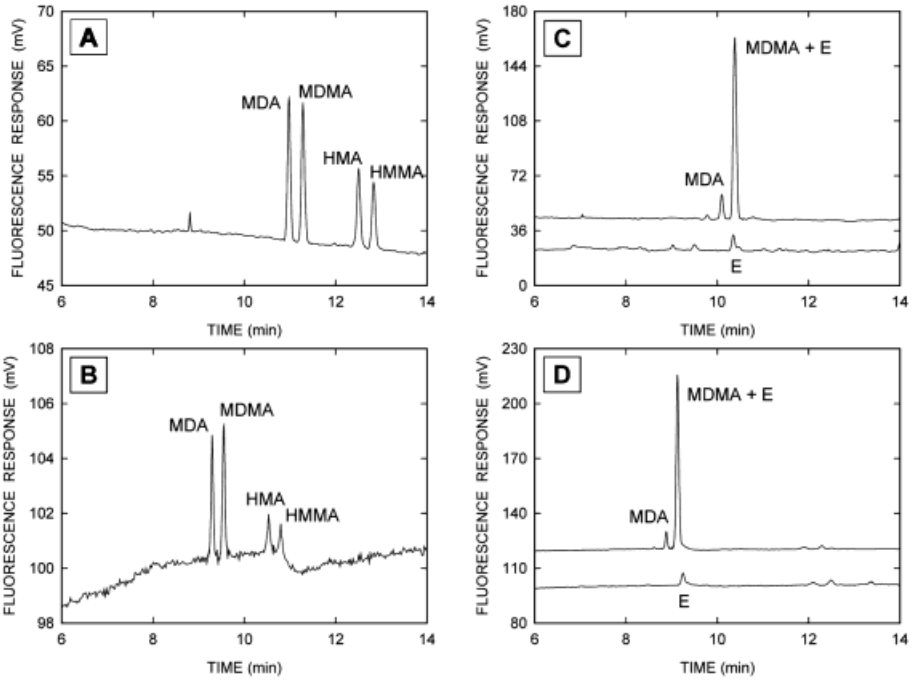

Figure 2. (A), (C) CZE-LIF and (B), (D) CZE fluorescence electropherograms obtained (A), (B) with an aqueous standard solution comprising MDA, MDMA, HMA, and HMMA (100 ng/mL each) and (C), (D) with injection of 10-fold diluted urines that were collected immediately before (lower graphs) and 12 h after (upper graphs) MDMA administration. The CZE-LIF and CZE fluorescence data were recorded using the ZetaLIF with a pulsed 266 nm laser and the Argos with an excitation wavelength of 256 nm (1 mm slit width). The wavelengths of the notch emission filters employed in the two detectors were 290 and 295 nm, respectively. Experiments were performed with a 75 mM KH_2PO_4 buffer that was adjusted to pH 2.5 using phosphoric acid, the applied voltage was 20 kV in all cases and the currents were (A), (C) 40 μA and (B), (D) 54 μA. Time intervals for sample injection were 1 s. The *y*-axis scales of the data of different instruments are not comparable. E refers to endogenous interference.

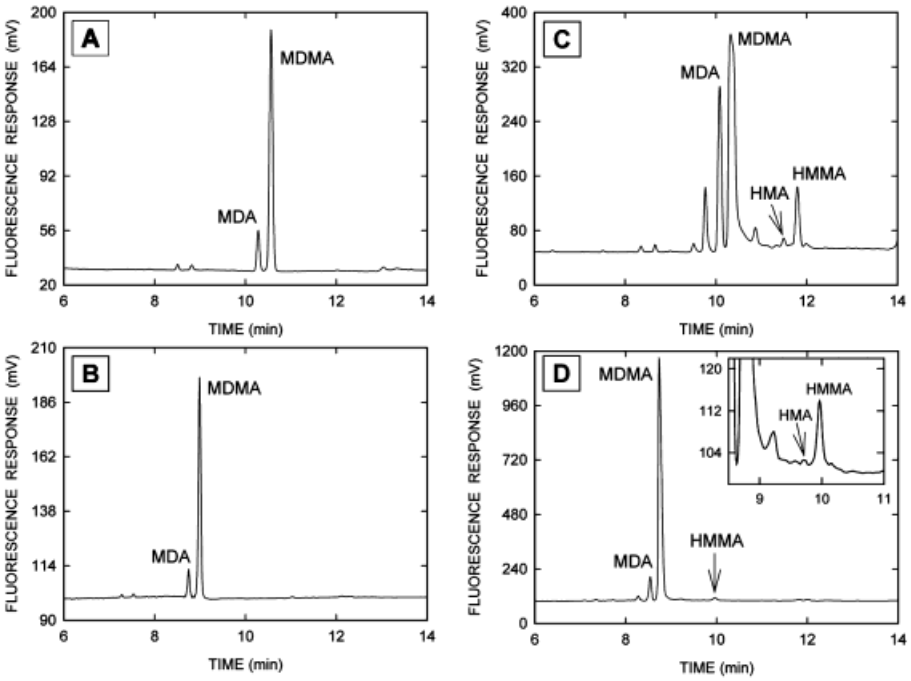

Figure 3. (A), (C) CZE-LIF and (B), (D) CZE fluorescence electropherograms obtained with (A), (B) a 10-fold diluted solid-phase extract of the 12 h urine of Fig. 2 and (C), (D) a twofold diluted extract of the same urine that was extracted after enzymatic hydrolysis with β-glucuronidase from bovine liver (for details see [18]). Instrumental conditions as for Fig. 2.

3.2 Urinary screening for cocaine consumption

Many electrokinetic capillary-based single- and multianalyte immunoassays that are based upon the separation of labeled antigens and antibody-antigen complexes and the LIF detection of labeled antigens have been developed and applied to urinary screening for drugs of abuse. In assays using the antibodies and fluorescein-labeled tracers of Abbott's FPIA reagent kits and LIF detection with an Ar-ion laser (excitation: 488 nm; emission: 520 nm), the peak of the free tracer (FT) is typically employed for data evaluation [2, 9, 19, 21]. As example, data obtained for a CZE-based immunoassay of benzoylecgonine, a metabolite of cocaine, are presented (Fig. 4A). The running buffer was composed of 50 mM disodium tetraborate (pH 9.3). With incubation of equal amounts of urine, tracer solution containing fluorescein-labeled benzoylecgonine and antiserum (25 μL each), and incubation for 12 min at room temperature, the peak height of FT was determined to be concentration-dependent. Using LIF detection with the Ar-ion laser, very simple electropherograms were obtained (Fig. 4A, same data as in [2]). The magnitude of the FT peak is increasing as the urinary benzoylecgonine concentration is increased. The opposite is true for the antibody-tracer complex (C, very small peak due to quenched fluorescence), this indicating that the competitive reaction is indeed monitored. The data presented as top graph in Fig. 4A were obtained with the undiluted external quality control urine No. 106 (urine of polydrug abuser) that tested positive for the cocaine metabolite benzoylecgonine (reported content: 2.46 μg/mL) and other drugs of abuse [21]. According to the information provided by the manufacturer, the antibody employed selectively responds to benzoylecgonine. The strong FT peak obtained with urine 106 suggests that the CE-based immunoassay can indeed be employed for screening of urinary benzoylecgonine in patient samples. The sensitivity limit of this screening assay is about 10 ng/mL, a value that compares well to that of 30 ng/mL reported for the commercial FPIA procedure on the TDxFLx apparatus of Abbott [2, 21].

With the Argos detector operated at 256 nm for excitation and a 495 nm notch filter on the emission side resulted in electropherograms that are more complex than those monitored by LIF with an Ar-ion laser. Nevertheless, the FT peak with a benzoylecgonine concentration-dependent height was recognized for the analysis of blank urines spiked with various amounts of benzyolecgonine and the detection limit was noted to be around 25 ng/mL (Fig. 4B). The FT peak was unambiguously detected for the analysis of undiluted, 5-fold and 10-fold diluted quality control urine 106 (Fig. 5A). Furthermore, having a 520 nm notch filter was found to provide a somewhat simpler electropherogram. This, however, at the expense of sensitivity (bottom graph in Fig. 5A). The same was found to be true for analysis of quality control urine 130 with a reported benzoylecgonine content of 450 ng/mL (Fig. 5B). Using

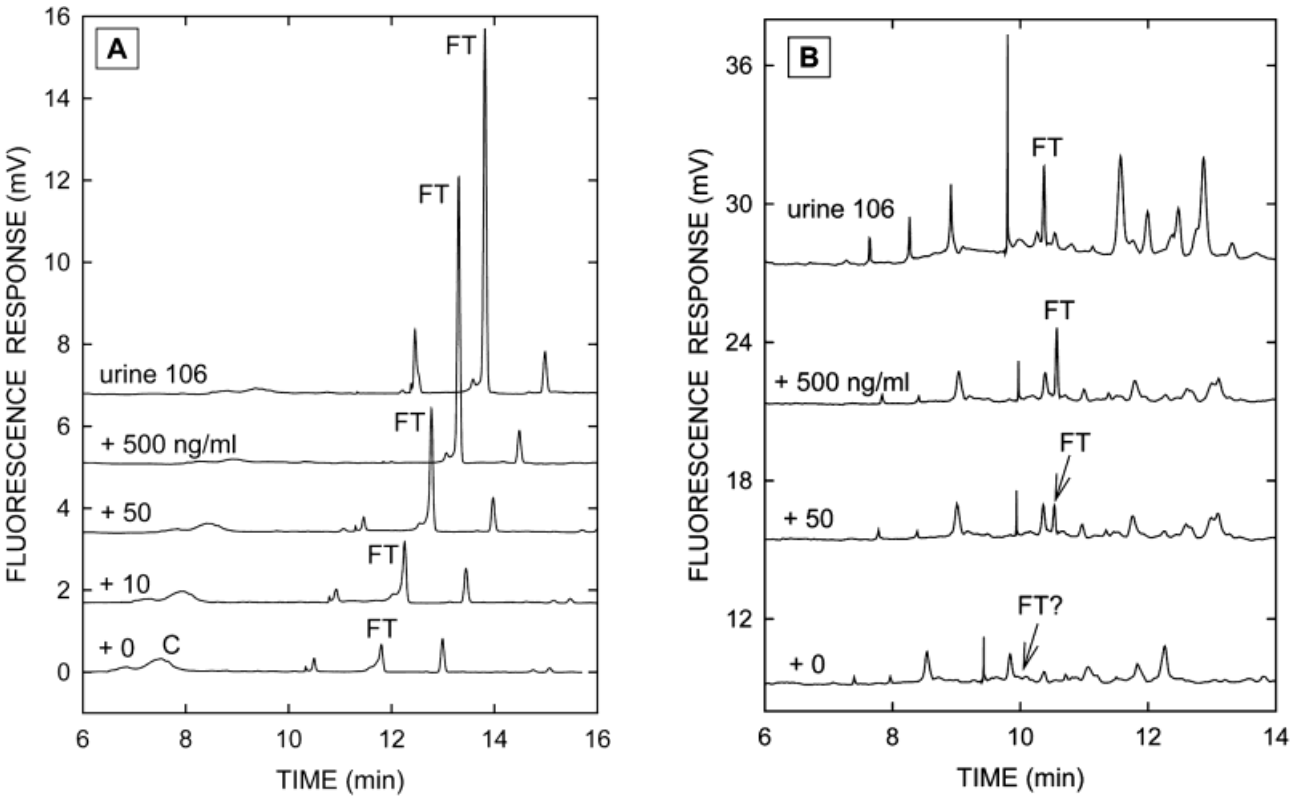

Figure 4. CZE immunoassay electropherograms obtained with (A) urine blank, urine blank spiked with 10, 50, and 500 ng/mL benzoylecgonine, and the external quality control urine 106 (from bottom to top , depicted with a 0.5 min *x*-axis shift and a 1.5 mV *y*-axis shift) and LIF detection with an Ar-ion laser and 520 nm band filter, and (B) with urine blank, urine blank spiked with 50 and 500 ng/mL benzoylecgonine, and urine 106 (from bottom to top, depicted with *y*-axis shift of 6 mV) and the Argos detector with 256 nm excitation and a 495 nm notch filter. The running buffer was composed of 50 mM disodium tetraborate (pH 9.3), the voltages (currents) were (A) 13 kV (87 μA) and (B) 20 kV (48 μA) and injection time intervals were 1 s in all cases. Peak identification: FT, free benzoylecgonine tracer; C, antibody-tracer complex.

480 nm for excitation, a very simple electropherogram with less sensitivity for FT was recorded (top graph in Fig. 5B).

3.3 Analysis of metabolites of acetylsalicylic acid in human urine

Analysis of salicylate and other metabolites in urine by CE with LIF using a 325 nm HeCd laser line was extensively studied in our laboratory [2, 4, 19]. The data presented in Figs. 6A and B are those obtained by CZE in a buffer composed of 6 mM disodium tetraborate and 10 mM disodium hydrogen phosphate (pH 9.1) using standards and a patient urine, respectively, and are the same as presented previously [19]. The patient urine is the same as the toxicological urine analyzed previously under various instrumental conditions [2, 4, 19]. The assay sensitivity for the three compounds is thereby visualized to be dependent on the emission band-pass filter employed. This was extensively discussed in [19], in which wavelength-resolved LIF is shown to be the best approach for detection of salicylate and metabolites in urine. Furthermore, increased sensitivity can be obtained *via* use of a notch filter. This is demonstrated with the data presented in Fig. 6C (from [2]), data that were produced with the same patient urine using micellar electrokinetic capillary chromatography (MECC, same phosphate/borate buffer with dodecyl sulfate micelles) and a 385 nm filter. Note that the signals for gentisic acid and salicyluric acid were limited by the range of the detector electronics. Furthermore, using a notch filter with a lower cutoff (*e.g.*, 366 nm) would have provided a somewhat larger signal for salicylate.

Data obtained with the Argos detector are shown in Figs. 7 and 8. Excitation was set to 256 nm and a 320 nm notch filter was employed on the emission side. Compared to the LIF data shown in Fig. 6, the Argos detector data are more in favor of monitoring salicylate. At equal concentrations, salicylate is shown to produce the high-

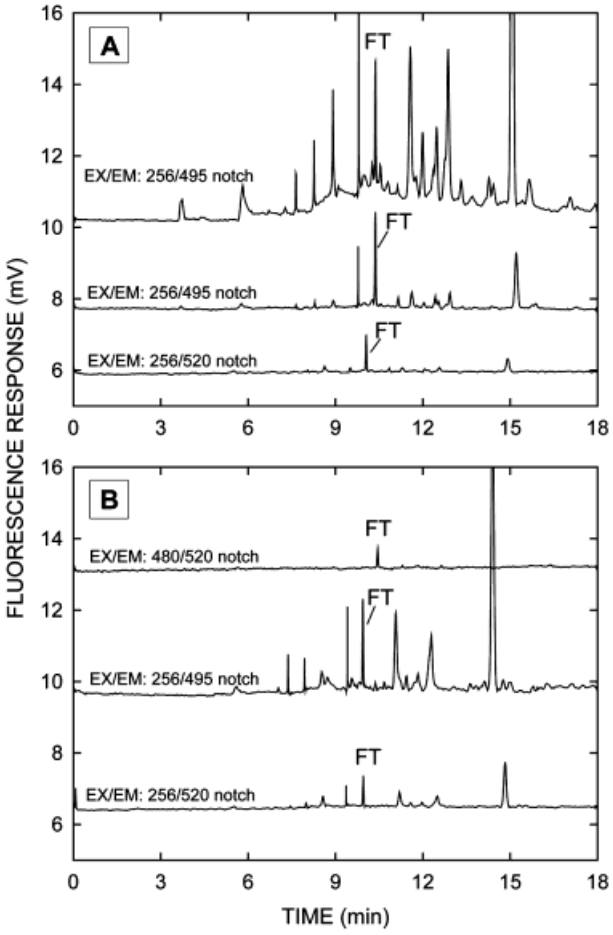

Figure 5. CZE fluorescence immunoassay electropherograms obtained with quality control urines (A) 106 and (B) 130. Panel A depicts data that were obtained with 5-fold diluted, 10-fold diluted and undiluted urine (from bottom to top) and having the optical arrangements as indicated for each graph. The data of panel B were obtained with undiluted urine and the indicated wavelengths. All other conditions as for Fig. 4B. For the sake of presentation, electropherograms are depicted with *y*-axis shifts.

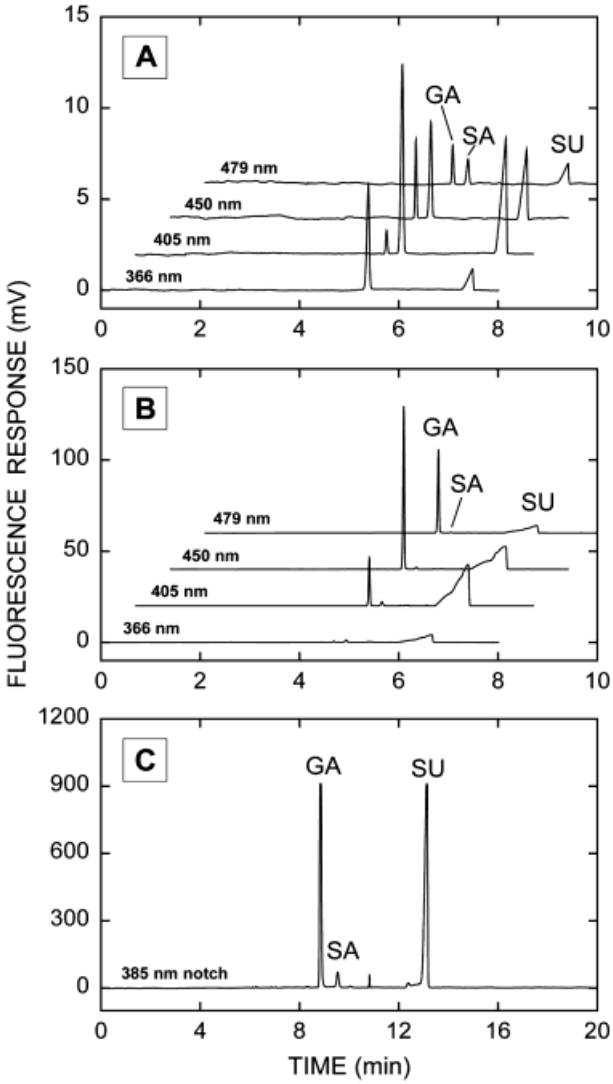

Figure 6. CZE-LIF electropherograms obtained with the 325 nm HeCd laser and four different band filters for (A) a standard solution containing 2 μg/mL GA, 80 μg/mL SA and 10 μg/mL SU and (B) the 30-fold diluted patient urine. The buffer was composed of 6 mM sodium tetraborate and 10 mM Na_2HPO_4 (pH 9.1) and the voltage was 21 kV (current, 55 μA). Panel C depicts MECC-LIF data of the 30-fold diluted patient urine measured with a 385 nm notch filter. The same buffer with the addition of 75 mM SDS was employed and the applied voltage was 15 kV (current, 70 μA). Peak identification: GA, gentisic acid, SA, salicylic acid, SU, salicyluric acid.

est peak (inset in Fig. 7A). This does not come as a surprise as the 325 nm line of the HeCd laser is too high for proper excitation of salicylate [19]. Detection limits for gentisic acid, salicylic acid and salicyluric acid were determined to be about 40, 20 and 100 ng/mL, respectively, values that are lower compared to those observed by LIF with the HeCd laser and similar to those obtained by LIF with a 257 nm laser [19]. Analysis of a urine blank resulted in one strong and a large number of small peaks (Fig. 7A). The compound producing the large peak was found to migrate on the front side of gentisic acid (Figs. 7B and C) as was previously monitored by LIF using the 257 nm line of a frequency-doubled Ar-ion laser and wavelength-resolved data gathering [19]. Under the MECC conditions (Fig. 8), this endogenous compound was found to coelute with gentisic acid which prohibits the determination of gentisic acid in that configuration. The data also reveal that salicyluric acid is producing a much nicer peak in the presence of the dodecyl sulfate micelles, a phenomenon that was observed previously [19].

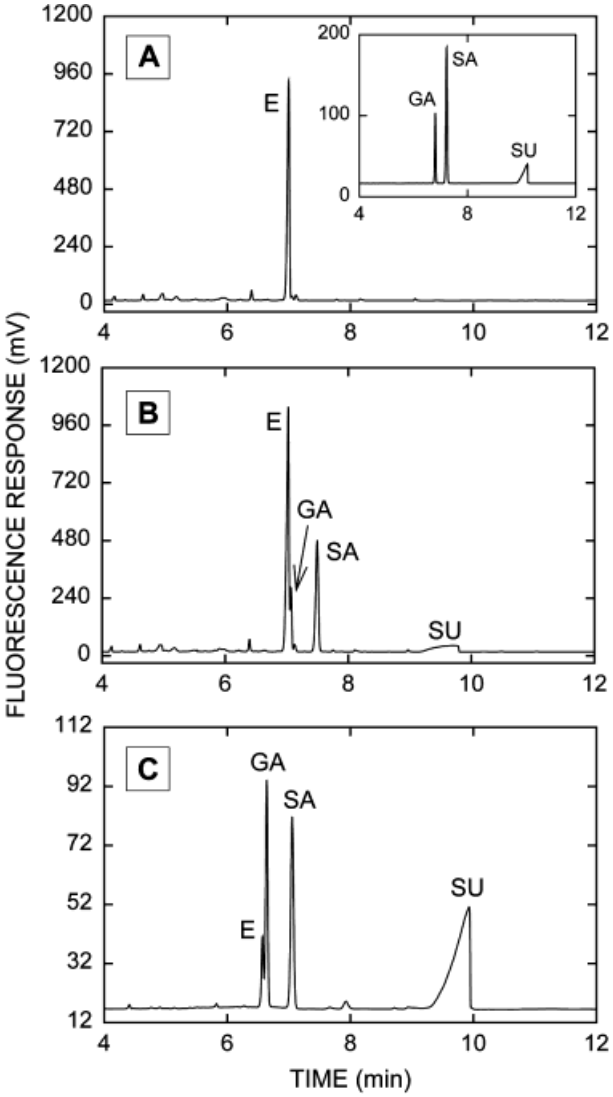

Figure 7. CZE fluorescence electropherograms obtained with an excitation wavelength of 256 nm and a notch filter of 320 nm for (A) 5-fold diluted urine blank, (B) 5-fold diluted urine blank that was fortified with GA, SA and SU (20 μg/mL each), and (C) the 100-fold diluted patient urine. Data obtained with a standard solution containing GA, SA and SU (10 μg/mL each) are presented as inset in panel A. The buffer was composed of 6 mM sodium tetraborate and 10 mM Na_2HPO_4 (pH 9.1). The voltages (currents) were 20 kV (22 μA). Peak identification: as for Fig. 6; E refers to endogenous interference.

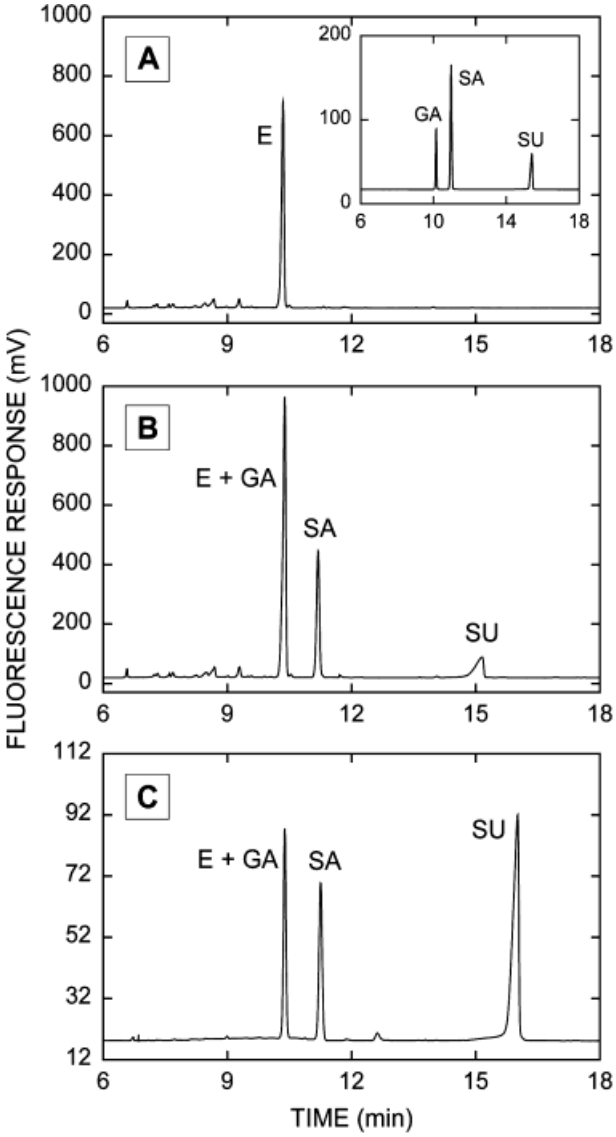

Figure 8. MECC fluorescence electropherograms obtained with an excitation wavelength of 256 nm and a notch filter of 320 nm for (A) 5-fold diluted urine blank, (B) 5-fold diluted urine blank that was fortified with GA, SA and SU (20 μg/mL each), and (C) the 100-fold diluted patient urine. Data obtained with a standard solution containing GA, SA and SU (10 μg/mL each) are presented as inset in panel A. The buffer was composed of 6 mM sodium tetraborate, 10 mM Na_2HPO_4 and 75 mM SDS (pH 9.1). The voltages and currents were 20 kV and 34 μA, respectively, in all cases. Peak identification as for Fig. 7.

3.4 ABZSO and $ABZSO_2$ in human serum

The Argos detector was recently employed for the monitoring of the albendazole metabolites in human serum and saliva using chiral CZE with a 20 mM Tris buffer (pH 7) containing 3% sulfated β-cyclodextrin [20]. With solute excitation at 280 nm (600 μm slit width) and a 320 nm notch filter, the sensitivity for ABZSO was found to be about equal, for $ABZSO_2$ much better and for oxfenbendazole (OxBZ, internal standard) lower compared to UV absorption at 225 nm. For analysis of ABZSO enantiomers, fluorescence detection was noted to provide higher selectivity and a detection limit of about 0.10 μg/mL per ABZSO enantiomer [20]. It was of interest to find out

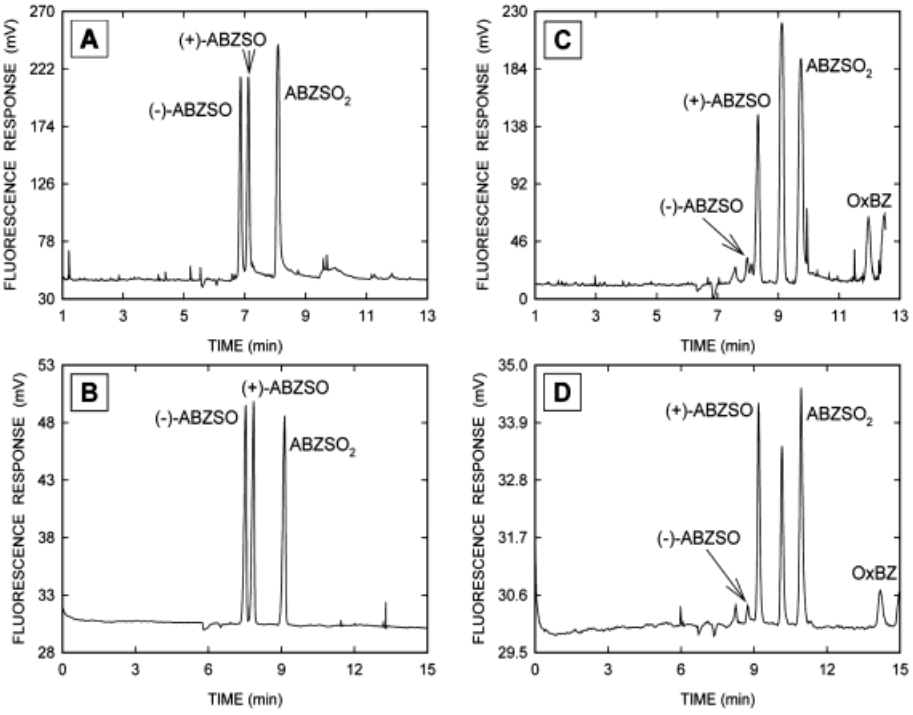

Figure 9. (A), (C) CZE-LIF and (B), (D) CZE fluorescence electropherograms obtained with (A), (B) an aqueous standard solution comprising 50 μg/mL ABZSO and (C), (D) an extract of a patient plasma. For analysis of the patient sample, OxBZ was added as internal standard. The CZE-LIF and CZE fluorescence data were recorded using the ZetaLIF with a pulsed 266 nm laser and the Argos with an excitation wavelength of 280 nm (1 mm slit width). The wavelengths of the notch emission filters employed in the two detectors were 290 and 320 nm, respectively. Experiments were performed with a 20 mM Tris buffer (pH 7) containing 3% w/v sulfated β-cyclodextrin, the applied voltage was 20 kV in all cases and the currents were (A), (C) 39 μA and (B), (D) 56 μA. Time intervals for sample injection were 1 s.

whether the ZetaLIF detector with the 266 nm pulsed laser and a notch filter of 290 nm provides better sensitivity for these compounds. Data obtained with an ABZSO standard solution and an extract from the plasma of a patient under albendazole pharmacotherapy (ABZSO plasma level of 2.08 μg/mL) are presented in Fig. 9. These data were generated with solute excitation at 280 nm (1 mm slit width) and a 320 nm notch filter.

Analysis of the standard solution revealed two peaks for the ABZSO enantiomers and an additional peak representing the $ABZSO_2$ impurity present in the standard substance [20]. For ABZSO, the ZetaLIF detector was found not to provide higher sensitivity compared to that obtained with the Argos. The higher noise prevented the analysis of ABZSO concentrations lower than 0.5 μg/mL. The same applies to the data that were registered with the plasma extract (compare electropherograms of Figs. 9C and D) which was prepared by liquid/liquid extraction as described in [20]. Furthermore, excitation at 266 nm for analysis of extracts of patient plasma samples revealed an additional peak between those of the two ABZSO enantiomers which makes quantitation of these compounds more difficult.

4 Concluding remarks

The data obtained for analysis of (i) urinary MDMA and its major metabolites, (ii) urinary metabolites of acetylsalicylic acid, (iii) benzoylecgonine in urine *via* a CE-based immunoassay, and (iv) ABZSO enantiomers and $ABZSO_2$ in plasma extracts suggest that the Argos 250B can be employed for the monitoring of these compounds at pharmacological and toxicological-relevant concentrations. The comparison of the XeHg lamp-based fluorescence and LIF data has to be understood in the light of a number of limitations. The sample volumes analyzed and the excitation/emission wavelengths employed in the different setups were similar but not equal. Furthermore, the detection cells of the various instruments and data averaging/filtering varied as well. All these aspects do not permit a straightforward comparison of sensitivity. Although LIF detection is typically more sensitive, further work is required to assess sensitivity and linear range of the various approaches. Nevertheless, for the monitoring of the solutes investigated, the presented data permit the conclusion that the Argos 250B with its versatility of wavelength selection for solute excitation in a wide range (200–800 nm) is an attractive alternative to LIF detection.

For fluorescence monitoring of drugs and metabolites in body fluids, solute excitation at low wavelength ($<$ 325 nm) can provide a large number of signals that might not lead to straightforward analytical data without having wavelength-resolved fluorescence detection as described elsewhere [10, 19].

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