ELSEVIER

Journal of Chromatography A, 924 (2001) 507–518

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Head-column field-amplified sample stacking in presence of siphoning
Application to capillary electrophoresis–electrospray ionization mass spectrometry of opioids in urine

Anita B. Wey, Wolfgang Thormann*

Department of Clinical Pharmacology, University of Berne, Murtenstrasse 35, 3010 Berne, Switzerland

Abstract

Capillary electrophoresis (CE) with head-column field-amplified sample stacking (FASS) in presence of a water plug inserted at the capillary tip is a robust approach providing a more than 1000-fold sensitivity enhancement when applied to low-conductivity samples that are analyzed in an integrated instrument. Employing modular systems comprising a small hydrodynamic buffer flow (siphoning) towards the capillary end and featuring UV absorption or electrospray ionization mass spectrometric (MS) detection, insertion of a water plug is demonstrated to deteriorate the performance of head-column FASS or making it unfunctional. Electroinjection in the absence of the water plug can be employed instead and is shown to provide a ng/ml sensitivity when applied to low conductivity samples. With some suction of sample into the capillary during electroinjection, contamination of the sample vial with buffer is thereby largely avoided. Electroinjection applied to the CE–ion trap MS–MS and MS–MS–MS analysis of twofold diluted urines, urinary solid-phase extracts and urinary liquid–liquid extracts is shown to provide much improved sensitivity compared to hydrodynamic injection of these samples. With electroinjection from diluted urine and urinary solid-phase extracts, the presence of free opioids and their glucuronic acid conjugates can be unambiguously confirmed in urines that were collected after single-dose administration of small amounts of opioids (tested with about 7 mg codeine and 25 mg dihydrocodeine, respectively). Thus, CE–multiple MS with direct electroinjection of opioids from untreated urines could prove to become a rapid and simple approach for unambiguous urinary testing of drug abuse. Procedures leading to the reduction of siphoning in modular CE setups are briefly discussed as well.

Keywords: Field-enhanced sample stacking; Morphine; Codeine; Codeine-6-glucuronide; Dihydrocodeine; Dihydrocodeine-6-glucuronide; Opioids

1. Introduction

In a recent contribution from our laboratory, head-column field-amplified sample stacking (FASS) in binary system capillary electrophoresis (CE) was shown to be a robust approach providing over 1000-fold sensitivity enhancement [1]. As solute concentration occurs at the capillary tip (Fig. 1), the method was originally termed field-amplified sample injection [2,3] or referred to as electroinjection [4] and electroextraction [5]. Head-column FASS is based on Ohm's law. After replenishing the capillary with running buffer, a short low-conductivity zone

*Corresponding author. Tel.: +41-31-6323-288; fax: +41-31-6324-997.

E-mail address: wolfgang.thormann@ikp.unibe.ch (W. Thormann).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00986-4

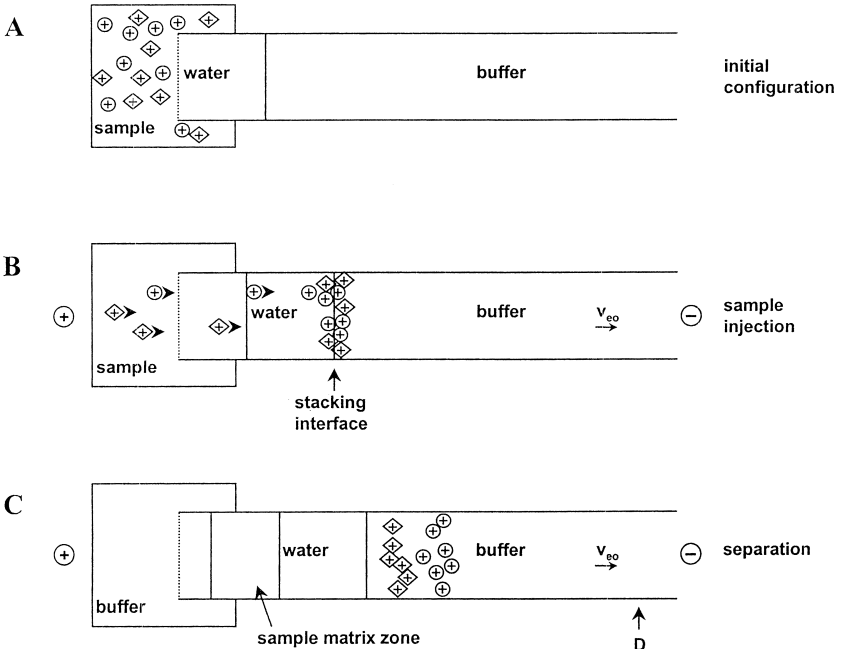

Fig. 1. Schematic representation of head-column FASS in a configuration without siphoning with (A) initial configuration prior to sample injection, (B) time point during electroinjection of sample and (C) time point during separation. D and v_{eo} refer to the point of detection and the electroosmotic flow, respectively.

(water plug) is introduced at the inlet side prior to electrokinetic sample injection from a sample solution of low conductivity. During electroinjection, charged solutes migrate rapidly through the water zone. Upon reaching the interface with the running buffer, their electromigrational transport is drastically decreased because the electric field within the water plug is much higher compared to that within the buffer. As a result, many charged solutes are effectively concentrated prior to their electrophoretic separation. Furthermore, little sample solvent is co-injected (sample matrix zone in Fig. 1C) because the net electroosmotic velocity is much smaller than local electrophoretic transport. Head-column FASS has no limited sample injection volume. Analytes from samples that are significantly larger than the total capillary volume can be injected [1,5,6]. Exhaustive injection from a sample vial [5] and injection from a continuously replenishing sample stream [7] have also been demonstrated. Furthermore, although the same sensitivity can be obtained by electroinjection in absence of the low-conductivity zone at the capillary tip [8,9], in binary CE the presence of the water plug was shown to provide much improved reproducibility [1,9]. Finally, head-column FASS was applied to the monitoring of ppb levels of drugs in small samples, including amiodarone and its metabolite desethylamiodarone in microliter amounts of human plasma [10], of opioids in plasma, serum and urine [6,9,11], of opiates in hair extracts [12], of abused drugs in hair of cocaine and ecstasy [3,4-methylenedioxymethamphetamine (MDMA)] users [13], of formoterol in a low-dosage dry syrup [14] and of metformin in plasma [15].

In recent work from our laboratory, in which the confirmation of urinary amphetamine and analogs [16] and of urinary opioids [17,18] by CE–ion trap mass spectrometry (CE–MS) was investigated, head-column FASS as described above and in Fig. 1 could not be implemented. The employed setup comprised the Prince sampler hyphenated to the atmospheric pressure electrospray ionization interface of the LCQ ion trap MS system, a configuration which was found to comprise siphoning of buffer towards the outlet that reduced both the residence times of solutes in the CE capillary and the likelihood of ion

penetration from the sheath liquid into the capillary [16]. Using hydrodynamic sample injection, this setup was determined to provide a sensitivity comparable to that obtained by UV absorption detection (about 5 μg/ml with direct sample injection and about 100–200 ng/ml when solutes are extracted from 2 ml of urine and reconstituted in 200 μl [17]), a limit of detection that is insufficient for many applications. Thus, application of head-column FASS to setups comprising a small amount of hydrodynamic flow towards the capillary outlet was evaluated. This paper reports (i) the impact of siphoning on the performance of head-column FASS and (ii) the use of electroinjection for CE–ion trap MS of opioids in urine and urinary extracts. The processes involved are characterized for a standard sample comprising eight compounds, namely dihydrocodeine (DHC), nordihydrocodeine (NDHC), dihydromorphine (DHM), nordihydromorphine (NDHM), codeine (COD), normorphine (NMOR), norcodeine (NCOD) and morphine (MOR), and for urines that were collected after self administration of COD and DHC containing pharmaceutical preparations.

2. Experimental

2.1. Chemicals, urine samples, blank matrices and standard solutions

DHC and its metabolites NDHC, DHM, NDHM and dihydrocodeine-6-glucuronide (DHC-6-G) were received from Mundipharma (Basel, Switzerland). NCOD and NMOR were purchased as methanolic solutions (1.0 mg/ml base) from Alltech (State College, PA, USA). MOR, COD, codeine-6-glucuronide (COD-6-G), morphine-3-glucuronide (MOR-3-G) and morphine-6-glucuronide (MOR-6-G) were kindly received from Dr. R. Brenneisen (Department of Clinical Research, University of Berne, Berne, Switzerland). All other chemicals were of analytical grade.

Two human urine samples that tested positive for opiates using immunological drug screening procedures [17] were analyzed. Urine u91 was collected after administration of one tablet of Pretuval C (Roche, Reinach, Switzerland, containing 30 mg pseudoephedrine, 20 mg dextrometorphan, 300 mg paracetamol and 250 mg ascorbic acid) and 30 drops of Resyl Plus (Novartis Consumer Health, Nyon, Switzerland; about 7 mg of COD) and urine u94 was collected during the 0–8 h time interval after administration of 75 drops of Paracodin (Knoll, Liestal, Switzerland; about 25 mg DHC). Our own urine was employed as blank and fortified urines were prepared by adding appropriate aliquots of stock solutions to urine blank. All urines were stored at −20°C. Stock solutions of free drugs (1 mg/ml) were prepared with methanol–water (50:50, v/v) containing 1% of formic acid. Conjugates were dissolved in water (20 μg/ml). Standard solutions were prepared by diluting appropriate aliquots of the stock solutions with water or with the sample solvent that was composed of 20 m*M* ammonium acetate and 20 m*M* acetic acid (pH 4.6). All solutions were stored in glass vials at −20°C.

2.2. Sample preparation

Urine pretreatment included dilution, solid-phase extraction or liquid–liquid extraction. Dilution was effected by mixing the urine 1:1 (v/v) with water. Solid-phase extraction was performed in a similar way as described previously [17,18] using disposable, mixed-mode polymer cartridges (Bond Elut Certify, No. 1211-3050, Varian, Harbor City, CA, USA) together with the Vac-Elut setup (Varian). Briefly, the cartridges were conditioned with 2 ml of methanol and 2 ml of water using vacuum aspiration without drying the sorbent bed. A 2-ml volume of urine (adjusted to pH 7 with 1 *M* KOH solution) was loaded onto and slowly drawn through the cartridges. Prior to elution of the adsorbed opioids with 1.5 ml of methanolic solution containing 30% of ammonia, the cartridges were sequentially washed with 2 ml of water, 1 ml of 0.1 *M* acetate buffer (pH 4) and 2 ml of methanol applying vacuum aspiration. The eluate was collected in a glass tube and evaporated to dryness at 35°C under a gentle stream of nitrogen. The residue was redissolved in 200 μl of water (for electroinjection) or of sample solvent (for hydrodynamic injection). For liquid–liquid extraction, the commercially available Toxi-Tube A system (Analytical Systems, Laguna Hills, CA, USA) comprising about 2 ml of an organic solvent mixture of CH_2Cl_2

and $C_2H_4Cl_2$, (pH 9) was employed. After adding of 2 ml of urine, gently shaking for about 1 min and centrifugation for 5 min at about 1500 *g*, 2 ml of the organic phase was transferred to a glass tube, two drops of 2 *M* acetic acid in ethyl acetate were added, and the solvent was evaporated in a water bath at 35°C under a gentle stream of nitrogen. The residue was redissolved in 200 μl of water (for electroinjection) or of sample solvent (for hydrodynamic injection).

2.3. CE–UV in an integrated system

CE was performed on a P/ACE 5510 capillary electrophoresis system (Beckman, Fullerton, CA, USA) equipped with an 87 cm (effective length 80 cm)×50 μm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA). A new capillary was first flushed with 1 *M* NaOH for about 30 min and capillary conditioning between runs was effected by flushing with running buffer for 3 min (application of 20 p.s.i. pressure at the inlet end; 1 p.s.i.= 6894.76 Pa). The running buffer was composed of 25 m*M* ammonium acetate adjusted to pH 9 with 1 *M* ammonia solution. Sample was either introduced hydrodynamically (applying pressure of 0.5 p.s.i.) or electrokinetically using head-column FASS as described previously [9]. In the latter case and before sample injection at 10 kV, the capillary tip was first dipped for 3 s into a vial containing water and, from a different vial with water, a plug of 0.31 mm length (application of 0.5 p.s.i. for 1 s) was introduced into the capillary. The run voltage was 30 kV (anode on injection end, current about 17 μA) and solute detection was effected by UV absorbance at 214 nm. All operations were computer controlled using the Beckman P/ACE station software (version 1.0).

2.4. CE–UV in a modular system

The Prince apparatus (Lauerlabs, Emmen, The Netherlands) connected to a UVIS 206 PHD fast-scanning multiwavelength detector and No. 9550-0155 on-column capillary detector cell (both from Linear Instruments, Reno, NV, USA) was used. The Model 206 detector was employed in the high-sensitivity monochrome mode at 210 nm. All data were read, evaluated and stored employing a Mandax AT 286 computer system and running the Model 206 detector software package version 2.0 (Linear Instruments) under Windows 286 version 2.1 (Microsoft, Redmont, WA, USA). Fused-silica capillaries of 80 cm (effective length 62 cm)×50 μm I.D. were purchased from Polymicro Technologies. The running buffer was composed of 25 m*M* ammonium acetate adjusted to pH 9 with 1 *M* ammonia solution. The capillary tip was not dipped into a vial containing water prior to introduction of the water plug (application of 35 mbar for 0.01 min) for head-column FASS. Sample was introduced electrokinetically (for details see text below) and the run voltage was 30 kV (anode on injection end, current about 18 μA).

2.5. CE–MS analysis

MS was performed on a Finnigan LCQ ion trap instrument (Finnigan MAT, San Jose, CA, USA) equipped with an electrospray ionization interface (Finnigan) that was run in the positive ion mode (3.5 kV at electrospray tip which also acts as the cathode of the CE system). Sheath gas (N_2) pressure was set at 20 arbitrary units and mixtures of methanol–water (60:40, v/v) containing 1% of acetic acid or 1% of formic acid at flow-rates of 3 μl/min or 5 μl/min, respectively, were used as sheath liquids. The temperature of the heated capillary was 200°C. The instrument was computer controlled using the XCalibur 1.0 software (Finnigan). A Prince apparatus (Lauerlabs) equipped with an 80 cm×50 μm I.D. fused-silica capillary (Polymicro Technologies) was interfaced. Sample was introduced hydrodynamically by applying a positive pressure of 70 mbar for 12 s or electrokinetically during 90 s at 10 kV (application of 10 kV to the anode and 3.5 kV to the cathode; 6.5 kV effective voltage). Separation was effected with the running buffer employed for CE–UV measurements mentioned above and by applying a voltage of 30 kV to the anode (26.5 kV effective voltage for separation). Full scan mass spectra were acquired in the mass range of 100–500 m/z. Automatic gain control (AGC) was employed using three microscans and a maximum injection time of 200 ms. MS–MS was performed using data dependent scans with an isolation width of 2 m/z and a relative collision energy of 35%. In these

experiments the instrument automatically switches to MS–MS as soon as a defined mass peak exceeds a predefined threshold. MS–MS–MS (MS^3) experiments were performed with an isolation width of 2 m/z and a relative collision energy of 35%.

3. Results and discussion

3.1. Head-column FASS using an integrated system (absence of siphoning)

An aqueous buffer that was previously reported to provide good resolution for separation of opioids and trouble-free operation of CE–MS [17,18] was employed throughout this work. It consists of 25 m*M* ammonium acetate adjusted to pH 9 with 1 *M* NH_3 solution. For the assessment of stacking conditions, a standard sample comprising eight opioids (Fig. 2) dissolved in water was applied. At pH 9, all eight opioids are cations [19]. For an evaluation without siphoning, The P/ACE 5510 as fully integrated CE system was employed. The data presented in Fig. 2A were obtained by a 20 s hydrodynamic injection at 0.5 p.s.i. of a sample comprising 10 μg/ml of each compound. All eight opioids are shown to be resolved and detected within about 6.5 min. Using electroinjection instead from the same sample was found to provide significantly higher peaks (Fig. 2B). No water plug was used for that experiment. At that sample concentration, head-column FASS with a deliberately added water plug of 0.31 mm length (application of 0.5 p.s.i. for 1 s) was noted to result in a comparable electropherogram as was obtained without the plug (Fig. 2C). Electropherograms obtained with opioid concentrations of 1000 and 100 ng/ml are presented in panels D and E, respectively, of Fig. 2. In all cases, the eight opioids are nicely separated. The recognition of MOR, however, can be critical. The buffer pH employed is very close to the isoelectric point (p*I*) value of MOR. At a slightly higher pH, morphine is likely to be either neutral or even negatively charged. Variations in the behavior of MOR were noted (Fig. 2C–E, Fig. 3) and are believed to originate from insufficient replenishing of the buffer vial and/or variations in buffer preparation.

For the data presented in Fig. 2, injection times were 20 s in all cases. These data already indicate a

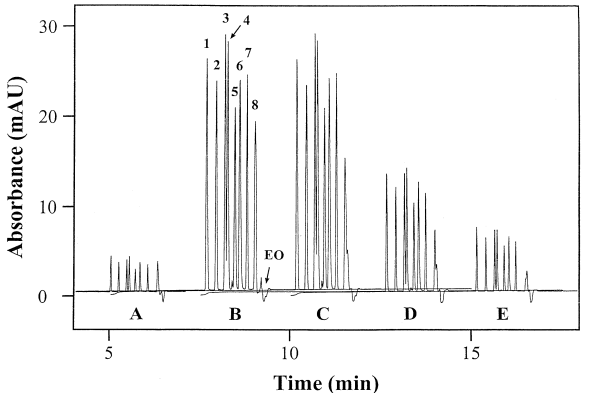

Fig. 2. CE–UV electropherograms of a standard mixture of eight opioids dissolved in water detected after (A) hydrodynamic injection for 20 s, (B) electroinjection at 10 kV for 20 s and (C–E) head-column FASS injection at 10 kV for 20 s using the P/ACE 5510. Data of panels C to E were obtained with a deliberately injected water plug prior to sample injection. Sample concentrations were (A–C) 10 μg/ml, (D) 1 μg/ml and (E) 100 ng/ml each. EO marks the fluid element transported by electroosmosis. The time axis is for panel A. Subsequent electropherograms are presented with an *x*-axis shift of 2.5 min. Key: 1, NDHC; 2, NCOD; 3, NDHM; 4, DHC; 5, NMOR; 6, DHM; 7, COD; 8, MOR.

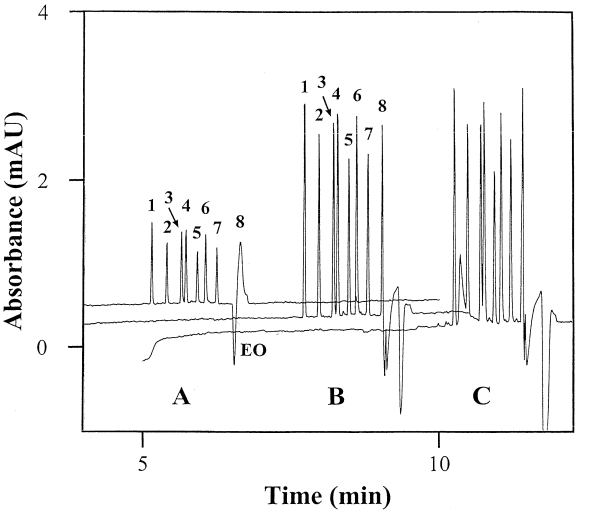

Fig. 3. CE–UV electropherograms of a standard mixture of eight opioids (10 ng/ml each) dissolved in water and detected after head-column FASS at 10 kV using injection times of (A) 20 s, (B) 50 s and (C) 80 s. Other conditions as for Fig. 2C–E.

tremendous sensitivity improvement using head-column FASS (compare panels A and E for which sensitivity enhancement is >100-fold). Optimization of the responses is further possible via increase of the injection time interval. The head-column FASS data depicted in Fig. 3 illustrate the impact of injection time for an aqueous sample solution containing 10 ng/ml of each component. Having a 20 s injection time (Fig. 3A), the response measured was found to be smaller than that shown in Fig. 2A. However, similar peak magnitudes were obtained for an injection time interval of 50 s (panel B of Fig. 3). With injections that lasted longer than 50 s, broader peaks were noted and peak heights did not become much higher (panel C of Fig. 3). Thus, using integrated instrumentation which does not exhibit siphoning, head-column FASS across a deliberately introduced water plug at the capillary inlet prior to sample injection is demonstrated to permit the detection of ppb levels of solutes and was, e.g., successfully applied to ppb drug monitoring in small amounts of biological samples [1,6,8–13].

3.2. Head-column FASS using a modular system (presence of siphoning)

Our initial efforts using head-column FASS with a water plug for the analysis of opioids by CE–MS using the Prince sampler did not work at all. Thus, the performance of head-column FASS was investigated with a modular setup comprising the Prince sampler together with an absorbance detector. The Prince apparatus features the possibility of applying hydrodynamic co- or counterflow (application of positive or negative pressure) during an experiment. In the context of other experiments [20], this configuration was observed to provide a hydrodynamic flow that is linearly dependent on applied pressure. Furthermore, at zero pressure and without application of power, a residual flow of 0.37 cm/min was noted [20]. Thus, the impact of siphoning on the performance of head-column FASS in presence of a water plug was evaluated.

Using integrated systems, sensitivity of head-column FASS was found to be dependent on the length of the applied water plug [1]. As the water zone length was increased, peak heights were observed to decrease. In principle, the length of the water plug is determined by three factors, capillary action (an effect that can be neglected in aqueous solutions but not in binary media [1]), deliberate pressure injection, and siphoning of the system. Siphoning in integrated systems is negligible, but not in our modular system using the Prince sampler (see above). Thus, data obtained with electroinjection without a water plug (panel A of Fig. 4) and with a deliberately added water plug of about 0.25 mm length (panel B of Fig. 4; injection: 0.01 min at 35 mbar) were found to be substantially different. The first electropherogram is nice and as expected, whereas the second is characterized with smaller peaks. The use of longer water plugs lead to even worse data. It appears that in this system ppb sensitivity cannot be reached with the water plug approach. This is in contrast to the data obtained on the P/ACE with a water plug length of 0.31 mm

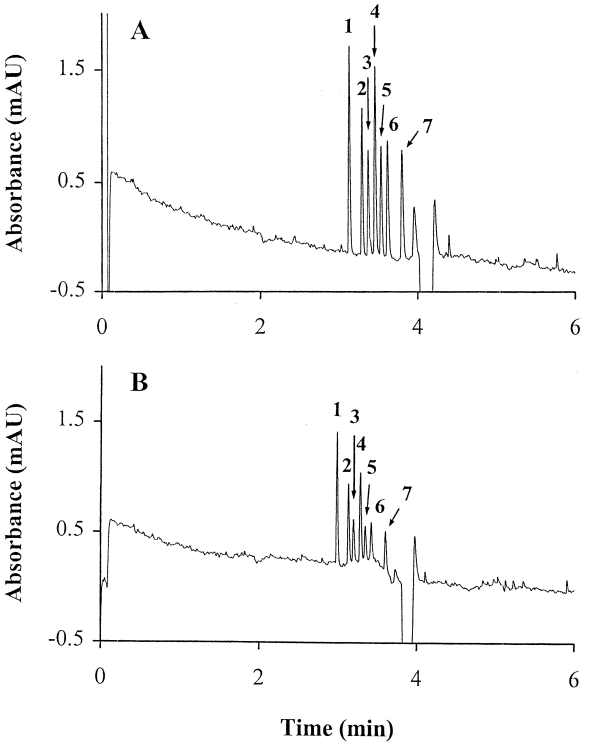

Fig. 4. CE–UV electropherograms of a standard mixture (50 ng/ml) dissolved in water obtained on the Prince apparatus for (A) electroinjection and (B) head-column FASS in presence of a water plug of about 0.25 mm. Injection occurred at 10 kV and 12 s in both cases. Key as for Fig. 2.

(Figs. 2C–E and 3A–C). Thus, it can be assumed that siphoning is elongating the water plug during the time interval in which the capillary tip is in contact with the water (a few seconds before and after deliberate injection of the water plug) and the sample solvent zone behind the water plug (mainly during electroinjection, see Fig. 1B) and thereby reducing the performance of head-column FASS. Furthermore, with suction of sample into the capillary during electroinjection, contamination of the sample vial with buffer should thereby be prevented which should have a positive influence on reproducibility [9]. For electroinjection without the water plug, a 12 s injection provided a fully resolved electropherogram (Fig. 4A). Using higher injection times (e.g., 30 s) was found to provide broader peaks of similar height that were incompletely separated. As discussed above for Figs. 2 and 3, MOR in Fig. 4 could not unambiguously be allocated.

3.3. Application of head-column FASS to CE–ion trap MS

Application of head-column FASS with a water plug of 0.3 mm (introduction: 70 mbar for 0.01 min) and electroinjection of the sample at 10 kV for 90 s did not work. Interruption of the current within the first minute after power application during the separation stage (Fig. 1C) was observed which prevented the completion of the experiments. As the electric field applied was comparable to that used for the CE–UV setup described above, siphoning in the case of the CE–MS configuration can be assumed to be stronger. In the employed coaxial sheath-flow arrangement featuring both a coaxial sheath liquid and a coaxial sheath gas with the electrospray tip being the cathode, the sheath gas [21] and possibly also the positive voltage applied to the cathode [22] could represent the sources for the additional co-flow of the

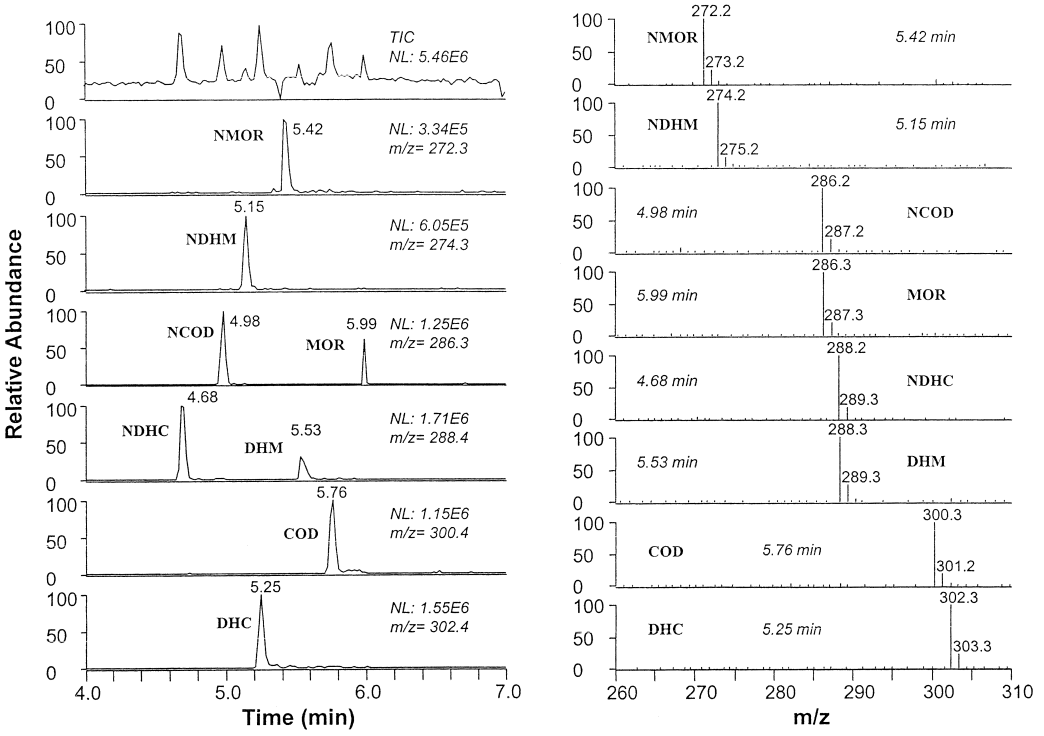

Fig. 5. CE–MS mass traces and the total ion current (TIC) electropherogram (left panel) and MS^1 mass spectra (right panel) obtained with an aqueous mixture of eight opioids (50 ng/ml each). Electroinjection occurred at 10 kV for 90 s without having a water plug. The sheath liquid with formic acid was used.

fluid. With the employed system having 0 V at cathode and no sheath gas, the performance could not be improved. Electroinjection (10 kV for 90 s) from an aqueous sample in absence of the water plug, however, was found to permit analysis of opioids on the ppb level. The CE–MS data presented in Fig. 5 were obtained for electroinjection from a sample composed of 50 ng/ml of each compound. Mass traces similar to those obtained after hydrodynamic injection of a sample comprising 20 μg/ml of each opioid were noted (400-fold sensitivity enhancement, for comparison refer to [18]). All eight opioids could be characterized by MS–MS. The obtained MS–MS spectra were found to be identical to those reported previously [18] and are thus not shown. Using an injection time interval of 90 s, an identification limit with the capability of measuring MS–MS spectra of 10 ng/ml was observed. Shorter injection times lead to decreased sensitivity (e.g., with a 30 s injection time interval, the abundance was about 30% only) and increased injection did not improve the sensitivity. Using the described and other CE–MS setups which are characterized with rather strong siphoning towards the capillary outlet [23], it can be concluded that a water plug should not be inserted and that a ≥1000-fold sensitivity increase (comparison with sample dissolved in buffer or another medium of similar ionic strength that was introduced hydrodynamically) can be reached employing electroinjection from a sample of low conductivity.

The reproducibility of the CE–MS system with electroinjection was assessed via analysis of a sample comprising the eight opioids (50 ng/ml each) in water. The intra-day RSD values ($n=3$) found for run times were <1.2% for all eight opioids, whereas the RSD values for the peak intensities were between 25 and 60%. RSD values of peak intensity ratios were noted to be considerably smaller but typically not <10%. Inter-day RSD values ($n=3$) for run times were determined to be <7% and for the intensities between 50 and 90%. These data compare favorably with those observed after hydrodynamic sample injection [18]. In the work described here, qualitative data were generated only. With inclusion of a deuterated internal standard that comigrates with the solute of interest, however, smaller RSD values for peak intensity ratios are expected and quantitative data could thus also be obtained. Using CE–ion trap MS with the LCQ and deuterated methylphenidate as internal standard, quantitation of urinary methylphenidate on the ppb level was reported by Bach and Henion [24]. In that approach, the high sensitivity was obtained by liquid–liquid extraction of 4 ml urine followed by reconstitution of the dried residue in 200 μl of water prior to electroinjection at 20 kV for 20 s.

3.4. Confirmation testing of urinary opioids by CE–ion trap multiple MS (MS^n) with electrokinetic sample introduction

For the determination and identification of opioids in urine, CE–MS analysis of diluted and extracted samples were performed with the LCQ ion trap MS system that is capable of measuring up to MS^9. As reported previously [17,18], the identity of a substance can be confirmed via gathering and comparing MS–MS or MS^3 spectra with those of standards. Aqueous standard solutions (10 μg/ml) of DHC, NDHC, DHM, NDHM, MOR, COD, NMOR, NCOD, DHC-6-G, COD-6-G, MOR-3-G and MOR-6-G were directly analyzed (via syringe inlet) and their MS, MS–MS and MS^3 (glucuronides only) spectra were stored in a computer library. This library is capable of comparing a selected spectrum with all stored spectra and the probability (%) of a match is automatically calculated by the computer. Using hydrodynamic sample injection, data obtained with blank urine, fortified blank urine at a concentration level of 10 μg/ml of each opioid, and the two volunteer urines have been discussed previously [18] and are summarized in Table 1. Using electroinjection without the water plug, the presence of opioids could be confirmed as well and this approach was found to provide higher sensitivity than with hydrodynamic sample injection (Table 1).

Selected CE–MS mass traces for urine u91 are presented in Fig. 6. Electroinjection from twofold diluted urine revealed the presence of the parent drug and its glucuronide (COD and COD-6-G, respectively). Their respective mass traces at m/z 300.4 and m/z 476.5 are depicted in the left panel of Fig. 6. The presence of these compounds could be confirmed as reported elsewhere using MS–MS and

Table 1
Confirmed presence of opioids in urines using CE–MS–MS or CE–MS[3] and different sample injections[a]

Urine sample	Compounds detected by CE–MS–MS or CE–MS[3]					
	Hydrodynamic injection (70 mbar/12s)[b]			Electroinjection (10 kV, 90 s)[c]		
	Twofold diluted urine	Liquid–liquid extract in sample solvent	Solid-phase extract in sample solvent	Twofold diluted urine	Liquid–liquid extract in water	Solid-phase extract in water
Blank urine	No opioids	No opioids	No opioids	No opioids	No opioids	No opioids
Blank urine fortified with eight unconjugated opioids (10 μg/ml each)	All eight opioids	All eight opioids	All eight opioids	All eight opioids	All eight opioids	All eight opioids
u91	No opioids	COD	COD, COD-6-G	COD, COD-6-G	NCOD, COD	NCOD, COD, COD-6-G (MOR)
u94	No opioids	DHC, NDHC	DHC, DHC-6-G	DHC, DHC-6-G (NDHC)	DHC, NDHC	NDHC, DHC, DHC-6-G

[a] For the compounds given in parentheses, detected mass traces provided too small peaks for identification with MS–MS.
[b] Data of Ref. [18].
[c] No water plug was used.

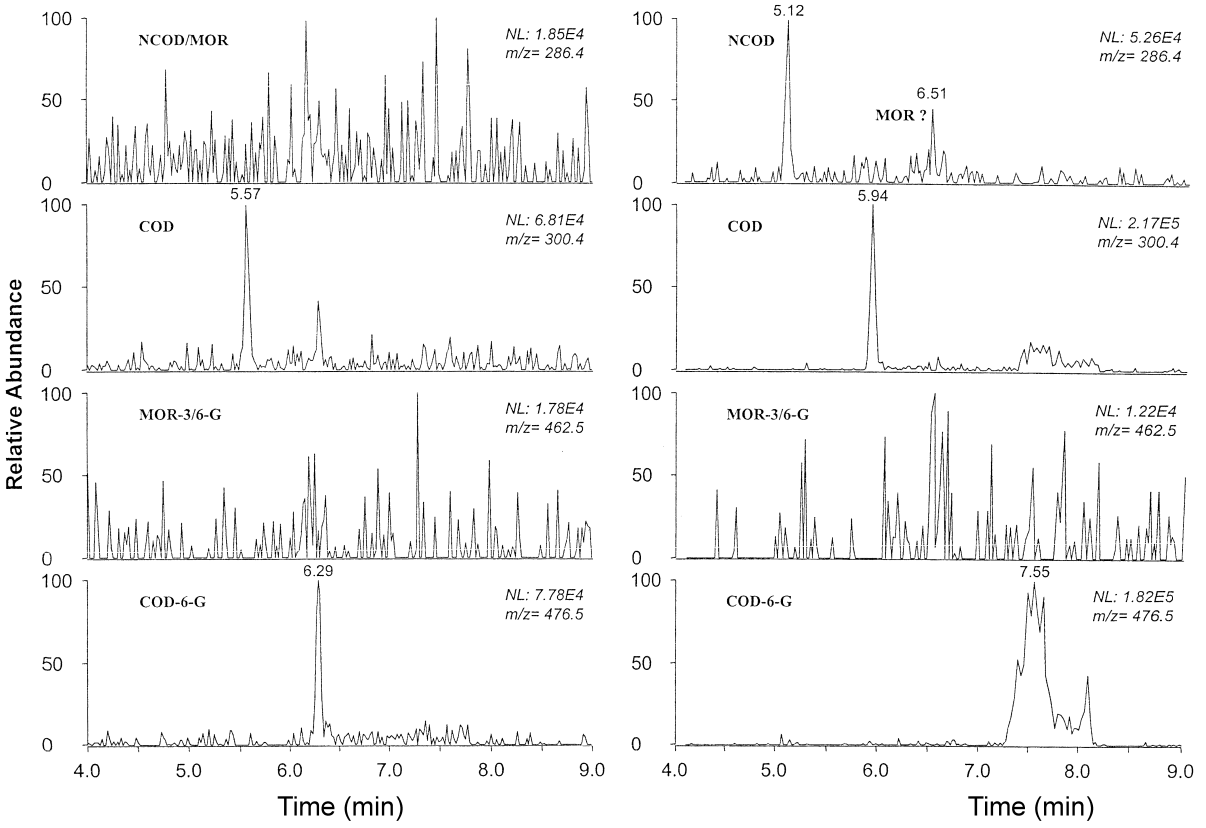

Fig. 6. Selected CE–MS mass traces obtained after electroinjection from twofold diluted urine u91 (left panel) and from a solid-phase extract of the same urine prepared in water (right panel). Other conditions as for Fig. 5.

MS^3, respectively [17]. The mass spectra were identical to those depicted previously and are thus not shown. Furthermore, NCOD and MOR (m/z 286.4), as well as the glucuronides of MOR (m/z 462.5), were not detected (left panel of Fig. 6). With electroinjection from a solid-phase extract, the presence of COD, COD-6-G and NCOD could be confirmed (right panel in Fig. 6). For MOR, a tiny peak was monitored after 6.51 min that could, however, not be analyzed by MS–MS. No peaks were seen for the glucuronides of MOR. Employing liquid–liquid extraction, COD and NCOD were detected only (data not shown). In that configuration, the extraction recovery for MOR is lower than for the solid-phase extraction procedure [18] (which explains the no show of the MOR peak) and COD-6-G is not extracted at all. Similar data were obtained for the analysis of urine u94 (Table 1). With hydrodynamic injection, no opioids could be detected after application of the diluted urines and fewer compounds were recognized for the analysis of urinary extracts (Table 1). Despite the sensitivity increase achieved with electroinjection, however, free and conjugated morphinoids expected to be present at or below the ppb level could not be detected by CE–MS. Further work is required to assess the detection limits of opioids after electroinjection from plain and diluted urines.

4. Concluding remarks

Head-column FASS in presence of the water plug is a robust approach providing a more than 1000-fold sensitivity enhancement when applied to low conductivity samples that are analyzed in an integrated

instrument. It is, however, demonstrated to become deteriorated in systems comprising a small hydrodynamic buffer flow (siphoning) towards the capillary end and even unfunctional in presence of increased siphoning. Using the Prince sampler, this could independently be demonstrated for instrumental combinations with a UV absorbance detector and the LCQ benchtop ion trap MS system with a coaxial atmospheric pressure electrospray ionization interface, respectively. Instead, electroinjection in absence of the water plug can be employed and is shown to provide a ppb sensitivity when applied to low-conductivity samples. With some suction of sample into the capillary during electroinjection, contamination of the sample vial with buffer is thereby largely avoided which should have a positive effect on reproducibility. Electroinjection applied to the CE–MS^n analysis of twofold diluted urines, urinary solid-phase extracts and urinary liquid–liquid extracts is shown to provide much improved sensitivity compared to hydrodynamic injection of these samples. For the former two kind of samples, free and conjugated opioids can thereby be injected and determined from urines that were collected after single-dose administration of small amounts of opioids (tested with about 7 mg COD and 25 mg DHC, respectively). These urines also tested positive for opiates using immunological drug screening procedures [17]. Thus, CE–MS^n with direct electroinjection of opioids from untreated urines could proof to become a rapid and simple approach for unambiguous urinary testing of drug abuse. Using CE with UV detection was recently shown to be applicable for this task as well [25]. However, UV detection is limited to absorbing substances and, for forensic case work, MS detection is accepted only and thus a preferred approach. Alternatively, instead of electroinjection of the sample at the capillary tip, hydrodynamic injection of large amounts of sample has also been reported to provide increased sensitivity for CE–MS of urinary morphine [26].

In order to be able to use a water plug in the CE–electrospray ionization MS configuration employed in our laboratory, siphoning would have to be minimized by one or several of the following aspects, namely (i) lowering of the sampler relative to the capillary outlet, (ii) application of a pneumatically controlled backpressure, (iii) reduction of the inner diameter of the capillary, (iv) establishing working conditions with reduced or even without sheath gas, and (v) using an assembly in which the electrospray tip is not being employed as the driving CE electrode (cathode or anode). Stabilization of the sample plug position by using a backpressure that was feedback controlled via maintenance of a minimum current in the CE capillary has recently been shown to be functional for head-column FASS sampling from a sample stream flowing across the capillary tip [27]. Such an approach, however, cannot be applied to an open capillary system, such as that employed in the hyphenation of CE with MS. Reproducible application of counterflow (negative pressure) with the Prince sampler of the magnitude required (<1 cm/min) is believed to be difficult if not impossible. Furthermore, using capillaries with an I.D. <50 μm would provide reduced siphoning and this possibly even without loss of assay sensitivity [28]. The use of the electrospray tip as driving electrode for CE was found to have an impact on electroosmosis (particularly at low pH) [22], an effect that can only be eliminated by modifying the electrode assembly. Thus, mounting the sampler on a table with height adjustment appears to be the simplest approach for reducing siphoning. The adjustment, however, is believed to be tedious [21] and is simply avoided by the use of electroinjection without the water plug.

Acknowledgements

This work was supported by grants from Mundipharma Medical Company, Basel, Switzerland and the Swiss National Science Foundation.

References

[1] C.-X. Zhang, W. Thormann, Anal. Chem. 68 (1996) 2523.
[2] R.-L. Chien, D.S. Burgi, J. Chromatogr. 559 (1991) 141.
[3] R.-L. Chien, D.S. Burgi, J. Chromatogr. 559 (1991) 153.
[4] K.R. Nielsen, J.P. Foley, J. Chromatogr. A 686 (1994) 283.
[5] E. van der Vlis, M. Mazereeuw, U.R. Tjaden, H. Irth, J. van der Greef, J. Chromatogr. A 687 (1994) 333.
[6] C.-X. Zhang, W. Thormann, Anal. Chem. 70 (1998) 540.
[7] R. Kuldvee, M. Kaljurand, Anal. Chem. 70 (1998) 3695.

[8] R.B. Taylor, A.S. Low, R.G. Reid, J. Chromatogr. B 675 (1996) 213.
[9] A.B. Wey, W. Thormann, Chromatographia 49 (1999) S12.
[10] C.-X. Zhang, Y. Aebi, W. Thormann, Clin. Chem. 42 (1996) 1805.
[11] A.B. Wey, C.-X. Zhang, W. Thormann, J. Chromatogr. A 853 (1999) 95.
[12] G. Manetto, F. Tagliaro, F. Crivellente, V.L. Pascali, M. Marigo, Electrophoresis 21 (2000) 2891.
[13] F. Tagliaro, G. Manetto, F. Crivellente, D. Scardella, M. Marigo, Forensic Sci. Int. 92 (1998) 201.
[14] J.Z. Song, J. Chen, S.J. Tian, Z.P. Sun, J. Pharm. Biomed. Anal. 21 (1999) 569.
[15] J.Z. Song, H.F. Chen, S.J. Tian, Z.P. Sun, J. Chromatogr. B 708 (1998) 277.
[16] A. Ramseier, C. Siethoff, J. Caslavska, W. Thormann, Electrophoresis 21 (2000) 380.
[17] A.B. Wey, J. Caslavska, W. Thormann, J. Chromatogr. A 895 (2000) 133.
[18] A.B. Wey, W. Thormann, J. Chromatogr. A 916 (2001) 225.
[19] C.-X. Zhang, W. Thormann, J. Chromatogr. A 764 (1997) 157.
[20] J. Caslavska, W. Thormann, J. Microcol. Sep. 13 (2001) 69.
[21] T.J. Thompson, F. Foret, P. Vouros, B.L. Karger, Anal. Chem. 65 (1993) 900.
[22] I.M. Lazar, M.L. Lee, J. Am. Soc. Mass Spectrom. 10 (1999) 261.
[23] W. Thormann, M. Lanz, J. Caslavska, P. Siegenthaler, R. Portmann, Electrophoresis 19 (1998) 57.
[24] G.A. Bach, J. Henion, J. Chromatogr. B 707 (1998) 275.
[25] W.-S. Wu, J.L. Tsai, Biomed. Chromatogr. 13 (1999) 216.
[26] J.L. Tsai, W.-S. Wu, H.-H. Lee, Electrophoresis 21 (2000) 1580.
[27] R. Kuldvee, P. Kuban, K. Vunder, M. Kaljurand, Electrophoresis 21 (2000) 2879.
[28] R.D. Smith, J.H. Wahl, D.R. Goodlett, S.A. Hofstadler, Anal. Chem. 65 (1993) 574A.