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Rapid analysis of furosemide in human urine by capillary electrophoresis with laser-induced fluorescence and electrospray ionization-ion trap mass spectrometric detection

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Abstract

Furosemide, a drug that promotes urine excretion, is used in the pharmacotherapy of various diseases and is considered as a doping agent in sports. Using alkaline electrolytes, analysis of furosemide by dodecyl sulfate based micellar electrokinetic capillary chromatography (MECC) and capillary zone electrophoresis (CZE) with laser-induced fluorescence detection (LIF, analyte excitation with the 325 nm line of a HeCd laser) is described. Data produced by injection of plain or diluted patient urines are confirmed with those obtained via analysis of urinary solid-phase extracts. CZE–LIF and MECC–LIF are thereby shown to permit unambiguous recognition of furosemide in urines collected after ingestion of therapeutic doses of this drug. This is in contrast to solute detection via UV absorbance for which the extraction of furosemide is required. MECC based electropherograms are somewhat more complex compared to those obtained by CZE–LIF, this suggesting that the latter approach is more suitable for rapid screening of urines with direct sample injection and LIF detection. Alternatively, capillary electrophoresis with negative electrospray ionization-ion-trap tandem mass spectrometry ($CE–MS^2$) is shown to permit the direct confirmation of furosemide in human urine. This approach is based upon the monitoring of the m/z 329.3→m/z 285.2 precursor–product ion transition. CZE–LIF and $CE–MS^2$ with injection of plain or diluted urine represent simple, rapid and attractive urinary screening and confirmation assays for furosemide in patient urines.

Keywords: Furosemide

1. Introduction

Furosemide is a potent, acidic (pK_a values of 3.8 and 7.5 [1]) diuretic drug that is widely used in the pharmacotherapy of a range of diseases, including congestive heart failure, chronic renal failure, hypertension and some types of oedema. Monitoring furosemide levels is used to check the patients' compliance and has been recommended in patients with impaired renal function and in premature infants since furosemide has been known to cause ototoxicity [2]. Based on its promotion of urine excretion (compared to normal excretion, renal excretion of salts and water can be as much as 40-fold increased [3]), furosemide is also a drug that is frequently employed to conceal the presence and misuse of performance enhancing substances or to achieve rapid weight loss and it is thus considered as a doping agent in sports which is, e.g., banned by the Medical Commission of the International Olympic Committee. After oral administration, furosemide is rapidly absorbed with a bioavailability of about 65%.

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Up to 90% of an intravenous dose is excreted in urine, mainly as unchanged drug and up to 14% as a glucuronide metabolite. Furthermore, 4-chloro-5-sulfamoylanthranilic acid (CSA) has been reported as another metabolite or as an analytical artifact produced during acidic extraction [4,5].

For analysis of furosemide in urine and other biofluids, many different screening and confirmation methods based upon gas chromatography and high-performance liquid chromatography have been developed and applied to patient samples and urines collected at sport events [1,2,5–8]. In the past few years, capillary electrophoresis (CE) of furosemide and other diuretics in absence and presence of micelles was investigated in several laboratories [9]. In the context of analysis of furosemide in urine, three CE-based assays could be found only. Jumppanen et al. reported a capillary zone electrophoresis (CZE) method that uses a 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer at pH 10.6 and UV detection at 220 nm [10], Sádecká and Polonský described a capillary isotachophoresis approach with conductometric solute detection [11], and Lalljie et al. developed a micellar electrokinetic capillary chromatography (MECC) assay employing a pH 9 borate buffer with dodecyl sulfate micelles and diode array detection [3]. With the first two assays, fortified blank samples were analyzed only, whereas the MECC assay was applied to urine samples collected from a healthy volunteer after ingestion of a single therapeutic dose (40 mg) of furosemide. All three methods have in common that they are based on the analysis of urinary extracts that were prepared by either solid-phase extraction (SPE) [10,11] or liquid–liquid extraction [3].

Recently, CE with laser-induced fluorescence (LIF) detection [12–14] and CE coupled to atmospheric pressure electrospray ionization mass spectrometry (CE–MS) [15–18] have been shown to permit direct analysis of drugs in body fluids thereby avoiding time consuming sample preparation. No reports describing the use of CE–LIF and CE–MS for analysis of furosemide was found in the literature. Furosemide fluoresces naturally [1,2,5–7] and should thus be detectable by LIF. Fluorescence is known to be optimal around pH 4.5, a condition under which furosemide is hydrolyzed to CSA and other products [5,6]. Thus, to prevent decomposition of furosemide, analysis of furosemide by CE–LIF and CE–MS was evaluated using alkaline buffers. This paper reports the first (i) CE–LIF and CE–MS data of furosemide and (ii) CE–LIF and capillary electrophoresis–ion-trap tandem mass spectrometry ($CE–MS^2$) based assays for analysis of furosemide in human urine. Data produced by injection of plain or diluted urine are compared to those obtained for analysis of urinary extracts and these assay formats are applied to the screening for and confirmation of furosemide in patient samples.

2. Experimental

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

All chemicals were of analytical grade. Furosemide and CSA were kindly provided by Hoechst (Zürich, Switzerland). Sodium dodecyl sulfate (SDS) was purchased from BDH (Poole, UK) and CAPS was obtained from Sigma (St. Louis, MO, USA). Two urines (referred to as urines J and W) of patients under pharmacotherapy with Lasix (Aventis, Zürich, Switzerland; 40–80 mg of furosemide per day), one urine of a patient without furosemide therapy (referred to as urine R) and 10 randomly selected patient urines were obtained from the Department of Nephrology, Inselspital, Bern, Switzerland. Our own, drug-free urines were used as blank matrices. Standard solutions of furosemide were prepared by diluting appropriate aliquots of a 1 mg/ml stock solution (in methanol or appropriate buffers) with 10-fold diluted running buffer of each method and urines were fortified via addition of aliquots of these standard solutions. All urines and solutions were stored at −20 °C.

2.2. Sample preparation

Urines were analyzed as received or after two- to 10-fold dilution with water. SPE was effected using disposable, mixed-mode polymer cartridges (Bond Elut Certify, No. 1211-3050, Varian, Harbor City, CA, USA) together with the Vac-Elut setup (Varian). The procedure employed is similar to that for barbiturates recommended by the manufacturer of

the disposable columns and was not optimized for furosemide. The cartridges were conditioned with 2 ml of methanol and 2 ml of 0.1 *M* phosphate buffer (pH 6) using vacuum aspiration without drying the sorbent bed. Volumes of 1.0, 2.5 or 5 ml of urine were mixed with 2 ml of 0.1 *M* phosphate buffer (pH 6). The mixture was loaded onto and slowly drawn through the cartridge. The cartridges were sequentially rinsed with 1 ml each of 0.1 *M* phosphate buffer (pH 6)–methanol (80:20), 1 *M* acetic acid and hexane by applying vacuum aspiration. Between the rinsing steps, the columns were dried under full vacuum for 5, 10 and 2 min, respectively. Different elution and reconstitution protocols were applied that led to differences in the furosemide recovery. Using MECC combined with UV detection, furosemide was eluted with 4 ml of methylene chloride into a glass tube, evaporated to dryness at 35 °C under a gentle stream of nitrogen and redissolved in 100 μl of running buffer. For 1 and 5 ml urine, recoveries of about 50 and 7%, respectively, were thereby obtained. For MECC with LIF detection, the residue was reconstituted in 1 ml of running buffer (recovery from 5 ml urine was about 25%). SPE extracts for CZE–LIF and CE–MS were prepared via elution with 4 ml of methylene chloride–methanol (1:4, v/v) and reconstitution in 250 μl of methanol prior to addition of 250 μl water. Independent of the urine volume applied, the recovery was determined to be about 80%.

2.3. Instrumentation and running conditions for CE–UV

Electrokinetic measurements were made in a laboratory-made instrument described previously [19] using a 70 cm (50 cm effective length)×75 μm I.D. capillary. Solute detection was effected with a Model UVIS 206 PHD (Linear Instruments, Reno, NV, USA) detector that was operated in the fast

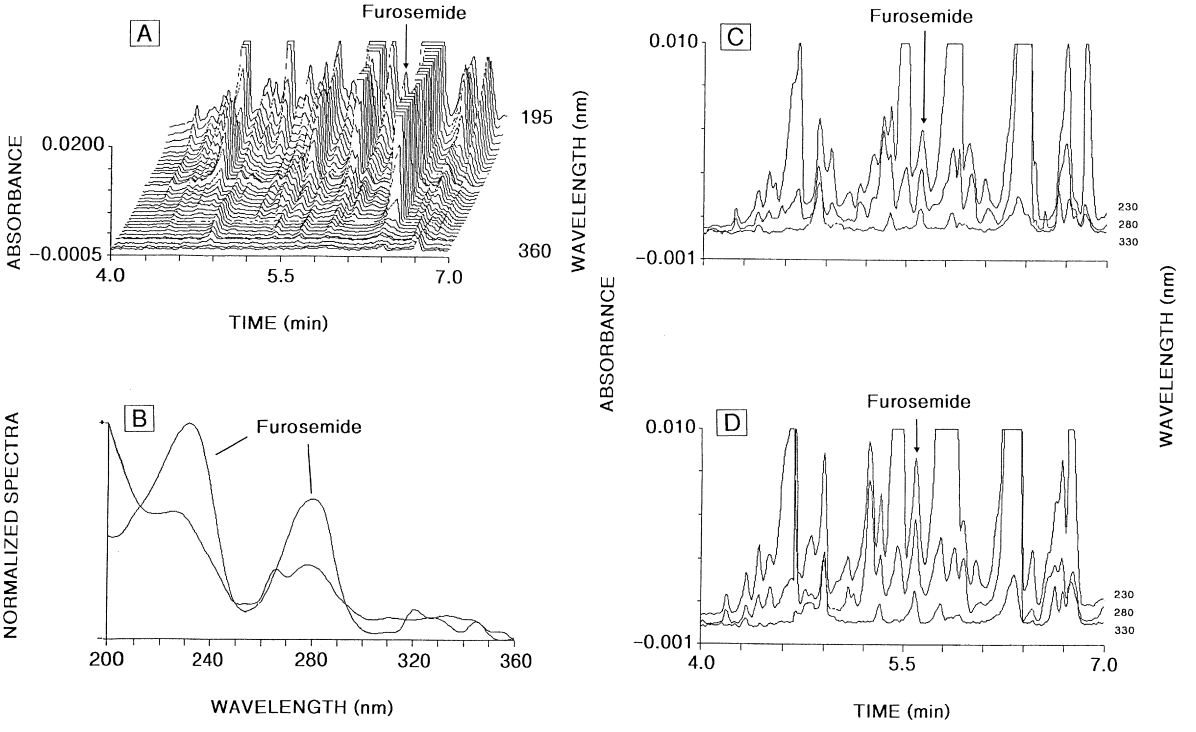

Fig. 1. MECC multiwavelength absorbance electropherograms obtained on a laboratory-made instrument and direct injection of (A, C) patient urine J and (D) the same patient urine fortified with 10 μg/ml furosemide. Panel B depicts the normalized absorbance spectrum of the peak assigned to furosemide (panels A, C) compared to that of the furosemide standard. Electropherograms were generated after 2 s injection and application of a constant 20 kV (current about 109 μA).

scanning mode between 200 and 360 nm (5 nm resolution). The MECC running buffer was the same as previously employed in our laboratory for analysis of other urinary drugs [19]. It was composed of 6 m*M* sodium tetraborate, 10 m*M* disodium hydrogenphosphate and 75 m*M* SDS (pH 9.2). Experiments were performed at ambient temperature.

2.4. Instrumentation and running conditions for CE–LIF

CE–LIF was performed on a P/ACE 5510 capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with a fused-silica capillary of 47 cm (40 cm effective length)×75 μm I.D. If not stated otherwise, samples were hydrodynamically injected by applying a positive pressure of 0.5 p.s.i. for 1 s (1 p.s.i.=6894.76 Pa). The capillary temperature was maintained at 35 °C (MECC) and 20 °C (CZE) and the carousel was at room temperature. Detection was effected by a LIF detector assembly (Beckman) powered by an air-cooled 325 nm HeCd laser (Model 4230NB, LiCONiX, Santa Clara, CA, USA) and equipped with 366 nm (for MECC) and 405 nm (for CZE) band pass filters. Data were evaluated using the P/ACE station software (version 1.0). The buffer employed for MECC was composed of 6 m*M* $Na_2B_4O_7$, 10 m*M* Na_2HPO_4 and 75 m*M* SDS (pH 9.2, same buffer as was used for UV detection, cf. Section 2.3), whereas the 0.06 *M* CAPS buffer titrated with 0.1 *M* NaOH to pH 10.6 of Jumppanen et al. [10] was used for CZE.

2.5. CE–MS instrumentation and running conditions

Mass spectrometry was performed on a Finnigan LCQ ion trap instrument (Finnigan MAT, San Jose, CA, USA) equipped with an electrospray interface (Finnigan) that was run in the negative ion mode (4.0 kV). Sheath gas (N_2) pressure was set at 20 arbitrary units. A mixture of methanol–water–concentrated ammonia solution (50:49:1, v/v) and an equivolume mixture of methanol–water at a flow-rate of 5 μl/min were used as sheath liquids for analysis of diluted urines and SPE extracts, respectively. The temperature of the heated capillary was kept at 200 °C. The instrument was computer controlled using the XCalibur 1.0 software (Finnigan). A Prince Instrument (Lauerlabs, Emmen, The Netherlands) equipped with fused-silica capillary of 80 cm×75 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA) was interfaced to the LCQ. Sample was introduced hydrodynamically by applying a positive pressure of 70 mbar for 12 s while keeping the voltage at the capillary tip (4 kV) and the sheath gas on. The background electrolyte (BGE) was prepared daily and was composed of 20 m*M* ammonium acetate adjusted to pH 9.5 with triethylamine. The applied voltage was 30 kV (26 kV for separation; current about 60 μA). Experiments were performed with a programmed instrument method comprising two different scan events that were applied alternately

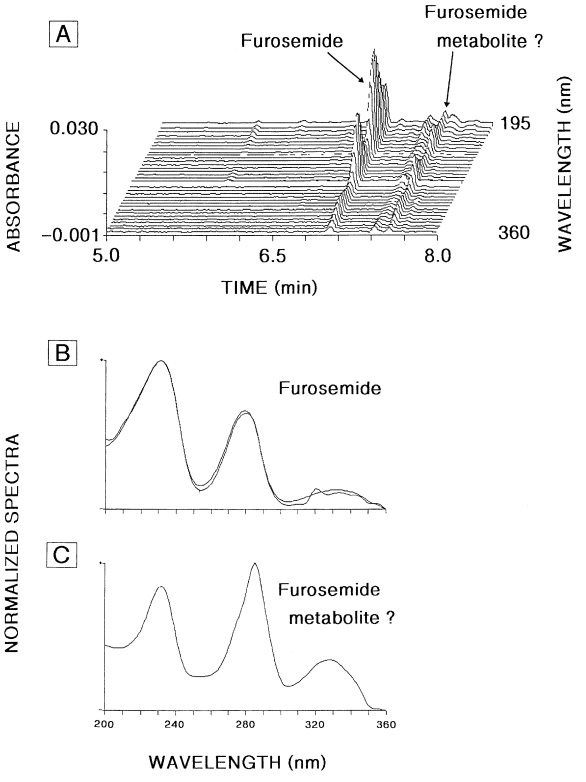

Fig. 2. (A) MECC multiwavelength absorbance electropherograms obtained on a laboratory-made instrument for the acidic SPE extract of 5 ml urine of patient urine J, (B) normalized furosemide spectrum compared to that of a standard and (C) spectrum of a possible furosemide metabolite. Sample was injected during 2 s and a constant 18 kV (current about 85 μA) was applied.

using automatic gain control (AGC). Selected reaction monitoring (SRM) using three microscans and a maximum injection time of 200 ms, a parent mass of 329.3, an isolation width of 2 Th, a relative collision energy of 35%, and a product mass of 285.2 was followed by the acquisition of a full scan mass spectrum in the mass range of 150–500 Th using three microscans and a maximum injection time of 400 ms. A whole cycle lasted no longer than 2 s.

3. Results and discussion

3.1. CE with optical detection

Using MECC with UV absorbance detection and direct urine injection was found to be unsuitable for monitoring of furosemide on a ppm concentration level. The data presented in Fig. 1 are those obtained for patient urine J with an estimated furosemide concentration of 6 μg/ml. At that level, the extracted normalized absorption spectrum was determined to be different compared to that of a standard (Fig. 1B). Even after spiking the urine with 10 μg/ml furosemide (Fig. 1D) incomplete spectral overlap was noticed (data not shown), whereas after fortification with 100 μg/ml, the spectral identity proof was complete (data not shown). Furthermore, extraction revealed the anticipated sensitivity as is documented with the data presented in Fig. 2. Moreover, a peak with a similar absorbance spectrum compared to that of furosemide was also detected (Fig. 2C). No efforts for identification of this possible furosemide metabolite or degradation product [20] were undertaken. Instead, MECC with LIF detection using a HeCd laser line of 325 nm for solute excitation and a 366 nm band filter for fluorescence detection was evaluated and found to be suitable to recognize the presence of urinary furosemide with a detection limit of about 1 μg/ml. The data presented in Fig. 3A are

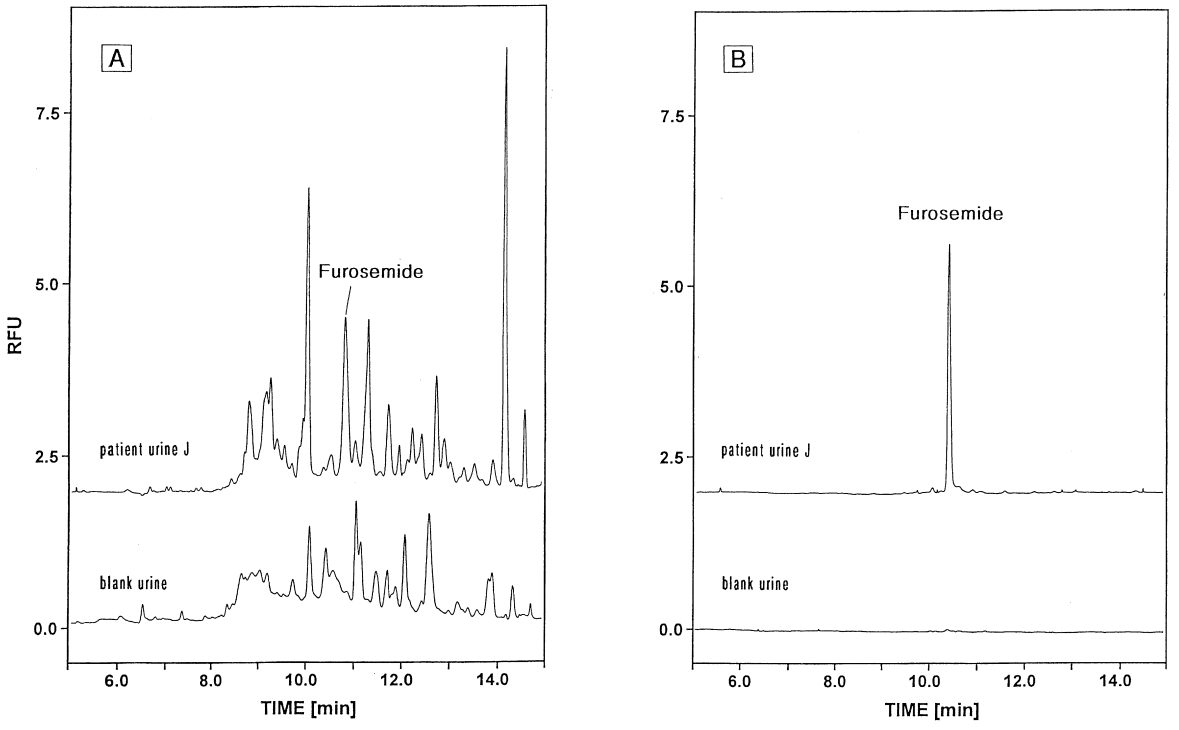

Fig. 3. MECC–LIF electropherograms obtained with a 366 nm bandpass filter for blank urine (lower graph) and patient urine J (upper graph) after injection of (A) plain urine and (B) SPE extracts prepared from 5 ml urine. Sample was injected at 0.5 p.s.i. for 1 s, the voltage applied was 8 kV (currents of 48 and 43 μA, respectively) and the cooling fluid temperature was kept at 35 °C. Graphs are presented in relative fluorescence units (RFU) with a 2 RFU *y*-axis shift.

those obtained for a urine blank (lower graph) and patient urine J which were analyzed without any sample pretreatment. Unambiguous identification of furosemide in the patient urine was thereby possible. Not surprisingly, extraction followed by analysis of the extract by MECC–LIF led to simpler electropherograms (Fig. 3B) and a much lower detection limit. Thus, MECC with LIF detection and direct urine injection could be employed for rapid recognition of furosemide present after ingestion of therapeutic amounts of the drug.

Using CZE with LIF and no sample pretreatment, the presence of 1 μg/ml of furosemide can be recognized (Fig. 4A). The data presented were obtained for a 405 nm emission. In accordance with the fluorescence spectrum of furosemide, the 405 nm filter was found to provide a higher response compared to those monitored with the band pass filters of 366 and 450 nm. Analysis of patient urine 3 did not reveal the presence of furosemide (Fig. 4A, second graph from bottom), whereas furosemide in patient urine J was clearly detected (Fig. 4A, second graph from top) and confirmed by spiking with 10 μg/ml of furosemide and reanalysis (Fig. 4A, top graph). Furthermore, data obtained for analysis of SPE extracts are presented in Fig. 4B. These data confirm the absence of furosemide in the blank urine and patient urine 3 (assessed with extracts prepared from 5 ml urine), and revealed the presence of furosemide in patient urine J (extract from 2.5 ml urine). Using 2.5 ml urine, this assay format was determined to permit the unambiguous detection of urinary furosemide concentration of 0.4 μg/ml. Comparison of the MECC data (Fig. 3) and CZE data (Fig. 4) suggests that the CZE assay is more suitable for rapid screening of urines with direct sample injection and LIF detection. Urines were also analyzed by CZE–LIF after two- to fivefold dilution with water. However, urine dilution was not found to provide an advantage.

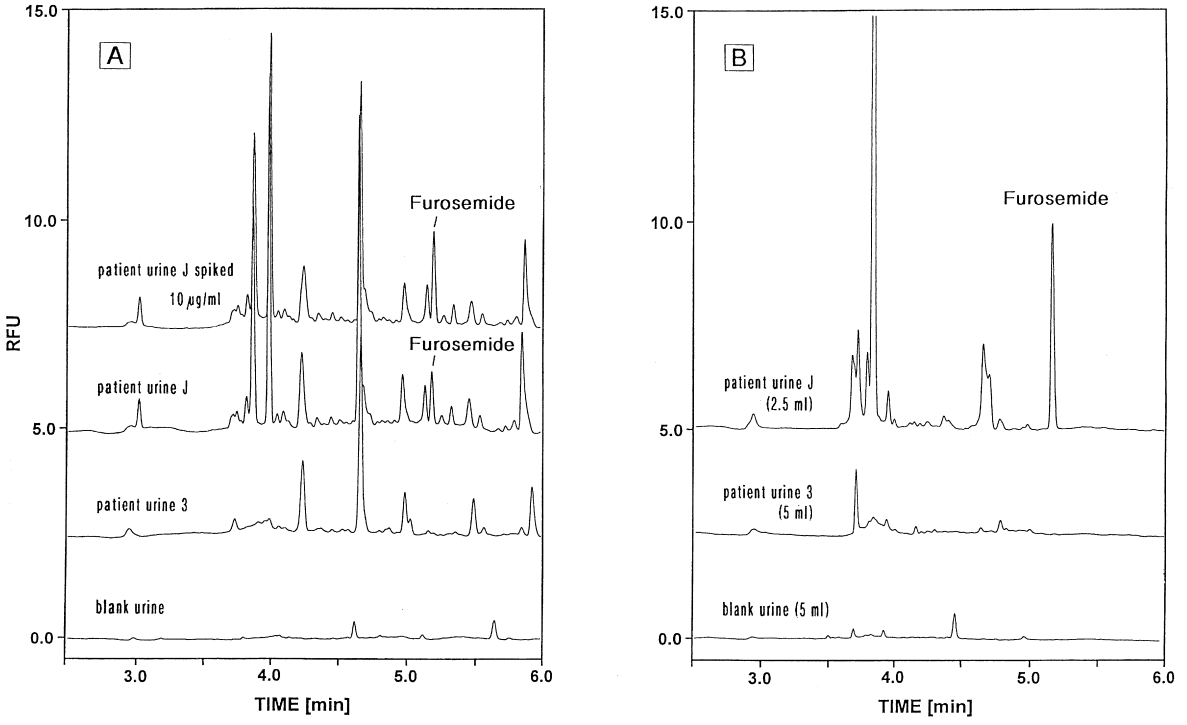

Fig. 4. CZE–LIF electropherograms obtained with a 405 nm bandpass filter for various urines after (A) direct urine injection and (B) SPE extract injection. Sample was injected at 0.5 p.s.i. for 1 s, the voltage applied was 20 kV (currents of about 45 μA) and the cooling fluid temperature was kept at 20 °C. Graphs are presented with a 2.5 RFU *y*-axis shift.

3.2. Identification and confirmation with CE–MS

For identification of urinary furosemide, diluted urines and urinary SPE extracts were analyzed by $CE–MS^2$ using the LCQ ion trap MS. A volatile running buffer composed of 20 m*M* ammonium acetate that was adjusted to pH 9.5 with triethylamine was employed. CE–MS data obtained with a standard solution of furosemide (10 μg/ml in 10-fold diluted running buffer) are presented in Fig. 5. Panel A depicts the mass trace for furosemide with *m*/*z* 329.3, whereas the mass spectrum is shown in panel B. Besides $[M-H]^-$ with *m*/*z* 329.3, ions with *m*/*z* values of $[M-H+n]$ where *n* equals 1, 2, 3 and 4 are detected as well. These masses originate mainly from the isotopes of C ($n=1$, with a relative abundance of 1.08% [21]), Cl ($n=2$, with a relative abundance of 32.5% [21]) and S ($n=1$ and 2, with relative abundances of 0.78 and 4.4%, respectively [21]) (for chemical structure of furosemide see panel A of Fig. 5). The abundance of the $[M-H+2]$ ion with *m*/*z* 331.1 is highest which is in agreement with the relative abundances. Furthermore, some fragmentation via loss of *m*/*z* 44 (neutral loss of CO_2) is also observed (*m*/*z* 285.3, 287.3).

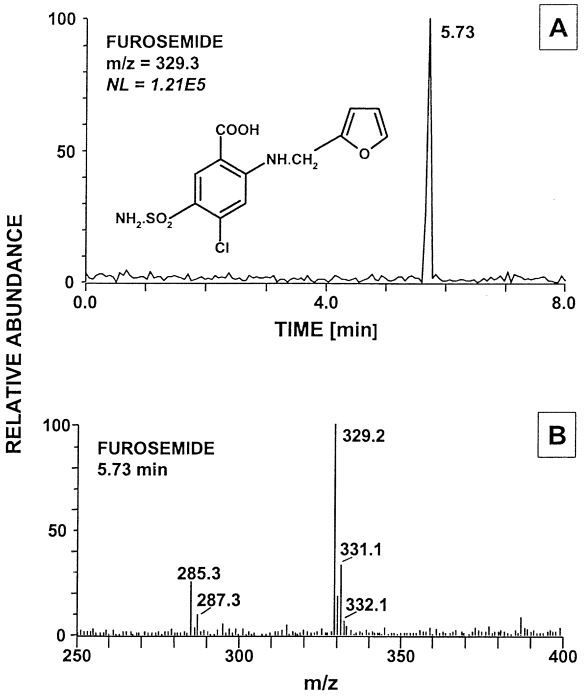

Fig. 5. CE–MS data of furosemide applied from a 10 μg/ml solution (in 10-fold diluted running buffer) with (A) mass trace for *m*/*z* 329.3 and (B) mass spectrum. Sample was injected with 70 mbar during 12 s and a constant voltage of 30 kV was applied.

The data obtained after injection of 10-fold diluted patient urine J are presented in Fig. 6. In the same run, two operational modes were employed alternately, namely SRM and full scan MS (cf. Section 2.5). The mass trace for furosemide (*m*/*z* 329.3) and the mass spectrum obtained are presented in panels A and B, respectively, of Fig. 6. The corresponding SRM data for the *m*/*z* 329.3→*m*/*z* 285.2 precursor–product ion transition (loss of CO_2) are depicted in panels C and D, respectively. In this tandem MS mode, the deprotonated furosemide ion was isolated with a 2 Th width and subjected to fragmentation with a collision energy of 35% and the fragment ion with *m*/*z* 285.2 was monitored. The presence of furosemide in patient urine J could thereby be unambiguously confirmed. Not surprisingly, the same was found to be true for analysis of twofold diluted urine (data not shown), for analysis of the SPE extract of patient urine J (Fig. 7), and for urine blank spiked with 1 μg/ml of furosemide (data not shown). To prevent an overload of the system, plain, undiluted patient urine was not applied [22]. The furosemide concentration in 10-fold diluted patient urine J is about 0.6 mg/ml (see above), a concentration that was found to be close to the detection limit when the sample is analyzed by MS only (Fig. 6A and B). Analysis of the SPE extract (Fig. 7) provided much increased responses and a mass spectrum comparable to that determined with the standard (compare Figs. 7B and 5B). Without extraction, SRM was found to permit detection of about 0.1 μg/ml of urinary furosemide (Fig. 6C). A somewhat better sensitivity is expected for operation with SRM as a single scan event. Reproducibility was determined to be comparable to that reported for opioids in the positive ion mode [22].

3.3. Screening of patient urines with CZE–LIF and CE–MS

The three patient urines for which the presence (urines J and W) or absence (urine R) of furosemide was known to the laboratory and the 10 randomly

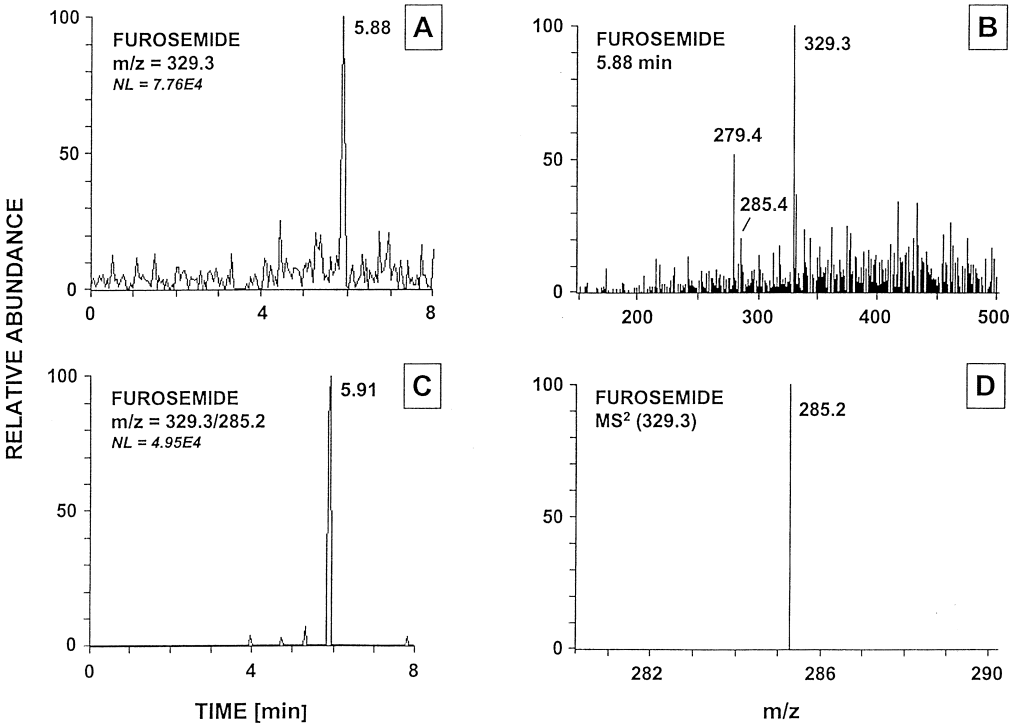

Fig. 6. (A) Mass trace, (B) mass spectrum, (C) SRM mass trace and (D) MS^2 mass spectrum of furosemide obtained by CE–MS of 10-fold diluted patient urine J.

chosen patient urines were analyzed by CZE–LIF and CE–MS/MS^2 as described above. All samples were analyzed as received (CZE–LIF) or after two- to 10-fold dilution with water (CE–MS/MS^2) and after SPE extraction from 5 ml urine and reconstitution of the dried extract in a total volume of 500 μl (CZE–LIF and CE–MS/MS^2; cf. Section 2.2). For urines J and W that stemmed from persons under furosemide pharmacotherapy, furosemide was recognized in all four approaches (for examples of electropherograms refer to Figs. 4, 6 and 7). As expected, furosemide was not detected in patient urine R. Similarly, for the 10 urines for which the history of furosemide treatment was unknown to the laboratory at the time of analysis, urines 2 and 7 were found to contain furosemide by CZE–LIF after injection of plain and extracted urine. Screening of the whole set using CE–MS/MS^2 revealed the presence of furosemide in the same two urines. No furosemide could be detected by both methods in the eight other urines (for examples of electropherograms refer to Fig. 4). Upon reporting, these screening data were found to agree with the drug history of the patients. Thus, CZE–LIF and CE–MS/MS^2 without sample extraction can be recommended for screening of patient urines. Except for one urine subjected to CE–MS, analysis of all samples was straightforward and detection times were stable. Analysis of urine 7 at various dilutions was found to require elongated run times for detection of furosemide (about 8 min instead of 5.9 min, Fig. 6). However, no changes were noted for analysis of the extract of that urine and for the two CZE–LIF runs. The origin of this behavior was not further investigated.

4. Conclusions

CZE–LIF and CE–MS^2 are demonstrated to be simple techniques that are suitable for rapid analysis of furosemide in human urine after ingestion of

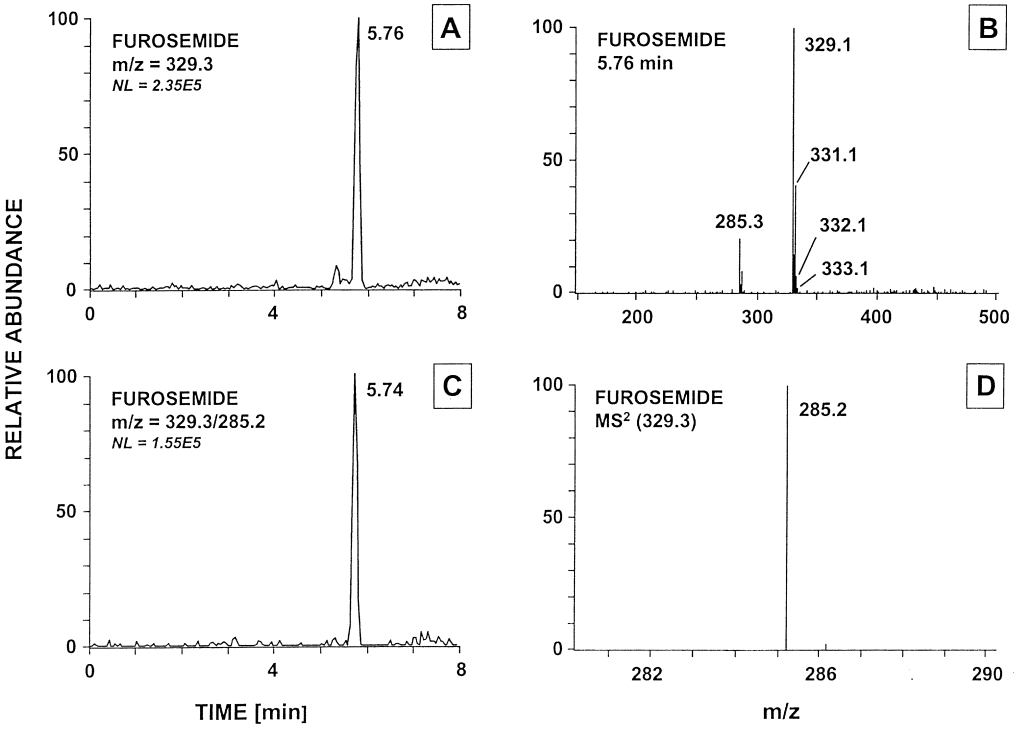

Fig. 7. CE–MS data obtained for the SPE extract prepared from 2.5 ml of urine J. (A) Mass trace, (B) mass spectrum, (C) SRM mass trace and (D) MS^2 mass spectrum.

therapeutic doses of this drug. Urines can be analyzed as received or after dilution with water which makes these assays attractive. This is a significant advantage compared to the use of CZE and MECC with UV detection, approaches that require drug extraction prior to analysis. MECC–LIF could also be used for analysis of untreated urine. Electropherograms, however, were found to be somewhat more complex compared to those obtained by CZE–LIF. CE–MS^2 provides a structural proof and is thus the most reliable among the investigated methods. Application of LIF with wavelength-resolved fluorescence detection, as was recently demonstrated for analysis of urinary salicylate and metabolites [13], would enhance the confirmation capability of CE–LIF. However, this kind of instrumentation is not commercially available. Without sample pretreatment and the experimental conditions described, detection limits for furosemide were noted to be at or slightly below the μg/ml level. This is somewhat better compared to the direct determination of bumetanide, a similar diuretic drug, in fortified urine using CZE with fluorescence detection [23], and is considered as adequate for clinical purposes, particularly for the assessment of compliance. Besides extraction with concomitant concentration, increased sensitivity could be reached with electrokinetic instead of hydrodynamic sample injection (as was demonstrated for urinary opioids [18]) and/or possibly also via lowering of the pH of the running buffer (for CZE–LIF only), approaches that remain to be investigated. Furthermore, CE–MS^2 with SRM as single scan event should provide enhanced sensitivity as well.

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References

[1] R. Ventura, J. Segura, J. Chromatogr. B 687 (1996) 127.
[2] S.P. Sood, V.I. Green, Z.M. Norton, Ther. Drug Monit. 9 (1987) 484.
[3] S.P.D. Lalljie, M.B. Barroso, D. Steenackers, R.M. Alonso, R.M. Jiménez, P. Sandra, J. Chromatogr. B 688 (1997) 71.
[4] A.C. Moffat (Ed.), Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids and Post-Mortem Material, 2nd ed., Pharmaceutical Press, London, 1986, p. 634.
[5] D.E. Smith, E.T. Lin, L.Z. Benet, Drug Metab. Dispos. 8 (1980) 337.
[6] A.L.M. Kerremans, Y. Tan, C.A.M. van Ginneken, F.W.J. Gribnau, J. Chromatogr. 229 (1982) 129.
[7] L.J.C. Love, J.J. Fett, J. Pharm. Biomed. Anal. 9 (1991) 323.
[8] A.A. Nava-Ocampo, E.Y. Velázquez-Armenta, H. Reyes-Pérez, E. Ramirez-Lopez, H. Ponce-Monter, J. Chromatogr. B 730 (1999) 49.
[9] M.-L. Riekkola, J.H. Jumppanen, J. Chromatogr. A 735 (1996) 151.
[10] J. Jumppanen, H. Sirén, M.-L. Riekkola, J. Chromatogr. A 652 (1993) 441.
[11] J. Sádecká, J. Polonský, J. Chromatogr. A 735 (1996) 403.
[12] U.B. Soetebeer, M.O. Schierenberg, J.G. Moller, H. Schulz, G. Grunefeld, P. Andresen, G. Blaschke, J. Chromatogr. A 895 (2000) 147.
[13] S. Zaugg, X. Zhang, J. Sweedler, W. Thormann, J. Chromatogr. B 752 (2001) 17.
[14] S. Zaugg, W. Thormann, J. Pharm. Biomed. Anal. 24 (2001) 785.
[15] W. Thormann, M. Lanz, J. Caslavska, P. Siegenthaler, R. Portmann, Electrophoresis 19 (1998) 57.
[16] S. Heitmeier, G. Blaschke, J. Chromatogr. B 721 (1999) 109.
[17] A. Ramseier, C. Siethoff, J. Caslavska, W. Thormann, Electrophoresis 21 (2000) 380.
[18] A.B. Wey, W. Thormann, J. Chromatogr. A 924 (2001) 507.
[19] J. Caslavska, S. Lienhard, W. Thormann, J. Chromatogr. 638 (1993) 335.
[20] T. Mizuma, L.Z. Benet, E.T. Lin, J. Chromatogr. B 718 (1998) 153.
[21] W.M.A. Niessen, in: Liquid Chromatography–Mass Spectrometry, Chromatographic Science Series, Vol. 79, Marcel Dekker, New York, 1999, p. 54.
[22] A.B. Wey, W. Thormann, J. Chromatogr. A 916 (2001) 225.
[23] E. Gonzáles, A. Becerra, J.J. Laserna, J. Chromatogr. B 687 (1996) 145.