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Direct determination of paracetamol and its metabolites in urine and serum by capillary electrophoresis with ultraviolet and mass spectrometric detection

Stefan Heitmeier, Gottfried Blaschke*

Institute of Pharmaceutical Chemistry, University of Münster, Hittorfstrasse 58–62, 48149 Münster, Germany

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Abstract

The use of capillary electrophoresis (CE) for the determination of paracetamol and its main metabolites in urine and serum is described. Due to its high efficacy, CE enables the analysis of drugs directly in complex matrices. Thus, simple, rapid and reliable assays could be developed that made use of some of the main advantages of this analytical technique. In order to prevent the peaks from tailing, a water zone was injected behind the sample. Occasionally occurring peak splittings of paracetamol were investigated and methods to suppress these splittings were developed. Paracetamol, its main metabolites, paracetamol glucuronide, paracetamol sulfate as well as paracetamol cysteinate and paracetamol mercapturate, as metabolites of the oxidative pathway were identified in urine using diode-array detection and coupling of the CE instruments to electrospray–mass spectrometry. The assays were validated. Their usefulness was demonstrated by applying them to the analysis of urine and serum samples of healthy volunteers as well as to urine samples from children under anticancer therapy.

Keywords: Paracetamol

1. Introduction

Paracetamol (acetaminophen, *N*-acetyl-*p*-aminophenol) is a widely used drug for the treatment of fever and pain [1]. Although the drug is very safe at therapeutic doses, overdoses are known to cause severe liver damage. After the application of low doses, paracetamol is primarily metabolized by conjugation to endogenous glucuronide and sulfate (Fig. 1). Only a small portion is oxidized by cytochrome P-450 enzymes, yielding the postulated reactive intermediate *N*-acetylbenzoquinoneimine (NAPQI), which is preferentially conjugated with glutathione and excreted as mercapturate and cysteinate conjugates. Excessive overdosage leads to the depletion of glutathione and, thus, to the binding of NAPQI to liver cell proteins, causing hepatic necrosis. Other metabolites, like 3-methoxy-paracetamol occur only in minor amounts [2].

The relief of postoperative or oncological pain often requires high doses of paracetamol. This has led, in particular in pediatric cases, to severe intoxications. Additionally, at high doses, paracetamol is one of the most frequently abused drugs for self poisoning [3–5]. In contrast, at low doses, paracetamol is a safe model compound for a non-invasive

*Corresponding author.

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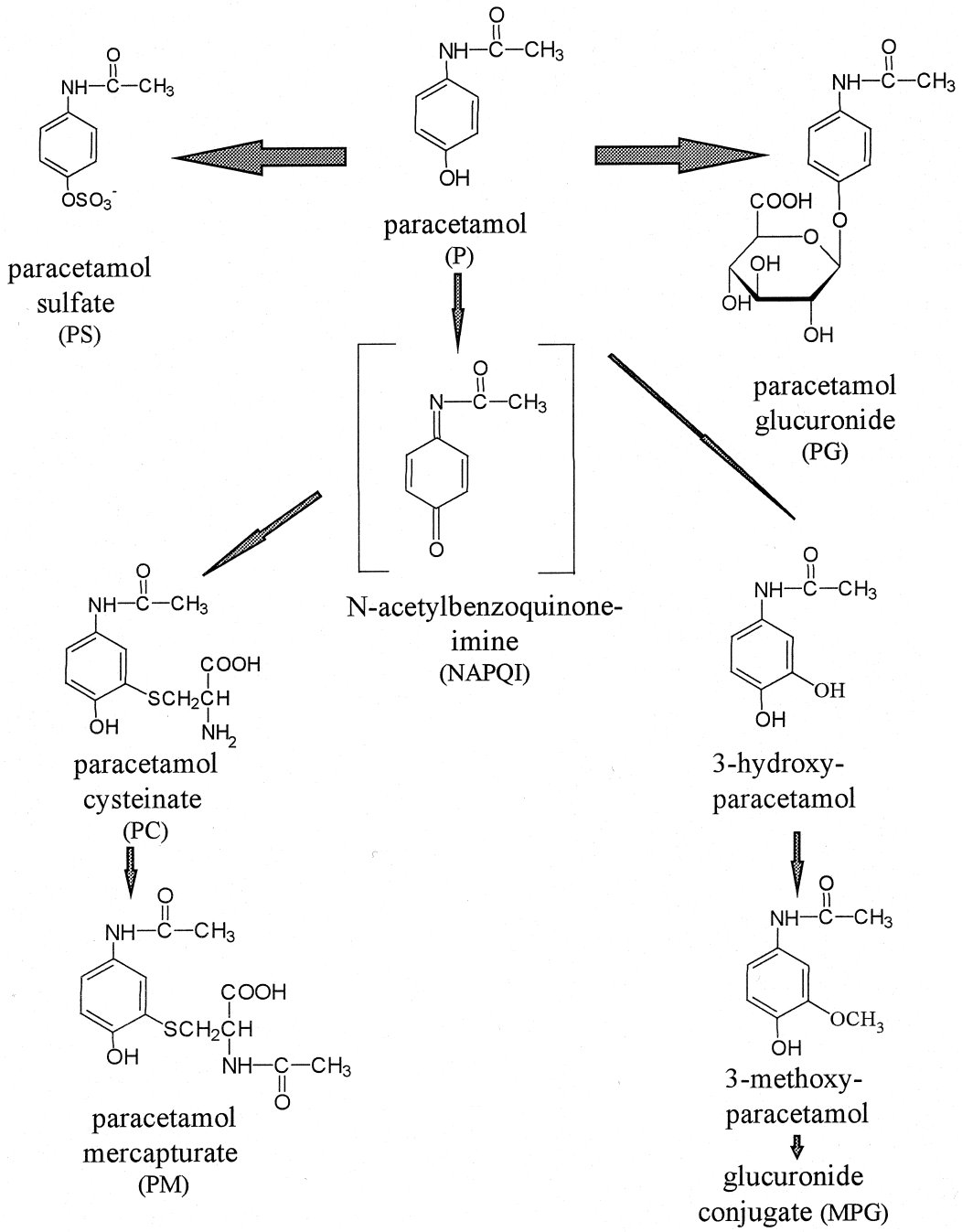

Fig. 1. Main metabolism pathways of paracetamol.

screening method to check a patient‘s glucuronidation and sulfation capacity as well as to investigate the influence of factors or compounds on the metabolic pattern in general [6–8].

Numerous analytical methods have been developed to analyze paracetamol in biological fluids, including colorimetry, spectrophotometry, thin-layer chromatography (TLC), gas chromatography (GC)

and high-performance liquid chromatography (HPLC) and several types of immunoassays [1,9]. For the simultaneous determination of paracetamol and its metabolites, mainly HPLC methods have been described [2,10–12]. However, most of these assays are complex and include the use of additives like ion pairing agents or require time-consuming sample preparation.

Capillary electrophoresis (CE) has been established as a powerful method for the analysis of drugs and their metabolites in body fluids [13–18]. In particular, charged phase-II metabolites, like glucuronides or sulfates, which are difficult to determine with other techniques, may be analyzed directly in biological samples due to the high efficacy of CE. At present, the direct determination of paracetamol itself in urine and plasma has been described [19–22].

In this paper, simple, rapid and reliable methods for the determination of paracetamol and its main metabolites in urine and serum, based on capillary zone electrophoresis (CZE), will be presented. They allow one to monitor the metabolic pattern and especially the conjugation products easily. On-line identification of the analytes in urine by diode-array detection (DAD) and CE–mass spectrometry (MS) coupling is shown and the application of the assays to samples from healthy volunteers as well as to clinical samples is presented.

2. Experimental

2.1. Chemicals and reagents

Paracetamol, paracetamol glucuronide and 3-acetamidophenol were purchased from Sigma–Aldrich Chemie (Deisenhofen, Germany). Paracetamol sulfate was synthesized as described previously [23]. The mercapturate and cysteinate conjugates were kindly donated by Novartis (Basel, Switzerland). 3-Methoxyparacetamol was prepared according to ref. [24]. The corresponding glucuronide was obtained after the biochemical reaction of 3-methoxyparacetamol and uridine-5′-diphosphoglucuronic acid in the presence of glucuronyl transferase.

Sodium tetraborate (borax), 0.1 *M* sodium hydroxide, sodium dihydrogenphosphate, disodium monohydrogenphosphate, sodium hydrogencarbonate, acetic acid, ammonium acetate, ammonium hydroxide and methanol were purchased from Merck (Darmstadt, Germany). 2-(Cyclohexylamino) ethanesulphonic acid (CHES) and 3-(cyclohexylamino)propanesulphonic acid (CAPS) were bought from Fluka (Buchs, Switzerland).

2.2. Apparatus and methods

The CE instrument used for the UV-detection experiments was a Beckman P/ACE 5510 (Beckman Instruments, Munich, Germany), equipped with a diode-array detector and GoldSoftware 8.10. Electrophoretic runs were performed in an uncoated fused-silica capillary (50 μm I.D., 40/47 or 50/57 cm effective/total length) filled with a buffer that usually consisted of 50 m*M* borax. pH-Values were adjusted by adding 0.1 *M* sodium hydroxide. The field strength was 500 V/cm. Between each run, the capillary was rinsed with 0.1 *M* sodium hydroxide for 1 min, followed by a rinse with the run buffer for 2 min. The temperature of the cooling system of the capillary was maintained at 20°C. Detection was carried out at 245 nm. Urine samples were introduced into the capillary by hydrodynamic injection with 0.5 p.s.i. (1 p.s.i.=6894.76 Pa) for 2 s, followed by the injection of water for 1 s. The serum samples were injected for 10 s.

The MS experiments were performed with a Grom capillary electrophoresis system 100 high voltage power supply (Herrenberg, Germany) with an uncoated fused-silica capillary (50 μm I.D., 44 cm length) filled with a buffer consisting of 50 m*M* ammonium acetate adjusted to pH 9.8 with 50 m*M* ammonium hydroxide. Hydrostatic injection (10 cm, 10 s) was used to introduce the samples into the anodic site of the capillary. A run potential of 15 kV was applied. An LCQ ion trap mass spectrometer (Finnigan, Branford, CT, USA) equipped with the matching commercially available electrospray interface (Finnigan) was used in the negative ion mode for the detection of the analytes. The sheath liquid, consisting of 50% methanol–49% water–1% ammonia was delivered at a flow-rate of 6 μl/min using a syringe pump.

2.3. Sample preparation

Urine samples were prepared by adding 20 μl of a 3-acetamidophenol solution containing 2 mg/ml in

water to 80 μl of human urine. Standard solutions were made by evaporating aliquots of the methanolic stock solutions of the analytes and reconstituting them in blank human urine.

Three different methods of sample preparation were investigated for the determination in serum samples:

- (a) Direct determination in a separation buffer (50 m*M* borax, pH 9.4, 100 m*M* SDS). For this method, no other preparation of the serum sample apart from the addition of the internal standard was necessary.
- (b) Determination after precipitation of the proteins: A 0.25-ml serum sample was mixed with 0.75 ml of acetonitrile at −20°C. After centrifugation for 10 min at 1200 *g*, 0.8 ml of the supernatant were evaporated under a stream of nitrogen. The residue was reconstituted in 50 μl of water.
- (c) Determination after ultrafiltration: To 50 μl of serum sample, 12.5 μl of a 3-acetaminophenol solution (80 μg/ml) in water was added. This mixture was filtered through a 30-kDa ultrafiltration membrane in an Eppendorf cap by centrifugation (10 min, 1200 *g*). The filtrate was analyzed as described above. Standard solutions were prepared by evaporating aliquots of methanolic solutions of the analytes under a gentle steam of nitrogen and reconstituting them in blank human serum.

2.4. Assay validation

The CE–UV assays developed in Sections 2.2 and 2.3(c) were validated.

Five blank urine samples, which were spiked with the analytes at concentrations of 7.5, 15, 45, 75 and 150 μg/ml for paracetamol, 12.5, 50, 200, 600 and 2000 μg/ml for paracetamol glucuronide, 18.75, 37.5, 150, 450 and 1500 μg/ml for paracetamol sulfate and 8, 12, 36, 60 and 120 μg/ml for paracetamol cysteinate and paracetamol mercapturate were analyzed five times to investigate the precision and accuracy. The absence of matrix interferences was confirmed by analysis of blank urine samples.

Five spiked blank serum samples at concentrations of 1.25 (paracetamol) or 2.5 (paracetamol glucuronide and paracetamol sulfate), 5, 10, 25 and 50 μg/ml per analyte were prepared according to Section 2.3(c) and analyzed five times to determine the precision and accuracy of the assay. No matrix interferences were observed when blank serum samples were analyzed.

3. Results and discussion

3.1. Development of the assays

Due to its phenolic function, paracetamol as well as its metabolites migrated as anionic species when basic separation buffers were used. Borax buffers were found to be the most suitable under these conditions. Other separation buffers containing phosphate, carbonate, acetate, CHES and CAPS were tested as well between pH values of 9 and 11. Although separations were obtained with all of them, only the performance with a CAPS buffer at pH 10.9 revealed results that were almost comparable to those obtained with a borax buffer.

For a simple assay in urine, conditions were found that allow the determination of paracetamol and its metabolites after direct injection of the sample without a need for prior sample preparation. High salt contents in urine samples might have caused peak-broadening based on electrodispersion if the separation buffers had low ionic strengths. Thus, in order to avoid effects caused by high and widely differing salt concentrations, buffers with high ionic strengths, like those of 50 m*M* borax or 100 m*M* CAPS, and short sample zones were used. A field strength of 500 V/cm was a good compromise between a shorter analysis time at higher voltages and minor Joule heating and, therefore, sharper peaks at lower voltages.

The impact of capillary length on the separation was investigated as well. Overloading phenomena and peak splitting phenomena were more pronounced in short capillaries. Therefore, 40/47 cm (effective/total length) or 50/57 cm capillaries were used. The optimal internal diameter of the capillary was determined to be 50 μm. Capillaries with wider internal diameters revealed a loss of peak separation

due to the complex matrix in urine. Similar problems occurred when bubble cell capillaries were used.

3.1.1. Choice of the injection site

In CE, samples can be injected at the anodic as well as at the cathodic site. After injection at the anodic site of an uncoated capillary, paracetamol in its anionic form was transported against its own migration direction towards the detection window near the cathode by the countermigrating electroosmotic flow (EOF). Thus, paracetamol and its metabolites were detected after the neutral compounds in urine, which migrated with the velocity of the EOF (Fig. 2A).

To determine paracetamol after injection at the cathode, the EOF was modified by using spermine tetrahydrochloride as a buffer additive. This resulted in reversal of the migration order of the analytes, with the neutral compounds being detected as the last peak (Fig. 2B).

However, due to the easier buffer preparation and the better separation of the metabolites, injection at the anodic site was preferred in this case.

3.1.2. Use of a water zone for peak focusing

When the samples were injected at the anodic site, a water zone was injected behind. This prevented peak tailing phenomena, as presented in Fig. 3A for paracetamol glucuronide and paracetamol sulfate.

This focusing of the peaks may be explained by stacking processes. Sample stacking procedures are routinely used to improve the limit of detection

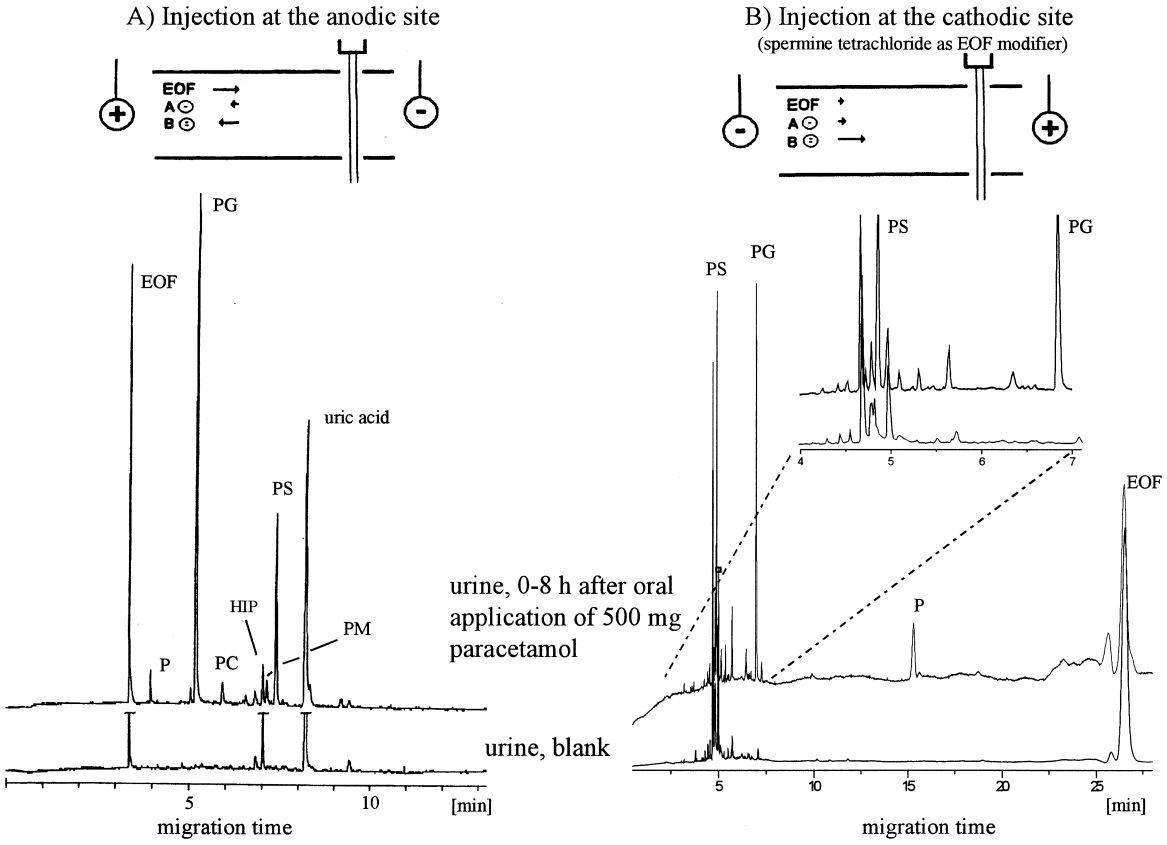

Fig. 2. Electropherograms of a urine sample, 0–8 h after oral application of 500 mg of paracetamol, and a blank urine sample after injection at the anodic (A) and the cathodic (B) site. Conditions: (A) buffer, 50 m*M* borax, pH 9.4; capillary, 40/47 cm (effective/total length); field strength, 500 V/cm; injection, hydrodynamically, 2 s. (B) Same as (A), apart from the addition of 10 m*M* spermine tetrahydrochloride to the buffer and the reversal of the field strength.

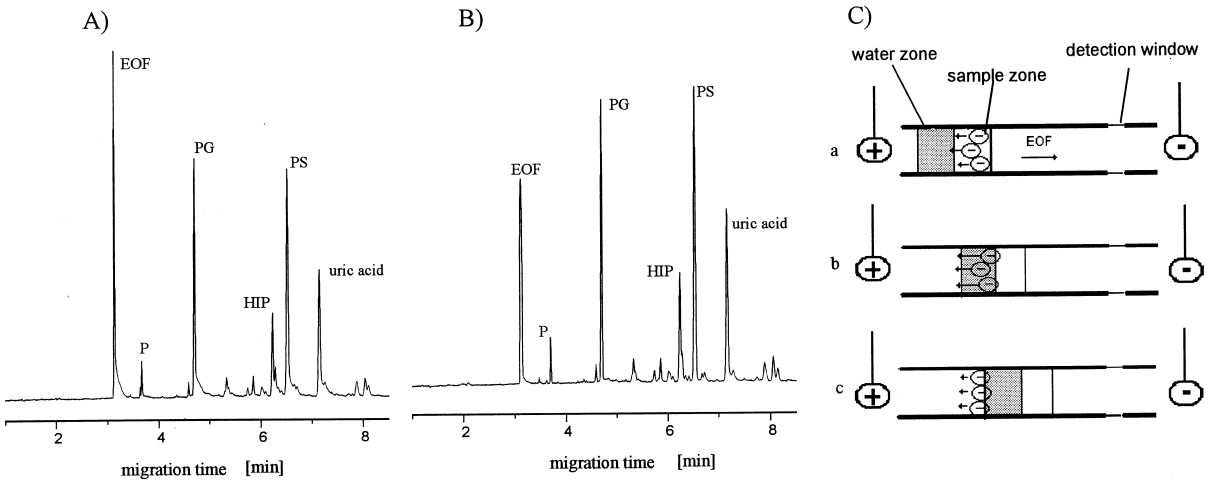

Fig. 3. Effect of the water zone injected behind the sample on the peak shape. For conditions in (A) and (B), see Fig. 2A, apart from the additional injection of a water zone for 1 s in (B). In (C), the effect of the water zone is discussed theoretically. Further explanations are in the text.

[25,26]. Due to the different ionic and field strengths in the sample zone and the water zone, the analytes were accelerated after the transition from the sample zone into the water zone (Fig. 3C, b). When entering the separation buffer, the analytes were decelerated (Fig 3C, c). This caused the focusing of their zones. Since the net migration of the anionic analytes was directed against their total migration direction, by sharpening the front boundary of the analyte zone, the peak tailing was suppressed.

3.1.3. Investigation of peak splitting phenomena

When 50 m*M* borax, pH 9.4, was used as the separation buffer for the analysis of aqueous standard solutions or biofluids, paracetamol occasionally occurred in two peaks (Fig. 4A, a). In further experiments with other phenolic compounds, such as phenol, *p*-aminophenol, *p*-nitrophenol and 2-naphthol, similar peak splitting phenomena were observed. With other separation buffer compounds, such as phosphate, carbonate or CHES, no splitting was found.

At first, this phenomenon was explained by the existence of different phenolic borate esters [27]. However, in this case, the peak splitting of paracetamol appears to be generated by electrochemical effects at the zone boundaries. In order to investigate this phenomenon, we used a standard method with a 50-m*M* borax buffer, pH 9.4, at 500 V/cm in a fused-silica capillary (40/47 cm, 50 μm I.D.) and an injection of an aqueous solution of paracetamol for 2 s and varied each of these conditions.

When paracetamol was dissolved in separation buffer (50 m*M* borax, pH 9.4; Fig. 4A, b) instead of in water, the peak splitting disappeared due to the absence of zone boundaries. This effect does not depend on the pH value of the sample zone as demonstrated using a buffer pH of 9.4 (Fig. 4A, b) and 7 (Fig. 4A, c) in the sample. In contrast, when a 50 mM phosphate buffer was used in the sample zone, the splitting was observed again (Fig. 4A, d) thus revealing that at a separation buffer 50 mM borax pH 9.4 the splitting of paracetamol could only be suppressed by using a buffer with the same borax concentration in the sample zone.

To overcome the peak splitting, for the direct analysis of urine samples, investigations were conducted with an aqueous solution of paracetamol (50 μg/ml) that included variations of the pH (Fig. 4A, b and c), ionic strength (Fig 4B) and injection time (Fig. 4C) of the sample as well as the pH (Fig. 4D) and ionic strength (Fig. 4E) of the separation buffer and the capillary length. The paracetamol concentration in the sample had almost no effect on the splitting between 5 and 100 μg/ml.

Optimized conditions were obtained in a 50/57

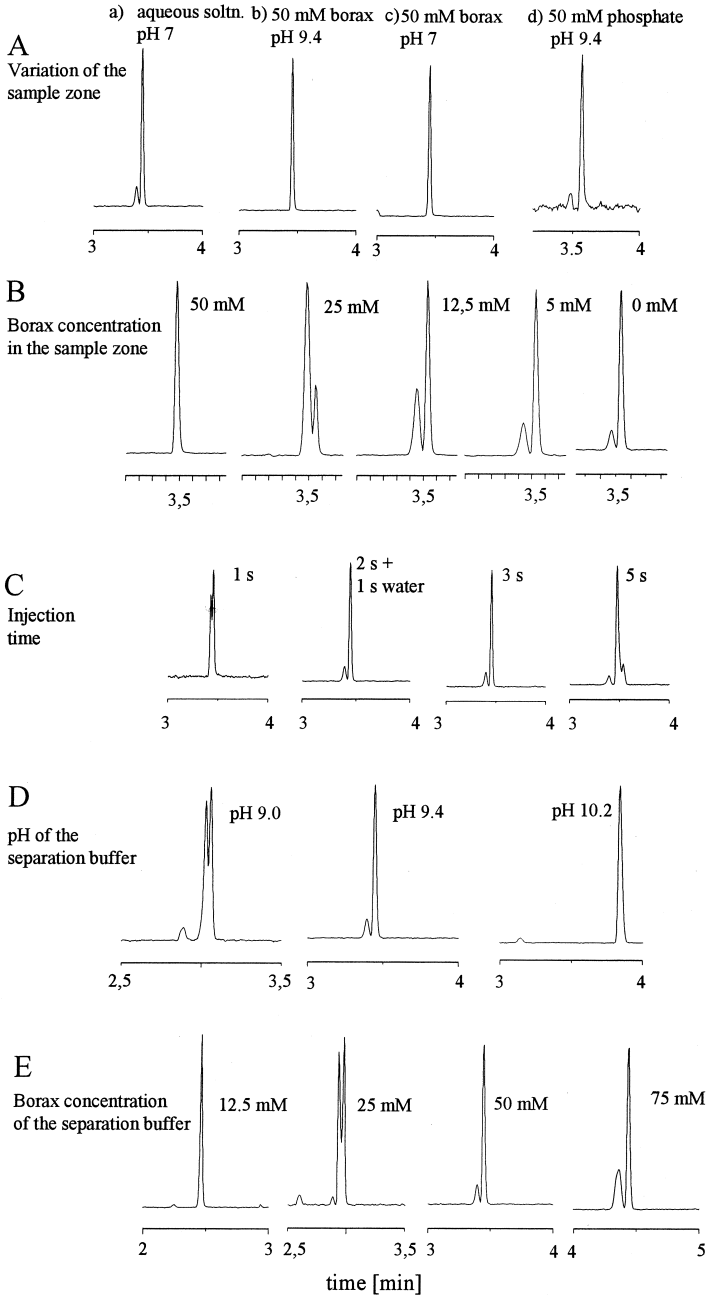

Fig. 4. Investigation of the peak splitting of paracetamol. Basing on the standard conditions (50 m*M* borax, pH 9.4, 40/47 cm capillary with 50 μm I.D., 500 V/cm, injection of an aqueous sample of paracetamol for 2 s), each of the parameters named at the beginning of each line was varied.

cm capillary with 50 m*M* borax, pH 10.6, as the separation buffer, when the sample was introduced for 2 s with a 1-s water zone.

3.1.4. Sample preparation for the determination of paracetamol and its main metabolites in serum

In contrast to urine samples, it is not the complexi-

ty of the matrix, but rather the proteins in serum samples that raise problems in biomedical assays. In uncoated capillaries, they adsorb on the capillary wall and thus affect the EOF and the separation. This causes non-reproducible results, so that it is impossible to determine the analytes directly by CZE.

Three different ways were examined in order to overcome these problems.

The proteins are known to form complexes with sodium dodecyl sulfate (SDS) at higher concentrations, and do not become adsorbed on the capillary wall. Therefore, when a micellar electrokinetic chromatography (MEKC) method is used, analytes can be determined in a migration window separated from the protein–SDS complexes, as described for paracetamol [20]. Experiments with a 50-m*M* borax buffer, pH 9.4, containing 100 m*M* SDS revealed that paracetamol, paracetamol glucuronide and paracetamol sulfate could be detected after direct injection. However, the reproducibility with normal rinse steps was not satisfactory. In the literature, a MEKC method with higher SDS concentrations and extensive rinsing procedures has been described [21]. As noted, time-consuming rinse steps were necessary to obtain acceptable reproducibilities. In this case, a direct analysis time became impractical because of the long total analysis time.

Another approach to solve the problems caused by proteins is their precipitation by organic solvents. After the addition of cold acetonitrile to the serum samples, the proteins were precipitated and removed by centrifugation. Since direct injection of the supernatant led occasionally to a breakdown of current, the solution was evaporated first, reconstituted in water and then analyzed in a separation buffer consisting of 50 m*M* borax. Under these conditions paracetamol and its main metabolites, paracetamol glucuronide and paracetamol sulfate, could be analyzed.

A suitable method to remove the proteins physically is ultrafiltration. The filtrates obtained with 30 kDa filtration membranes were analyzed directly. Since the drug–protein bonds are kept intact, this is the only method to measure the free concentration of paracetamol and its metabolites in serum. Whether the determined absorbances are applied to a standard curve obtained by adding the standard solutions to the blank serum filtrate or to native blank serum, the free as well as the total concentration can be determined.

In our experiments, standard solutions were prepared of native blank serum samples. The ultrafiltration step was performed afterwards. Therefore, the total concentrations were determined in this case (Fig. 9).

3.2. Peak identification

Beside spiking biological samples with the authentic compounds and glucuronidase–sulfatase experiments to identify glucuronides and sulfates, detection modes were used that collect additional data on the detected compounds.

With a diode-array detector, on-line UV spectra (200–400 nm) were recorded of each peak. Fig. 5 shows the electropherograms of a urine sample, 5–7 h after the administration of 1000 mg of paracetamol (2 tablets) (A) and of a blank urine sample (B). The UV-spectra of the compounds in the urine sample were plotted, together with the spectra of the authentic compounds. Good correlations were found between the reference spectra and the UV spectra of the corresponding peaks in urine. Paracetamol and its metabolites, as well as hippuric acid and uric acid, could be identified by this procedure.

Even more specific data for peak identification can be obtained by coupling the CE system to an electrospray mass spectrometer [28–30]. In order to avoid the appearance of non-volatile compounds in the MS equipment, the non-volatile borax buffer was substituted by a volatile 50 m*M* ammonium acetate buffer, pH 9.8. To the sheath liquid, ammonia was added so that the acidic compounds were entirely ionized and could be detected in the negative ion mode. In Fig. 6 the direct analysis of a urine sample by CE–ESI–MS is presented. Paracetamol and its metabolites were identified by the peaks in the reconstructed ion electropherograms (RIEs) of their deprotonated species (Fig. 6A). The mass spectra recorded at the apex of these peaks are shown in Fig. 6B. Apart from the permanent mass peaks of acetate ($M-1=59$, $2M-1=119$, $3M-1=179$), the expected mass peaks of the analytes had the highest abundance.

Under CE conditions, the endogenous creatinine, as a neutral compound, migrated with the EOF. In

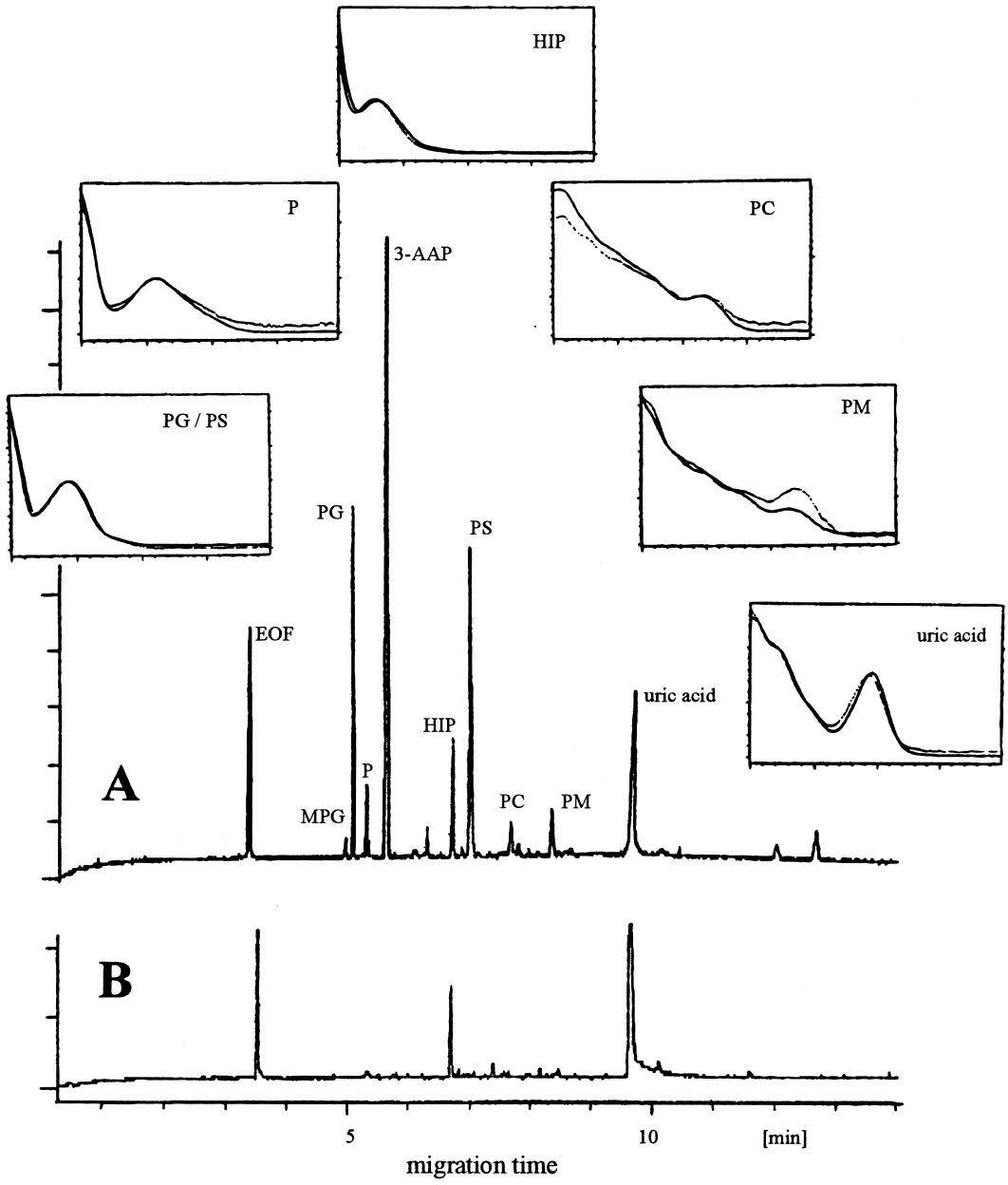

Fig. 5. Electropherograms of a urine sample, 5–7 h after the administration of 1000 mg of paracetamol to a healthy volunteer (A) and of a blank urine sample (B). Conditions: buffer, 50 m*M* borax, pH 10.6; field strength, 500 V/cm; capillary, 50/57 cm; injection, hydrodynamically, 2 s+1 s water zone. The UV spectra were recorded with a DAD (200–400 nm). The UV spectra of the peaks in urine are compared to the corresponding reference substances.

the spray in contact with the alkaline sheath liquid, it was deprotonated and could be detected in the negative ion mode. Thus, it served as an EOF marker.

Although changes had to be carried out to transfer the CE–UV method into a suitable CE–MS method, the migration order of the peaks remained almost constant. A buffer pH of 9.8 was chosen due to

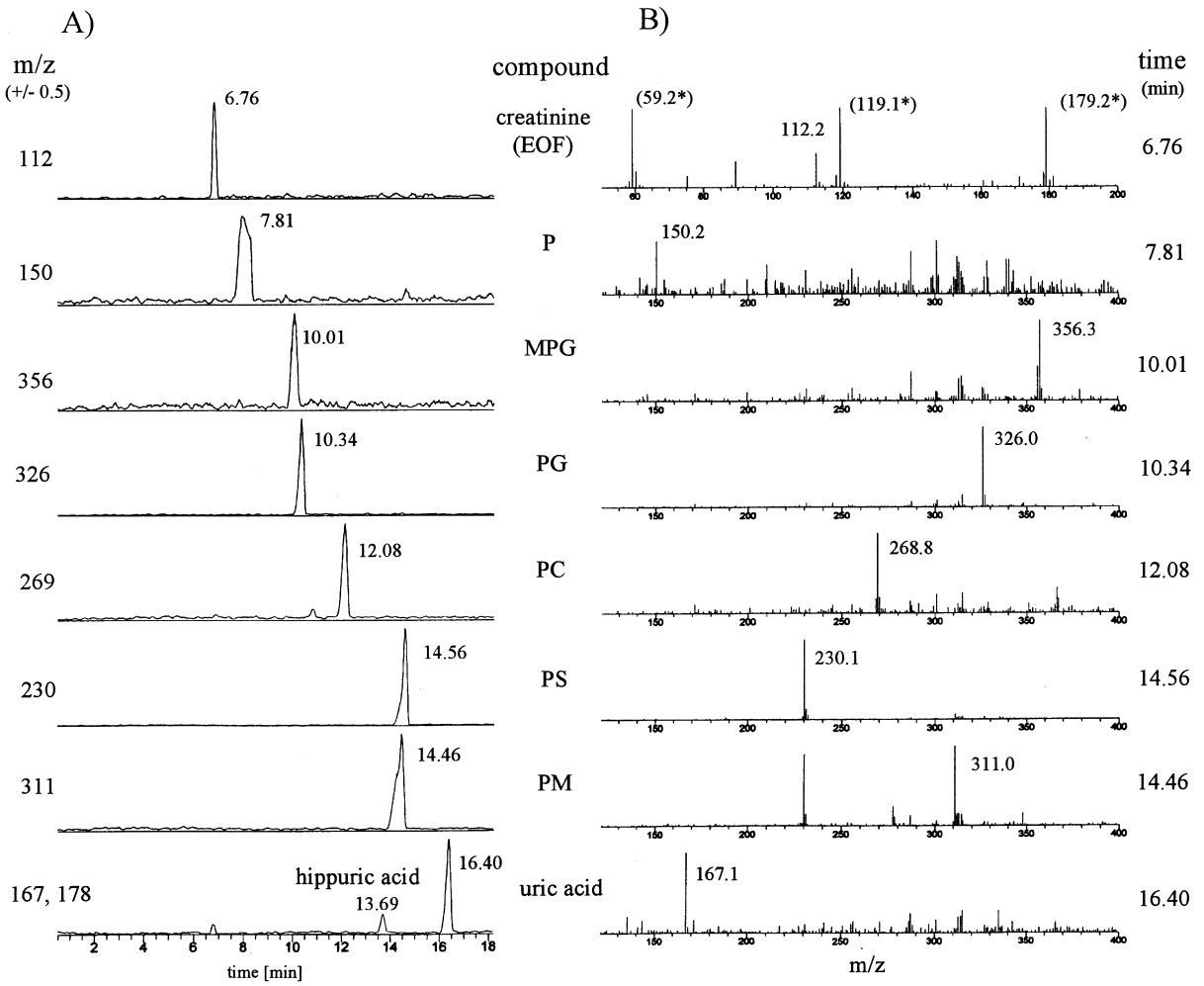

Fig. 6. Direct analysis of urine samples after the application of paracetamol by CE–ESI–MS. In (A), reconstructed ion electropherograms (RIEs) of the deprotonated species of paracetamol and its metabolites are presented. (B) shows the mass spectra at the apex of the peaks in the RIEs. Conditions are as described in the text.

CE–UV experiments with ammonium acetate buffers at pH 8.5–10.5, which revealed that this pH gave the best separations in CE–UV experiments with comparable migration times to that of the borax buffer, pH 9.4.

Due to its high selectivity, rapidness and simplicity, this method is very useful for the investigation of complex samples, for example, in cases of unknown intoxications or multi-medicated patients.

3.3. Validation of the assays

3.3.1. Linearity

Standard solutions with five different concentrations in the range between 7.5 and 150 μg/ml for paracetamol, 12.5 and 2000 μg/ml for paracetamol glucuronide and 18.75 and 1800 μg/ml for paracetamol sulfate, 8 and 120 μg/ml for paracetamol mercapturate and paracetamol cysteinate in urine

Table 1
Typical calibration parameters (mAU vs. μg/ml) of paracetamol and its metabolites in urine

Compound	Slope (mean±SD)	Intercept (mean±SD)	Correlation coefficient (*r*)
P	2.059±0.0121	0.0018±0.0025	0.9997
PG	0.906±0.0059	0.0069±0.0062	0.9996
PS	1.161±0.0022	0.0063±0.0017	0.9998
PC	1.108±0.0054	0.0111±0.0031	0.9993
PM	0.943±0.0057	0.0123±0.0028	0.9991

samples and five different concentrations in the range between 1.25 and 50 μg/ml for paracetamol, paracetamol glucuronide and paracetamol sulfate in serum samples were analyzed. 3-Acetaminophenol was used as an internal standard to enhance the reproducibility of the injection system. Linear correlations were found between the concentrations and the corrected peak areas. The parameters of the linear regression for paracetamol and its metabolites are given in Tables 1 (urine samples) and 2 (serum samples).

3.3.2. Precision and accuracy

Blank urine and serum samples spiked with five different concentrations of paracetamol, paracetamol glucuronide, paracetamol sulfate, paracetamol cysteinate and paracetamol mercapturate (last two only in urine samples) were analyzed five times, respectively. The concentrations were calculated from the corrected peak area ratios of the analytes and the internal standard. For drugs in biological fluids, precision and accuracy should always be within ±15%, except at the lower limit of quantification, where they should not deviate by more than ±20%. The results are shown in Tables 3 (urine samples) and 4 (serum samples). The calculated values for precision and accuracy are all within the commonly accepted limits.

3.3.3. Sensitivity

The limits of detection, defined as the concentration where the signal-to-noise ratio is 3:1, were found to be 2 (urine) and 0.5 μg/ml (serum) for paracetamol, 5 (urine) and 1 μg/ml (serum) for paracetamol glucuronide and paracetamol sulfate. The limits of quantification were 7.5 (urine) and 1.25 μg/ml (serum) for paracetamol, 8 μg/ml for paracetamol cysteinate and paracetamol mercapturate in urine, 12.5 (urine) and 2.5 μg/ml (serum) for paracetamol glucuronide, and 18.75 (urine) and 2.5 μg/ml (serum) for paracetamol sulfate, when defined as the lowest concentration that can be measured with acceptable precision and accuracy.

3.4. Application to human samples

In order to demonstrate the applicability of the assay, it was used to analyze urine samples of a healthy volunteer after the application of 1000 mg of

Table 2
Typical calibration parameters of paracetamol and its metabolites in serum (AU vs. μg/ml)

Compound	Slope (mean±SD)	Intercept (mean±SD)	Correlation coefficient (*r*)
P	0.0571±0.00068	0.0366±0.017	0.9991
PG	0.0218±0.00054	0.0297±0.014	0.9962
PS	0.0163±0.00035	0.0020±0.0041	0.9972

Table 3
Precision and accuracy of paracetamol and its metabolites in urine ($n=5$)

Compound	Concentration added (μg/ml)	Concentration found (μg/ml)	RSD (%)	Accuracy (%)
P	7.5	7.70	13.7	102.9
	15	14.55	6.6	97.0
	45	43.81	2.4	97.4
	75	76.01	1.4	101.4
	150	148.8	0.6	99.2
PG	12.5	12.23	7.7	97.8
	50	47.77	4.6	95.5
	200	199.7	1.7	99.9
	600	602.5	1.5	100.4
	2000	1989	0.7	99.4
PS	18.75	16.06	14.1	85.6
	37.5	40.08	5.7	106.9
	150	154.3	1.5	102.8
	450	455.1	1.3	101.1
	1500	1505	1.5	100.3
	8	9.53	12.4	119.1
PC	12	14.47	3.0	96.5
	36	38.01	1.7	105.6
	60	58.95	1.4	98.2
	120	119.2	1.2	99.3
PM	8	9.32	13.3	116.5
	12	12.27	3.9	102.3
	36	36.78	1.6	102.2
	60	59.15	1.6	98.6
	120	120.4	1.6	100.4

Table 4
Precision and accuracy of paracetamol and its metabolites in serum ($n=5$)

Compound	Concentration added (μg/ml)	Concentration found (μg/ml)	RSD (%)	Accuracy (%)
P	1.25	1.02	8.3	81.6
	5	4.76	1.6	95.1
	10	10.48	4.2	104.8
	25	25.19	1.8	100.8
	50	49.27	0.7	98.6
PG	2.5	2.75	9.3	110.2
	5	5.24	3.9	104.7
	10	10.82	2.9	108.2
	25	25.25	2.6	101.0
	50	48.87	0.8	99.7
PS	2.5	2.34	7.1	93.7
	5	5.54	5.8	110.8
	10	9.90	6.4	99.0
	25	24.98	2.9	99.9
	50	50.16	1.3	100.3

paracetamol. Electropherograms A and B in Fig. 5 show the analysis of a urine collection interval, 5–7 h (A) and a blank urine (B). In both samples, the endogenous compounds hippuric acid (HIP) and uric acid as well as neutral substances like creatinine (migrating with the EOF) were detected. Additionally, in electropherogram A, paracetamol, paracetamol glucuronide, paracetamol sulfate, paracetamol cysteinate, paracetamol mercapturate and 3-methoxy-paracetamol glucuronide could be found.

The excretion rates of the metabolites in different urine collection intervals of three healthy volunteers after the application of paracetamol are presented in Fig.7. They confirm the possible interindividual differences in the metabolic pattern of paracetamol and its main metabolites. The analyses of the collection intervals in Fig. 7A and B were carried out five times yielding error bars which indicate that the assay worked with satisfactory precision under these typical bioanalytical conditions. In the diagram in Fig. 7C, analysis of the minor metabolites paracetamol cysteinate and paracetamol mercapturate is included. In relation to the main metabolites, paracetamol glucuronide and paracetamol sulfate, paracetamol cysteinate and paracetamol mercapturate were excreted in very small amounts.

Deviating from this, the analysis of randomly collected urine samples from different patients under oncological therapy, who receive different cytostatics and were co-medicated with paracetamol show higher ratios of these metabolites (Fig. 8). These are probably caused by the higher doses that had to be applied to the patients and may be used as indicators for an increasing risk of intoxication due to the higher amount of NAPQI metabolically formed in the liver.

The application of the assay in serum is presented in Fig. 9. In (A) and (B), electropherograms of serum samples are presented, which were taken from a healthy volunteer 0 and 60 min after the administra-

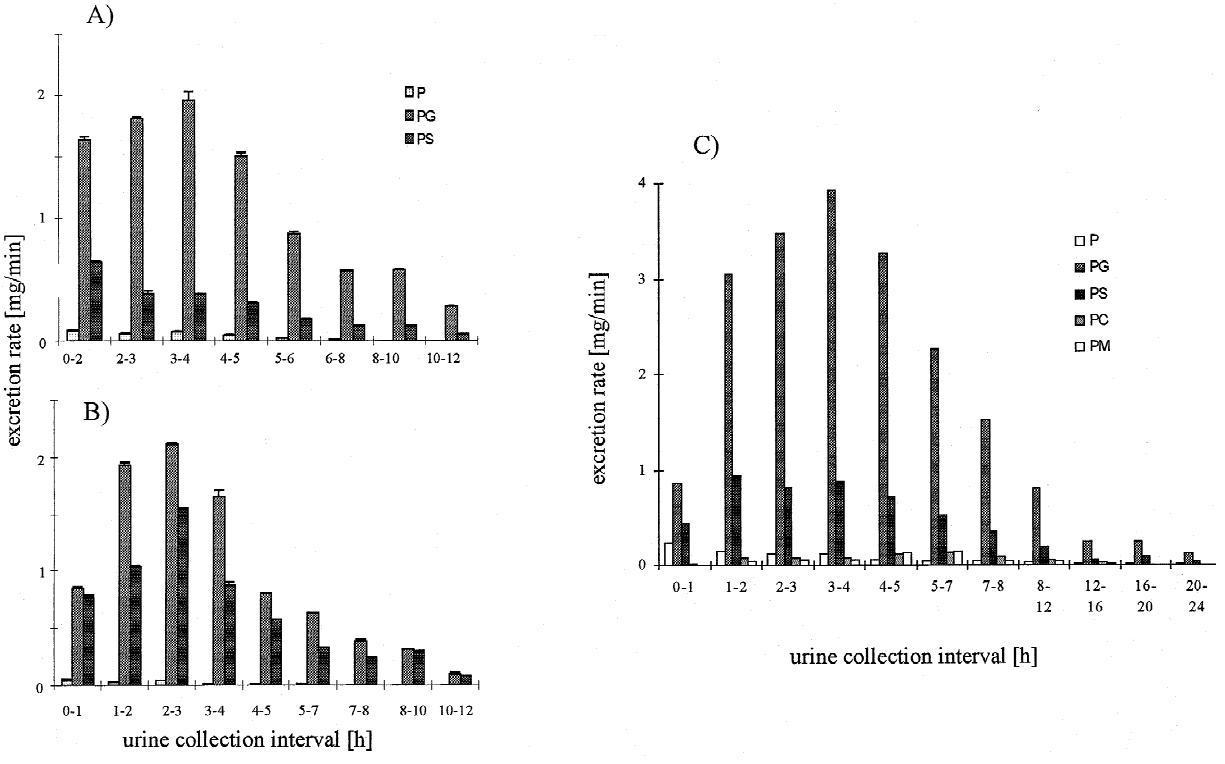

Fig. 7. Determination of the excretion rates of paracetamol and its metabolites in urine collection intervals after the administration of 500 mg (A and B) and 1000 mg (C) to three different healthy volunteers.

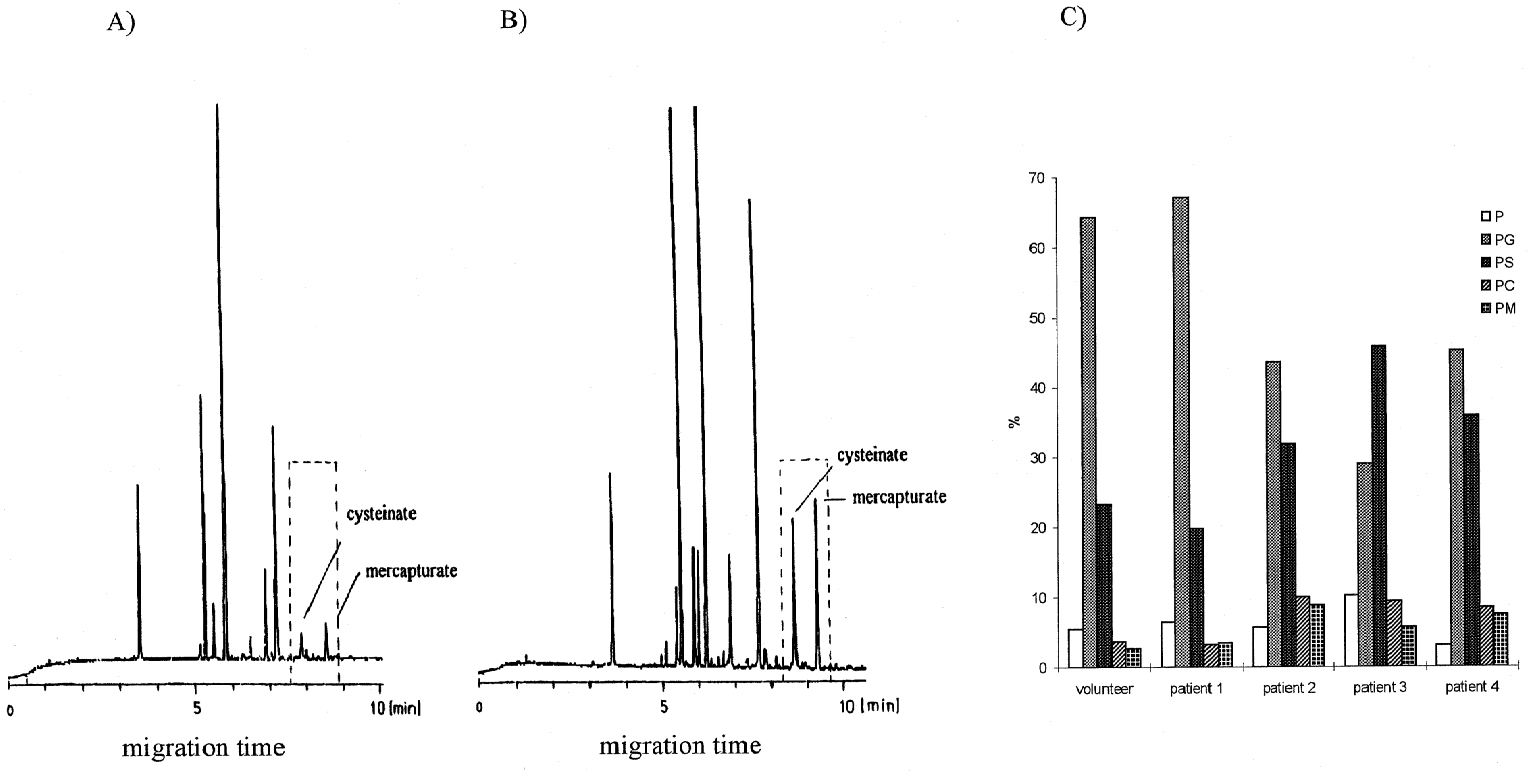

Fig. 8. Comparison of samples from a healthy volunteer with urine samples from patients in anticancer therapy. (C) shows the metabolic pattern of a healthy volunteer and different patients under oncological therapy.

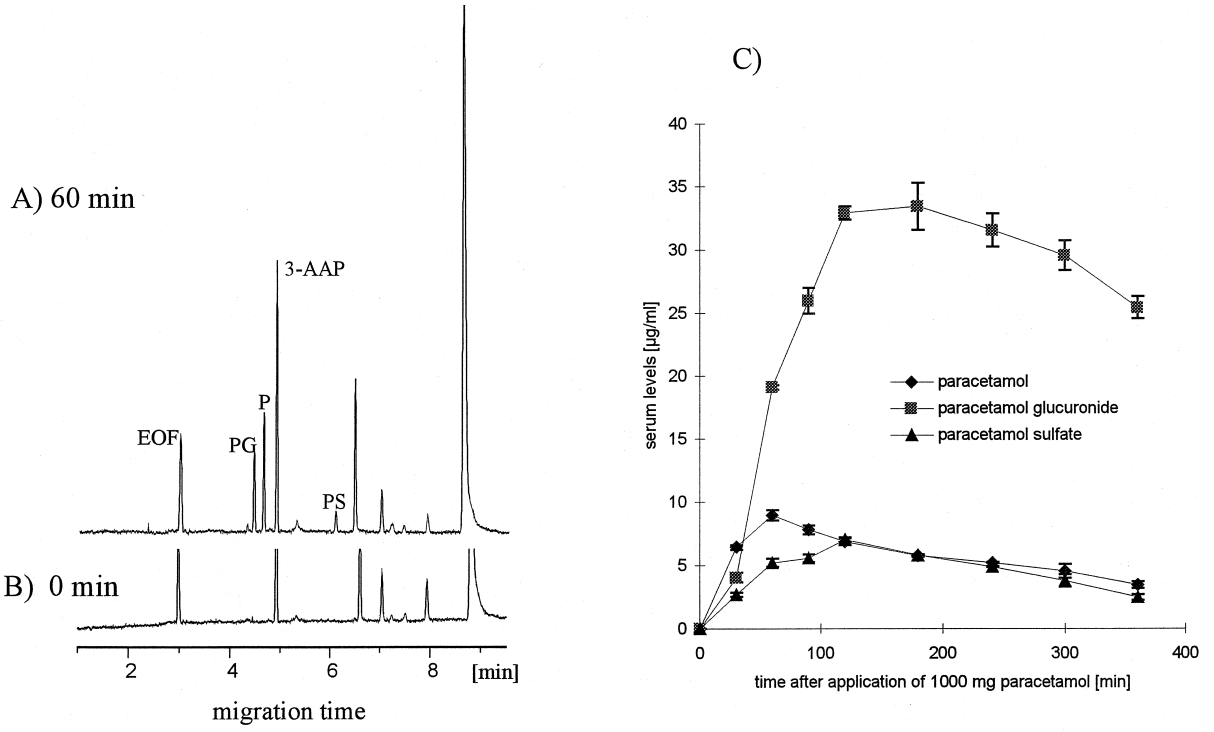

Fig. 9. Electropherograms (A and B) of serum samples obtained 0 (B) and 60 min (A) after oral administration of 1000 mg of paracetamol to a healthy volunteer (for conditions: see Fig. 5, apart from: capillary, 40/47 cm). (C) shows the serum levels of paracetamol and its main metabolites from 0 to 6 h after the administration.

tion of 1000 mg of paracetamol. In Fig. 9C, the serum levels of paracetamol, paracetamol glucuronide and paracetamol sulfate and their time-dependence are presented.

4. Conclusions

In this paper, the capabilities of CE for the determination of drugs and their metabolites in body fluids are demonstrated. In urine samples, paracetamol and its main compounds could be determined directly by a simple, rapid and reliable assay. In particular, the good separations of the conjugates reveal the advantages of this analytical method for the investigation of phase-II metabolites. The injection of a water zone behind urine samples prevented the peaks from tailing and, thus, supported the direct determination in complex media like urine.

The use of CE–MS and CE–DAD on-line detection accelerates and ensures peak identification in electropherograms, especially when urine samples of patients with co-medications, like patients under anticancer therapy, have to be analyzed.

In order to analyze paracetamol and its metabolites in serum, ultrafiltration was found to be the most suitable preparation to solve the problems that arose.

The assays will be used for further studies with clinical samples.

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