Analysis of Buprenorphine, Norbuprenorphine, and Their Glucuronides in Urine by Liquid Chromatography–Mass Spectrometry

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Abstract

Buprenorphine is used for the treatment of chronic pain and also in treatment of heroin addiction as an alternative to methadone. As the availability of buprenorphine increases, so does the risk for abuse and the pressure on forensic and clinical laboratories to analyze for it. Buprenorphine and its dealkylated metabolite are excreted in urine, almost exclusively as glucuronides. The aim of the present study was to evaluate electrospray liquid chromatography tandem mass spectrometry (LC–MS–MS) for the rapid screening and quantitation of buprenorphine and its metabolites in urine. Three approaches were evaluated: (1) direct injection of diluted urine for measurement of glucuronides, (2) direct injection of diluted urine after enzymatic hydrolysis for the quantitation of buprenorphine and norbuprenorphine, and (3) quantitation of buprenorphine and norbuprenorphine after enzymatic hydrolysis and solid-phase extraction (SPE). One hundred six samples were subjected to procedure 1 and, when positive, further quantitated using procedure 2. Only samples with low analyte concentrations (< 20 µg/L) were subject to SPE. Concentrations of buprenorphine and norbuprenorphine in patients (N = 16) ranged between 31 and 1080 µg/L and 48–2050 µg/L, respectively. In suspected abusers (N = 33), the ranges were 2.3–796 µg/L and 5.0–2580 µg/L. In four of the authentic samples, both the buprenorphine and norbuprenorphine concentrations were below the 20-µg/L cutoff. We concluded that LC–MS–MS analysis of the glucuronides provided an adequate screening method, but that the direct method for quantitation sometimes had to be complemented with a concentration by SPE, providing increased sensitivity, thus lowering the cutoff from 20 to 1 µg/L urine.

Introduction

Buprenorphine is an opioid used for the prevention or treatment of moderate to severe chronic pain with therapeutic doses of 0.3–0.6 mg in the form of sublingual tablets. In some European countries including Sweden, buprenorphine is also used in treatment of heroin addiction as an alternative to methadone. The doses are then higher from 1 up to 32 mg/day. In humans, buprenorphine is metabolized by N-dealkylation to norbuprenorphine. Both the metabolite and the parent drug undergo extensive conjugation to glucuronides that are excreted in the urine (1). Reported urine concentrations of free buprenorphine are in the low nanogram range (2), and thus a hydrolysis step is recommended before analysis. Feng et al. (3) reported the quantitative hydrolysis of buprenorphine-glucuronide (B-3-G) using β-glucuronidase from *E. coli* after 2 h of incubation at 37°C and at pH 6.8. Their study did not include evaluation of the hydrolysis of norbuprenorphine-glucuronide (norB-3-G). However, Lisi et al. (4) optimized the hydrolysis of norB-3-G in a urine sample from a volunteer who was administered 0.2 mg buprenorphine. The hydrolysis required very high concentrations of the enzyme β-glucuronidase from *H. pomatia*, even at a norB-3-G concentration as low as 6 µg/L.

There are only a few reports on urine concentrations of buprenorphine from either drug addicts or patients. Vincent et al. (5) reported concentrations from 1.0 to 3.3 µg buprenorphine/mg creatinine and 0.6–7.0 µg norbuprenorphine/mg creatinine in five buprenorphine abusers. Tracqui et al. (6) also reported postmortem urine concentrations of 4–1000 µg/L (median 196 µg/L) in 20 fatalities involving buprenorphine. Hand et al. (7) reported mean concentrations measured by radioimmunoassay (RIA) ranging from 16 to 157 µg/L urine in five patients receiving 0.4–2 mg/day. The authors report that urinary concentration of buprenorphine increased with increased dose, even though there was a marked variation.

Several methods to determine buprenorphine and norbuprenorphine in biological matrices using detection techniques such as radioimmunoassay (8), electron capture gas chromatography (GC) (9,10), and liquid chromatography (LC) with electrochemical detection (2,11) have been published. Also, a variety of mass spectrometric (MS) methods have been published (3,12–17). In recently published methods for therapeutic monitoring the detection modes have been tandem MS coupled either to LC (15,17) or GC (14). Postmortem blood concentrations have been reported using electrospray LC–MS (6,18). Polettini et al. (17) measured not only buprenorphine

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and norbuprenorphine, but also B-3-G in plasma samples using LC–MS–MS. The methods for urine, though, have all been directed towards the free (or liberated) analytes using either GC–MS with electron impact ionization (3,5) or LC–MS with electrospray (13).

The aim of the present study was to evaluate electrospray LC–MS–MS for the rapid screening and quantitation of buprenorphine and its metabolites in urine. Three approaches and combinations of these were evaluated: (1) direct injection of diluted urine for measurement of glucuronides, (2) direct injection of diluted urine after enzymatic hydrolysis for the quantitation of buprenorphine and norbuprenorphine, and (3) quantitation of buprenorphine and norbuprenorphine after enzymatic hydrolysis and solid-phase extraction (SPE).

Materials and Methods

Chemicals and reagents

The reference materials buprenorphine, norbuprenorphine, buprenorphine-d_4, and norbuprenorphine-d_3 where purchased from Cerilliant (Austin, TX). B-3-G and norB-3-G were isolated from urine by collection of high-performance LC fractions around the retention time for each analyte. The eluates were used to optimize the MS–MS parameters by direct infusion. β-Glucuronidase (*E. coli*, 200 U/mL) was purchased from Roche (Mannheim, Germany). Millipore Ultrafree MC 0.22-µm filters were purchased from Millipore AB (Sundbyberg, Sweden). All inorganic chemicals were of analytical grade, and all organic solvents were of gradient or analytical grade.

Instrumentation

The LC–MS–MS analysis was performed on a PerkinElmer series 200 chromatography system consisting of two 200 micropumps, a "hot-pocket" column oven, a 200 autosampler, and a SCIEX API 2000 MS–MS instrument (Applied Biosystems, Toronto, ON, Canada) equipped with an electrospray interface (TIS). Ion spray voltage was set to 5000 V. Nitrogen was used as nebulizer gas (25 psi), auxiliary gas (50 psi heated to 300°C), curtain gas (30 psi), and as collision-activated dissociation gas (set on 5). A 50- × 2.1-mm Zorbax phenyl analytical column with 3.5-µm particle size (Agilent Technologies, Kista, Sweden) was used. Mobile phase A was a 10:10:80 mixture of acetonitrile/methanol/20mM ammonium formate buffer (pH 3.0), and mobile phase B was a 35:35:30 mixture. The system was run in a linear gradient from 75% A-phase to 10% A-phase during 5 min, followed by a 1-min equilibration with 90% A-phase. The total flow rate was 0.25 mL/min. The column oven was set at 30°C. A 10-µL aliquot of the samples was injected. Chromatograms were evaluated with Analyst v. 1.2 software. The mass spectrometric details of the method are listed in Table I.

Table I. Retention Times, Transitions, and MS–MS Parameters for Each Analyte and Internal Standard*

Analyte	Rt (min)	Transition (*m/z*) Q1	Q3	DP (Q1) (V)	CE (Q2) (V)	CXP (Q2) (V)
			468.4	100	5	23
Buprenorphine	4.20	468.4	414.2	100	45	21
			396.3	100	50	10
			414.1	80	5	15
Norbuprenorphine	3.05	414.1	101.2	80	55	18
			644.4	100	5	35
B-3-G	2.73	644.4	468.1	100	53	20
			590.1	110	5	30
NorB-3-G	1.71	590.1	414.2	110	50	20
Buprenorphine-d_4	4.17	472.3	472.3	100	5	23
Norbuprenorphine-d_3	3.03	417.2	417.2	80	5	21

* Focusing potential, entrance potential, and dwell times were 350 V, 11 V, and 100 ms, respectively, for all transitions. DP = declustering potential, CE = collision energy, and CXP = collision cell exit potential.

Samples

Urine samples from 16 patients receiving Subutex® in a detoxification program were sent to our laboratory for buprenorphine analysis. Urine samples from 106 cases sent to our laboratory from the Swedish police or a Swedish prison and probation services, where buprenorphine was requested, were also analyzed. Urine samples from patients and authentic cases were collected in plastic tubes without any preservative, shipped to the laboratory, and stored at 4°C until analyzed. The urine creatinine concentration was determined with the Jaffé method on an Advia 1650 from Bayer AB (Gothenburg, Sweden).

Analytical procedures

Direct analysis of glucuronides and free buprenorphine and norbuprenorphine. Aliquots of the urine samples were centrifuged through 0.22-µm filters, and 100 µL of the filtrate was transferred to a vial prepared with 25 µL internal standard (buprenorphine-d_4/norbuprenophine-d_3, 0.4 µg/mL). One hundred microliters of mobile phase buffer (20mM ammonium formate buffer, pH 3.0) was added. The vials were then capped and mixed. A 10-µL aliquot was injected.

Quantitation of buprenorphine and norbuprenorphine after enzymatic hydrolysis. One milliliter of the urine samples was transferred to a 10-mL glass tube. Twenty-five microliters of internal standard (buprenorphine-d_4/norbuprenorphine-d_3, 4.0 µg/mL), 300 µL 0.5M BIS-TRIS propane buffer (pH 6.8), and 40 µL β-glucuronidase were added. The tubes were capped and incubated in a water bath (with orbital shaking) at 37°C for 20 h. An aliquot of the samples were then centrifuged through the 0.22-µm filter, and 100 µL of the supernatant was subsequently transferred to a vial. A 10-µL aliquot was injected.

Together with the authentic samples controls at 20 and 200 µg/L, a hydrolysis control composed of pooled samples diluted to a concentration of approximately 100 µg/L of the glucuronides were analyzed.

Quantitation of buprenorphine and norbuprenorphine after enzymatic hydrolysis and SPE. Hydrolysis of 1.0 mL urine was performed as described previously, and the pH was then adjusted to 6.1 with 4 mL of 0.2M phosphate buffer. SPE was performed as described by Vincent et al. (5), but with lower solvent volumes in the wash steps because only half the sample

volume was used. BondElute Certify columns were activated and conditioned with 2 mL of methanol followed by 2 mL of 0.2M phosphate buffer (pH 6.1). The sample was drawn through the column, which was then rinsed with 2 mL of deionized water, 2 mL of 0.1M HCl, and 2 mL of methanol. The column then was dried for 5 min (10 in. Hg), and the analytes were eluted with 2 mL of a mixture of dichloromethane/2-propanol (80:20) containing 2% ammonia (25%). The eluate was evaporated in a TurboVap at 40°C and 5 psi nitrogen. The residue was reconstituted in 100 µL of mobile phase A. A 10-µL aliquot was injected.

Retention times and measured transitions for each analyte are listed in Table I. MS–MS parameters for each analyte were optimized by infusion of standards dissolved in mobile phase. Calibration curves were performed by addition of standard solutions to 5-mL batches of negative urine. From the batches, 1.0 mL was pipetted for each calibration level, and the calibrators were treated as authentic samples. Calibrations were performed in duplicates. Final calibrator concentrations for the direct injection methods were 10, 25, 50, 100, 250, 500, and 1000 µg/L for buprenorphine and norbuprenorphine. For the SPE method, the calibrator concentrations were at 1.0, 5.0, 10, 25, and 50 µg/L.

Method validation

The hydrolysis step was performed with β-glucuronidase from *E. coli* as proposed by Feng et al. (3). The efficiency for norB-3-G hydrolysis was tested with different amounts of enzyme (40–120 µL) and at different incubation times from 0.5 to 24 h at pH 6.8 as proposed by Feng et al. (3), as well as the manufacturer of the enzyme.

The sensitivity for the screening method was estimated by correlating the peak areas for the glucuronides before hydrolysis to the concentrations of the free analyte after hydrolysis (in the 16 patient samples). In this way, equations describing the relationship between B-3-G and buprenorphine ($y = 173{,}055x - 868$, $r = 0.99$) and norB-3-G and norbuprenorphine ($y = 113{,}000x - 1793$, $r = 0.99$) were obtained. The point where the regression line crosses the x-axis ($y = 0$) could be an estimate of the thresholds for B-3-G and norB-3-G.

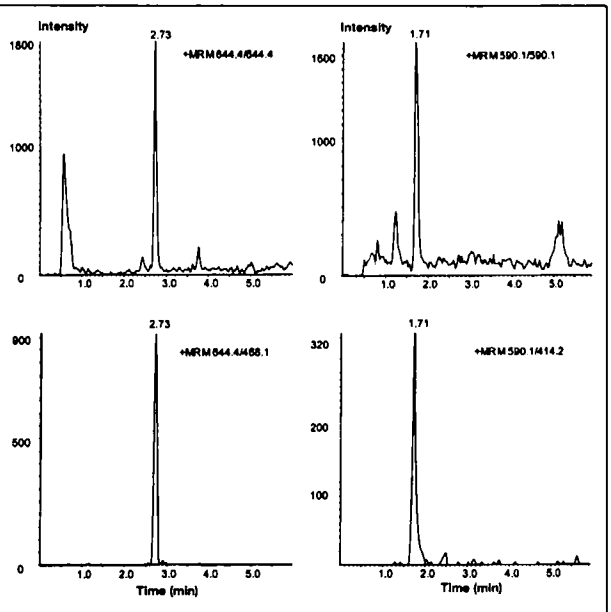

Figure 1. Ion chromatograms from direct injection of the hydrolysis control before hydrolysis. B-3-G transitions are *m/z* 644/644 and 644/468, and norB-3-G transitions are 590/590 and 590/414.

LOQ was considered the concentration where the coefficient of variation was lower than 15% ($N = 5$).

The within-day and between-day imprecisions for the direct method were estimated by analysis of control samples at 20 and 200 µg/L, as well as a hydrolysis control (authentic sample) in the same batch ($N = 5$) and on different days over a period of two months ($N = 10$).

Extraction recovery for the SPE method was estimated by comparing peak areas from extracted standards ($N = 5$) at 1.0 and 10.0 µg/L with those from standards prepared directly in mobile phase A. Within-day imprecision was estimated by analysis of 5 control samples at 1.0 and 10 µg/L.

Results

Linear calibration curves were established for both the direct and SPE methods. For the direct method, equations were $y = 0.990x + 0.0540$ ($r = 0.999$) for buprenorphine and $y = 0.701x + 0.0381$ ($r = 0.999$) for norbuprenorphine. For the SPE method, the equations were $y = 0.890x - 0.0024$ ($r = 0.999$) for buprenorphine and $y = 0.778x + 0.0004$ ($r = 0.998$) for norbuprenorphine. Chromatograms from the analysis of the hydrolysis control are shown in Figures 1 and 2. Chromatograms from a positive sample containing low concentrations of buprenorphine and norbuprenorphine are shown in Figures 3–5. The estimated thresholds for B-3-G and norB-3-G were 5 and 16 µg/L, respectively. These were also confirmed by results from authentic samples (see Figure 3). The hydrolysis of norbuprenorphine at different enzyme concentrations is shown in Figure 6. Imprecision data for the two quantitation methods are shown in Tables II and III. The extraction recoveries of the SPE method were 84% and 98% for buprenorphine at 1.0 and 10.0 µg/L, respectively. For norbuprenorphine, the recoveries were 77% and 91% at 1.0 and 10.0 µg/L, respectively. The relative intensities for the product ion transitions compared with the molecular ion was 1.4% and 1.6% for transitions *m/z* 468/414 and 468/396, whereas for norbuprenorphine the transition *m/z* 414/101 showed a 3.4% intensity. Even though the molecular ions showed prominent peaks well below 20 µg/L LOQ for the direct method and the one of 1 µg/L for the SPE method, they were limited by the criteria to have visible peaks for the respective product ions.

As urine samples were analyzed both before and after hydrolysis, the concentration of unconjugated buprenorphine and norbuprenorphine was measured. Four patients had concentrations of free buprenorphine above the 20-µg/L cutoff, whereas all but two had higher concentrations of free norbuprenorphine. Urine concentrations of buprenorphine and norbuprenorphine from patients are shown in Table IV, and concentrations in urine samples from authentic cases are shown in Table V. For samples that had concentrations of either

buprenorphine or norbuprenorphine lower than 20 µg/L, the quantitation results from the SPE method are also included.

Discussion

The approach to determine the glucuronides both in the direct injection, as well as after hydrolysis, gives the unique opportunity to evaluate the efficiency of the hydrolysis step in each and every sample. Thus, a traditional hydrolysis control is not of paramount importance. The hydrolysis of norB-3-G showed a much slower rate than B-3-G (Figure 6), which may be important when measuring ratios of the parent to metabolite, as one tends to overestimate the ratio if the hydrolysis is in-

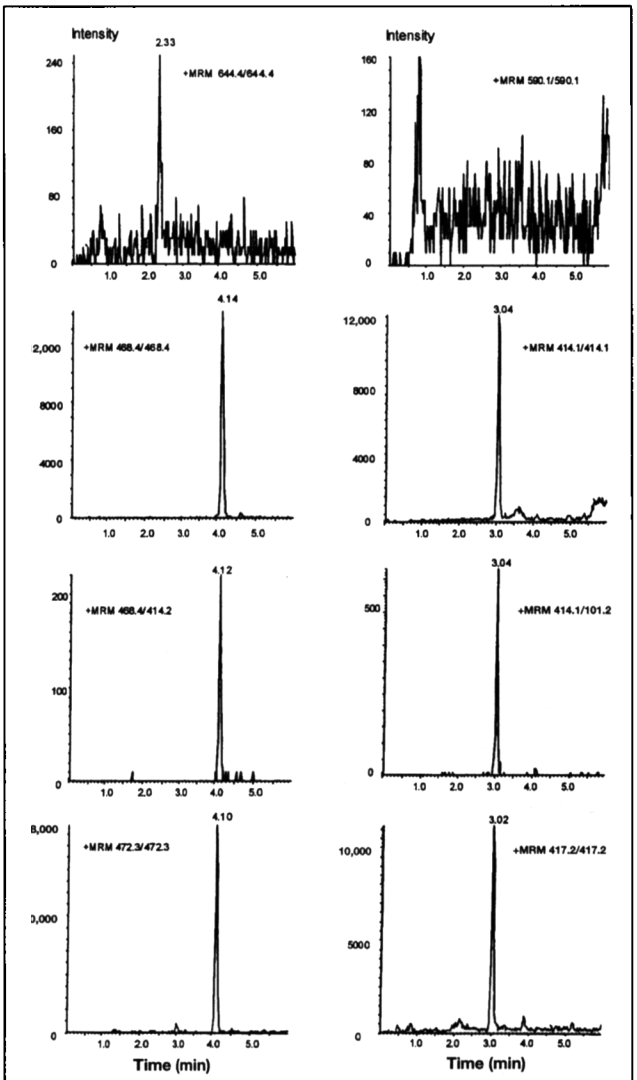

Figure 2. Ion chromatograms from direct injection of the hydrolysis control after enzymatic hydrolysis. B-3-G transition 644/644 is shown together with 468/468 and 468/414 for buprenorphine and *m/z* 472/472 for the internal standard buprenorphine-d_4. norB-3-G transition 590/590 is shown together with 414/414 and 414/101 for norbuprenorphine and *m/z* 417/417 for the internal standard norbuprenorphine-d_3. There are no peaks at the retention times for norB-3-G (1.71 min) and B-3-G (2.73 min), indicating complete hydrolysis.

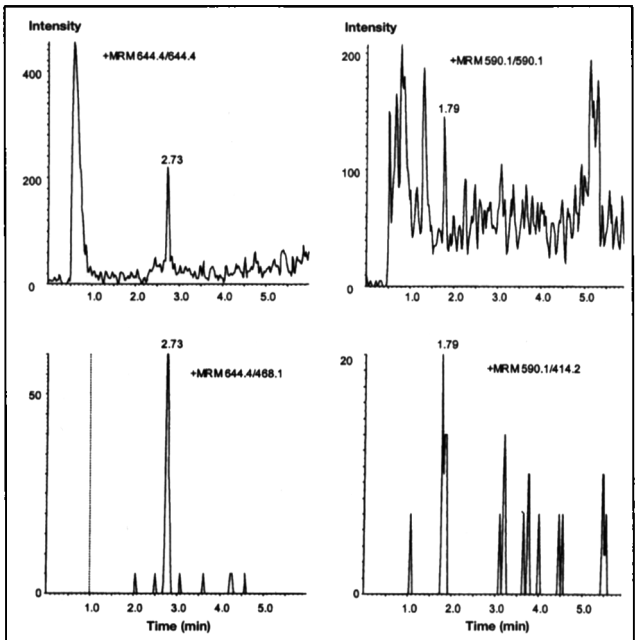

Figure 3. Ion chromatograms from direct injection of sample 4 before hydrolysis. B-3-G transitions are *m/z* 644/644 and 644/468, and norB-3-G transitions are 590/590 and 590/414.

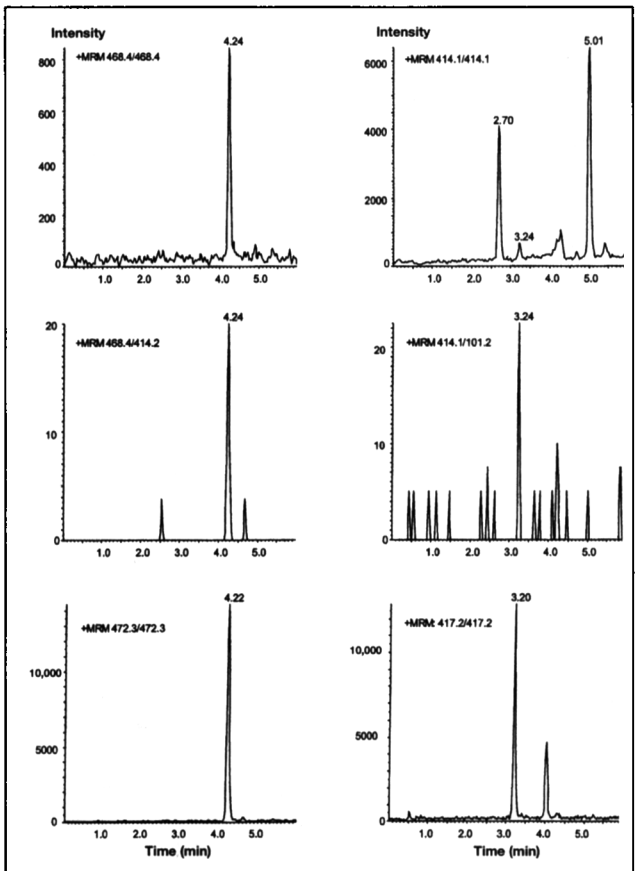

Figure 4. Ion chromatograms from direct injection of sample 4 after enzymatic hydrolysis. Transitions *m/z* 468/468 and 468/414 for buprenorphine are shown together with *m/z* 472/472 for the internal standard buprenorphine-d_4. Transitions *m/z* 414/414 and 414/101 for norbuprenorphine are shown together with *m/z* 417/417 for the internal standard norbuprenorphine-d_3.

complete. This phenomenon is well known for the hydrolysis of the glucuronides of morphine and codeine (19). Increasing the amount of enzyme in the incubation mixture did improve the recovery of norbuprenorphine, but because the difference was less than 15%, we chose to use the lowest amount of enzyme. Because of the direct injection, it was more important to minimize the amount of possible interferences in the sample. Even though the hydrolysis may not be complete, the repeated analysis of an authentic sample over 2 months showed a variation of less than 8% (see Table III).

The detection of glucuronides also adds another dimension to identification. Josefsson et al. stated that working with LC–MS one can choose to measure several analytes to confirm the intake of a drug instead of measuring several ions (or transitions) for just one analyte (20). This is especially worthwhile for substances in which the fragmentation is limited.

Some of the published methods for buprenorphine deal with the difficulties in obtaining adequate fragmentation for LC–MS–MS (15,17). The molecular ions of both buprenorphine and norbuprenorphine are readily formed, but seem very stable under varying conditions in the collision cell. This may be because of the complex ring structure that is able to accommodate the excitation energy to a point where the molecule literally shatters with no significant high mass fragment ions surviving. Polettini and Huestis (17) overcame that problem by using the parent ion for quantitation, in other words letting the molecular ion pass through the mass filters to the detector as in single MS mode. Moody et al. (15), however, achieved a 20% abundance of the *m/z* 396 product ion providing sufficient sensitivity to analyze subnanogram buprenorphine concentrations in plasma samples using multiple-reaction monitoring (MRM) (15). The experiments in these two reports were performed on differently designed triple-quadrupole instruments with unique collision cells using different collision gases (nitrogen or argon). The use of the heavier argon as collision gas might account for the more pronounced fragmentation achieved by Moody et al. (15). In our applications, however, a LINAC™ collision cell and nitrogen gas were used. As did Polettini and Huestis (17), we

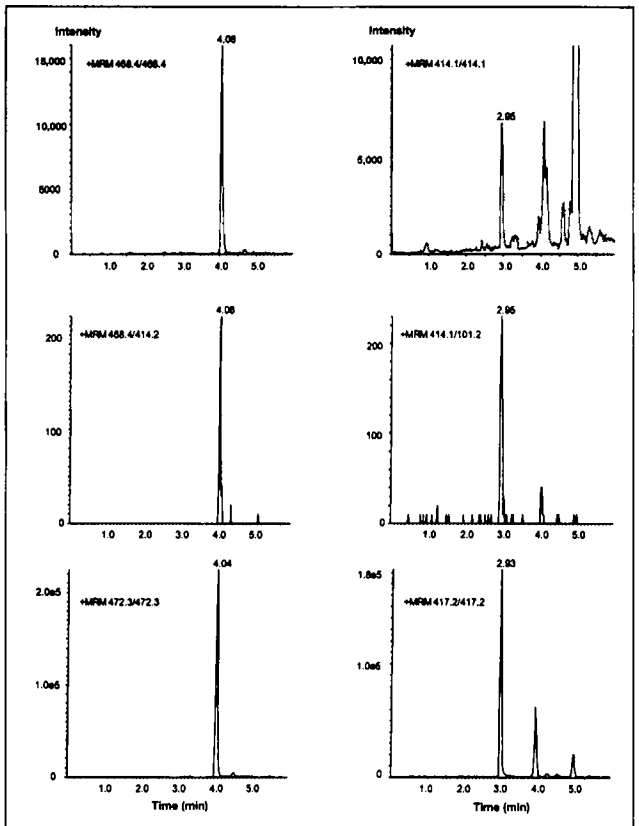

Figure 5. Ion chromatograms from sample 4 after enzymatic hydrolysis and SPE. Transitions *m/z* 468/468 and 468/414 for buprenorphine are shown together with *m/z* 472/472 for the internal standard buprenorphine-d_4. Transitions *m/z* 414/414 and 414/101 for norbuprenorphine are shown together with *m/z* 417/417 for the internal standard norbuprenorphine-d_3. Concentrations of buprenorphine and norbuprenorphine are 8.1 and 5.0 µg/L, respectively. After SPE, the peak intensities were approximately 10 times as high as those from direct injection (see Figure 4).

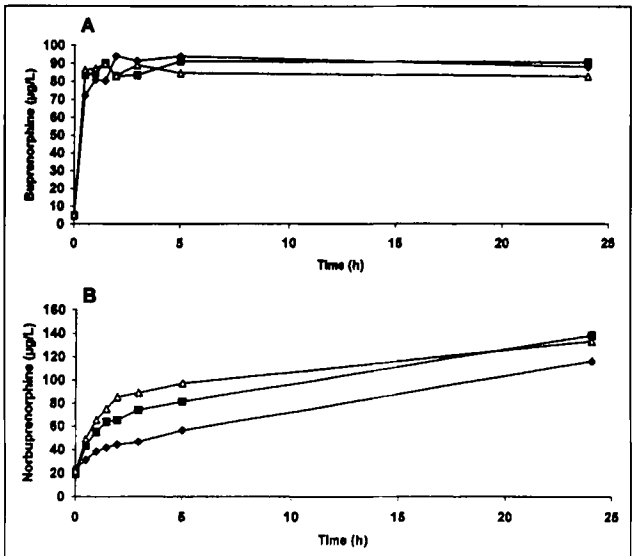

Figure 6. Buprenorphine (A) and norbuprenorphine (B) concentrations after hydrolysis with different amounts of β-glucuronidase. Incubation in a waterbath with orbital shaking at 37°C. (u) 40 µL enzyme, (n) 80 µL enzyme, and (Δ) 120 µL enzyme.

Table II. Within-Day Imprecision for the Quantitation Methods ($N = 5$)

Analyte	Added Conc. (µg/L)	Mean (µg/L)	Accuracy (%)	CV (%)
Controls (SPE method)				
Buprenorphine	1	1.16	116	9.5
Buprenorphine	10	10.4	104	2.1
Norbuprenorphine	1	1.23	123	13.3
Norbuprenorphine	10	10.7	107	9.4
Controls (direct method)				
Buprenorphine	20	18.8	94	1.9
Buprenorphine	200	191	96	3.2
Norbuprenorphine	20	22.2	111	5.0
Norbuprenorphine	200	222	111	6.5
Authentic sample (direct method)				
Buprenorphine	–	93.1	–	4.0
Norbuprenorphine	–	146	–	3.6

used the surviving parent ion for buprenorphine, norbuprenorphine, and their internal standards for quantitation, but also measured the transitions for one or two product ions to strengthen the identity. However, the relative intensities compared with the molecular ion were only a few percent. The glucuronides, though, showed prominent fragmentation caused by deconjugation, as can be seen in Figure 1.

Concentrations in patients and abusers showed a considerable overlap, but no patients had lower concentrations than 30 µg/L, whereas some abusers showed as low as 2.3 µg/L of buprenorphine. Hand et al. (6) also reported such low concentrations in abusers at a drug clinic. Forty-three of their 60 positive cases had concentrations lower than 25 µg/L (measured with RIA). These and our low concentrations probably reflect buprenorphine administration several days before sampling. Vincent et al. (5) reported urinary concentrations higher than 20 µg/L, even after three days of abstinence. Tracqui et al. (6) reported buprenorphine concentrations of 4–1033 µg/L in 20 fatalities attributed to buprenorphine overdose. Because concentrations in urine were high, one cannot rule out residual buprenorphine concentrations as an acute overdose well might leave a negative urine sample. In approximately 10% of our cases, both the buprenorphine and norbuprenorphine concentrations were below the 20-µg/L cutoff. This threshold seems appropriate for testing of patients, but the direct quantitation method may suffer from low sensitivity.

Thus, in cases in which the peaks for B-3-G or norB-3-G are very small, one should consider to proceed with the more sensitive SPE method instead of injecting the diluted urine. This could then be a suitable combination for general drug screen, as it could detect buprenorphine abuse several days after last use.

The possibility of LC–MS–MS to inject samples without pretreatment or time-consuming extractions is very tempting, but although MRM analysis with compound-specific transitions al-

Table III. Between-Day Imprecision for the Quantitation by Direct Injection (N = 10)

Analyte	Added Conc. (µg/L)	Mean (µg/L)	Accuracy (%)	CV (%)
Controls				
Buprenorphine	20	20.1	100.8	7.3
Buprenorphine	200	198	99.0	6.2
Norbuprenorphine	20	21.7	108.6	6.8
Norbuprenorphine	200	201	100.5	7.5
Authentic sample				
Buprenorphine	–	96.3	–	7.6
Norbuprenorphine	–	146	–	6.2

Table IV. Urine Concentrations of Buprenorphine and Norbuprenorphine, Daily Dose, and Creatinine Concentrations from 16 Patients Under Ongoing Subutex Treatment for Heroin Dependence

Patient	BUP (µg/L)	NorBUP (µg/L)	Daily Dose (mg)	Creatinine (g/L)	BUP/ Creatinine (µg/g)	NorBUP/ Creatinine (µg/g)
1	36	48	1	0.6	60	80
2	40	142	6	0.5	80	284
3	174	690	8	1.7	102	406
4	543	1510	8	2.8	194	539
5	87	473	10	0.7	124	676
6	52	274	12	0.5	104	548
7	61	60	12	0.8	76	75
8	538	2050	12	2.4	224	854
9	72	559	12	0.9	80	621
10	222	611	14	1.6	139	382
11	537	1020	14	2.1	256	486
12	45	265	16	0.6	75	442
13	35	204	16	0.6	58	340
14	31	116	16	0.2	155	580
15	433	646	24	0.5	866	1290
16	1080	1700	32	1.5	720	1130

Table V. Urine Concentrations of Buprenorphine, Norbuprenorphine, and Creatinine in Authentic Samples for which Auprenorphine Analysis had been Requested and Screened Positive for the Glucuronides*

	Direct Method		SPE Method		
Sample no.	BUP (µg/L)	NorBUP (µg/L)	BUP (µg/L)	NorBUP (µg/L)	Creatinine (g/L)
1	2.9	4.8	2.3	7.3	1.2
2	4.9	17.4	5.7	9.5	0.9
3	5.0	40.1	5.1	32.5	0.6
4	7.6	7.8	8.1	5.0	1.4
5	9.2	35.4	7.7	24.2	0.2
6	9.5	16.9	7.9	12.9	1.6
7	9.9	29.0	8.8	25.1	0.5
8	11.4	27.2	5.3	20.3	0.8
9	21.1	107			1.2
10	23.6	41.4			1.9
11	30.0	78.8			1.2
12	32.1	72.6			1.0
13	40.7	69.4			2.6
14	41.0	129			1.4
15	55.3	221			1.8
16	56.3	64.6			2.2
17	77.7	21.2			1.5
18	83.5	114			1.1
19	138	287			2.4
20	148	447			2.2
21	151	410			0.5
22	169	333			1.0
23	215	232			0.7
24	218	33.3			2.6
25	295	1620			2.5
26	321	766			0.9
27	349	35.8			1.9
28	365	48.7			3.6
29	410	352			0.6
30	457	2580			2.4
31	501	65.4			3.2
32	650	180			0.6
33	796	212			1.2

lows accurate determinations at very low concentrations, there is a risk for ion suppression in the ES interface causing reduced analyte ionization. In urine analysis, this is especially true for less retained polar analytes such as the glucuronides because polar components and salts in the matrices might coelute. However, with SPE sample preparation, most of these components can be eliminated and the analytes concentrated.

Conclusions

We conclude that the methods presented can be used for screening and quantitation of buprenorphine and its metabolites in urine. The direct injection of diluted urine and measurement of conjugates with LC–MS–MS serves as a rapid and reliable procedure that can be followed by the hydrolysis of a new aliquot of urine. Depending on the results from the screening, either a direct quantitation by injection of the diluted hydrolysate or a further concentration of the sample by SPE can be performed.

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