Evaluation of Buprenorphine CEDIA Assay versus GC–MS and ELISA using Urine Samples from Patients in Substitution Treatment

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Abstract

As buprenorphine becomes more clinically used in heroin substitution treatment, there is an increasing need for methods suitable for high-volume screening. In this study, a new immunochemical test based on CEDIA technology was evaluated for the use in clinical urine drug testing. The method was compared with an existing ELISA method and a gas chromatography–mass spectrometry (GC-MS) method on urine specimens from patients in heroin substitution treatment. The precision of the CEDIA assay was < 9% both within- and between-day at levels at and above the cutoff limit of 5 pg/L. The concordance in qualitative results with an existing ELISA method was 96.8%. The CEDIA measuring range was extended by diluting urine samples 100-fold with saline, and the results agreed well (slope of regression line was 1.09, \( r^2 = 0.968 \)) with GC-MS. The sensitivity of CEDIA in detecting authentic specimen containing buprenorphine at levels ≥ 5 pg/L was 99.5%. Cross-reactivity causing false-positive response was discovered in patients receiving prescribed dihydrocodeine. The urine concentration of total buprenorphine in urine from patients prescribed daily doses between 0.2 and 24 mg ranged from 0.5 to 2900 pg/L. The concentration of the metabolite norbuprenorphine was usually higher, and the median ratio of buprenorphine to norbuprenorphine was 0.23 (95% were below 1). We conclude that the CEDIA assay is suitable for application in high-volume screening of buprenorphine for urine drug testing.

Introduction

Buprenorphine (Subutex®) is used as an analgesic drug and for detoxification and substitution therapy of opioid dependence (1,2). Since 1996, starting in France, the use of buprenorphine in substitution therapy has increased and spread to over 30 countries, many in Europe but also in Asia, Australia, and the Americas. Buprenorphine offers an attractive alternative to methadone, with benefits including increased safety for respiratory depression, suppressed heroin use, and possibility of longer dosing interval (2). However, in combination with the increased medical use, buprenorphine also occurs on the black market as an illicit drug (3,4), and fatalities due to poly-drug use have been reported (5,6).

Buprenorphine is metabolized by dealkylation to norbuprenorphine primarily by the cytochrome P450 isozyme CYP 3A4 (7). Both buprenorphine and norbuprenorphine are conjugated with glucuronic acid and subsequently excreted in urine during the course of several days (8). In urine drug testing, parent buprenorphine has become the primary analytical target compound, and less focus has been devoted to the metabolite norbuprenorphine. The proportion of the two compounds in urine has not been well-documented in patient material. The activity of the CYP 3A4 isozyme is subjected to variability influenced by both genetic and environmental factors, which will determine the proportion of norbuprenorphine that is being formed and subsequently excreted in urine (9,10).

As a consequence of increased legal and illicit use of buprenorphine, there is a need for an analytical method for toxicological monitoring of patients. In addition, an analytical method is necessary for compliance monitoring during substitution therapy, which has been integrated in many methadone programs. A number of methods for urinary determination of buprenorphine are available, including radioimmunoassay, enzyme-linked immunosorbent immunoassay (ELISA), thin-layer chromatography, high-pressure liquid chromatography, gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), and liquid...
Materials and Methods

Chemicals

Buprenorphine, morphine, morphine-3-glucuronide, codeine, norbuprenorphine, dihydrocodeine, hydrocodone, buprenorphine-d$_4$, and norbuprenorphine-d$_3$ were obtained as methanol solutions from Promochem GmbH (Wesel, Germany). Codeine-6-glucuronide, dihydrocodeine-6-glucuronide, dihydromorphine, dihydromorphine-3-glucuronide, dihydrocodeine-6-glucuronide, dihydrocodeinone, and dihydrocodeinone were obtained as solid material from Lipomed GmbH (Bad Säckingen, Germany) and dissolved in pure water or methanol. Working solutions were prepared by dilutions with blank urine or saline (0.9%). The experiment for obtaining cross-reactivity data used typically 4–7 prepared solutions for each compound using linear regression analysis. For compounds with low cross-reactivity, one data point was used.

Helix pomatia β-glucoronidase/arylsulfatase (supplied in liquid form) was obtained from Roche GmbH (Mannheim, Germany); acetic anhydride was from Fluka (Buchs, Switzerland); methanol, ethyl acetate (suprasolve grade), sodium acetate, ammonia (32%), and acetic acid were from Merck GmbH (Darmstadt, Germany); acetonitrile was from Riedel-de Haen (Seelze, Germany); and 4-dimethylaminopyridine (crystalline grade) was from Sigma Chemical (Taufkirchen, Germany). All chemicals were of analytical grade unless otherwise stated.

Urine samples

Urine samples were collected from outpatients in heroin substitution treatment with methadone, buprenorphine, or dihydrocodeine assumed to be in steady-state. In total, 1552 samples were obtained from approximately 600 patients, consisting of 70% males and having an age range of 18–54 years. The samples were collected under supervision and sent to the laboratory for routine urine drug testing. Three different criteria were used for inclusion in various parts of the study: prescription of Subutex with known daily dose, prescription of other substitution medication (methadone or dihydrocodeine), or a specific request of urine buprenorphine testing. The ethics committee at the Karolinska Institute approved the study.

CEDIA assay

Reagents for the CEDIA buprenorphine test were supplied by Microgenics (Fremont, CA). The test was performed on Hitachi 911 and 912 (Roche Diagnostics, Indianapolis, IN) instruments with a test protocol supplied by Microgenics and used a sample volume of 10 μL. Calibrators (levels: 0, 5.8, 21.3, 51.5, and 78.2 μg/L) and controls (levels: 3.9, 6.4, 40.2, and 65.1 μg/L) supplied by Microgenics were used. During the study, calibration was done on a daily basis together with four different quality control samples every 25th sample and following calibration.

Reagent for opiate CEDIA screening (cutoff 300 ng/mL) was obtained from Microgenics and was applied on a Hitachi 911 instrument according to kit instructions.

ELISA assay

The ELISA assay of buprenorphine was performed using reagents and calibrators from Diagnostix (Mississauga, ON, Canada). The procedure was performed manually according to kit instructions, and the final result was obtained by using a Labsystems Multiskan (Helsinki, Finland) plate reader (450 nm). A cutoff limit of 5 μg/L was used. Calibrating points used were 0, 5, 10, and 20 μg/L.

GC–MS procedure

A 3-μL aliquot of the urine sample was fortified with 0.05 mL internal standard solution (methanol containing 50 ng buprenorphine-d$_4$ and 100 ng norbuprenorphine-d$_3$), 0.3 mL of 1.7 M acetate buffer, pH 4.75 (pH was controlled to be 4.5–5.0), and 30 μL (3000 Fishman units) of Helix pomatia enzyme. The mixture was vortex mixed and incubated overnight in a water bath at 37°C (or at 2 h at 56°C). This procedure was documented to be optimal for the hydrolysis of both buprenorphine and norbuprenorphine (21).

The analytes were then extracted using a solid-phase extraction (SPE) procedure. The mixed phase (nonpolar/SCX) SPE cartridge (SPE DAU 3 mL, Varian, Darmstadt, Germany) was activated by treatment with 0.2 mL methanol, followed by application of the sample. The procedure was performed using a vacuum manifold (approximate flow 1 mL/min). Washing was performed with 0.5 mL 0.1 M acetic acid and subsequently 0.5 mL methanol. The cartridge was vacuum dried with aspiration for 5 min. Subsequently, the elution of analytes was performed with 0.8 mL freshly prepared methanol/ammonia (9:1, v/v). Thereafter, 25 μL of 4-dimethylaminopyridine (0.5 mg/mL in acetonitrile) was added, and the extract was evaporated to dryness under nitrogen (22). The dry residue was redissolved in 100 μL acetic anhydride, vortex mixed, screwed, and incubated for 5 min in a 500 W microwave oven. The reaction mixture was evaporated at 65°C in a heating block under nitrogen, the residue redissolved in 70 μL of ethyl acetate, and 1 μL was injected into the GC–MS.

The final extract was analyzed using a QP5050 Shimadzu GC–MS using automated splitless injection (280°C) (autosampler AOC20s). The column was a 15-m (0.25-mm i.d., 0.25-μm film thickness) DB-5MS (J&W Scientific, Promochem GmbH) with helium (grade 5.0) as carrier gas. Initial temperature was 130°C for 1 min, then increased 30°C/min to a final temperature of 300°C. The instrument operated in the selected ion monitoring mode measuring m/z 420 (quantitation ion), 452, and 453 (qualifier ions) for buprenorphine; m/z 424 (quantitation ion), 456, and 457 (qualifier ions) for buprenorphine-d$_4$; m/z 440 (quantitation ion) and 422 (qualifier ion) for norbuprenorphine; and m/z 443 (quantitation ion) and 425 (qualifier ion) for norbuprenorphine-d$_3$.

Standards were prepared in blank pooled urine covering the
The limits of detection (LOD) and quantification (LOQ) were calculated according to DIN 32645 (23) from the lower end of the calibration curves using B.E.N. software version 2.03 (Arvecon GmbH, Heidelberg, Germany). Linearity for buprenorphine and norbuprenorphine was shown in the range from 2 to 2000 µg/L with this software. This measuring range was extended by 1:5 dilution of urine with saline. For buprenorphine, the LOD was 0.5 µg/L, the LOQ 1.5 µg/L, and the correlation coefficient \( r^2 = 0.9998 \). For norbuprenorphine, the LOD was 2 µg/L, the LOQ 6 µg/L, and the correlation coefficient \( r^2 = 0.9985 \). Quality control of the GC–MS method was performed using certified urine control material (Medichem, Stuttgart, Germany) in every series with target values at 18.1 µg/L buprenorphine and 84.8 µg/L for norbuprenorphine. The observed mean value for buprenorphine was 19.4 µg/L [coefficient of variation (CV) 9.0%, \( n = 13 \)] and for norbuprenorphine 84.9 µg/L (CV 9.2%, \( n = 12 \)). The method has been part of the GTFCh (German Society for Toxicological and Forensic Chemistry) proficiency test programme since 2001 and gives comparable results to other laboratories using GC–MS and LC–MS methods. Representative chromatograms are shown in Figure 1.

### Results

**Validation of the CEDIA assay**

The within- and between-day variability in quantification at 5 different levels was studied using the 4 control samples and the cutoff calibrator supplied by Microgenics. At the levels exceeding the 5 µg/L cutoff limit, a variability lower than 11% was observed (Table I).

The CEDIA calibrators were compared to laboratory made urine standards used for the GC–MS assay in the concentration range 3–50 µg/L, and the recovery was 92% (\( n = 5 \), CV 4.0%). The result from CEDIA analysis of the certified urine control material used for the GC–MS method with assigned value of 18.1 µg/L buprenorphine was 17.5 µg/L (mean, \( n = 8 \), between-day CV 3.0%).

The CEDIA response curve in the measuring interval was documented using calibrators from Microgenics covering the range from 0 to 78.2 µg/L (Figure 2).

The agreement of the CEDIA and ELISA assays was studied in 221 samples (Table II). There was a 96.8% agreement in qualitative results between the methods. In three samples (2.7% of all positives), CEDIA produced a false-positive

<table>
<thead>
<tr>
<th>Assigned Value (µg/L)</th>
<th>Within-Day</th>
<th>Between-Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed mean value (µg/L)</td>
<td>CV (%)</td>
</tr>
<tr>
<td>3.9</td>
<td>3.1</td>
<td>17.0</td>
</tr>
<tr>
<td>5.8</td>
<td>6.2</td>
<td>6.3</td>
</tr>
<tr>
<td>6.4</td>
<td>6.4</td>
<td>4.1</td>
</tr>
<tr>
<td>40.2</td>
<td>40.9</td>
<td>2.7</td>
</tr>
<tr>
<td>65.1</td>
<td>64.7</td>
<td>11.0</td>
</tr>
</tbody>
</table>

**Figure 1.** Chromatograms from the GC–MS analysis of two authentic patient samples: patient prescribed 0.4 mg Subutex daily and had a concentration of buprenorphine of 9.5 µg/L and of norbuprenorphine of 56 µg/L (creatinine was 286 mg/dL) (A) and patient prescribed 4 mg Subutex daily and had a concentration of buprenorphine of 1.9 µg/L and of norbuprenorphine of 21 µg/L (creatinine was 46 mg/dL) (B).
response as the GC–MS confirmation was clearly negative. In one sample, the CEDIA result was false negative as the sample contained 26 ng/mL of buprenorphine according to GC–MS.

The agreement between the CEDIA and GC–MS methods in quantifying buprenorphine was studied in the range from 0 to 6000 μg/L using 300 samples collected from 72 patients with known Subutex doses. The CEDIA measuring range was extended by dilution of samples 10- or 100-fold with saline. The slope of the regression line was 1.09 with an intercept of −13 on the CEDIA axis (Figure 3A). The median ratio between CEDIA and GC–MS was 0.96 (n = 300). In the sample with the highest concentration of buprenorphine, a considerable and reproducible mismatch between methods was observed (GC–MS 2936 μg/L and CEDIA 5990 μg/L). In the undiluted samples, it was again observed that the quantitative results of the two methods were correlated, but low values from CEDIA were observed in the upper part of the measuring range.

By pooling data for all samples with a buprenorphine concentration > 5 μg/L according to GC–MS or ELISA (n = 400), the sensitivity of CEDIA for detecting positive samples at the cutoff level of 5 μg/L was calculated to be 99.5%.

A total of 1011 samples from about 400 different patients who were not receiving prescribed buprenorphine but received other heroin substitution treatments was used to estimate specificity of the CEDIA assay and to estimate frequency of abuse in this population. Thirty samples (3.0%) were found to be positive by CEDIA at the cutoff level of 5 μg/L. One of these had detectable buprenorphine by GC–MS but at a low level (0.6 μg/L together with 80 μg/L norbuprenorphine). Fourteen of these 30 samples were from patients prescribed dihydrocodeine and contained high amounts of this substance and metabolites (according to GC–MS), indicating a cross-reactivity. An additional 20 samples selected from different patients prescribed dihydrocodeine in
daily doses of 14–80 mg were therefore investigated, and all were false positive in the CEDIA assay with responses ranging from 5 to 45 lag/L. In 15 of the other false-positive samples (1.5%), no cause for the response could be revealed in this investigation. However, 14 of these samples were out of range in the CEDIA opiate immunoassay, which could indicate a cross-reactivity from codeine (see Table III). Most of these samples had a low (5.0–9.1 μg/L) CEDIA response for buprenorphine in combination with a high creatinine concentration.

The cross-reactivity of various analogue substances was studied (see Materials and Methods) in urine matrix and the data is presented in Table III. In immunoassays, one has to consider that different cross-reacting substances, when present simultaneously and at different concentrations, do not always add to the assay response in an arithmetical or predictable way (24). We therefore conducted an experiment with a saline sample spiked with reference materials to the following concentrations: 7.14 mg/L dihydromorphine-3-glucuronide, 7.14 mg/L dihydro-morphine-6-glucuronide, 14.3 mg/L dihydronorcodeine, 14.3 mg/L dihydrocodeine-6-glucuronide, 14.3 mg/L codeine, 14.3 mg/L dihydromorphine, and 14.3 mg/L dihydronormorphine. The CEDIA assay gave a result of 5.2 μg/L, which corresponds to the expected result if the total cross-reactivity would be calculated from the individual substance and its cross-reactivity at the given concentrations.

As can be seen in Figure 4A, urine samples from patients on Subutex therapy may contain levels of buprenorphine below the 5 μg/L cutoff limit. In this material, 4.7% of the samples had such low levels, giving a calculated sensitivity for detecting buprenorphine intake of 95.3%.

By applying a 2 μg/L cutoff limit, an increase in sensitivity to 98.3% could have been obtained. However, when doing that, the rate of false-positive results for CEDIA increased from 3% to 15%, which was observed in urine samples from patients receiving substitution treatments other than Subutex and may be the result of more complex sample matrices that contain additional drugs in high concentrations.

**Pharmacokinetic observations**

The concentration of buprenorphine and norbuprenorphine in the 300 samples from patients prescribed Subutex ranged from 0.5 to 2936 μg/L and 4.0 to 4462 μg/L, respectively (Figure 4). A majority (51%) of the samples had a buprenorphine concentration below 100 μg/L. After correcting the urine concentrations with creatinine, a dose-dependent urinary excretion of both buprenorphine and norbuprenorphine could be observed (Figure 5).

### Table III. Cross- Reactivity of Related Substances in the CEDIA Assay

<table>
<thead>
<tr>
<th>Substance</th>
<th>Tested Concentration Range (mg/L)</th>
<th>Linear Regression (r²)</th>
<th>Cross-Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>0.78-50</td>
<td>0.990</td>
<td>0.010</td>
</tr>
<tr>
<td>Morphine-3-glucuronide</td>
<td>50</td>
<td>—</td>
<td>0.002</td>
</tr>
<tr>
<td>Norcodeine</td>
<td>50</td>
<td>—</td>
<td>0.005</td>
</tr>
<tr>
<td>Codeine</td>
<td>6.25-50</td>
<td>0.997</td>
<td>0.016</td>
</tr>
<tr>
<td>Codeine-6-glucuronide</td>
<td>6.25-100</td>
<td>0.991</td>
<td>0.010</td>
</tr>
<tr>
<td>Dihydrocodeine</td>
<td>12.5-100</td>
<td>0.995</td>
<td>0.014</td>
</tr>
<tr>
<td>Dihydrocodeine-6-glucuronide</td>
<td>6.25-100</td>
<td>0.995</td>
<td>0.010</td>
</tr>
<tr>
<td>Dihydromorphine</td>
<td>12.5-100</td>
<td>0.983</td>
<td>0.007</td>
</tr>
<tr>
<td>Dihydromorphine-3-glucuronide</td>
<td>12.5-100</td>
<td>0.992</td>
<td>0.005</td>
</tr>
<tr>
<td>Dihydromorphine-6-glucuronide</td>
<td>12.5-100</td>
<td>0.974</td>
<td>0.005</td>
</tr>
<tr>
<td>Dihydronormorphine</td>
<td>12.5-100</td>
<td>0.989</td>
<td>0.004</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>6.25-100</td>
<td>0.999</td>
<td>0.013</td>
</tr>
<tr>
<td>Dihydronorcodeine</td>
<td>12.5-100</td>
<td>0.998</td>
<td>0.004</td>
</tr>
<tr>
<td>Norbuprenorphine</td>
<td>0.31–5.0</td>
<td>0.984</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**Figure 4.** The distribution of urinary buprenorphine and norbuprenorphine concentrations obtained from 72 patients receiving from 0.2 to 24 mg buprenorphine per day.
Buprenorphine concentrations were generally lower than norbuprenorphine with a median and mean value of the ratio of 0.23 and 0.34, respectively, and was found not to be related to the dose (Figure 6 and Table IV). Fifteen samples from 8 patients had a ratio > 0.99. One patient with a constant dose of 4 mg/day produced 6 of 12 samples with a ratio between 1.21 and 3.16. The ratio between buprenorphine and norbuprenorphine was found to be variable within some individuals, whereas in others, the ratio appeared to be more stable (Figure 7).

Of the 300 urine samples from Subutex-treated patients, 21% were found to be positive for opiates. These samples were from 44% of the patients, indicating relapse into heroin use during treatment. No difference existed between opiate positive and negative subgroups regarding dose (median dose 8.0 for both), buprenorphine/norbuprenorphine ratio (Table IV), or buprenorphine and norbuprenorphine excretion.

### Table IV. Ratio of Buprenorphine to Norbuprenorphine in Opiate Negative and Positive Subgroups

<table>
<thead>
<tr>
<th></th>
<th>All Samples</th>
<th>Opiate Negative</th>
<th>Opiate Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.34</td>
<td>0.34</td>
<td>0.33</td>
</tr>
<tr>
<td>Median</td>
<td>0.23</td>
<td>0.22</td>
<td>0.24</td>
</tr>
<tr>
<td>5% Conf. limit</td>
<td>0.08</td>
<td>0.07</td>
<td>0.09</td>
</tr>
<tr>
<td>95% Conf. limit</td>
<td>0.99</td>
<td>1.07</td>
<td>1.03</td>
</tr>
<tr>
<td>N</td>
<td>300</td>
<td>237</td>
<td>63</td>
</tr>
</tbody>
</table>

Discussion

This work demonstrates that the newly developed CEDIA assay for buprenorphine in urine is well suited for application in clinical testing and thereby provides the first high-volume screening test for buprenorphine. It was demonstrated that the quantitative results are in good agreement with GC-MS and that the measuring range can be extended by simply diluting the urine sample with saline. A 1:10 dilution was performed in 44% of the samples and 1:100 dilution in 3.7%. This possibility might be useful from the aspect of finding a urine sample that has been spiked with buprenorphine and are likely to contain much higher concentrations. Proof of tampering by lack of norbuprenorphine or an abnormally high buprenorphine/norbuprenorphine ratio can be obtained from the confirmation analysis in such cases. The observation of low values for buprenorphine at the high end of the CEDIA measuring range, as compared to GC–MS, was surprising and might suggest that a more limited measuring range should be used. Tenfold dilution could be performed at responses above 50 to improve that quantitative correlation to GC–MS. A modified test with an extended measuring range (0–750 μg/L) would cover 96.3% of samples.

From a qualitative aspect, the CEDIA assay compares both with the existing ELISA test and GC–MS at the cutoff level at 5 μg/L. Unwanted cross-reactivity in samples from patients receiving dihydrocodeine was discovered but always with a response ≤ 45 μg/L. Several metabolites of dihydrocodeine were studied, but all displayed a rather low cross-reactivity. According to present knowledge of dihydrocodeine metabolism (25,26), the main metabolites were included in the cross-reactivity experiment. It is most likely that several dihydrocodeine metabolites contributed to the observed cross-reactivity in the CEDIA assay. Because the slope of the CEDIA/GC–MS regression line was close to 1 and the cross-reactivity of norbuprenorphine (Table III) was low, it can be concluded that the CEDIA response corresponds to the sum of buprenorphine and conjugated buprenorphine only. Norbuprenorphine does not contribute to the response in the CEDIA assay. This feature of having buprenorphine as the main analyte is similar to the ELISA assay (19).

Cirimele and co-workers (19) validated the ELISA assay used for comparison, against an LC–MS procedure. False-positive results were observed at the 2 μg/L but not at the 5 μg/L cutoff limit in that study, which is in some contrast to our findings. However, this might simply be related to the differences in material studied. The rate of false-positive results was low for...
both assays. One advantage with the CEDIA assay is the better precision that can be achieved at the cutoff limit. However, it has been observed in the authors' laboratories that the precision of the CEDIA buprenorphine test during routine use is not as good as most other CEDIA urine tests for drugs of abuse. Both GC–MS and LC–MS methods have been published for buprenorphine and norbuprenorphine measurement (16–18,20). The procedure described in this paper has the novel feature of using acetylation with 4-dimethylaminopyridine as the catalyst. This enabled a high yield of acetyl derivatives that had favorable GC and MS characteristics. The present CEDIA assay was primarily intended for use with a 5 μg/L cutoff limit. In this study, a majority (96%) of the samples from patients being prescribed buprenorphine was above this limit. The biological noise in samples from other substitution treatments (mainly methadone) was significant because of unspecific reactivity or from other cross-reacting substances, and application of 2 μg/L as cutoff resulted in an unacceptably high rate of false-positive results. However, it is our experience that when monitoring Subutex-treated patients, a cutoff limit of 2 μg/L can be applied. In the material studied, this would have decreased the false-negative results by 75%, from 12 to 3 out of 300. In the study by Cirimele and co-workers (19), it was concluded that a 2 μg/L cutoff limit was preferable over a 5 μg/L cutoff limit because of minimal false-negative results. Because buprenorphine is available on the black market and is a subject of non-medical use, there is a need for including buprenorphine in general toxicology screening. The optimal cutoff limit for this application is unknown and cannot be estimated from present data. However, the observation that patients receiving prescribed buprenorphine may test negative may indicate that a lower cutoff level than 5 μg/L should be used in compliance testing. A 1 μg/L cutoff limit was previously proposed (13).

One essential clinical effect of buprenorphine is to replace and reduce heroin intake. It was therefore, unexpected to find a rather high opiate-positive rate (21%) in the samples from Subutex-treated patients. However, according to the meta-analysis of buprenorphine made by Mattick and co-workers (2), the reduction of heroin use is dose-dependent and is observed at high doses (8 mg/day). However, because the mean dose in this study was 8 mg/day, both in the whole group and in the subgroup with opiate positive samples, this was somewhat surprising.

The median ratio of buprenorphine and norbuprenorphine was found to be 0.23. However, there was a significant inter- and intra-individual variability. Because the elimination kinetics are slower for norbuprenorphine (27), the ratio is likely to be dependent on the time between sampling and dose intake. From this, one could expect high ratios to be related to low concentration. This was not observed in this material. Another factor is variability in activity of the enzyme responsible for the formation of norbuprenorphine. The CYP 3A class of enzymes is subjected to influence from both genetic and environmental factors, and a significant variability of norbuprenorphine formation is therefore to be expected (9). The observed buprenorphine/norbuprenorphine ratios and the dose-dependent urinary excretion are in agreement with a recent report (28).

In conclusion, this study has shown that the CEDIA buprenorphine assay is suitable for high-volume screening of urine. The optimal cutoff limit for buprenorphine cannot be determined at present and will require future studies. At present we would recommend the use of a 5 μg/L cutoff limit. In connection with monitoring of patients on a low dose or in the case of dilute urine sample, a 2 μg/L cutoff may be considered. In monitoring patients for buprenorphine, caution has to be taken for weak positive samples in combination with a positive opiate test as it may be due to cross-reactivity from dihydromorphone and possibly codeine.

References


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