Buprenorphine is a potent partial opioid agonist that is analyzed in urine to (i) monitor adherence to maintenance or detoxification therapy and (ii) detect illicit use. Buprenorphine analysis is commonly conducted on urine by immunoassay, but is subject to cross-reactivity from other drugs/drug metabolites, including morphine, codeine and dihydrocodeine. This study reports false-positive buprenorphine analysis [Thermo Fisher Scientific cloned enzyme donor immunoassay (CEDIA)] in patients who denied unauthorized buprenorphine use prior to sampling, but who had been prescribed amisulpride. In two cases, confirmatory analysis by liquid chromatography–tandem mass spectrometry was negative (<0.5 μg/L) for buprenorphine and metabolites and positive for amisulpride. Although the cross-reactivity of amisulpride and sulpiride in the CEDIA buprenorphine assay is low (estimated at 0.003 and 0.002%, respectively), it remains a significant consideration given the likely high concentrations of these compounds in urine relative to the low cutoff of the buprenorphine assay. Neither amisulpride nor sulpiride was listed as potential sources of interference on the CEDIA data sheet when this work was performed. These findings highlight the importance of confirming immunoassay-positive buprenorphine results using a more selective analytical technique.

**Introduction**

Buprenorphine (Figure 1) is a potent partial opioid receptor agonist that is used in low doses for pain relief (<1 mg/d), but also in higher doses (2–32 mg/d) for the treatment of opioid dependence. Illicit use of buprenorphine has also been reported (1). Buprenorphine analysis in urine is requested to (i) monitor adherence to the maintenance treatment of opioid dependency and to opioid detoxification therapy, and (ii) detect illicit use of the drug.

After sublingual dosage, buprenorphine is quickly and extensively metabolized by hepatic N-dealkylation to norbuprenorphine, and both buprenorphine and norbuprenorphine are conjugated with glucuronic acid prior to urinary excretion (2, 3). Urinary concentrations of unconjugated (free) buprenorphine are low (4, 5). The Substance Abuse and Mental Health Services Administration (SAMHSA) and the UK Laboratory Guidelines for Legally Defensible Workplace Drug Testing (2003) advise a positive urine cutoff of 5 ng/mL for total buprenorphine (i.e., buprenorphine plus metabolites) (6). Therefore, buprenorphine analysis by different immunoassay methods is subject to variation, depending upon which target immunogen the antibody was raised against and the degree of cross-reactivity with other buprenorphine metabolites (7).

The measurement of buprenorphine by cloned enzyme donor immunoassay (CEDIA; Thermo Fisher Scientific CEDIA, Hemel Hempstead, UK) is subject to cross-reactivity from tramadol (9) and from opioids such as morphine, codeine and dihydrocodeine (8). Other structurally unrelated compounds that are likely to be present in urine from heroin users, including quinine used to cut illicit heroin, may also interfere. Recently, cross-reactivity was encountered in the CEDIA urine buprenorphine assay that was traced to the presence of the antipsychotic amisulpride (Figure 1). Further study has shown that interference from sulpiride (Figure 1) may also be encountered in clinical samples.

**Methods**

**Materials and reagents**

Buprenorphine, norbuprenorphine, buprenorphineglucuronide, norbuprenorphine glucuronide, buprenorphine-Δ4 and norbuprenorphine-Δ4 were from LGC Standards (Teddington, UK). (+)-Amisulpride, (+)-sulpiride and β-glucuronidase (Helix pomatia) were from Sigma Aldrich (Poole, UK). CEDIA reagents were supplied through Thermo Fisher Scientific.

**CEDIA assays**

Urine samples were processed for opiates, methadone metabolite (EDDP), cocaine, benzodiazepines, barbiturates, amphetamines, cannabinoids and buprenorphine according to the manufacturer’s instructions by using an automated analyzer (Olympus AU640, Beckman Coulter, UK). Sample integrity was assessed by the measurement of creatinine (Jaffé method) using the same analyzer.

**HPLC–UV and LC–MS–MS**

Urinary total (free and conjugated) buprenorphine and norbuprenorphine were measured using solid-phase extraction (SPE), followed by liquid chromatography–tandem mass spectrometry (LC–MS–MS). Briefly, samples were analyzed by mixed-mode SPE (Phenomenex Strata Screen CTM columns) before and after hydrolysis (β-glucuronidase in 1.0 mmol/L sodium acetate; pH 5.0, 37°C, 12 h). The eluate was evaporated to dryness under a stream of nitrogen (40°C) and reconstituted in methanol. Extracts were analyzed using isocratic strong cation exchange (SCX) high-performance liquid chromatography (HPLC; Waters Spherisorb S5SCX), followed by positive mode atmospheric pressure chemical ionization (APCI)–MS–MS (TSQ Quantum Access, Thermo Fisher Scientific). This method was cross-validated using Heathcontrol Drugs of Abuse Scheme external quality assessment (EQA) samples (Sample numbers: 238, 247–249) containing various concentrations of buprenorphine and buprenorphine metabolites.
Urinary amisulpride and sulpiride were measured in the laboratory by either (i) HPLC with ultraviolet absorption detection (UV) (Case 1), or (ii) by a recently developed LC–MS–MS method (Case 2). For HPLC–UV, methyl tert-butyl ether extracts of samples and calibration standards prepared in analyte-free urine (200–500 mg/L amisulpride) were analyzed by SCX–HPLC with detection at 240 nm. For LC–MS–MS, samples were diluted (1 + 9, v/v) with deionized water and assayed against extracts of plasma calibration solutions (11). Liquid–liquid extraction into butyl acetate–butanol (9 + 1, v/v) was followed by SCX–HPLC and positive mode APCI–MS–MS (TSQ Quantum Access, Thermo Fisher Scientific).

Clinical Background

Patient 1
A 31-year-old male with a long history of solvent, alcohol and illicit substance misuse had been diagnosed with paranoid schizophrenia at the age of 18. Treatment was complicated by occasional methadone and heroin use. A urine drug screen in a local laboratory (CEDIA) was positive for buprenorphine. He admitted to regular cannabis use and frequent heroin use until five weeks before the urine test. He also admitted occasional alcohol use, but consistently denied using buprenorphine, or awareness that it was or had been available at the center where he was being treated.

Patient 2
A 30-year-old male started using illicit heroin at the age of 17. Some seven years later, he started buprenorphine (Subutex) maintenance therapy, but methadone was substituted after he was found to be injecting buprenorphine. Aged 28, he was prescribed amisulpride. A year later, the prescribed amisulpride dose was 600 mg/d. He admitted to occasional illicit heroin use while on methadone, but relapsed into more regular heroin use and methadone prescription was discontinued. Aged 29, he was prescribed buprenorphine: 2 mg/d for five days. He discharged himself from the detoxification clinic and over the next three days said he used illicit buprenorphine (reported as 2 mg/d) and benzodiazepines. He was subsequently admitted to the hospital, and over the ensuing months discharged himself and was re-admitted many times, but claimed that, other than smoking cannabis and drinking alcohol, he did not take illicit drugs, including buprenorphine. However, a random urine sample tested positive for buprenorphine (CEDIA) at a local laboratory.

Patient and external quality assessment samples
Random urine samples from Patients 1 and 2, and from further patients prescribed amisulpride, but not buprenorphine, were supplied for analysis. At the time of sampling, Patient 1 was prescribed clozapine (800 mg/d), amisulpride (150 mg/d), venlafaxine (300 mg/d), hyoscine hydrobromide (600 µg/d), metformin (2,550 mg/d), simvastatin (40 mg/d), fenofibrate (160 mg/d) and aspirin (75 mg/d), and admitted to using cannabis; Patient 2 was prescribed amisulpride (600 mg/d), diazepam (12.5 mg/d), zopiclone (15 mg/d) and diclofenac (50 mg/d), and admitted to using cannabis. HeathControl Drugs of Abuse Scheme (LG C Standards) urinary buprenorphine EQA specimens were analyzed as appropriate.

Assessment of amisulpride and sulpiride CEDIA cross-reactivity
Urine from a healthy volunteer that tested negative for buprenorphine on CEDIA was reanalyzed using this same technique after the addition of amisulpride or sulpiride (50–1,000 µg/L of each compound). The relative concentrations at which the cross-reactants provided an apparent CEDIA buprenorphine concentration > 5 µg/L were used to estimate percentage cross-reactivity for each compound.

Results
The results obtained on the analysis of the samples were as follows. Patient 1: creatinine, 2.6 mmol/L; CEDIA buprenorphine, < 5 µg/L; LC–MS–MS buprenorphine and norbuprenorphine (total, i.e., after hydrolysis of conjugates), both < 0.5 µg/L; and HPLC–UV amisulpride, 22 µg/L. Patient 2: creatinine, 20.1 mmol/L; CEDIA buprenorphine, 8.0 µg/L; LC–MS–MS buprenorphine and norbuprenorphine (total, i.e., after hydrolysis of conjugates); both < 0.5 µg/L; LC–MS/MS amisulpride, > 5 µg/L (after 10-fold dilution in deionized water). In both cases, CEDIA was positive for cannabinoids; for Patient 2, benzodiazepines were also present. All other CEDIA assays were negative.

Of nine urine samples from three patients prescribed amisulpride but not buprenorphine, three (HPLC–UV amisulpride concentrations of 7–22 µg/L) showed apparent buprenorphine concentrations < 5 µg/L on CEDIA. The remaining samples (HPLC–UV amisulpride concentrations of 168–1,380 µg/L) provided apparent buprenorphine results of 5–46 µg/L on CEDIA. The highest of these results was from a sample from a patient prescribed 1,200 mg/d of amisulpride.

A urine sample from a patient prescribed 600 mg/d of sulpiride (Heathcontrol sample number 275) showed an apparent buprenorphine result (CEDIA) of 11.5 µg/L. The same sample was negative for buprenorphine, norbuprenorphine and conjugated metabolites by LC–MS–MS, but had a sulpiride concentration > 5 µg/L when analyzed by LC–MS–MS after 10-fold dilution in deionized water.

CEDIA cross-reactivity
The analysis of urine to which amisulpride or sulpiride had been added showed positive cross-reactivity on the CEDIA buprenorphine assay (approximately 0.003 and 0.002%,
False-Positive Buprenorphine by CEDIA in Patients Prescribed Amisulpride or Sulpiride

Discussion

Neither amisulpride nor sulpiride were listed as potential cross-reactants in the CEDIA buprenorphine immunoassay when this work was undertaken. Amisulpride and sulpiride are usually given orally at doses in the range 400–1,200 and 600–1,600 mg/d, respectively (12), and are excreted largely unchanged (95%) in urine and bile (3). Although the cross-reactivity of these compounds in the CEDIA buprenorphine assay was very low (estimated at 0.003 and 0.002% for amisulpride and sulpiride, respectively), cross-reactivity remains a significant consideration, given the likely high concentrations of these compounds in urine from patients prescribed these drugs (of the order of 1 g/L in some cases) relative to the low cutoff (5 µg/L) of the buprenorphine assay. Of course, there may also be a contribution from amisulpride or sulpiride metabolites in patient samples.

In both case studies, cross-reactivity from opiate class compounds (morphine, codeine and dihydrocodeine) was excluded by a negative CEDIA opiate class result and the presence of amisulpride was confirmed by chromatographic analysis. LC–MS-MS analysis for buprenorphine and metabolites confirmed the absence of all analytes (limit of detection: 0.5 µg/L). For Case 1, despite a positive CEDIA buprenorphine result at a local laboratory, the further urine sample analyzed in the authors’ laboratory had a CEDIA buprenorphine below the cutoff. However, the prescribed amisulpride dose in this patient was relatively low (150 mg/d) and the urine sample provided was very dilute (creatinine: 2.6 mmol/L).

The analysis of buprenorphine by LC–MS-MS is complicated by (i) low analyte concentrations (especially of unconjugated buprenorphine); (ii) poor ionization of the analytes using either electrospray ionization (ESI) or APCI; and (iii) poor fragmentation of the protonated molecular ([M + H]+) ions. Many methods have been reported for both buprenorphine and norbuprenorphine (13–15) and for the direct analysis of their conjugated metabolites (16–19). However, appropriate sample preparation and chromatographic steps are important for a robust assay.

Buprenorphine is primarily excreted in urine as buprenorphine glucuronide, unconjugated norbuprenorphine and norbuprenorphine glucuronide (3–5). The buprenorphine CEDIA assay has minimal cross-reactivity (<0.015%) with norbuprenorphine and norbuprenorphine glucuronide, and can provide false negative results in the final stages of buprenorphine detoxification (5). Furthermore, norbuprenorphine has a longer plasma half-life and is present in larger amounts in urine than the parent compound, and thus a urinary norbuprenorphine/norbuprenorphine glucuronide assay gives a more sensitive indication of buprenorphine use. At the doses used for the treatment of opioid dependence, buprenorphine metabolites may be detected for up to one week following the last dose (20). Much of the drug excreted after two or three days will be excreted as metabolites, and therefore may not be detectable using immunoassay alone.

The importance of accuracy in drugs of abuse assays performed for clinical purposes, let alone those performed for medico-legal purposes, cannot be over-emphasised. When substances are detected using one analytical method, especially one that is susceptible to interference such as immunoassay, and the patient denies use, a more selective method should be used to confirm or refute the findings. There may also be situations in which the failure to detect an analyte due to the use of an inappropriate test may bring the therapeutic relationship into doubt, as discussed previously for buprenorphine in the later stages of an opioid detoxification regimen.

Conclusions

CEDIA remains a useful tool with which to monitor urine from clients prescribed buprenorphine, or if buprenorphine abuse is suspected, because the assay is rapid and simple to perform. However, particular caution is needed when interpreting weakly positive results (5–30 µg/L), because such results may be achieved by cross-reactivity from opioids or other drugs alone. A selective method such as LC–MS-MS can be used not only in confirmation work, but also when monitoring the later stages of opioid detoxification therapy when total buprenorphine in urine is low. The ability to detect norbuprenorphine and norbuprenorphine glucuronide is valuable in such circumstances, and also serves to increase the detection window from the time of last use.

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References


Figure 2. CEDIA buprenorphine assay: amisulpride and sulpiride cross-reactivity. Lines were fitted using quadratic equations \( y = -0.000002x^2 + 0.0537x - 1.197 \) for amisulpride and \( y = -0.00001x^2 + 0.0338x - 2.0212 \) for sulpiride; each point represents single analysis. The dashed line represents the 5 µg/L CEDIA cutoff.


