Comparison of CEDIA® Buprenorphine II Assay vs. on Market CEDIA ® Buprenorphine Assay in Human Urine

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INTRODUCTION

Buprenorphine
Buprenorphine is a semi-synthetic opioid derived from the opioid alkaloid thebaine. It is widely used clinically as an analgesic and as a substitute therapy for opioid dependence. It is considered a relatively safe opioid because of its agonistic-antagonistic characteristics on opiate receptors. Correlation between dose and therapeutic effects are not linear, indicating a ceiling on the effects in patients.
In the human body, buprenorphine is primarily metabolized by N-dealkylation into norbuprenorphine, which is pharmacologically active, and both buprenorphine and norbuprenorphine are conjugated to glucuronide. About 10% each of conjugated buprenorphine and of conjugated norbuprenorphine, but only 1 – 3% of the non-conjugated substances are eliminated in the urine. After a single dose overall 95% is eliminated from the body within 6 days.

Opiate maintenance therapy
Subutex® and Suboxone® are the commonly prescribed drugs containing buprenorphine in opiate maintenance therapy. Main daily doses range within 2 and 16 mg, up to 32 mg. However, buprenorphine is also quite common on the illicit market.
In substitution therapy regular toxicological analyses of patients' urine samples is mandatory, proving the opiate addiction, the correct taking of the prescribed substitution drug and the additional consumption of non-prescribed and/or illicit substances. Immunoassays may provide quick results without the need of time-consuming sample preparation.


Buprenorphine


Norbuprenorphine

OBJECTIVE

Up to now, commercially available buprenorphine immunoassays have detected either only buprenorphine and buprenorphine glucuronide, or only non-conjugated buprenorphine and norbuprenorphine.
The objective of the present study was to evaluate a homogeneous enzyme immunoassay currently in development that can detect buprenorphine, norbuprenorphine and in addition their conjugated metabolites in human urine.
For this purpose urine samples of patients in opiate maintenance therapy were analyzed using the commercially available Thermo Scientific™ CEDIA Buprenorphine assay and the Thermo Scientific™ CEDIA Buprenorphine II assay which is in development. Results were compared to GC-MS analyses.

MATERIALS AND METHODS

Urine Samples
Routinely collected urine samples from 120 opiate maintenance therapy patients were included in this study.
Fifty patients received buprenorphine-substitution (daily dose 2 to 28 mg), 50 patients received methadone substitution (daily dose 20 to 160 mg) and 20 patients received morphine substitution (daily dose 120 to 900 mg). Urine samples were stored at – 20 ° C.

Immunoassays
Urine samples were analysed on a Thermo Scientific™ Indiko™ Plus benchtop analyser using the commercially available CEDIA Buprenorphine assay according to the manufacturer's instructions, and the CEDIA Buprenorphine II assay, which is currently in development at Thermo Fisher Scientific.
The on market CEDIA Buprenorphine assay has a cut-off of 5 ng/mL using quality control samples ±40% of the cut-off.
The CEDIA Buprenorphine II assay has a cut-off of 10 ng/mL using quality control samples ±25% of the cut-off.

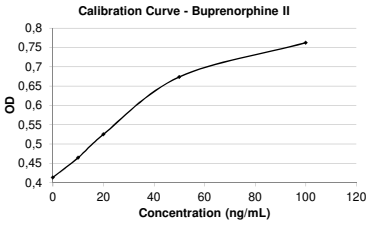
Sample preparation
For enzymatic hydrolysis, 2.0 ml of urine samples were mixed with 25 µl of enzyme solution (β-glucuronidase, Roche Diagnostics, Mannheim) and incubated for 16 hours at 38 ° C.
Hydrolysed urine samples were mixed with 2 ml 0.1 M phosphate buffer (pH 6.0), and 50 µl internal standard (buprenorphine-d4 and norbuprenorphine-d3, each 1.0 mg/l in methanol). SPE was performed on Spe-ed Scan ABN columns (200 mg/3 ml, Applied Separations, Allentown, PA). The column was conditioned with 2 ml methanol and 1 ml 0.005 M hydrochloric acid. Loading of sample was accomplished at a flow rate of 1.0-1.5 ml/ min. The column was washed with 2 ml distilled water, 1 ml 0.005 M hydrochloric acid, centrifuged for 5 min (4500 g) and dried under nitrogen stream. Elution was performed with 2.0 ml of dichloromethane, propan-2-ol and ammonium hydroxide (80/20/8, v/v/v). The eluate was evaporated to dryness at 60 ° C. 50 µl pyridine and 100 µl acetic anhydride were added for derivatization at 60 ° C for 60 min. After evaporation of excess of derivatizing reagent, the residue was reconstituted in 50 µl ethyl acetate.

GC-MS Analysis of Buprenorphine / Norbuprenorphine
The GC-MS system consisted of a HP7890 GC device with a HP5975C inert XL mass-selective detector (Agilent Technologies, Palo Alto, CA), using a DB-XLB column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, Agilent Technologies). Carrier gas helium at a flow rate of 1.0 ml / min. Injection volume 2 µl (splitless), injection temperature 250 ° C. Temperature program: 50 ° C, hold 1 min; increase to 150 ° C with 25 ° C/min, to 320 ° C with 10 ° C/min, hold for 8 min and to 330 ° C in 20 ° C/min, hold for 7.5 min. Electron impact ionization was performed (70 eV). The mass spectrometer was operated in selected ion monitoring (SIM) mode at m/z 443 (quantifier ion), 440 (quantifier ion), 408, 366, 485, 422 for norbuprenorphine and norbuprenorphine-d3 and m/z 424 (quantifier ion), 420 (quantifier ion), 456, 452, 412, 408, 398, 394 for buprenorphine and buprenorphine-d4, respectively. Recording and analysis of mass spectral data by HP MS ChemStation software G1034C version D01.00 (Agilent Technologies). Limit of detection (LOD) 2.0 µg/l, lower limit of quantification (LLOQ) 6.0 µg/l for both buprenorphine and norbuprenorphine.

GC-MS, LC-MS/MS General Unknown Screening
Urine samples were enzymatically hydrolyzed for cleavage of phase II metabolites (see above).
For SPE, a generic mixed-mode strategy was applied using ABN columns (200 mg/3 ml; Applied Separations). The eluate was split in two aliquots, which were submitted to LC-MS/MS and GC-MS analysis, respectively. The GC-MS sample was analysed in the native form and after acetylation.
GC-MS analysis were performed as described above, scanning from 50 to 600 m/z. For compound identification obtained mass spectra were matched to the "Maurer/Pfleger/Weber 2011" and the "Designer Drugs 2016" mass spectral libraries (both Wiley).
LC was performed on a Eurosphere C18 column (100 × 2 mm, 5 µm, Knauer, Berlin). A QTRAP 3200 system (Sciex, Framingham, MA) was used for ESI-MS/MS in positive ion mode using data-dependent acquisition control. The obtained MS/MS spectra were matched to the "Wiley Registry of Tandem Mass Spectral Data, MSforID" (Wiley).

RESULTS

Figure 1. CEDIA Buprenorphine II Calibration Curve and Controls Recovery



Controls	Expected Conc. (ng/mL)	Observed Conc. * (ng/mL)	% Recovery
Low Control	7.5	7.6	100.9
High Control	12.5	14.3	114.4

* Results from n = 6 replicates

Table 1. CEDIA Buprenorphine vs. GC-MS

		GC-MS	
		+	-
Bup	+	50	21
	-	0	49

Positive Agreement	Negative Agreement	Overall Correlation
100%	70%	82.5%

Table 2. CEDIA Buprenorphine II vs. GC-MS

		GC-MS	
		+	-
Bup II	+	50	1
	-	0	69

Positive Agreement	Negative Agreement	Overall Correlation
100%	98.6%	99.2%

Table 3. Discordant sample in CEDIA Buprenorphine II Assay compared to GC-MS

ID	Bup II IA	GC-MS (ng/mL)			Daily Bup Dose (mg)	Daily Morphine Dose (mg)	GC-MS / general unknown screening
	SQ (ng/mL)	Total Bup	Total Norbup	Total Bup/ Norbup			
N099	10.1	0	0	0	0	500	Morphine, normorphine, nicotine, caffeine

Table 4. CEDIA Buprenorphine II 5-day precision

n=10				
Calibrators / Controls	Expected Conc. (ng/mL)	Mean Observed Conc. (ng/mL)	SD	%CV
Low Control	7.5	9.1	1.19	13.15 %
Cut-off	10	12	1.06	8.88 %
High Control	12.5	14.1	0.99	7.04 %

CONCLUSIONS

The CEDIA Buprenorphine II detects the presence of free buprenorphine, free norbuprenorphine, conjugated buprenorphine and conjugated norbuprenorphine accumulated and does not differentiate between the parent drug and its metabolites.
The set cut-off of 10 ng/mL is therefore comparable to the CEDIA Buprenorphine assay cut-off of 5 ng/mL.

The data presented here demonstrates that the CEDIA Buprenorphine II assay has an excellent correlation to GC-MS.
CEDIA Buprenorphine II assay has improved specificity when compared to on market CEDIA Buprenorphine assay that shows a concentration-dependent cross-reactivity to opiates.

Slow-release oral morphine has become more and more common in opiate maintenance therapy programs in several European countries. It is to be expected that the highly specific CEDIA Buprenorphine II assay is therefore a reliable tool for urine sample analyses of patients even receiving extreme doses of morphine.

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