Validation of a homogeneous immunoassay for Buprenorphine testing in oral fluid samples

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Introduction

Buprenorphine (Bup) is a semi-synthetic opioid and partial μ-agonist derived from thebaine. Bup is used globally in pain therapy and in the treatment of opioid dependency. Bup screening normally is performed in urine to assess compliance of patients in maintenance therapy or to detect illicit use.

Oral fluid (OF) is increasingly accepted as a suitable alternative matrix to test for drugs of abuse in clinical, drug treatment, workplace and other settings. This is mostly because of ease of collection and less risk for adulteration.

However, little is known about the required sensitivity of the screening methods. Especially with regard to compliance testing in substitution therapy false negative Bup results must be avoided.

In this study we therefore analysed OF samples from 500 Bup patients with different and known Bup doses applying a sensitive reference method (UPLC-MS/MS, LoQ 0.1 ng/mL) and a homogenous immunoassay at different cutoffs.

Methods

Patients

500 presumed Bup positive OF samples from 500 different Bup substitution patients (dose range 0.6 to 28 mg/d for 475 patients, Fig. 1) and 50 Bup presumed negative OF samples from patients in methadone maintenance therapy were collected with the Greiner Bio-One saliva collection device in different outpatient clinics. % OF concentration of the OF/buffer mixture was determined spectrophotometrically on an OLYMPUS® AU680 using the Greiner saliva quantification kit.

UPLC-MS/MS

Bup in OF was quantified on a Waters Acquity UPLC connected to a Waters Xevo-TQ-S. Gradient separation was performed within 6 min on Waters BEH Phenyl 1.7 µm, 2.1 x 150 mm column kept at 60°C at a flow rate of 0.55 mL/min. Mobile Phase A was 20 mM ammonium formate + 0.1% FA and mobile phase B was MeOH + 0.1% FA. The instrument was operated in ESI positive and SRM mode . Three transitions were recorded for Bup , Bup-d4, Norbup and Norbup-d3. Matrix calibration for Bup and Norbup was from 0.025 to 20 ng/mL (n = 16).

Sample preparation

100 µL OF/buffer was fortified with 10 µL internal standard (= 0.5 ng/mL Bup-d4 and Norbup-d3) and then protein precipitated with 50 µL sulfo salicylic acid (20%) and 600 µL ACN. Phase separation was achieved by adding 100 µL 10 M ammonium formate and centrifuging. The organic supernatant was evaporated into 10 µL ethylenglycol at 45°C under N2-stream. The residue was dissolved in 80 µL H2O + 10 µL MeOH and 5 µL was injected into the UPLC system.

Immunoassay

OF samples were analysed with the CEDIA Bup Assay (Thermo Fisher Scientific) on an OLYMPUS® AU680 (Beckman Coulter) according to the manufacturer. Calibrators were prepared with reference material (LGC Standards) at 0.0, 0.2, 0.5, 1.0, 1.5, 2.0 and 4.0 ng/mL in 50% OF/buffer. The CEDIA does not cross-react with the metabolite NorBup.

Results

All 500 OF samples from patients in Bup substitution therapy were true positive for Bup and Norbup with UPLC-MS/MS at a cutoff of 0.1 ng/mL. The dosing range was between 0.6 and 28 mg/d (Fig. 1). For the 500 OF samples the following Bup concentration distribution was obtained from UPLC-MS/MS: 0.1 to 1.0 ng/mL: 90, >1.0 to 4.0 ng/mL: 115, >4.0 to 20 ng/mL: 96 and >20 ng/mL: 199 samples (Fig. 2). NorBup concentrations were lower: 0.1 to 1.0 ng/mL: 186, >1.0 to 4.0 ng/mL: 192, >4.0 t0 20 ng/mL: 122 samples (Fig. 3).

The European Workplace Drug Testing Society recommends a Bup cutoff at 5 ng/mL. At this cutoff only 276 samples of all 500 OF samples were positive (55.2 %) with LCMS. With the CEDIA Bup Assay 221 of these samples were found positive also. Only 3 samples were detected as false positive and 55 samples as false negative [Tab. 1)]. Reducing the UPLC-MS/MS cutoff to 1 ng/mL resulted in 410 pos and 90 neg samples. CEDIA found 363 of these accordingly positive (88.5%) and 87 samples resp. [Tab. 2)].

Only 3 samples were false positive and 47 were false negative. Lowering the cutoff to 0.5 ng/mL for the immunoassay only [Tab. 3)] gave a good agreement (95.6%) with UPLC-MS/MS positive results at the 1 ng/mL cutoff. Only 18 OF samples were false negative. However, the false positive rate increased to 4.4% (22 samples).

To exclude the influence of matrix effects on the immunoassay performance (cross-reactivities) 50 Bup true negative OF samples (UPLC-MS/MS <0.1 ng/mL) were tested with the homogeneous immunoassay. Only one sample tested false positive with the immunoassay at cutoff 0.5 ng/mL (Fig. 4).

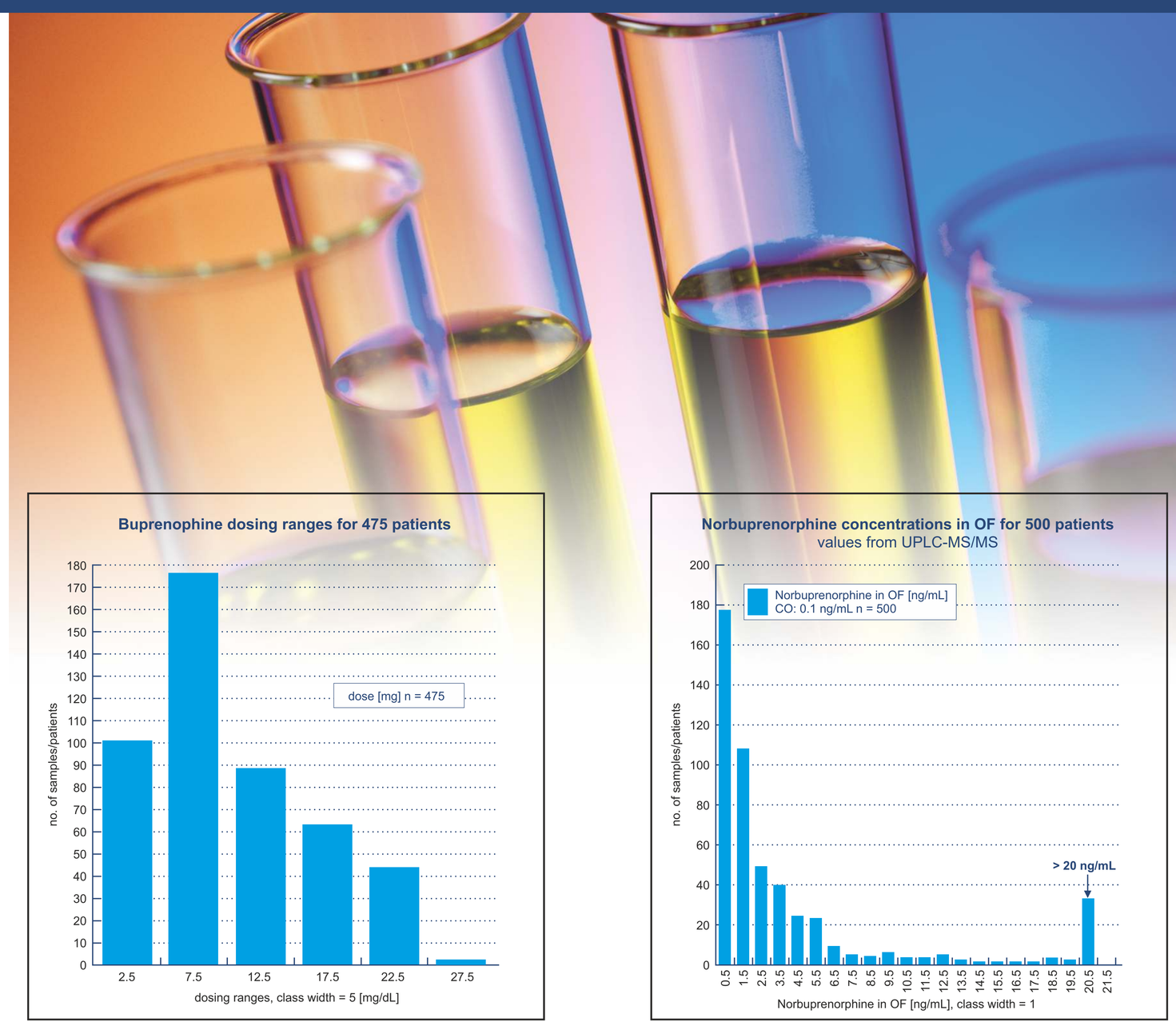

FIGURE 1: Bup dose ranges for 475 patients in Bup maintenance therapy.

FIGURE 3: Bup concentrations in 500 OF samples analysed with UPLC-MS/MS. Calibration range was from 0.025 to 20 ng/mL.

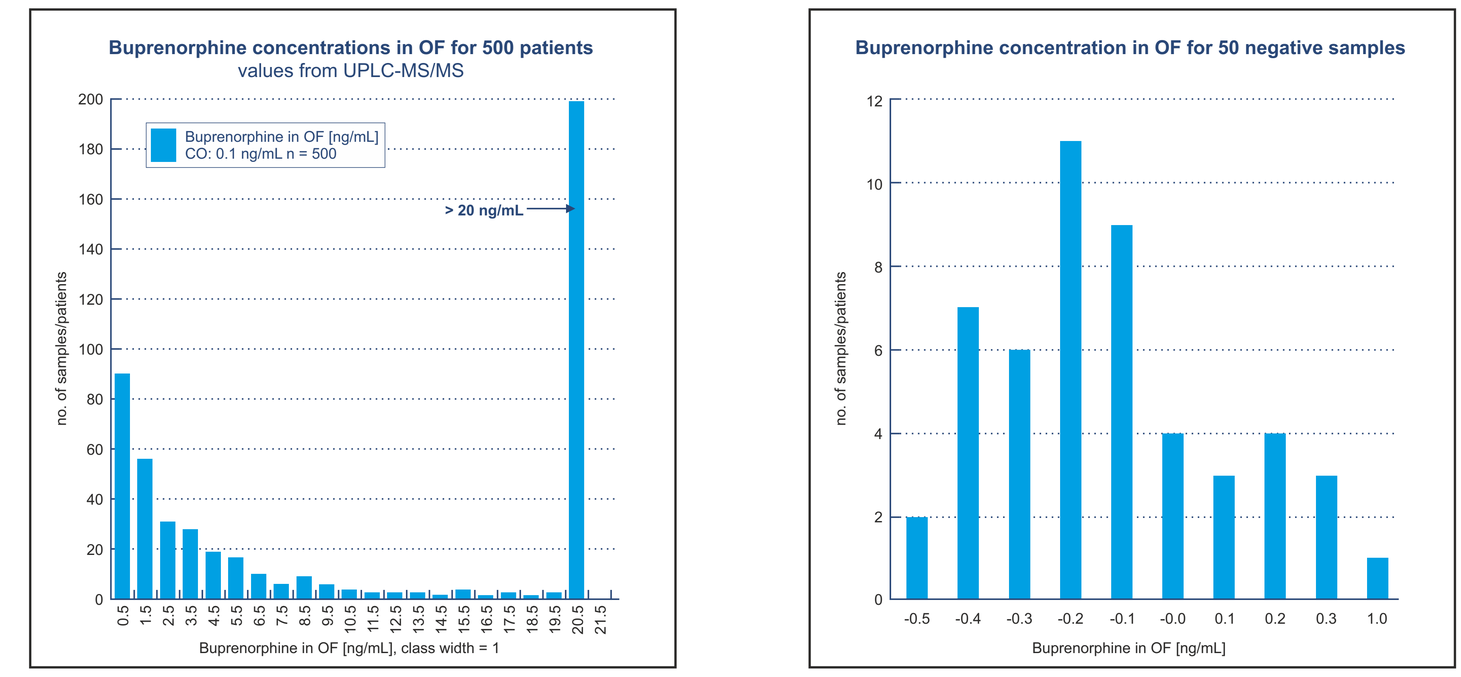

FIGURE 2: Bup concentrations in 500 OF samples analysed with UPLC-MS/MS. Calibration range was from 0.025 to 20 ng/mL.

FIGURE 4: Immunoassay response of the 50 true neg samples (UPLC-MS/MS: Bup <0.1 ng/mL). Immunoassay cutoff = 0.5 ng/mL

cutoff = 5 ng/mL	LC-MS/MS +	LC-MS/MS -
CEDIA +	221	3
CEDIA -	55	221

TABLE 1: Comparison of results from OF samples for Bup at cutoff 5 ng/mL

cutoff = 1 ng/mL	LC-MS/MS +	LC-MS/MS -
CEDIA +	363	3
CEDIA -	47	87

TABLE 2: Comparison of results from OF samples for Bup at cutoff 1 ng/mL

cutoff = 1 ng/mL cutoff = 5 ng/mL	LC-MS/MS +	LC-MS/MS -
CEDIA +	392	22
CEDIA -	18	68

TABLE 3: Comparison of results from OF samples for Bup at cutoff 1 ng/mL for LC-MS/MS and. cutoff = 0.5 ng/mL for CEDIA

Conclusions

1.) Compliance testing in Bup maintenance therapy demands a cutoff at 0.1 ng/mL for OF samples.

2.) In general NorBup concentrations in OF are lower than Bup concentrations.

3.) Screening for Bup abuse in OF can be performed with the CEDIA immunoassay at a cutoff of 0.5 ng/mL to spot Bup concentrations >1.0 ng/mL.

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