A Fast and Sensitive Screening Method for Buprenorphine and Norbuprenorphine in Urine and Whole Blood by Solid Phase Extraction and Ultra High Performance Liquid Chromatography/Time-Of-Flight Mass Spectrometry (UPLC/TOFMS)

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AIMS: Buprenorphine (i.e. Subutex®, Suboxone® or Temgesic®) is an emerging drug in substitution therapy of opioid addicts in Denmark. Screening for buprenorphine by radioimmunoassay (RIA) has previously been applied in our laboratory, but the buprenorphine RIA kit is no longer available. Thus, the objective of this study was to develop a sensitive, fast and accurate screening method for buprenorphine.

METHODS: Buprenorphine (MW 468.3114) and norbuprenorphine (MW 414.2644) were extracted by mixed mode cat ion exchange columns, Isolute HCX-3 (130 mg/3 mL), by a modified method (1). Internal standards D4-buprenorphine (MW 472.3365) and D3-norbuprenorphine (MW 417.2833) were added to all samples prior to extraction. Urine (2 mL) was added 1 mL 1 M acetate buffer, pH 5.5 and 25 µL beta-glucuronidase/aryl sulphatase and hydrolyzed overnight at 40°C. Whole blood (0.5 mL) was added 2.7 mL 0.1 M KH₂PO₄ buffer, pH 6, mixed, sonicated 10 min and centrifuged 10 min at 3600 rpm. Gilson ASPEC XL4 (Gilson, Villiers-le-Bel, France) with positive pressure adjustment (Biolab, Aarhus, Denmark) was used for automated solid-phase extraction. The SPE columns were conditioned with 1.5 mL methanol and 1.5 mL 0.1 M KH₂PO₄ buffer. Sample (3 mL) was loaded, followed by washing steps consisting of 1 mL 0.1 M KH₂PO₄ buffer, 1 mL 1 M acetic acid, and 1 mL methanol in consecutive order. After drying 10 min, the analytes were eluted with ammonium hydroxide (25% aq.) -acetonitrile -ethyl acetate (2:10:88, v/v). After evaporation at 45°C, the blood extract was reconstituted in 100 µL methanol and the urine extract was reconstituted in 2 mL methanol. The UPLC/TOF/MS conditions: UPLC (Acquity, Waters, Milford, MA, USA) 1.5 min gradient elution with water-formic acid (0.1%) and acetonitrile (75:25) to (50:50) on an Acquity BEH C18, 2.1 x100 mm column, 1.7 µm at 50°C. The TOFMS (LCT Premier XE, Micromass, Manchester, UK) was operated in electropositive W-mode (ESI+).

RESULTS: The retention time was 0.85 min for norbuprenorphine and 1.23 min for buprenorphine, and the total UPLC run time was 3 minutes. The mass accuracy was found to be better than 5 ppm and resolution higher than 10,000. The linear range of the instrument method was 0.00005 to 0.1 mg/L for buprenorphine and 0.0005 to 0.1 mg/l for norbuprenorphine. Accuracy was verified by including spiked samples in each run. The limit of detection (LOD) in blank matrix was found to be 0.0001 mg/L in blood and 0.0005 mg/L in urine for buprenorphine, and 0.001 mg/L blood and 0.005 mg/L urine for norbuprenorphine. Thus, the LOD of buprenorphine in blood was found to be adequate for detection of buprenorphine in the therapeutic range; 0.0001 to 0.001 mg/L.

Urine from authentic criminal and autopsy cases were applied for method comparison; 18 positive and 5 negative RIA-buprenorphine urine samples were confirmed by UPLC/TOFMS.

CONCLUSIONS: A UPLC/TOFMS method for fast separation and accurate detection of buprenorphine and norbuprenorphine was obtained.

Keywords: Buprenorphine, Norbuprenorphine, UPLC/TOF-MS

(1) H. Klinke and K. Linnet: Performance of four mixed-mode solid-phase extraction columns applied to basic drugs in urine. The Scandinavian Journal of Clinical & Laboratory Investigation (In Press)