AM-p-10

Screening by immunoassay and confirmation & quantitation by GC-MS of buprenorphine and norbuprenorphine in urine, whole blood and serum

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EMIT-Screening of urine samples: Urine samples were screened using EMIT II enzyme immunoassay. The method was modified by using Dade Behring VIVA analyzer with Microgenics reagents. Calibrators were prepared in drug-free urine. One GC-MS confirmed positive clinical sample and one negative clinical sample were used as control samples. The cut-off level was 1,5 ng/ml.
True positive, true negative, false positive and false negative results were calculated after comparison the EMIT II and GC-MS results. From screened samples there were 8 false positive out of 29 positive samples. These false positive samples were also analysed for opiates with GC-MS method. It was observed that buprenorphine EMIT II screening assay had cross-reactivity with other opiates such as morphine, codeine, pholcodine and tramadol at the high levels. All screened negative samples were true negative.

ELISA Screening of blood samples: The whole blood samples were screened by enzyme-linked immunosorbent assay (ELISA, Cozart Microplate Elisa). The method for whole blood samples was modified from the screening method for urine. Samples, controls and calibrators were all whole blood, but Microplate EIA Kit was originally meant for urine samples. Calibrators were prepared in drug-free whole blood. A positive clinical sample was used as a control sample. The limit of detection was 1,0 ng/ml.
True positive, true negative, false positive and false negative results were calculated after comparison the ELISA and the GC-MS results. From screened samples there were 11 false positive out of 36 positive samples. There wasn't common reason to explain the false positive results. All screened negative samples were true negative. Opiate positive blood samples (14 samples) were also screened by immunoassay for buprenorphine and analysed with the GC-MS method. It was shown that all these analysed samples were negative in screening and in quantitation analysis.

GC-MS confirmation: A sensitive gas chromatography-mass spectrometry method has been developed and validated for simultaneous determination of buprenorphine (BU) and norbuprenorphine (NBU) in serum, whole blood and urine samples. The urine sample had to be hydrolyzed first to release buprenorphine and norbuprenorphine from their glucuronide conjugates. The optimal incubation conditions of the enzymatic hydrolysis in 1 ml urine were 50 µl ß -glucuronidase and incubation at 37°C overnight. The drugs were extracted from basic solutions (pH ~9) by liquid-liquid extraction with toluene containing deuterated buprenorphine (BU-d4) as an internal standard. After evaporation of the solvent the extracted compounds were silylated by MSTFA (N-methyl-N-tri-methylsilyl-trifluoroacetamide). The samples were

analysed with the GC-MS. Separation was carried out on a DB-35MS fused silica capillary column using helium as a carrier gas. Selected ion monitoring (SIM) was used in the electron impact mode.

The method was found to be sensitive (the limits of quantitation (LOQ) of 0,5 ng/ml for both buprenorphine and norbuprenorphine). The accuracy and precision of the method at the concentration level 0,5 ng/ml were: 9,4 % (BU) / 4,6 % (NBU) for accuracy; 5,3 (BU) / 13,0 (NBU) intra-day assay; 7,9 (BU) / 27,7 (NBU) inter-day assay. The linearity range for both analytes were 0,5-15 ng/ml.

The GC-MS method and immunological screening by EMIT II has been accredited by Finnish Accreditation Service. All described methods have been used in routine analysis for serum, whole blood and urine in clinical samples and samples from drunken driver for several years.

KEYWORDS: *Buprenorphine, Immunoassay, GC/MS*

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