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Development of a fully automated screening system for the determination of basic compounds in urine by on-line extraction – high performance liquid chromatography with photodiode-array detection

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AIM: Systematic toxicological analysis based on GC, HPLC and immunological methods is usually performed in plasma/serum and urine. Due to a larger time window of detection, substances with very short half-lives in blood, such as psilocin, scopolamine and morphine are more effectively identified in urine. We therefore aimed to develop an analytical screening method to identify basic compounds in urine. To keep sample pre-treatment and costs to a minimum, we aimed to develop a method characterized by fully automated on-line extraction using common HPLC equipment.

METHODS: A weak cation exchange material (StrataX-CWTM, 20 × 2.0 mm, Phenomenex) was used for the on-line extraction of basic drugs from urine. The samples were loaded onto the extraction column with 0.01 M phosphate buffer pH 6.0. Switching valves were employed to wash the extraction column in the forward and back flush modes with ACN/H20 (90/10, v/v) and water, respectively, and to elute the extracted analytes into two coupled analytical columns (LunaSCXTM, 150 × 4.6 mm, Phenomenex) for isocratic separation.
The mobile phase consisted of 0.05 M phosphate buffer pH 2.3 and ACN/H20 (90/10, v/v). Compound identification was carried out by spectra comparison (λ= 185-380) with a spectra library and by relative retention times set to an internal standard (IS). The method was validated using a performance control test (PCT) consisting of six different analytes which represented the following groups of interest: alkaloids (scopolamine), amphetamine derivatives (methylene-dioxyamphetamine (MDA)) opiates (codeine, and morphine), opioids (2-ethyliden-1,5-dimethyl-3,3-diphenylpyrrolidene (EDDP)) and the IS neostigmine bromide. In addition the analysis of real biological specimens was evaluated and compared to an automated urine screening system (RemediTM-HS, BioRad).

RESULTS: The analytical HPLC-DAD system allowed simple and reliable analysis of toxicologically relevant compounds in urine. Urine samples were successfully extracted by fully automated on-line extraction using switching valves. With the combination of a lipophilic and a hydrophilic wash step a good clean-up was achieved by removing interferences prior to elution. Recovery of the PCT-analytes was > 73-97%. The results for the intra-assay precision ranged from 0.4-7.2% and linearity for the analytes was obtained from 0.1-15.0 µg/mL (R2 > 0.995) for codeine, EDDP, IS and morphine, 0.1-5.0 µg/mL for MDA (R2 = 0.993) and 0.25-15.0 µg/mL for scopolamine (R2 = 0.993), respectively. The method showed sufficient selectivity/specificity and the lower limit of detection was 0.1 µg/mL (S/N >3) and 0.25 µg/mL (S/N >3) for scopolamine. The analysis of real toxicological samples and the comparison of the results with those obtained from an existing urine screening method proved the applicability of the method for routine diagnosis.

CONCLUSION: A fully automated method for the qualitative determination of basic compounds in urine has been developed and validated. The system allowed simple analysis of urine samples at low costs and has been successfully validated and applied to routine clinical toxicology. The analysis of real biological specimen showed applicability of the system for drugs of abuse screening, clinical toxicological and forensic applications.

KEYWORDS: *On-line extraction, HPLC-DAD, Screening method, Urine, Basic compounds*

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