Screening of basic drugs in urine by GC-NPD, GC-MS and HPLC-DAD: automation and comparison of solid phase extraction columns

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AIMS: Screening of basic drugs is performed on a routine basis by manual solid phase extraction. The objective of this study was to transfer the method to positive pressure automated solid phase extraction. Different mixed mode solid phase columns were tested and extraction efficiency and reproducibility was evaluated by GC and HPLC.

METHODS: Human urine was spiked with thirteen different basic drugs and extracted by Gilson ASPEC XL4 automated solid phase extraction. Mixed-mode columns tested were; Applied Separations ABN (3 ml/130 mg); Isolute HCX-3 (3 ml/130 mg); Oasis MCX (3 ml/60 mg); and Bond Elut Certify (3 ml/130 mg). Sample pre-treatment. Urine (2 ml) was added 1 ml 1 M acetate buffer pH 5.5. Conditioning: 1.5 ml methanol and 1.5 ml 0.1 M phosphate buffer pH 6. Sample load was followed by wash steps; 1 ml 0,1 M phosphate buffer, 1 ml 1 M acetic acid, 1 ml methanol. The columns were dried 10 minutes and eluted with 3 ml eluent (2% ammonium (25% aq.) and 10% acetonitrile in ethyl acetate). The extract was evaporated, dissolved in methanol, divided in two fractions and quantified by GC-NPD and HPLC-DAD. GC-NPD analysis was performed on an Ultra-2 capillary column. GC-MS full scan semi-quantitative analysis was performed on a HP-5MS capillary column. HPLC gradient separation (10% to 64% acetonitrile in phosphate buffer pH 3) was performed on a Pursuit C18, 3µm column.

RESULTS AND CONCLUSIONS: The manual extraction conditions were transferred to the Gilson system with the same buffers, solvents and amounts. Due to the low boiling point of solvents, the flow rates of aspiration and dispensing were lowered to avoid formation of interfering bubbles in tubing. Air push after addition of solvents, buffers and samples were reduced from 2 to 0.2 ml in the conditioning steps. Due to the higher viscosity of aqueous solutions, air push after addition of buffers should be higher compared to solvents. Thirteen compounds were extracted from spiked urine at the concentrations 0.5 and 1.5 mg/L: Amphetamine, cetobemidone, mepivacaine, methadone, mirtazapine, sertraline, chloroprothixene, fentanyl, olanzapine, haloperidol, amlodipine, zopiclone and quetiapine. Amlodipine was detected by HPLC only. The GC-recoveries of all drugs were negatively affected by hydrochloric acid–methanol addition prior to evaporation; this was assigned to poor vaporization and degradation in the injector of the precipitated salts. However, the HPLC-recovery of amphetamine decreased from 95% to 75% when the addition of hydrochloric acid–methanol was omitted. At the same extraction conditions, all solid phase columns obtained sufficient extraction recoveries >50% for the tested compounds; however Isolute HCX-3 had the most reproducible recoveries (CV < 20%) and best correlation between HPLC and GC recoveries. The purity of the extracts was comparable and verified by GC-MS. There was observed late eluting impurities from the Oasis MCX column on GC-MS. Isolute HCX-3 was chosen for further work.

KEYWORDS: *Automated Solid Phase Extraction, Urine, Drugs*

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