

Microwave assisted acid hydrolysis of urine as a variation of GC-MS based Systematic Toxicological Analysis (STA)

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AIMS: The sample preparation of the GC-MS based Systematic Toxicological Analysis (STA) according to Maurer (Maurer,H.H.,2004, Clin.Chem.Lab.Med.,42, 1310-1324) has been proved to be very efficient for urine drug screening. The procedure includes acid hydrolysis of the urine for cleavage of conjugates, followed by liquid-liquid extraction at pH 8-9 and acetylation. In order to establish the STA in their routine screening procedure, the authors developed a microwave assisted acid hydrolysis procedure instead of hydrolysis of the urine under reflux for 15 min.

METHODS: A microwave oven MLS 1200 MEGA by Milestone (purchased by EMLS-GmbH, Leutkirch) with temperature measurement and control was used. Firstly, 2.5 mL of blank urine were stocked with morphine-6-glucuronide and diazepam (resulting concentrations 2500 and 1000 µg/L, respectively). 1 mL of 37% hydrochloric acid was added. The urine was filled up into QS-50 quartz inserts with quartz caps which were placed into the PTFE standard vessels of the rotor. Distilled water was added to the vessels surrounding the quartz inserts. For microwave assisted hydrolysis, different time profiles, temperatures and power levels were tested. After hydrolysis, the sample was basified with about 1.5 mL of 10 M aqueous sodium hydroxide and 2 mL of 2.3 M aqueous ammonium sulphate to obtain a pH 8-9. Then, morphine-d3 and diazepam-d5 were added as internal standards in equimolare concentrations as morphine-6-glucuronide and diazepam, respectively. The solution was extracted with 5 mL of a dichloromethane-isopropanol-ethyl acetate mixture (1:1:3, v/v/v). After centrifugation (2 min, 1500 g), the organic layer was evaporated to dryness. The residue was acetylated (with 100 µL of acetic anhydride-pyridine (3:2, v/v), 5 min at 440 W in a household microwave) and analyzed by full scan GC-MS. The work-up, excepting the hydrolysis, was in accordance with the STA procedure of Maurer. The peak areas of the hydrolysis product morphine and the standard morphine-d3 as well as remaining diazepam and diazepam-d5 were compared and the extent of hydrolysis was determined as percent of the stocked concentrations. Secondly, several urine samples from authentic cases were worked up by hydrolysis under microwave irradiation as well as under reflux and the efficiency of hydrolysis of the both procedures was compared.

RESULTS AND DISCUSSION: The use of the quartz inserts that fit into standard vessels of the rotor facilitated the cleaning of the equipment and avoided carry over in the PTFE standard vessels through diffusion into the vessel walls. Therefore, this procedure combines the purity of quartz, ensuring no “memory” effects, with the performance capabilities and safety of the fluoropolymer vessels. Under microwave irradiation at temperatures of 135-140 °C for 5 min, 85% hydrolysis of morphine-6-glucuronide to morphine was obtained, unaffected by the selected power level (250 to 600 W). The proposed procedure revealed also a more than 90% hydrolysis of diazepam to the respective benzophenone. Therefore, these conditions were chosen to compare the two procedures (microwave and reflux) using authentic urine samples. Comparison of the peak area ratios lead to similar results for e.g. morphine, codeine, papaverine and hydrolyzed oxazepam. This is in accordance with a study of Kraemer et al. (Proceeding, 1997, GTFCh-Symposium Mosbach, 200-204), who also compared acid hydrolysis under microwave irradiation and reflux, but the use of a household microwave oven beard the risk of explosion, the distribution of the electric field was not homogeneous and a temperature control was not possible.

CONCLUSION: Microwave assisted acid hydrolysis in quartz inserts is a safe and reproducible alternative to the conventional reflux based hydrolysis within the STA procedure for urinalysis. It showed comparable results than the standard procedure. The temperature control assured a high reproducibility, as all samples are processed under equivalent temperature conditions.

KEYWORDS: *STA, Microwave, Hydrolysis, Urine*

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