Improvement of sample preparation for the STA - Acceleration of acid hydrolysis and derivatization procedures by microwave irradiation

Thomas Kraemer, Armin A. Weber and Hans H. Maurer

Abstract

For systematic toxicological analysis by GC-MS, acid hydrolysis, extraction at pH 8-9 and acetylation have been proved to be a very effective sample preparation for urine samples. To reduce the relatively long working-up time, microwave irradiation was used to accelerate the acid hydrolysis and the acetylation. For the evaluation of the efficiency of the microwave assisted sample preparation, we have analyzed several authentic urine samples using standard hydrolysis (15 min) and microwave assisted hydrolysis (5 min). The extracts after both hydrolysis procedures were each derivatized by standard acetylation (30 min) and microwave assisted acetylation (5 min). These 4 different samples were analyzed by full scan GC-MS. Comparison of the peak areas of different analytes showed that the hydrolysis as well as the acetylation under microwave irradiation lead to similar results as under standard conditions. However, because of the high costs and the risk of explosion during the microwave assisted hydrolysis, we recommend classical hydrolysis under reflux combined with microwave assisted acetylation. Therefore, the efficiency of the acetylation was additionally tested using a mixture of derivatizable drugs. The results of both studies showed that microwave assisted sample preparation for the STA is at least 3 times faster than conventional sample preparation. Therefore, there should be no more reason to renounce hydrolysis and/or derivatization for reasons of time.

1. Introduction

For systematic toxicological analysis by GC-MS, acid hydrolysis, extraction at pH 8-9 and acetylation have been proved to be a very effective sample preparation for urine samples [1-3]. To reduce the relatively long working-up time, we used microwave irradiation to accelerate the acid hydrolysis and the acetylation according to methods described by Lagana et al. [4], and by Thompson and Dasgupta [5-6]. In the following, studies on the efficiency of microwave assisted in contrast to conventional sample preparation are described.

2. Experimental

2.1 Chemicals and reagents

All chemicals used were obtained from E. Merck, Darmstadt (Germany) and were of analytical grade.

2.2 Apparatus

A Hewlett Packard (HP, Waldbronn, Germany) 5890 Series II gas chromatograph was used in combination with an HP 5989B MS Engine mass spectrometer and an HP MS ChemStation (DOS series) with HP G1034C software. The GC conditions were as follows: splitless injection mode; column, HP-1 capillary (12 m x 0.2 mm I.D., cross linked methylsilicone, 330 nm film thickness); injection port temperature, 280°C, carrier gas, helium; flow-rate 1 mL/min; column temperature, programmed from 100-310°C at 30°/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full scan mode; EI ionization mode: ionization energy, 70 eV; ion source temperature, 220°C; capillary direct interface heated at 260°C.

For microwave irradiation a normal household microwave oven from Panasonic (NN 3256) was used at a power level of 440 Watts.

2.3 Urine samples

A urine sample pooled from authentic clinical cases was used for the comparison of the efficiency of the different sample preparation procedures under routine conditions.

2.4 Acid hydrolysis of urine for cleavage of conjugates

A 5 mL volume of urine was mixed with 100 µL of internal standard (methaqualone/methanol: 0.1 µg/µL) and then hydrolyzed with 1.5 mL of 37% HCl for 15 min under reflux or for 5 min under microwave irradiation in a special, pressure proof microwave vessel from Berghof (Eningen, Germany). After transfering the sample into a centrifuge tube pH 8-9 was adjusted (2 mL of 10 mol/L aqueous NaOH, 5 mL of 30% aqueous ammonium sulphate). This solution was extracted with 5 mL of a dichloromethane-isopropanol-ethyl acetate mixture (1:1:3; v/v/v). After phase separation by centrifugation (5 min, 1500g), the organic layer was evaporated to dryness. The residue was dissolved in 100 µL of methanol and transfered into a sample vial (procedure according to Ref. 1 and 2).

2.5 Acetylation of urine extracts and drug solutions

The urine extracts or the methanolic solutions (see below) were evaporated and then acetylated with 100 µL of acetic anhydride-pyridine (3:2; v/v) for 30 min in a heating block (60°C) or for 5 min under microwave irradiation. After

evaporation of the derivatization mixture, the residue was dissolved in 50 µL of methanol and 0.5-1 µL were injected into the GC [1,2].

Methanolic solutions (0.1 µg/µL) of amphetamine, dihydrocodeine, dobutamine, maprotiline, metoprolol, morphine, oxprenolol and paracetamol were acetylated as described before.

2.6 Gas chromatographic-mass spectrometric determination

For the evaluation of the efficiency of the microwave assisted sample preparation, we have analyzed a urine sample pooled from authentic cases using standard hydrolysis (15min, Hy) and microwave assisted hydrolysis (5 min, MW-Hy). The extracts after both hydrolysis procedures were each derivatized by standard acetylation (30 min, Ac) and microwave assisted acetylation (5 min, MW-Ac). These 4 different samples were analyzed by full scan GC-MS. The ratios of the peak areas of the drugs and/or metabolites in relation to that of the internal standard methaqualone were determined in the corresponding reconstructed mass chromatograms. The peak area ratios of the microwave treated samples were compared with that of the standard extracts. The efficiency of the acetylation was additionally tested using a mixture of derivatizable drugs (cf 2.5). Again, the peak area ratios of the microwave treated samples were compared with that of the standard derivatization.

2.7 Statistics

All analyses were repeated at least six times. Mean values, standard deviations and coefficients of variation were calculated. Significant differences in the acetylation procedures were tested by a t-test.

3. Results and discussion

Comparison of the peak areas of different analytes in a pooled urine sample showed that the hydrolysis as well as the acetylation under microwave irradiation lead to similar results as under standard conditions. Fig. 1 shows the efficiency of microwave assisted sample preparation in comparison with the standard STA procedure. The peak areas of the different drugs and/or metabolites in relation to that of the internal standard are given for the four tested procedures (cf 2.6). The peak area ratios of the standard STA procedure were set to 100%. Since the microwave assisted procedures were several times faster (hydrolysis 3 times, acetylation 6 times), these procedures should be prefered for rapid sample preparation especially in emergency cases. However, since the microwave assisted hydrolysis needs expensive pressure proof vessels (about 450 DM/piece plus 3 DM per analysis for special washers), since explosions during hydrolysis under microwave irradiation can occur, and since saving of time was o nly 10 min,

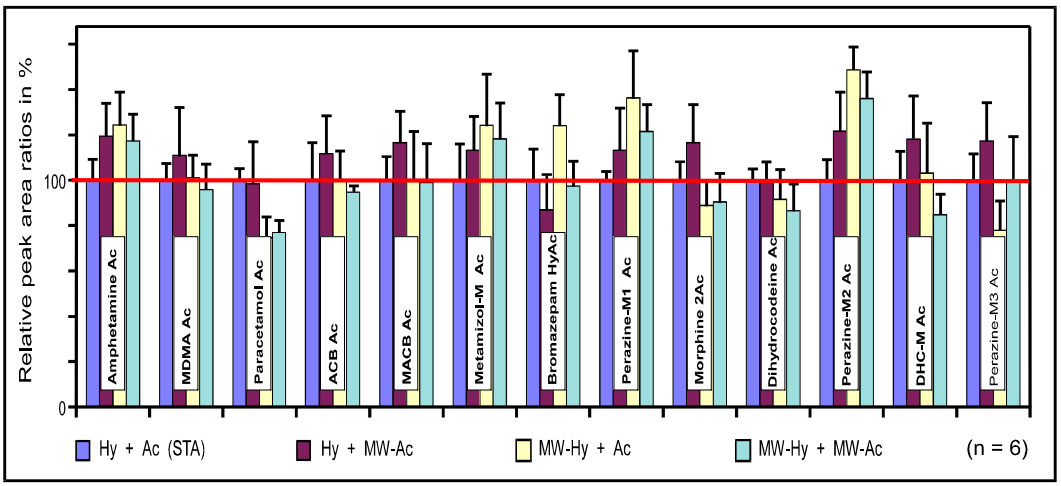

Fig. 1: Efficiency of microwave assisted sample preparation in comparison with the standard STA procedure. The peak areas of the different drugs and/or metabolites in relation to that of the internal standard are given for the four tested procedures (cf 2.6). The peak area ratios of the standard STA procedure were set to 100%. The acronyms are defined in 2.6.

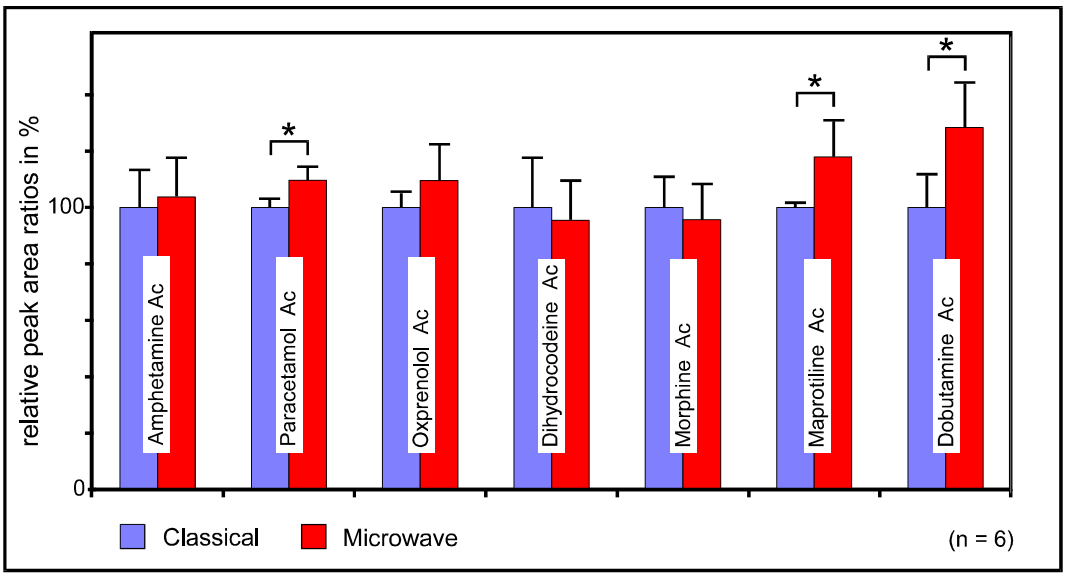

Fig. 2.: Peak areas of the selected drugs in relation to that of the internal standard given for the standard acetylation (30 min, set to 100%) and the microwave assisted procedure (min 5). Significant differences are marked with an asterisk.

we prefer conventinal hydrolysis under reflux followed by microwave assisted acetylation. Therefore, we evaluated the efficiency of the microwave assisted acetylation for further relevant drugs. In Fig. 2 the peak areas of the selected drugs in relation to that of the internal standard are given for the standard acetylation (30 min, set to 100%) and for the microwave assisted acetylation (5 min). Significant differences ($p<0,05$) could be found only for paracetamol, maprotiline

and dobutamine. In these cases the microwave assisted acetylation was even better than the normal procedure.

4. Conclusions

Comparison of the peak areas of different analytes showed that the hydrolysis as well as the acetylation under microwave irradiation lead to similar results as under conventional conditions. Since the microwave assisted hydrolysis saves only 10 minutes, since the costs are relatively high and since explosions can occur, we recommend classical hydrolysis under reflux followed by microwave assisted acetylation, thus saving 25 minutes. Therefore, there should be no more reason to renounce hydrolysis and derivatization for reasons of time.

5. References

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Univ.-Prof. Dr. Hans H. Maurer
Institute of Pharmacology and Toxicology
Department of Toxicology
University of Saarland
D-66421 Homburg (Saar)