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## **Detection of bendroflumethiazide metabolites in human urine using liquid chromatography-tandem mass spectrometry: application to urinary excretion study**

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### **Abstract**

An LC-ESI-MS/MS method is described for identification of bendroflumethiazide metabolites in urine after oral administration of drugs. Identification of three bendroflumethiazide metabolites was carried out by comparing their changes in molecular mass and product ion spectra with those of the parent drug. Analysis of urine samples obtained from two healthy volunteers after ingestion of Tensionorme<sup>®</sup> indicated that the parent drug and its three metabolites could be detected up to 50 hours after administration. The excretion study showed that bendroflumethiazide was metabolized to aminotrifluoromethylbenzendisulfonamide, hydro-flumethiazide and 3-4 deshydroflumethiazide.

### **Introduction**

Bendroflumethiazide (BMF) is a diuretic mainly used in the treatment of hypertension, chronic and acute heart failures and cirrhosis [1]. Diuretics are banned in sports by the World Anti-Doping Agency (WADA). There are a few data on the detection of BMF and its metabolites associated with doping analysis [2-4]. In addition, no reports on the urinary excretion profiles of this diuretic and its metabolites have been so far described. The present work describes a sensitive and specific an LC-ESI-MS/MS method for identification of BMF metabolites in urine after oral administration of drugs.

### **Experimental**

Excretions study and purification:

The study was approved by the local Ethics Committee of the Charles Nicolle Hospital (Tunis). Urinary samples were obtained from two healthy volunteers (22 and 26 years old) who signed a written consent form. After clinical examination and biochemical tests the volunteers received two tablets of Tensionorme<sup>®</sup> 2.5 mg (Lisapharm, France) and urine was collected at different time-points up to 50 h post-dose. Samples were then extracted by BackerBond spe C-18 (3 mL/200 mg) cartridges (J. T. Baker, Holland).

LC-ESI-MS parameters:

Agilent HPLC coupled to Quattro micro (Micromass, UK); ionization mode: ESI; MRM transitions: 420>289 (BFM), 330>239 (hydroflumethiazide), 318>214 (4-aminotrifluoromethylbenzendisulfonamide), 328>248 (deshydro-flumethiazide), 381>189 (IS, mefruside); high voltage electrodes: 3.2 kV; source and desolvation temperature: 120°C and 400 °C, respectively; nebulisation gas pressure/desolvation gas flow: 7 bar/650L/h; collision gas: 2·10<sup>-3</sup> mbar; column: Zorbax C-8 (2.1 × 150 mm, 5 µm), mobile phase: ammonium acetate (A) - acetonitrile (B) [5mM, pH = 4.5] (10:90) at 0.3 mL/min, 20 % of B from 0 to 2 min, followed by a 14 min gradient to 80% B; then 80% B for 2 min.

## Results and Discussion

The total ion chromatograms of excretion urine after ingesting of two tablets of Tensionorme® (2x 2.5 mg) were compared with blank urine to detect suspicious peaks. Identification of BMF metabolites was performed by comparing their retention-times, differences between molecular masses ( $\Delta m$ ) and ESI-MS/MS spectra with those of the parent drug. This investigation showed that BMF is converted to three metabolites namely 4-aminotrifluoromethylbenzendisulfonamide (ATFB, Figure 1A), hydroflumethiazide (HFMS, Figure 1B) and 3-4 deshydroflumethiazide (Figure 1B).

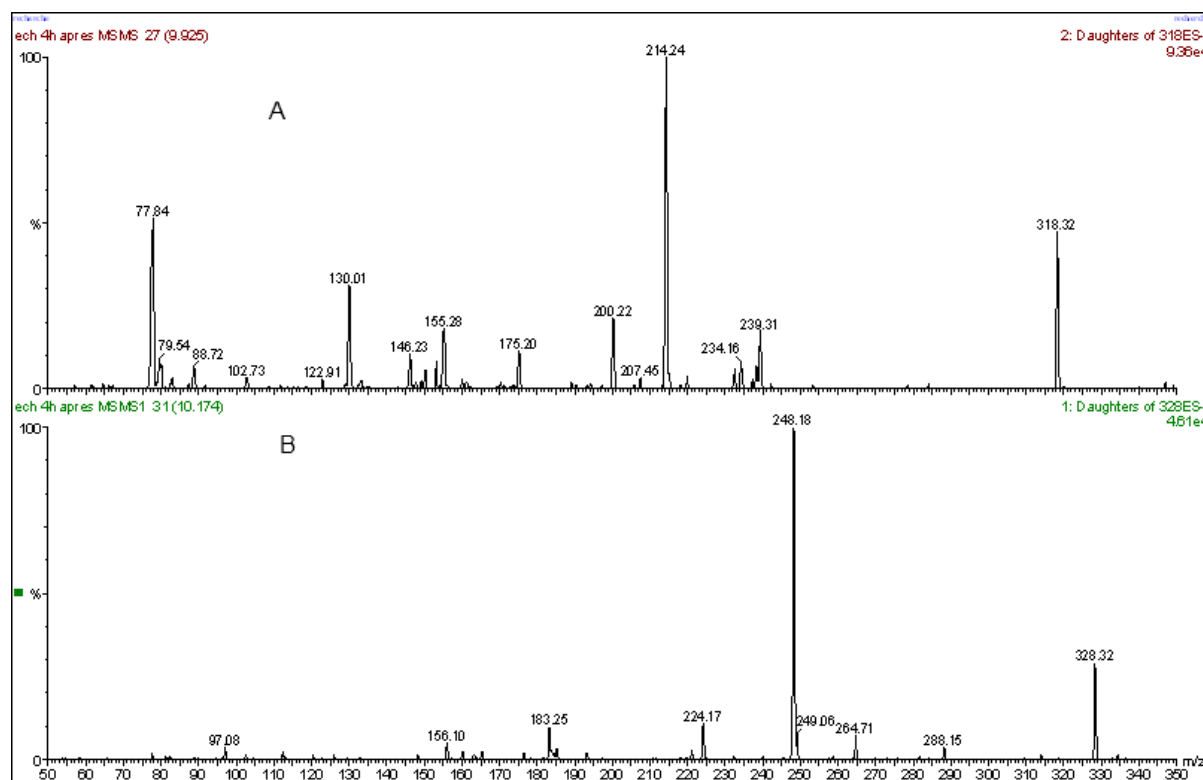


Figure 1: Negative electrospray product ion spectra at 15 eV collision energy of (A) ATFB and (B) 3-4 deshydroflumethiazide (B)

HFM was only detected in urine at a few time points for the two volunteers and therefore the elimination profiles of HFM could not be reliably characterized. The urinary excretion profiles for BMF, ATFB and deshydroflumethiazide are shown in Figures 2, 3 and 4, respectively.

The maximum urinary concentration of BMF (Figure 2) was reached 4 hours after intake of the drug for the two volunteers while that of ATFB was reached after 28 h (Figure 3). For BFM and ATFB, the maximum concentrations ranged between 1180 and 1950 ng/mL and varied between 15 and 44 ng/mL, respectively.

Therefore, the results indicate that bendroflumethiazide is substantially excreted as unchanged drug. This study indicates also that for both tested subjects BFM concentration (Figure 2) is still higher than 250 ng/mL until 28 h whereas ATFB concentration was found to remain lower 50 ng/mL during excretion. The excretion profile for deshydroflumethiazide is presented in Figure 4. Metabolites were substantially excreted during the first 12 h after intake and the concentration remains significant until the 28 h post administration. It's worth to note that the urinary concentration of BMF is 10 to 50 times higher than ATFB 24 h after intake of the drug indicating that the parent drug should be used as primary target compound for the detection of the abuse of this diuretic in doping analysis.

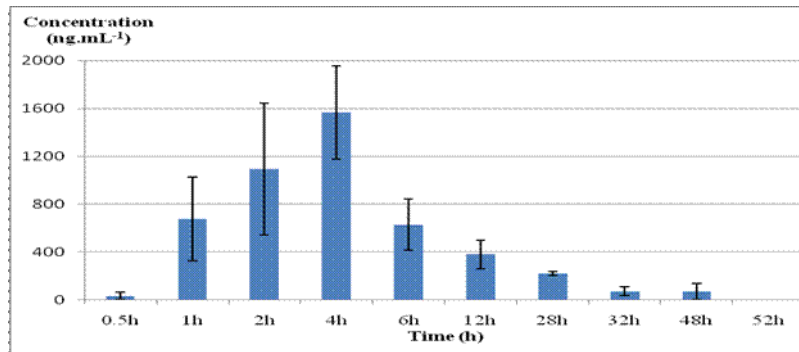


Figure 2: Excretion profiles of BMF after oral administration of the drug for the two volunteers

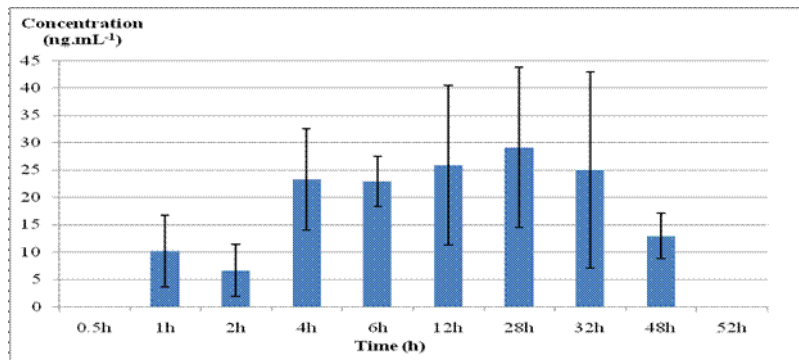


Figure 3: Excretion profiles of ATFB after oral administration of the drug for the two volunteers

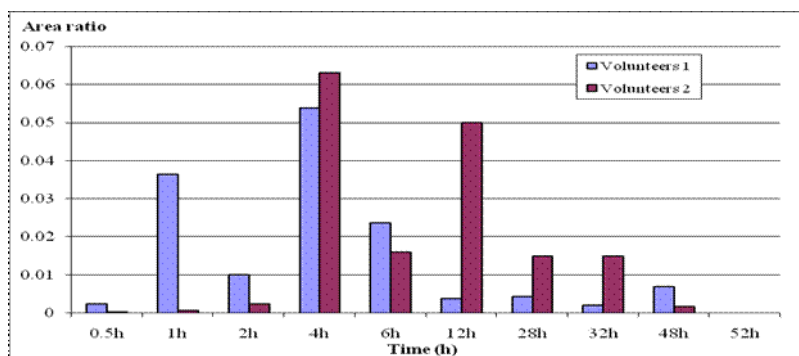


Figure 4: Excretion profiles of deshydroflumethiazide after oral administration of the drug for the two volunteers

## Conclusions

Analysis of samples taken after ingestion of two tablets of Tensionorme® showed that BMF is converted to ATFB, HFM and 3-4 deshydroflumethiazide. The maximum concentration of BMF and ATFB was reached in 4 and 28 h after oral administration, respectively. Furthermore, the excretion study showed that the urinary concentration of BMF was 10 to 50 times higher than ATFB until 28 h after intake of the drug. It was shown that the parent compound should be used as primary target for the detection of BMF abuse in doping analysis.

## References

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