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Detection of urinary phase-I and phase-II metabolites of ephedrine and oxilofrine by LC-MS/MS

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Introduction

Stimulants are prohibited in sports by the World Anti-Doping Agency (WADA) for use in competition. Ephedrine acts indirectly sympathomimetic and has central stimulating properties. It is prohibited when its concentration in urine is higher than 10 µg/mL. Oxilofrine (p-OH-ephedrine) acts direct sympathomimetic on α- and β-receptors and has indirect sympathomimetic effects, too. Ephedrine is used therapeutically as decongestant in cold medicines whereas oxilofrine is used in the therapy of hypotony.

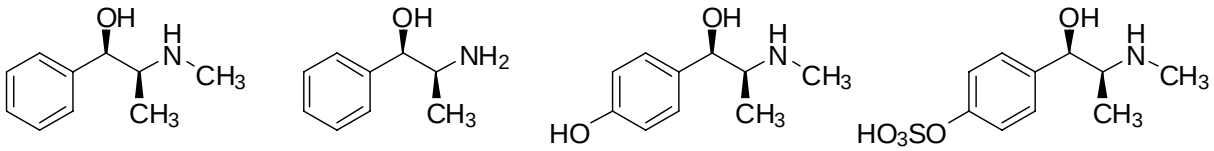

Figure 1: Structures of ephedrine (1), norephedrine (2), p-OH-ephedrine (3) and p-OH-ephedrine sulfoconjugate (4)

N-Demethylated ephedrine (figure 1 (2)) has been described as main metabolite of ephedrine (figure 1 (1)) and was found in urine after oral uptake besides the unchanged drug. In addition, hippuric acid, benzoic acid and a metabolite resulting from oxidative desamination of the side chain are decribed as metabolites with lower incidence. Aromatic hydroxylation or excretion of phase-II metabolites are not yet reported. For oxilofrine (figure 1 (3)) a conjugated metabolite was described by Kauert et al. (1988), but it was not characterised in detail weather it is conjugated with sulfuric acid or glucuronic acid.

In the course of our investigations, two excretion studies were performed. Urine specimen were analyzed after uptake of ephedrine and oxilofrine, respectivley. The main focus of these

studies was the identification and characterization of their metabolites with attention on the conjugated metabolites by the help of LC-MS/MS.

Materials and Methods

For excretion study I, 32 mg of oxilofrine (as HCl salt) were taken orally and urine was collected for 3 days followed by one morning urine. In this excretion study, the detection time of the unconjugated and the sulfoconjugated derivative was determined and mapped. Excretion study II was performed with one tablet of ephedrine-HCl (20 mg) orally, and urine was collected for 2 days followed by morning urines the following 5 days. The known main phase-I metabolite norephedrine and additionally the hydroxylated metabolite of ephedrine and its sulfoconjugate were monitored and excretion profiles of them were mapped.

For determination of the *p*-hydroxylated ephedrine and its sulfoconjugated derivative, urine samples were prepared and determined as described by Orlovius et al. (2009) and Parr et al. (2007). Analytes were determined using ion transitions employing multiple reaction monitoring: d_3-ephedrine as ISTD (*m/z* 169-151); sulfoconjugated *p*-hydroxyephedrine (*m/z* 262-164); unconjugated p-hydroxyephedrine (*m/z* 182-149).
Preparation and determination of the urine specimen for unchanged ephedrine and its nor-derivative was performed as described by Thevis et al. (2003). Analytes were determined using ion transitions employing multiple reaction monitoring: d_3-ephedrine as ISTD (*m/z* 169-151); ephedrine (*m/z* 166-148); norephedrine (*m/z* 152-134). Calibration curves for quantification were performed for all analytes. p-OH-Ephedrine sulfoconjugate was synthesised in house (Orlovius et al. (2009)) and its structure was proven by MS/MS and NMR-measurement.

Results and Discussion

In excretion study I, a major sulfoconjugated phase-II metabolite of the unchanged oxilofrine could be determined in urine. The structure of this metabolite was assigned as phenolic sulfoconjugate (Figure 1 (4)). It was confirmed by synthesis of the reference material and characterized by LC-MS/MS (Figure 2) and NMR measurement (data not shown).

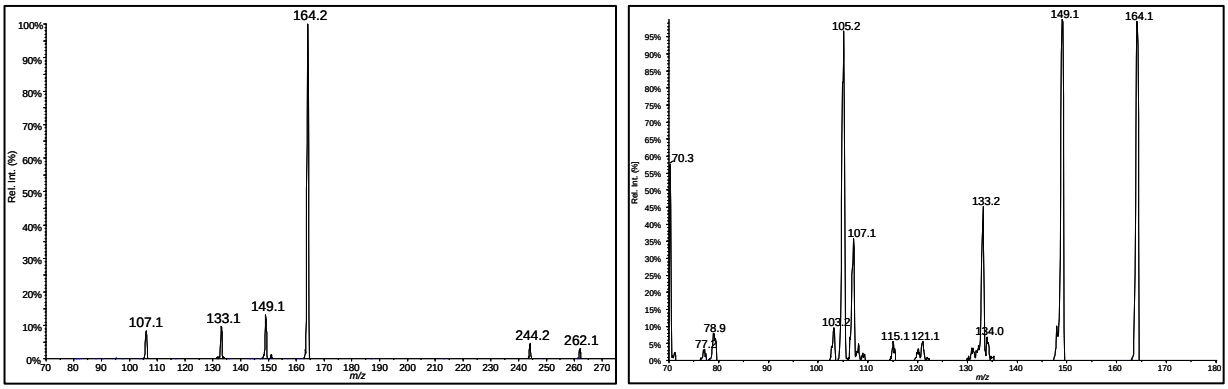

Figure 2: Product ion spectrum of phenolic p-OH-ephedrine-sulfate (left, $[M+H]^+$=262) and oxilofrine (right, $[M+H]^+$=182)

Elimination kinetics of the unchanged drug and its conjugate are displayed in Figure 3. The conjugate could be detected even longer than the parent compound (67 h vs. 43 h).

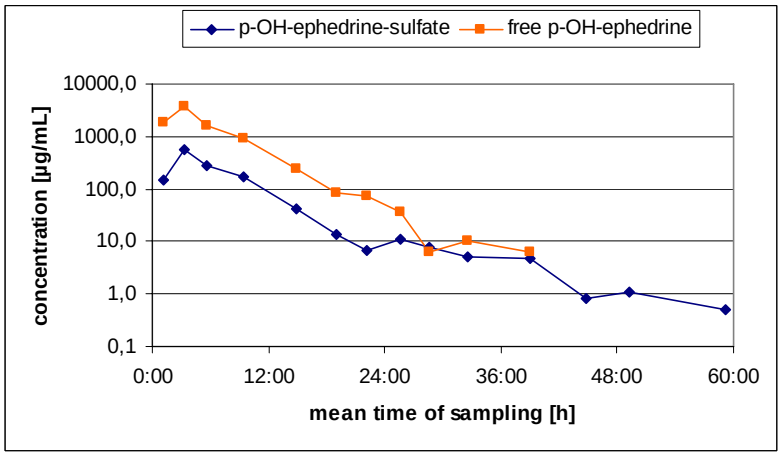

Figure 3: Excretion study I: p-OH-ephedrine and its sulfoconjugate after uptake of 32 mg of oxilofrine

Excretion study II resulted in a metabolite which was not described up to now. A *p*-hydroxylated metabolite of ephedrine and its sulfoconjugated derivative were proven in urine specimen after uptake of ephedrine. The detection time of the *p*-hydroxylated ephedrine was shorter (18 h) than that of its sulfoconjugated compound (147 h). While the parent compound ephedrine (Figure 1 (1)) could be detected for 51 h, the N-nor-derivative (Figure 1 (2)) could be detected even longer (75 h). Only in the first urine sample after uptake of ephedrine, the threshold (10 µg/mL) for ephedrine exceeded.
Elimination kinetics of the unchanged drug and its metabolites are displayed in Figure 4.

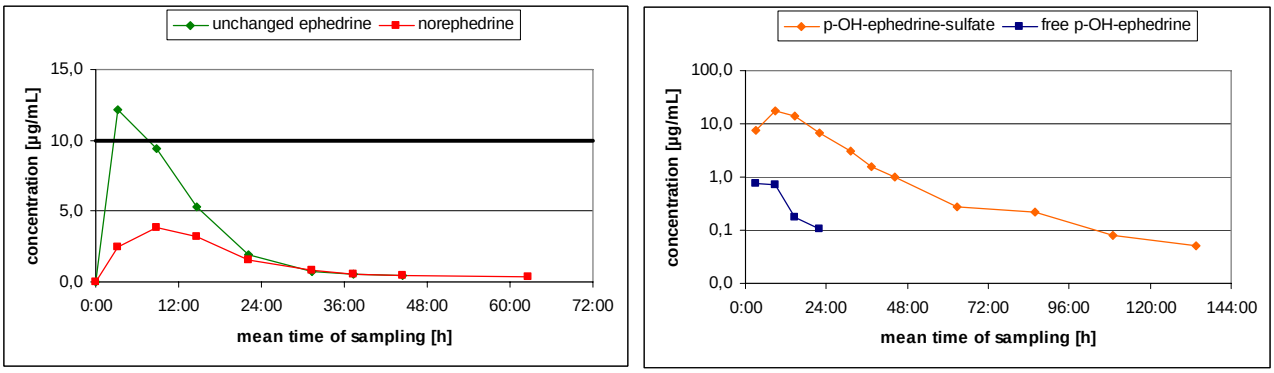

Figure 4: Excretion study II: unchanged ephedrine and its nor-derivative (left; black line illustrates the threshold for ephedrine in urine) and p-OH-ephedrine and its sulfoconjugate (right) after uptake of 20 mg ephedrine

In order to distinguish whether an adverse analytical finding of p-OH-ephedrine is due to intake of ephedrine or p-OH-ephedrine itself, the concentration of p-OH-ephedrine and the presence of ephedrine in the urine sample should be considered.

Acknowledgements

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