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Reactions of Salbutamol and Reproterol with urine matrix components - preliminary results

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Introduction

According to the IOC Medical Code beta-2-sympathomimetic agonists are prohibited in sports due to their anabolic and stimulating side effects, but the administration of Salbutamol, Salmeterol and Formoterol is permitted by inhalation [1].

Reactions of the beta-2 agonists Salbutamol and Reproterol in urine are investigated.

For the free excreted Salbutamol a cut-off limit of 100 ng/ml of urine for in-competition and of 1000 ng/ml for out-of-competition testing is set by the IOC [1]. From earlier studies the reduced stability of Salbutamol is well known. During this study its stability in urine during the storage was investigated.

In a second study the question if 2-[3-theophyllinyl(7)-propyl]-4,6,8-trihydroxy-1,2,3,4-tetrahydroisoquinoline is a metabolite of Reproterol or an artifact should be answered. According to the literature Reproterol is excreted as free and conjugated 2-[3-theophyllinyl(7)-propyl]-4,6,8-trihydroxy-1,2,3,4-tetrahydroisoquinoline [2-4]. But this derivative can also be detected after analysis of urines spiked with Reproterol itself [5].

Salbutamol – kinetics of the decomposition

Experimental:

The stability of Salbutamol during the storage was investigated: A urine from an excretion study with 4 mg of Salbutamol (Salbulair[®], 1 tablet, 0-8-hours) as well as a urine spiked with 500 ng/ml of Salbutamol were stored for one year at +4°C and -18°C with and without the addition of sodium azide (1 g/l) as stabilizer. Additionally spiked urines from five different persons with 500 ng/ml of Salbutamol each were analysed after storage at +4°C, after 2, 4, 7, and 11 days. All samples were prepared according to the method for anabolic steroids with direct hydrolysis [6].

Results and Discussion:

For all urines the same results were obtained (Fig. 1, Fig. 2): Already after 1 month only about 15% of the Salbutamol found at the beginning were recovered. No further decomposition even after one year of storage was detected. Temperature and stabilisation did not influence the recovery in this experiment.

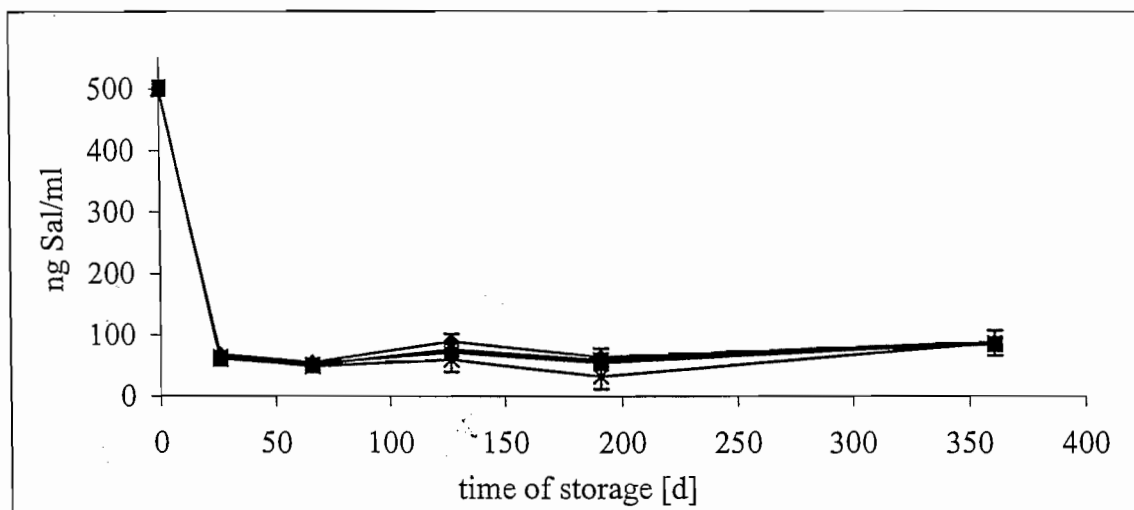


Fig. 1: Decomposition of Salbutamol in spiked urines during the storage: concentration versus time

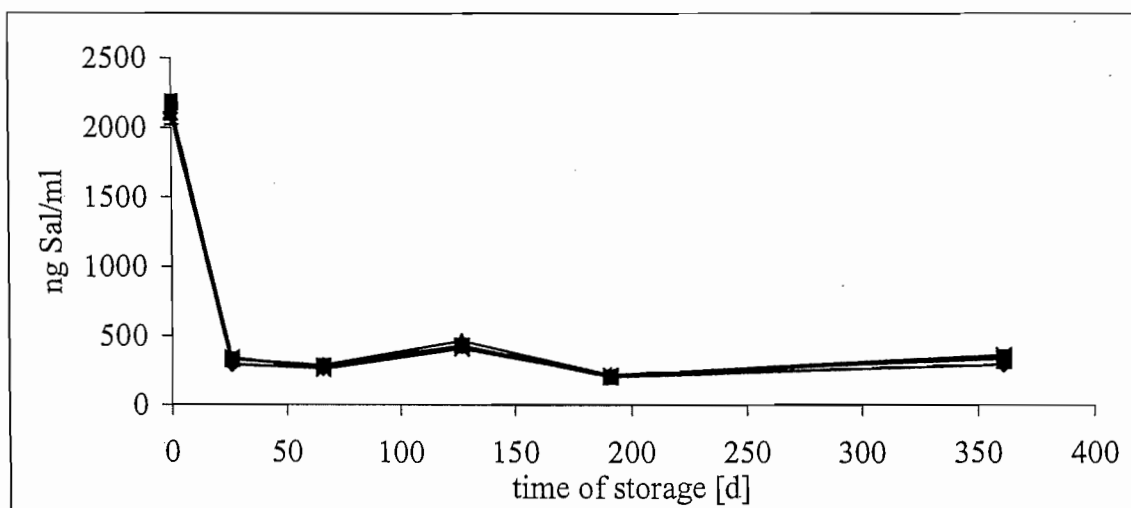


Fig. 2: Decomposition of Salbutamol in excretion study urines during the storage: concentration versus time

Fig. 3 shows the kinetics of the decomposition of Salbutamol in the urines from five different persons. The function, which describes this kinetics, was regressed as an exponential curve with an asymptote (maximum likelihood).

The equation found for the native urine stored at +4°C was:

$$A_{369,rel} = 90 + 129 * e^{-\frac{\ln 2}{1,62} * t} \quad \text{or} \quad \frac{dA_{369,rel}}{dt} = -\frac{\ln 2}{1,62} * (A_{369,rel} - 90).$$

A possible explanation for this is a reaction with first order kinetics or pseudo-first-order kinetics, which ends before all of the Salbutamol has reacted. Until now it was not possible to identify any product of this reaction.

During the storage at -18°C a significantly quicker reaction can be observed and after separation from the sediment a weakly significantly enhanced asymptote and a significantly decreased variance of the values at the beginning.

A scientific explanation for these results has to be found in further studies.

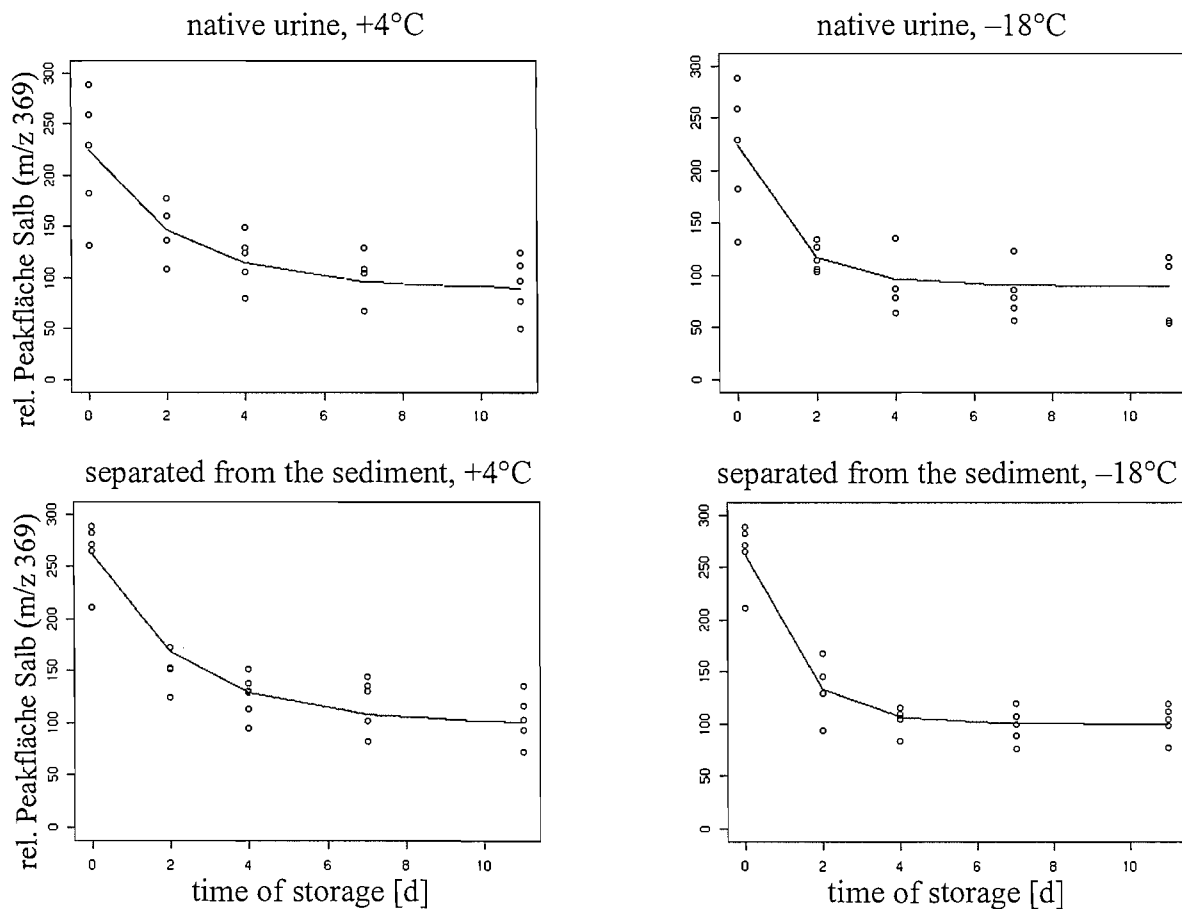


Fig. 3: Stability of Salbutamol in human urine (spiked urine, 500 ng/ml) of different persons during the storage at different conditions

Reproterol – Tetrahydroisoquinoline derivative as metabolite or artifact

Experimental:

To follow up the question whether the Tetrahydroisoquinoline is a metabolite of Reproterol or an artifact two experiments were performed:

The urines from an excretion study with 20 mg of Reproterol were spiked with 1 mg/l of deuterated formaldehyde (DCDO) immediately after excretion and analysed with and without hydrolysis with glucuronidase/ arylsulfatase from *Helix pomatia* at pH 5.2 (16h at 37°C). After extraction with 5 ml of TBME and 1 ml of t-butanol at pH 9.6 (NaHCO₃/K₂CO₃, 2:1) under addition of NaCl (saturation) the organic layer was evaporated to dryness and the residue derivatised with 100 µl of MSTFA/NH₄I/ethanethiol (1000:2:3) for 20 min at 60°C. 3 µl of the mixture were injected into the GC/MS.

In the second experiment the urines from the excretion study were analysed with LC/MS/MS without any sample preparation.

Results and Discussion:

In the samples from the first experiment 96±2% of the Tetrahydroisoquinoline were found as deuterated and 4±2% as undeuterated derivative.

During the analysis of the urines Reproterol itself as well as conjugated Reproterol can react with the deuterated and with undeuterated formaldehyde, which is excreted with the urine. The Tetrahydroisoquinoline is inert against an exchange of hydrogen and deuterium atoms. The explanation of the results must be that at least 96% of the excreted Reproterol are excreted as free and/or conjugated Reproterol and that the Tetrahydroisoquinoline is not a metabolite of Reproterol but an artifact which is generated during the sample preparation or during the storage in the bladder with urinary formaldehyde.

Without any sample preparation LC/MS/MS detected immediately after the excretion (sampling of the elimination urine) Reproterol only, after 2 days of storage at +4°C both, Reproterol and the Tetrahydroisoquinoline, and after 7 days of storage solely the Tetrahydroisoquinoline. Only the free fraction was analysed fraction because of the formation of the Tetrahydroisoquinoline during the enzymatic hydrolysis.

Summary

During the storage of the urine samples beta-2-agonists can react leading to reduced concentrations of the detected parent compound. In case of Reproterol the mechanism and the product of the reaction could be identified. The Tetrahydroisoquinoline derivative of Reproterol can be used to detect Reproterol in urine samples [5]. For Salbutamol the reaction of the decomposition is unknown until now. The kinetics of this reaction can be described by an exponential function (first order kinetics) with a half-life of the reacting Salbutamol of 1.6 days. This should be considered if samples were stored and analysed later. Especially for the analysis of the B-samples the decomposition of Salbutamol is important. The confirmation of the concentration found in the A-sample and the exceeding of the cut-off-limit might not be possible after the storage.

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