PROJECT REVIEW

”Development of analytical methods for the quantitative determination of beta-agonists and determination of detection times after therapeutic use”

Beta-agonists are substances frequently used for the treatment of asthma. These drugs are most frequently administered by inhalation of aerosol, powder or nebulised solution. Besides the main pharmacological effect, at higher doses, side effects of the use of these products result in anabolic action. Hence, beta-2-agonists might be misused for their stimulating effect on respiration and growth promoting action when administered in higher doses.

As a result, WADA has prohibited the use of these drugs in sports. Exceptions are formoterol, salbutamol, fenoterol, salmeterol and terbutaline which can be used by athletes if a proper medical justification (TUE) is issued.

Although this group of medication is frequently declared by athletes on the doping control forms these substances are not always detected. In addition, no minimum required performance levels (MRPL) for laboratories have been presently issued by WADA. Information about the excretion of beta-2-agonists in urine after the use by different administration routes could result in the establishment of MRPL levels based upon scientific evidence.

Therefore this project will focus on the administration of different beta-2-agonists by different administration routes. In this project 5 beta-2-agonists will be administered (i.e. salbutamol, formoterol, fenoterol, salmeterol and terbutaline) of which 2 of them will be administered both by inhalation and orally. All these studies will be conducted following a strict research protocol as approved by an ethical committee.

As the supposed outcome of this research is a MRPL level, the first step of this project is to develop and validate quantitative analytical methods for all administered beta-2-agonists (except salbutamol for which these methods already exist).

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Results and Conclusion

The goal of this project was to investigate which concentrations can be detected in urine after therapeutical inhalation of β2-agonists.

For this purpose a quantitative GC-MS method was developed for the detection of salbutamol. For salmeterol, formoterol and terbutaline LC-MS/MS methods were developed and validated. Since no isotopic labeled internal standard was available for fenoterol, only a semi quantitative LC-MS/MS method could be developed.

After the validation, the developed methods were used to investigate the urinary excretion of the β2-agonist after oral administration of 4 mg of salbutamol (Ventolin®) and by inhalation of: Ventolin® (800 µg salbutamol), Serevent® (100 µg salmeterol), Oxis® (18 µg formoterol), Bricanyl® (500 µg terbutaline) and DuoventHFA® (100 µg fenoterol). After oral administration of salbutamol urinary concentrations reached 2626 ng/mL. After inhalation of a high therapeutic dose of salbutamol, the threshold of 1000 ng/mL was almost exceeded for one volunteer (994 ng/mL).

The results show that the concentration of salmeterol and formoterol reach a maximum after 1 and 3 hours after ingestion. The highest salmeterol concentration detected was 1.27 ng/mL. For formoterol highest concentration was 11.4 ng/mL. After inhalation of terbutaline and fenoterol highest concentration detected were 197 ng/mL and 58.3 ng/mL, respectively.

These concentrations were then compared with routine samples in which an above mentioned β2-agonist was found. In this way samples containing salmeterol (n=45). Formoterol (n=82), terbutaline (n=8) and fenoterol (n=3) were collected and quantified using the developed methods. Concentrations in these samples could not be related to the use of abnormal high doses of β2-agonists.

Publications/Presentations

Presentations:

- Presenting “Excretion Studies with β2-Agonists” at the VI Latin American Workshop in Doping Analysis, November 8 to 11, Asunción- Paraguay, 2009.
- Presenting “Excretion Studies with β2-Agonists” at the 28th Workshop on dope analysis, 2010, Cologne, Germany.

Publications:

- Excretion Studies with β2-Agonists, K. Deventer, W. Van Thuyne, O.J. Pozo, P. Van Eenoo, F.T. Delbeke, Proceedings of the 28th Workshop on dope analysis, 2011, Cologne, Germany.
- Quantitative detection of inhaled salmeterol in human urine and relevance to doping control analysis, Submitted to Drug Testing and Analysis.

- Quantitative detection of inhaled formoterol in human urine and relevance to doping control analysis, in preparation.
- Quantitative detection of inhaled terbutaline in human urine and relevance to doping control analysis, in preparation.