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Analytical Methodology for the Detection of b2-agonists in Urine by GC/MS
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Analytical methodology for the detection of β_2-agonists in urine by GC/MS

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A comprehensive gas chromatographic-mass spectrometric procedure for detection of the β_2-agonists bambuterol, clenbuterol, fenoterol, formoterol, reproterol, salbutamol, salmeterol and terbutaline in urine is described. The method is based on an enzymatic hydrolysis with β-glucuronidase from *Helix pomatia*, followed by a solid-phase extraction procedure using Bond Elut Certify columns. The influence of urine pH in the extraction recovery has been studied and pH 9.5 was found to give the best recovery and cleaner extracts. After pH adjustment, the sample was applied to the pre-conditioned cartridges and after a washing step, the β_2-agonists were eluted with a mixture of chloroform and isopropanol (80:20, v/v) containing 2% ammonia.

A derivatization study was performed with different derivatizing agents aiming to obtain derivatives with high selectivity to be used in the GC/MS analysis. Trimethylsilylation was compared with different agents and the role of some catalysts was evaluated. Acylation, methylation, combined trimethylsilylation and acylation, as well as the formation of cyclic methylboronates were also studied. Sterical hindering caused by different substituents at the nitrogen atom of the β-ethanolamine lateral chain of β_2-agonists molecules is mainly responsible for differences in the abundance of the derivatives obtained. The use of catalysts produces an increase in the derivatization yield, especially in compounds with low sterical hindering (substituents with primary and secondary carbon atom). Cyclic methylboronates were formed from bambuterol, clenbuterol, formoterol, salbutamol and salmeterol. Due to the hydroxy substituents in an inadequate position for ring formation, this procedure was not effective for

fenoterol and terbutaline. For screening purpose (i.e. sports drug testing), derivatization with MSTFA is recommended as a comprehensive derivatization technique for β_2-agonists due to its lower by-product formation.

A validation procedure for qualitative analysis of β_2-agonists in urine was performed. Selectivity of the method showed that no interfering peaks were observed for most of the compounds at the retention time of the β_2-agonists. Extraction recoveries ranged from 68.1% to 103.7% in urine samples. Detection limits from 0.5 to 5 $ng.mL^{-1}$ were obtained using selected-ion monitoring. Intra-assay precision ranged between 2.3% and 13.8% for all compounds except for fenoterol. The evaluation of the International Olympic Committee (IOC) criteria for compound identification was fulfilled by the compounds studied. The optimised method was successfully used in urine samples obtained from excretion studies of healthy volunteers.

References

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R. Ventura, L. Damasceno, M. Farré, J. Cardoso and J. Segura, Analytical methodology for the detection of β2-agonists in urine by gas chromatography-mass spectrometry for application in doping control. Anal. Chim. Acta 2000 (in press).

This presentation was dedicated to the memory of Eckhard Nolteernsting. Among the contributions of Eckhard to doping control, the authors would like to point out his contribution to the analysis of β-adrenergic drugs. His pioneering work has been the basis of many subsequent developments.