

***“Development of an analytical method for the detection of the misuse of the peptidic substance thymosin  $\beta$ -4 (TB4) as doping substance”***

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**Project Overview:**

Based on intelligence and confiscation data and on the monitoring of the black market for emerging doping agents the growth promoter thymosin beta 4 maybe misused in sports for doping purposes. The aim of this research project is the development of a method for the detection of misuse of this substance in urine. This goal should be achieved by means of the use of high resolution high accuracy mass spectrometry (HRMS) and Ion Mobility HRMS (IM-HRMS). As TB4 is an endogenous substance, reference values of athletes and non-athletes should be established to be able to differentiate between normal levels and atypical concentrations

**Results and Conclusions:**

A method enabling the accurate quantification of TB4 from human urine was established using isotope-dilution liquid chromatography- high resolution/high accuracy mass spectrometry. Following solid-phase extraction, a limit of quantification (LOQ) of 0.5 ng/mL was accomplished, enabling the analysis of TB4 at physiological concentrations in human urine. In order to assess urinary levels of the endogenous compound, a total of 122 urine samples from non-elite athletes, 103 routine doping control samples, and 54 long-term stored doping control samples from team sports athletes was analyzed, yielding results that indicated a broad range of urinary TB4 concentrations from below the assay's LOQ (0.5 ng/mL) up to 430 ng/mL. In all sets of samples, approximately 30% of the specimens did not contain measurable TB4 levels. Further, for the large majority of samples (97%), urinary TB4 remained below 60 ng/mL. In the light of these pilot study data, urine samples containing high TB4 concentrations could be considered as atypical and might warrant follow-up studies. However, as various factors are known that influence mRNA expression and/or plasma TB4 concentrations (e.g. viral infection, muscle injury, psoriasis) further studies are required to investigate if subsequently increased urinary concentrations of TB4 are observed and how these would influence potential future urinary threshold levels for TB4. With the currently available dataset, the suggestion of a threshold value does not seem sensible but suspiciously high concentrations could warrant a remark, leading to target controls, intelligence and investigation activities and long-term storage of the respective sample.