

## **PROJECT REVIEW**

### **“Immunological detection of tetracosactide in serum for anti-doping control purpose”**

**J. de Ceaurriz, F. Lasne, C. Buisson, J. Nugier, X. Morge** (Agence Francaise de Lutte contre le Dopage, Chatenay-Malabry, France)

The project deals with the developpment of immunological methods for the screening and the confirmation analysis of tetracosactide in serum.

In vitro and in vivo immunological studies will be performed to develop immunological tools and their performance will be compared to that of a spectrophysical method using a Q trap instrument.

Immunoextraction from serum prior to immunological analysis will be scheduled for confirmation analysis but not for screening analysis.

The aim is the specific detection of tetracosactide in serum at concentration level as low as 100 pg/ml or less in order to offer a relevant detection window.

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### **Results and Conclusions:**

Tetracosactide is a synthetic peptide which exercises the same biological effects as endogenous Adreno CorticoTropin Hormone (ACTH) produced by anterior pituitary gland. Its structure reproduces the first 24 amino acids of ACTH which is composed of 39 ones.

A screening method by ELISA was developed and validated for detection of Tetracosactide in plasma. The principle of the method was to use an ELISA kit (Enzyme Immunossay Kit from Peninsula Laboratories, San Carlos, USA), that reacts with the part 1-24 of ACTH (and thus Tetracosactide), after total elimination of endogenous ACTH from the tested plasma samples. In such conditions, ELISA became specific for Tetracosactide. The sample preparation consisted in removing most of the proteins and any trace of ACTH from plasma. This was achieved by cation-exchange chromatography (CM Sephadex ®).

The preanalytical conditions were shown to be very important. Only EDTA plasma samples must be analysed and if the plasma samples could not be frozen just after collection, it was essential to quickly ship them to the laboratory in refrigerated conditions.

The detection limit of the method was 13 pg/mL.

In parallel, a confirmation method by LC-MS/MS (Thermo) Triple Quad, ESI was developed and validated. The preparation of plasma included cation-exchange chromatography (CM Sephadex ®) and solid-phase extraction (Oasis® HLB).

The detection limit of the method was < 50 pg/mL.