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Detection of Synacthen® in Human Plasma with LC-ESI-MS/MS after Immunoaffinity Purification

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Extended abstract*

Corticotropin (adrenocorticotropic hormone, ACTH) is an endogenous peptide hormone of 39 amino acid residues, secreted by the pituitary gland and an important part of stress response of the organism. It activates the adrenal cortex for secreting corticosteroids such as hydrocortisone. This effect can be used for masking an administration of corticosteroids by reducing the suppression level of hydrocortisone. Although the properties of ACTH regarding a direct elevation of athlete's performance are marginal[1], the organism indirectly evolves a widespread spectrum of effects using endogenous corticosteroids such as e.g gluconeogenesis, lipolysis and anti-inflammatory activity. Due to the fact that only the first 24 amino acid residues of the N-terminus are needed for complete biological activity[2], a synthetic three kDa-corticotropin called Synacthen® was synthesized in the 1960s[3] for diagnostic[4] and therapeutic[5] purposes.

Because of its activating and masking effects Synacthen® belongs to the WADA List of Prohibited Substances (group S2 Hormones and Related Substances)[6]. Predominantly, it is detected by ELISA or RIA. Those methods offer the possibility of cross reactions. Additionally, they often are not able to provide the opportunity of distinguishing ACTH and

* For further details, please refer to:
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Synacthen®. Furthermore, analytical interest mainly emphasizes the conditions affected by Synacthen® but not Synacthen itself.[1, 4, 7]

We present a method using immunoaffinity chromatography for isolating and purifying Synacthen® followed by LC-ESI-MS/MS analysis (Figure 1 and 2). Distinction between Synacthen® and human ACTH is possible. According to an expected plasma level after administration of Synacthen® Depot the target limit of detection was set up to 100 fmol/mL of plasma. The procedure was validated regarding specificity, linearity, recovery (60%), lower limit of detection (LLOD,100 fmol/mL) and precision at LLOD (13.4%).

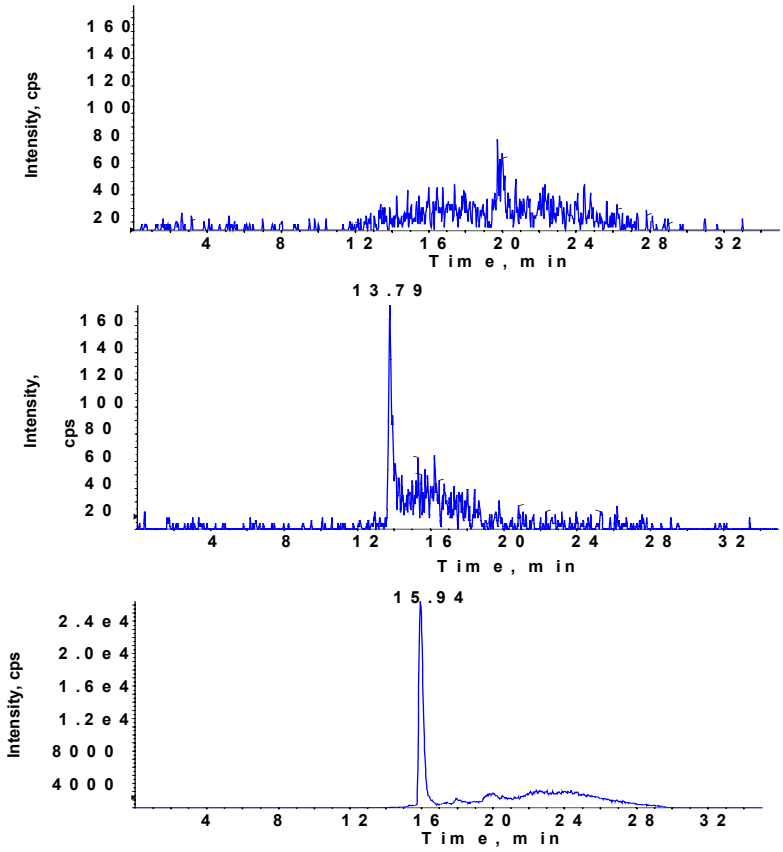

Figure 1: a) blank plasma, b) plasma sample fortified with 0.1 pmol/mL Synacthen®, c) ISTD (= murine ACTH, 5 pmol/mL)

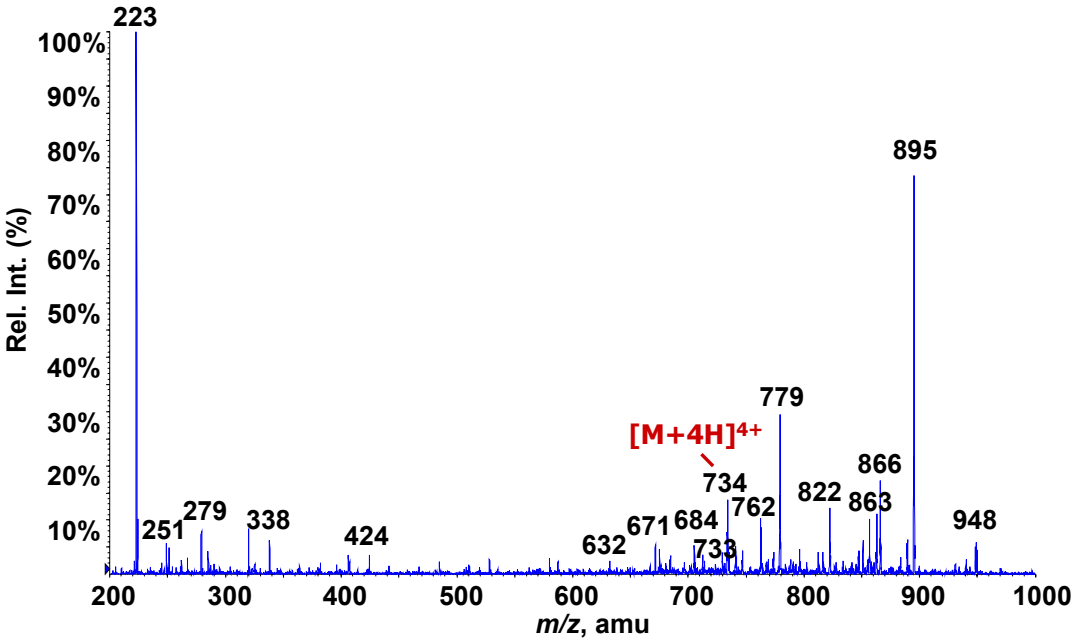

Figure 2: **ESI product ion scan spectrum of the quadruply charged molecular ion $[M+4H]^{4+}$ of Synacthen®.**

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