

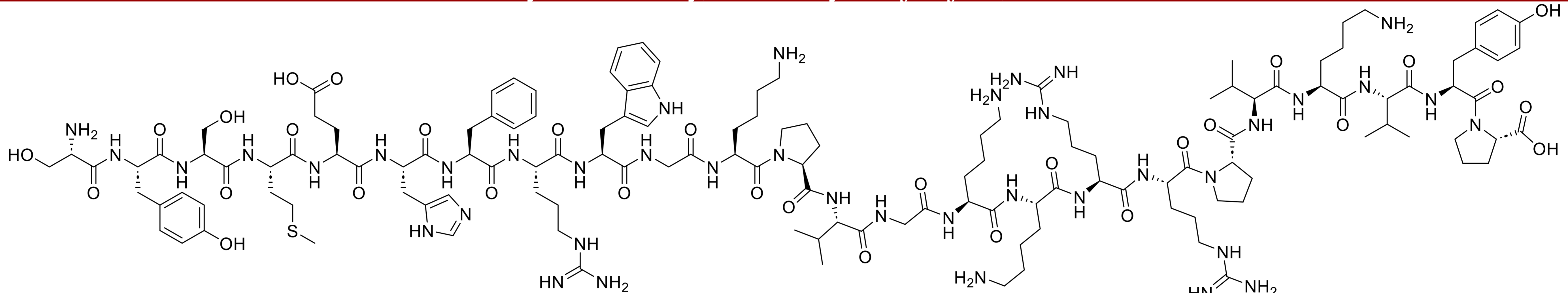
EVALUATION OF TETRACOSACTIDE PEPTIDE IN GALENIC FORMULATIONS FOR RAPID ADRENOCORTICOTROPHIC HORMONE STIMULATION TEST



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H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-OH

Figure 1: Chemical structure and amino acid sequence of tetracosactide.

Background and Importance

- The rapid adrenocorticotrophic hormone test (**ACTH test**) is a commonly performed test in the hospitals and outpatient clinics. Injection of **tetracosactide (TCS)** stimulated secretion of cortisol from the adrenal glands.
- The resulting cortisol level is compared to the baseline ACTH level before the test. If the cortisol level does not increase 30-60 minutes after the application, it indicates adrenal cortical atrophy.

Aim and Objectives

- The aim was the **quantitative and qualitative evaluation** of TCS peptide in a solution with a concentration of 5 µg/ml, filled in glass and plastic containers and stored under different conditions, using multiple methods.

Materials and Methods

- The first two relatively simple methods, **Qubit 4 fluorometer**, the **Bradford method**, did not provide the desired results. We assume that these methods were **not sensitive enough** for our sample with a concentration of 5 µg/ml.
- In the end, we used the ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry (**UHPLC-HRMS**), which proved to be **sensitive and highly selective**.

Results

- TCS has **eight basic centers** in its structure, so both TCS and each impurity were differently charged in an acidic medium.
- With UHPLC-HRMS we have identified **11 impurities**. The highest proportion was represented by impurity with the increased mass of 16 Da (**tetracosactide sulphoxide**).

Conclusion and Relevance

- To perform a rapid ACTH test, it is sufficient to load the patient with **1 µg of TCS**.
- **UHPLC-HRMS** method is **highly selective** and allows identification of TCS and each impurity.
- **Glass container** stored in the **refrigerator** and in **darkness** is the optimal solution for ensuring stability of TCS

Stimulation tests	Suppression tests
<ul style="list-style-type: none"> - rapid ACTH test - multi-day ACTH test - insuline tolerance test - metyrapone test 	<ul style="list-style-type: none"> - dexamethasone tests (1 mg, 2 mg and 8 mg)

Table 1: Adrenal function tests to evaluate the function of adrenal glands.



Figure 2: Mass Spectrometer Q Exactive Plus Orbitrap with HESI source and Liquid Chromatography UHPLC Thermo Scientific Ultimate 3000.

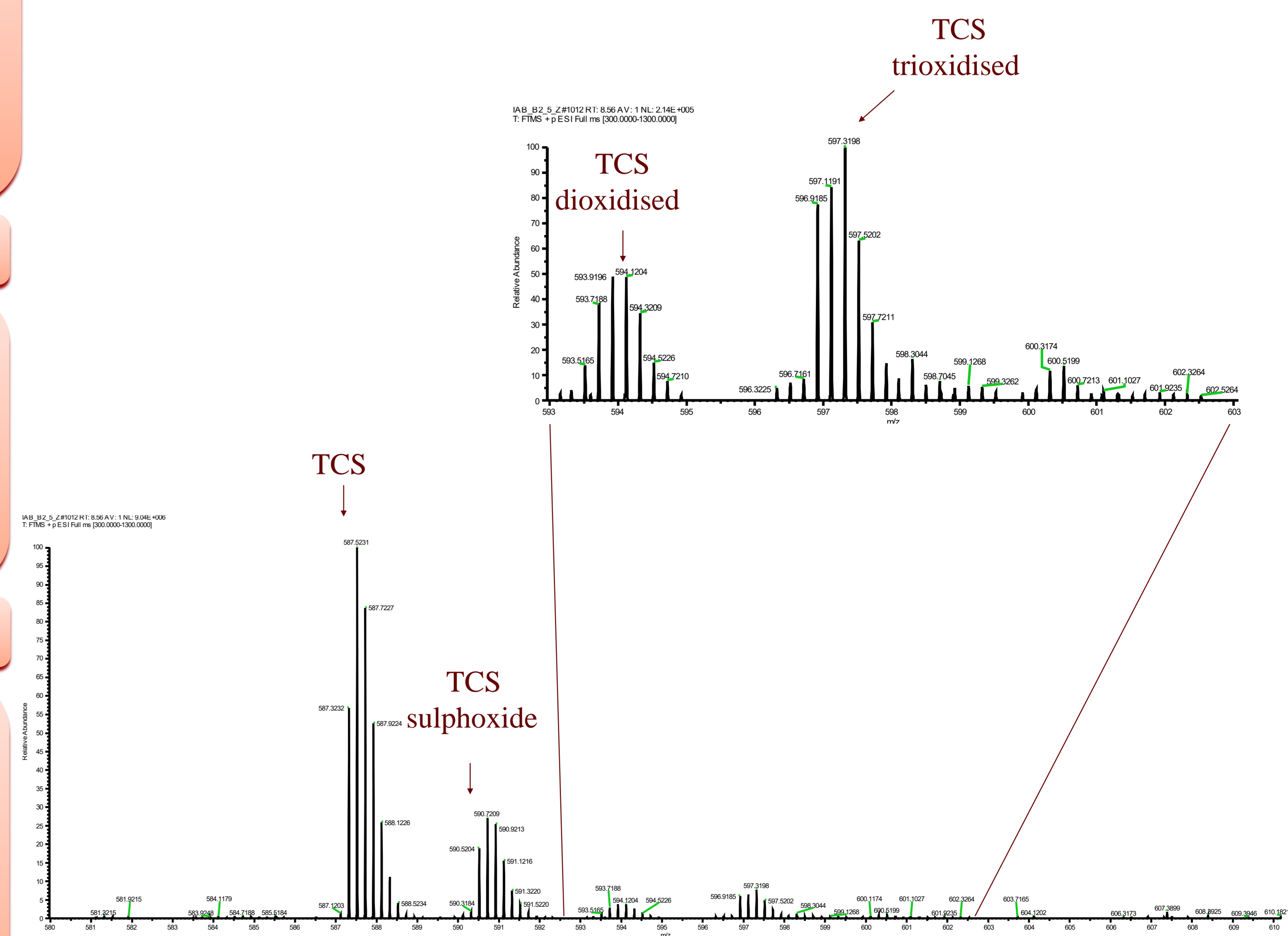


Figure 3: TCS and its impurities with a charge +5 analyzing with HRMS.

