

**CON-PRA-047**

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## **CONFIRMATORY METHODS**

### **The special case of estrogens**

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#### **• Context**

Prepubertal children are a very vulnerable population on the endocrine disruption point of view, because at a critical stage of development during which the endogenous production of steroid hormones – especially estrogens – is extremely very low. However, available data on this topic remains controversial. Serum concentrations in such populations are often almost undetectable with conventional estradiol assays, because they are below the detection limit of the methods used. Because the knowledge of the endogenous hormone profiles is of primary importance within the scope of risk assessment, their re-evaluation using sensitive and specific technique such as mass spectrometry was mandatory. GC-MS/MS appeared as the most suitable technique to reach the sub-ng.L<sup>-1</sup> level and a preliminary developed method was improved in order to be able to quantify estrogens in complex matrices such as serum.

#### **• General objective(s)**

The goal of this practical training is to demonstrate the suitability of GC-MS/MS for the detection and identification of natural estrogens as representative of small and non-polar compounds, as well as to present the strategy and different parameters to consider during the development of a confirmatory method.

#### **• Pedagogical objectives**

✓ Propose an appropriate analytical strategy for measuring the target compounds taking into account their physico-chemical properties: rationale for choosing sample preparation procedure, chromatographic separation method, ionisation technic and acquisition parameters.

#### **• Main items**

Estrogens/ Sample preparation/ Derivatisation/ EI-NCl/ MS<sup>n</sup>/ Triple quadrupole

#### **• Pedagogical tools**

✓ PowerPoint slide show

#### **• Duration**

✓ 1 hour

#### **• Pre-requisite**

✓ Basics of mass spectrometry (CON-THE-010, CON-THE-020 and CON-THE-030)