

**CON-PRA-052**

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## **CONFIRMATORY METHODS**

### **LC-MS<sup>n</sup> data analysis**

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#### **• Context**

Analysis and interpretation of raw data generated by LC-MS<sup>n</sup> instruments are crucial elements for finally delivering correct results. These items appear of even higher importance for laboratories working in a regulatory context, where imposed analytical criteria have to be verified and fulfilled. Thus, a perfect understanding of the typical results obtained by these techniques and a greater awareness of the potential tricks associated to this measurement methods, as well as the main key for their interpretation are required.

#### **• General objective(s)**

The goal of this hands-on session is to propose a real case study for illustrating a typical data analysis procedure applied to a set of raw data files generated by LC-MS and LC-MS/MS from real biological samples. The objective will be for the trainees to go through a full data analysis process, including validation of the injection sequence (notion of quality controls including external and internal standards, blank sample, fortified sample), detection of unknown target analytes (notion of target signal to monitor), unambiguous identification of these substances (notion of identification criteria to fulfil), and final quantitative estimation of the concentration (notion of calibration curve and quantification). All this exercise will be based on dedicated computers equipped with appropriate softwares and fit-for-purpose template files.

#### **• Pedagogical objectives**

- ✓ To know the 3 chronological steps typically required for a correct data analysis process
- ✓ To know the nature and role of 4 main quality control flags typically used for ensuring the validity of a sequence of injection
- ✓ To be aware about the benefits usually observed in LC-MS/MS compared to LC-MS in terms of signal specificity with direct impact on sensitivity, as well as identification and quantification performances
- ✓ To be aware about the strong influence of the sample preparation procedure on the final measurement performances

#### **• Main items**

- ✓ Short introduction, validation of the injection sequence, detection of unknown target compounds, unambiguous identification through the fulfilment of regulatory criteria, quantification, final discussion related to the comparison of LC-MS versus LC-MS/MS and to the influence of various sample preparation procedures.

#### **• Pedagogical tools**

- ✓ PowerPoint slide show
- ✓ Practical exercise on dedicated computers (laptops) equipped with the appropriate software for data processing and fit-for-purpose template files for the identification and quantification issues

#### **• Duration**

- ✓ 3 hours

#### **• Pre-requisite**

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