THE "LTQ-ORBITRAP": AN INSTRUMENT DEDICATED TO MULTI-FUNCTIONAL SEARCH IN MOLECULAR CHEMISTRY

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The physico-chemical analysis of the chemical residues from treatments (veterinary drugs) or from chemical contaminants (toxins, organic pollutants) is today essentially performed by means of analytical methods based on separative techniques (chromatography) coupled to detectors releasing spectral informations in relation with the physico-chemical characteristics of the compounds of interest. Mass spectrometric techniques evolved rapidly within the few last years during the beginning of this new century in particular in connection with the hyphenation to liquid chromatographic instruments. Several technologies from the mass spectrometric technique are nowadays commercially available and they expand their usefulness in different fields of applications.

The liquid chromatography coupled to tandem triple quadrupole mass spectrometer (LC-MS/MS) is one of those techniques widely used for confirmatory purpose when monitoring for presence of one or several chemical compounds in biological matrices. LC-MS/MS, performed in multiple reaction monitoring (MRM) mode, allows a specific and selective molecular identification and also gives the option to quantitate one or several of these compounds when properly selected (or targeted).

The MRM mode is particularly efficient in a targeted screening of an exact number of compounds, but is definitively not designed to screening for numerous untargeted molecules. To detect the presence of untargeted compounds a mass screening in full scan mode is absolutely needed. In this case, the instrument is capable of extracting the mean of the signals obtained over a large range of the mass spectrum. The characteristics of the signals are then depending on the adjusted parameters of both the interfacing source and of the spectral data acquisition.

This kind of LC-MS/MS instrument is competitive when monitoring for known targeted compounds but cannot analyze in details non-described molecules, those for which additional information obtained by other techniques are needed (high resolution mass spectrum, mass extraction/purification and molecular structural mass analysis).

In relation to its tasks for controlling veterinary drug residues in food from animal origin and for studying pharmaco- and toxico-kinetic behaviors of drugs, the Laboratory for Research & Studies on Veterinary Drugs and Disinfectants (LERMVD) has to face different issues such as:

- Evaluating and identifying the nature of molecules bearing relevant biological effects (bacterial growth inhibition, genotoxic effects, oestrogenic effects, …) when found into the biological extract but not fully identified in regard to a library of known compounds.
- Evaluating and identifying the unknown compounds in suspected biological samples when compared with the profile of blank samples (suspicion of frauds or misuse).
- Identifying through a non-radioactive methodology the metabolites generated from a known compound in a biological sample (inter-species pharmacokinetic study, comparison of in vitro/in vivo metabolisms).
- Screening and confirming the presence of known compounds when no suitable targeted method is developed for that compound and/or when no reference substance is available. Then, bibliographical data including chemical structure and formula might be useful.

- Validation of the presence of a specific substance based on its spectral pattern without any need of a standard reference substance to compare with (ie. by means of structural identification only).

To undertake such issues, it must be assumed that substances to be screened have not to be targeted at all and that the analytical system must allow:

- To monitoring a full scan spectrum in as high mass resolution as possible for the different peaks,
- To identify the compounds of interest within the full scan mass spectrum,
- To analyze the compound of interest under a sufficiently high chromatographic resolution in order to separate from co-elution substances.
- To obtain a reliable information on the molecular mass of the compound of interest. Estimation of exact mass in Dalton (mass error lower than 2 mDa) is mandatory to extract the molecular formula.
- To obtain informative data on the molecular structure of the substance of interest extracted from a structural analysis in mass spectrometry (by fragmentation).
- To collect repeatable information and to store full scan spectra available for further investigation in case of new information acquired.

This lecture will shortly display two significant examples extracted from studies performed at AFSSA-LERMVD and implementing the LC-MS hybrid “LTQ-Orbitrap” and shedding light on analytical problems such as molecular identifications of unknown substances for which only “high mass resolution” instruments coupled to systems with a high capacity in post-analyzing the memorized full-scan spectral data can give rise to a fruitful conclusion.