Modern targeted and untargeted LC-MS/MS screenings using low resolution systems

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Scope of the lecture:
An overview and a discussion about different recent LC-MS/MS screening concepts for clinical and forensic toxicology

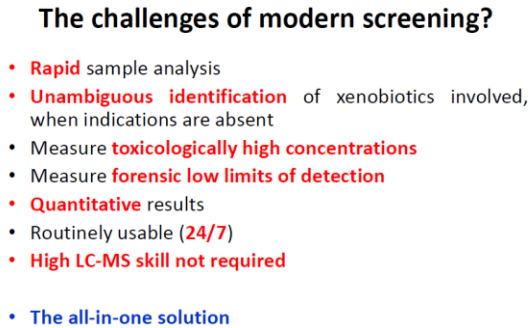
Learning objectives:
1. Provide participants on overview on recent state of the art of targeted and untargeted LC-MS/MS screening approaches
2. Provide participants on overview on the main advantages and drawbacks of these different approaches
3. After the lecture, the participants should be able to critically decide which screening approach should be used depending on the situation

Extended abstract:

Context:

Clinical and forensic toxicologists expect from a screening procedure the unambiguous identification of the xenobiotics involved in intoxication cases, even when they have no clues to guide the search. But, the challenge of modern screening analyses is to measure toxicologically high concentrations with the expectation of forensic low limits of detection also being possible. Additionally, there is a need for rapid sample analysis and for quantitative results. The challenges of modern screening are depicted in figure 1:

Figure 1: the challenges of modern screening



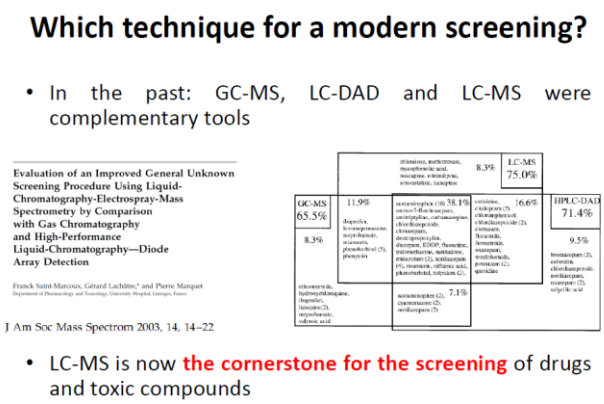
Automated immunoassays generally represent a first approach and provide a result in a few minutes, but these techniques allow, for most of them, only a class-diagnostic, notwithstanding the limited number of classes available. On the contrary, chromatographic techniques coupled to specific detectors such as MS or UV-diode array detectors cover a very large panel of relevant compounds. Nevertheless, the limited specificity of UV spectra (since several compounds can have similar UV-spectra), their variability as a function of pH and the fact that a lot of compounds present poor or no UV absorbance, make HPLC-UV-DAD not very specific, reliable, nor universal. Thus, very few UV spectrum libraries are commercially

available.

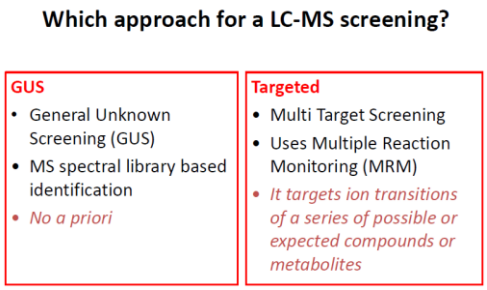
On the contrary, due to its widespread availability and its high specificity, gas chromatography/mass spectrometry (GCMS) has been considered as the gold standard technique for GUS in toxicology. It is based on electron ionization (EI) with standard conditions (70eV) for which very large EI-mass spectra libraries exist. However, GC-MS presents some weak points. It requires time-consuming extraction procedures and sometimes cleavage of conjugates prior to extraction. Drugs or metabolites can be detected in their native form only if they are thermally stable, volatile, and mildly or nonpolar. Furthermore, derivatization and artifact formation significantly complicates the identification process.

The role of LC-MS has become increasingly important in analytical laboratories for routine applications, particularly therapeutic drug monitoring, and forensic and clinical toxicology. While it was considered as a useful complement to immunoassays, LC-DAD and GC-MS, LC-MS is now recognized as the cornerstone for the GUS of drugs and toxic compounds. Figure 2 illustrates the place of LC-MS/MS.

Figure 2: LC-DAD, GC-MS and LC-MS/MS for screening procedures



For such an application, two approaches are classically possible: (i) untargeted (General Unknown Screening; GUS) (ii) targeted screenings, as illustrated on figure 3



Untargeted screenings:

For the purpose of clinical and/or forensic toxicology, LC-MS/MS screenings should ideally be untargeted, meaning they do not involve any pre-selection of analytes. Various methods for untargeted screenings have been developed in recent years.

Sample preparation strategies for untargeted screenings can be simple dilution or protein precipitation, liquid-liquid extraction, solid-phase extraction or salting out-assisted liquid-liquid extraction procedures.

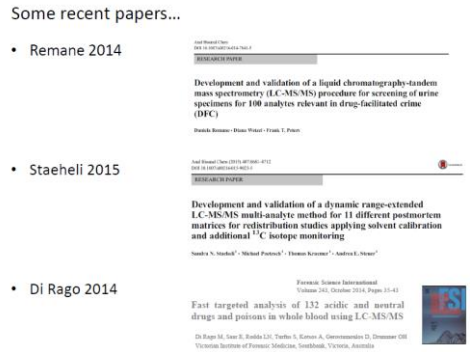
Recent detection concepts generally involve information-rich fragment ion spectra that are generated in collision cells after selection of pertinent precursor ions. Most LC-MS/MS screening approaches use collision cell-induced fragmentation to record information-rich product ion spectra (PIS) for identification. Data-independent acquisition (DIA) has become increasingly popular. Briefly, with DIA, collision cell-induced fragmentation spectra are recorded independently from any survey scan using broad precursor isolation widths.

In recent years, several reference libraries containing hundreds or up to thousand reference compounds have been developed using different fragmentation types and stages of MS.

There are no generally accepted validation procedures for untargeted screenings. Most of time, validated qualitative parameters such as LOD, recovery and selectivity are evaluated. Sometimes, full quantitative method validation for a subset of compounds can be performed.

Targeted screening:
Only a limited number of targeted screening procedures covering a subsequent amount of analytes out of different drug classes have published up to now. Shah *et al* have porposed a screening method for the analysis of hair samples covering more than 200 substances relevant in forensics and doping. Di Rago *et al.* focused on drugs with acidic and neutral structure resulting in a screening method for 132 drugs and poisons. An approach for 100 relevant analytes was established by Remane *et al.* Staeheli *et al.* published an approach covering a wide range of forensically relevant compounds.

Figure 4: recent published targeted screening procedures



Different acquisition modes have been proposed to reduce false positive and false negative (FP/FN) reporting. These are summarized in figures 5 and 6.

Figure 5: approaches to reduce FP/FN reporting

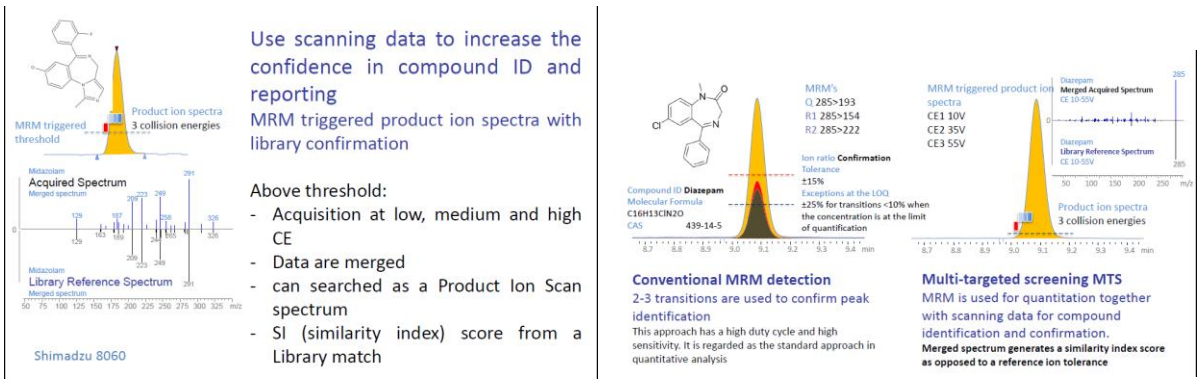
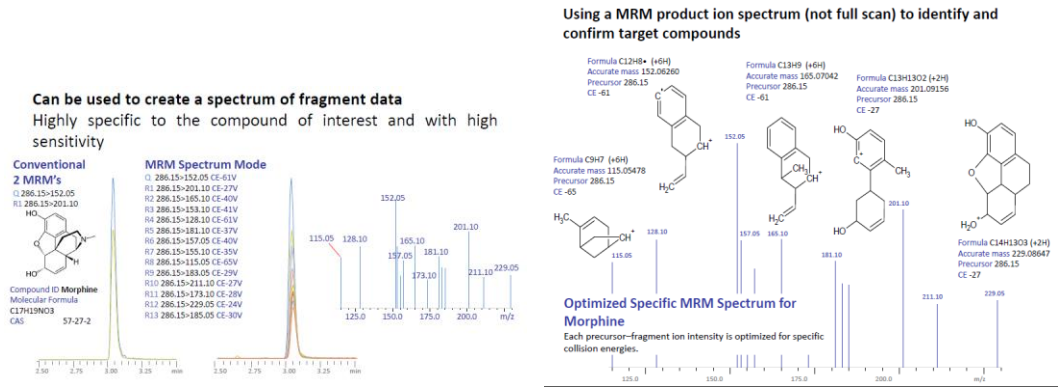
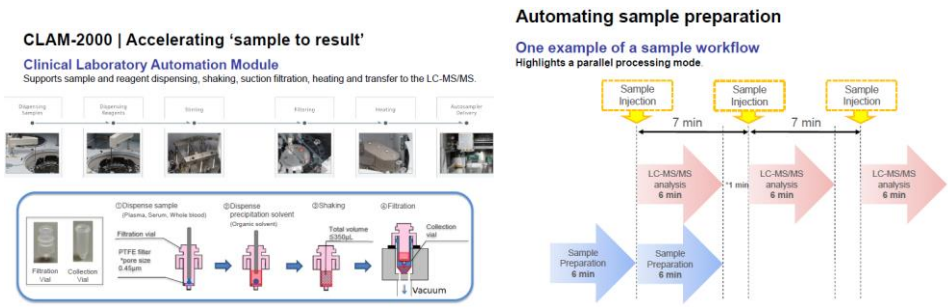


Figure 6: MRM Spectrum mode



Depending on the matrix, Sample preparation strategies for targeted screenings can be simple dilution or protein precipitation, liquid-liquid extraction, solid-phase extraction or salting out-assisted liquid-liquid extraction procedures. System for on-line sample preparations are now available to improve the performances and the workflow. A short description of the CLAM 2000 commercialized by Shimadzu is given on figure 7.

Figure 7: CLAM-2000 (Shimadzu®) for automated sample preparation



Method validation experiments are mandatory to ensure unambiguous identification and accurate quantification results. They include the evaluation of selectivity and specificity in terms of testing for linearity, accuracy and precision. Interferences from possible other drugs and analytes, several stability issues, carry-over and dilution integrity have also to be evaluated.

Conclusion:

A screening is usually seen as the first analysis carried out when the nature or the presence of a drugs is totally unknown, which is particularly useful in clinical and forensic toxicology. Using former LC-MS/MS systems, a screening usually precedes more specific analyses allowing the quantitation of the molecules. However, with increasing the performances of LC-MS/MS systems, it is now feasible to simultaneously detect and quantify. Additionally, recent automated sample preparation can make targeted screening easier and can greatly improve the workflow.

Key references:

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Some author's contributions to the field:

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