MOVING FROM NON-TARGETED TO TARGETED ON THE SAME INSTRUMENT

Sophie Mompelat & Dominique Hurtaud-Pessel

Residues and Contaminants Analysis Unit, Anses-Fougères Laboratory, France

Outline

Scope

What we call targeted (TA) and non-targeted analysis (NTA)

HRMS : a real Swiss Army Knife

Main issues in TA and in NTA

Some antidotes for reliable results

Conclusions

Scope

Analysis of veterinary drugs and other contaminants in food matrices

Surveillance and official control

Focus on HRMS (even though other tools exist !)

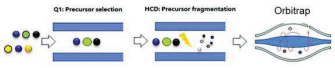
HRMS capabilities: targeted and/or non-targeted analysis in a single run !!!

Methodological approaches for the HRMS analysis of contaminants in food:

What we call targeted (TA) and non-targeted analysis (NTA)

Different ways of thinking depending on where we look from:

The scientific operator's intention		*versus*	The HRMS instrument process during acquisition	
Known molecules we are intentionally looking for	**Targeted**		**Targeted**	Selective: SIM (SRM), Full scan + target MS/MS (ddMS², MRM and PRM - parallel reaction monitoring)
Unknown molecules - we don't know « the name » of the compounds we are looking for (e.g. residue markers of the exposure to a xenobiotic)	**NTA**		**NTA**	Full scan mode Full scan combined with fragmentation mode (variable data independent analysis, vDIA) SIM + neutral loss

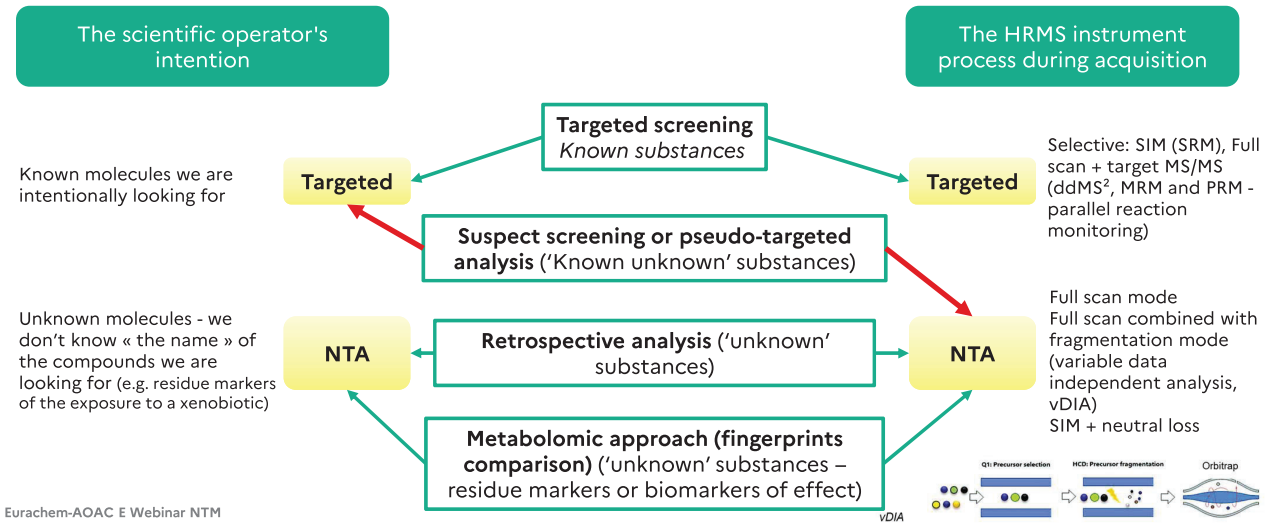


vDIA

What we call targeted (TA) and non-targeted analysis (NTA)

anses

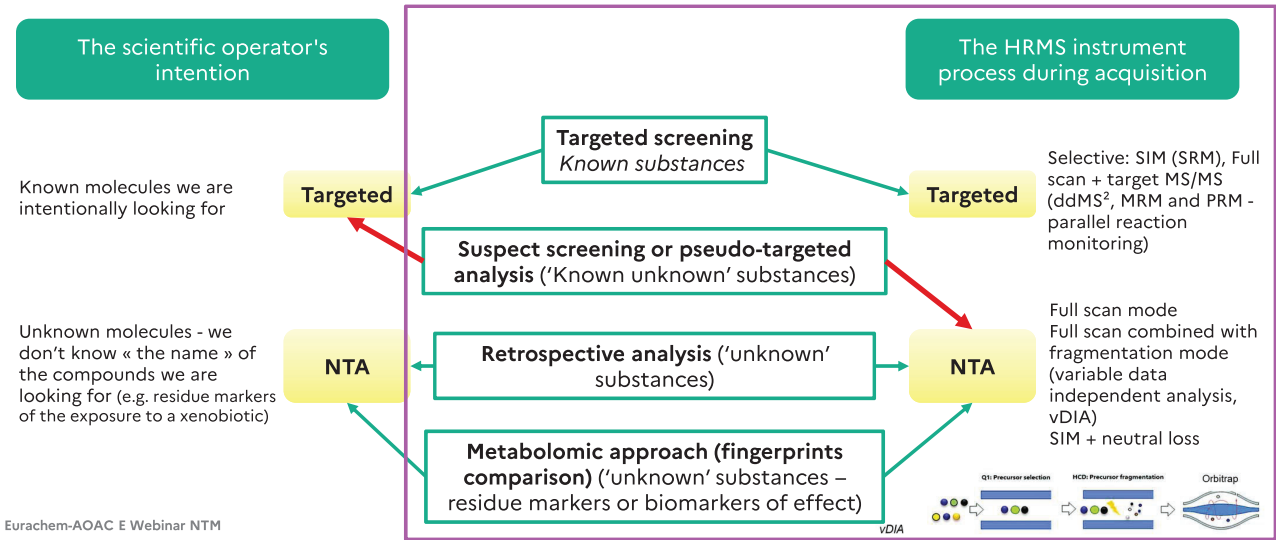
Different ways of thinking depending on where we look from:



What we call targeted (TA) and non-targeted analysis (NTA)

anses

Different ways of thinking depending on where we look from:



Eurachem-AOAC E Webinar NTM

HRMS : a real Swiss Army Knife

Targeted screening
Known substances (MS/MS)

→ Control and Surveillance

Non-targeted analysis

Suspect screening or pseudo-targeted analysis ('Known unknown' substances)

Retrospective analysis ('unknown' substances)

Metabolomic approach (fingerprints comparison) ('unknown' substances – residue markers or biomarkers of effect)

→ Surveillance and early warning system

- New emerging substances
- Remanent markers of contamination/exposure
- Contaminant mixtures

→ Exposure & Risk evaluation

Main issues in Targeted analysis (Full scan-ddMS²)

Selective ion acquisition modes

Automated selection of ions and process

Great specificity, selectivity and sensitivity may be achieved with HRMS

Performances can meet the official guidances

Major bottleneck = sample preparation to eliminate interfering substances (matrix effects)

Main issues in Non-Targeted Analysis

anses

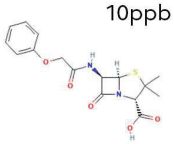
Suspect screening or pseudo-targeted analysis ('Known unknown' substances) and Retrospective analysis ('unknown' substances)

Some compounds may require a high degree of sensitivity and selectivity

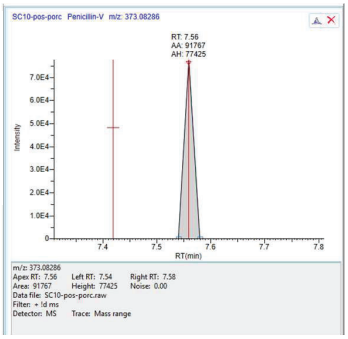
Analytes with poor recoveries or poor reproducibility in some matrices

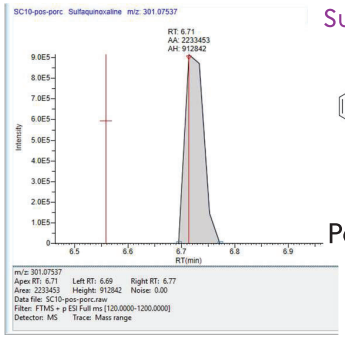
Matrix effects when running multiclass/multimatrix methods

Penicillin-V
10ppb

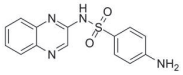


Pork meat





Sulfaquinoxalin
10ppb



Pork meat

Main issues in Non-Targeted Analysis

anses

Suspect screening or pseudo-targeted analysis ('Known unknown' substances) and Retrospective analysis ('unknown' substances)

Annotation: based on empirical or predicted data
Number of open-access reference spectra is limited

Identify and quantify a substances without a reference standard is complex
(not available, or too expensive for all standards to be introduced in a QC routine sample)

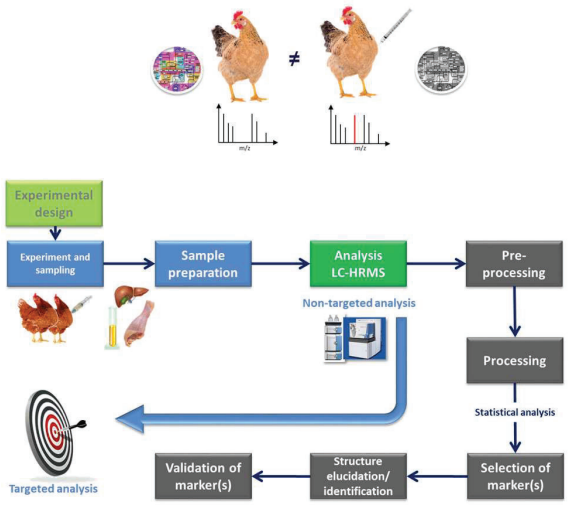
Identification confidence: Need to define different level of identification confidence (e.g. level 1-5 according to Shymanski *et al.* 2014)

Evaluate performances of NTA methods is complex. **Guidance?**

No equivalent guidelines (as Reg. (EU) 2021/808 for targeted) available for NT methods
=> application for routine or official control is difficult
Many sources of variation (pre-analytical, analytical, and post-analytical steps)
=> Need for normalisation/standardization strategies: currently under construction

Main issues in Non-Targeted Analysis

Metabolomic approach (fingerprints comparison) ('unknown' substances – residue markers or effect biomarkers)



- Needs a rigorous methodology to avoid any bias at the different stages
- Risk of loosing information at each stages of the workflow (type of sample extraction, data processing, filtration steps, etc.)
- Time consuming (annotation, validation)
- Identify and quantify substances without reference standard + no target/suspect list
- Workflow validation (QC, identification levels, etc.) in case of biomarkers of effects
- Validate a targeted method from discovered markers without the identity of marker residues

Some antidotes for reliable results

Suspect screening or pseudo-targeted analysis ('Known unknown' substances) and Retrospective analysis ('unknown' substances)

Sample preparation optimisations

Identification: identification points

Mass accuracy, Fragmentation - Full scan +AIF, relative intensities (ion ratio), isotopic fit, adducts, metabolites, IMS (ccs), experimental and predicted Rt, presence of heteroatoms
=> To priorise the different parameters and to propose a unified identification strategy for NTA

Quantification: estimation of concentrations from the precursor ion

External calibration (matrix matched calibration standards) - Standard addition - Isotopic dilution - etc.
Prediction strategies - *Predicting Ionization Efficiency ; machine learning predictor*

Some antidotes for reliable results

anses

Suspect screening or pseudo-targeted analysis ('Known unknown' substances) and Retrospective analysis ('unknown' substances)

Method performances evaluation

Calculation of false positive and false negative rates

False positive rate => measured on 21 blank samples
For 1 analyte,
presence of a signal at expected m/z and expected retention time on Full scan => **RT + Parent**
Presence of a signal at expected m/z and expected retention time on Full scan + presence of a fragment ion at expected m/z => **RT + Parent + Frag**

In FullMS/v-Dia

False positive rate (FP%)		
Compound	RT+Parent	RT+Parent+Frag
Example : Oxytetracycline	*14*	*0*
Example: Flunixin	*0*	*0*
Example: Josamycin	*24*	*5*
Number cpd giving false positive / Number total cpds studied	52 / 154	17 / 154
% compound giving FP	34%	11%

Some antidotes for reliable results

anses

Suspect screening or pseudo-targeted analysis ('Known unknown' substances) and Retrospective analysis ('unknown' substances)

QA/QC

Different types of QC:

QC Reagent blank => check for absence of contamination

QC Matrix blank sample => check for absence of contamination/ check interferences

QC Fortified matrix blank (with n analytes) => check of the analytical procedure (detection capability and recovery

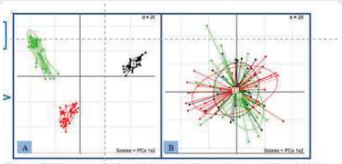
Certified referenced materials

Some antidotes for reliable results

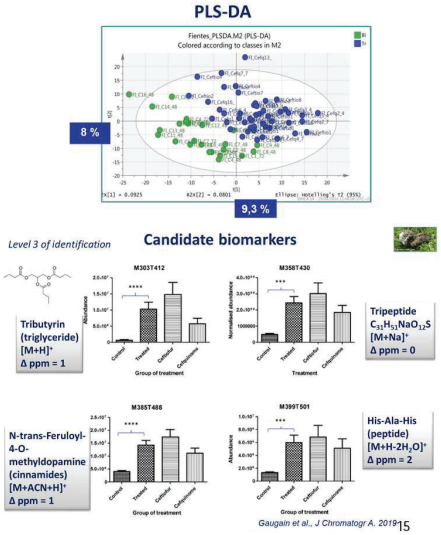
Metabolomic approach (fingerprints comparison) ('unknown' substances – residue markers or effect biomarkers)

QA/QC importance to validate the relevant detected markers (normalisation intra/interbatches)

DATA PREPROCESSING	Droppings	Liver	Eggs
Number of QC samples	14	9	21
Number of batches	2	1	3
Number of extracted metabolites	1398	1734	3633
Type of normalisation	Linear intra and inter-batch	Linear intra-batch	Linear intra and inter-batch
Analytical CV threshold (%)	30	30	50
Number (and %) of retained metabolites after analytical CV filtration	1160 (83 %)	852 (49 %)	2098 (58 %)



Eggs : PCA score-plot from all metabolites before any filter before and after intra/interbatch normalisation



Gaugain et al., J Chrom A, 2019

Some antidotes for reliable results

Metabolomic approach (fingerprints comparison) ('unknown' substances – residue markers or effect biomarkers)

To validate the identity of marker(s) with reference standards

When identification is not feasible: Possibility to create a predictive/classification model (PLS-DA, logistic regression, etc.), although quite complex to validate (robustness) and to implement in routine analysis

To prove the specificity of the found marker(s) when they are not the parent compounds (additional experimental studies)

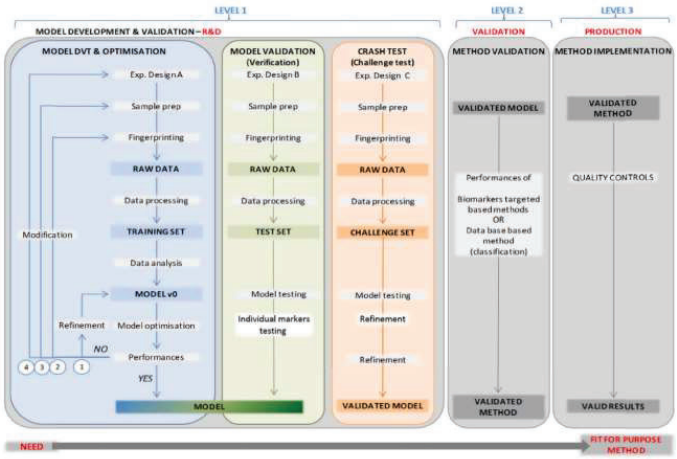


Fig. 5. Generic scheme for development and validation of untargeted based methods (Dervilly-Pinel et al., 2018).

C. Cloteau et al., Food Control 148 (2023) 109601

Conclusions

HRMS is real Swiss Army Knife with many possibilities from targeted to non-targeted analysis

Lots of potential

Some drawbacks

Lots of possible solutions and new ones to explore

Expectations

Technical expectation: expand the linear dynamic range of HRMS devices & further automation of processing

To share NTA MS data with the scientific community to create the most comprehensive and reliable database of suspect lists (contaminants, their metabolites, their degradation/transformation products)

To propose harmonized and unified validation/performance assessment guidelines adapted to the development of multiclass/multimatrix NTA methods

Acknowledgements

Pierrick Couëdor, Murielle Gaugain, & all ARC Unit