

**VALIDATION OF
NON-TARGETED
METHODS**

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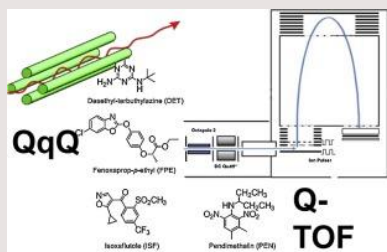
Outline

- **Introduction**
- **What is Non-Targeted Methods?**
- **Approaches**
- **Issues to be addressed**

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Introduction

FACT



Mass Spectrometry

- Dominant Analytical Technique (Last 20 years)
- Low Resolution
- High Resolution

Routine Analysis

- Pesticides
- Organic Contaminants
- Veterinary Drug Residues
- etc

Research

- Development of new methodologies
- Emerging Contaminants
- Authenticity – Food Fraud
- Biomarkers

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Introduction



Why?

- Simultaneous detection and quantification of analytes
- Low detection limits
- Capability of detection unknown compounds, especially for HRMS
- Minimizes cost and time of analysis
- High selectivity and strong identification criteria, especially in tandem or/and high-resolution mass analyzers

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Introduction



As a result ...

- ✓ Many official methods convert to MS-based methods
- ✓ Even the more complicated mass spectrometry techniques become more user-friendly
- ✓ New sophisticated methodologies developed, with complicated analytical challenges, such as authenticity, emerging pollutants, new biomarkers etc.
- ✗ More complicated methodologies become more advanced technical competence is needed.
- ✗ There are a lot of fit for purpose guidelines for target analysis but very few for non-target analysis
- ✗ New guidelines are necessary in order to be used non-target analysis for official control.

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What is Non- Targeted Method?

There are not an "official" definition

Non-Target Screening: Non-targeted analysis procedure without limitation to **pre-selected** substances. All substance that can be measured by chromatography and mass spectrometry by the applied analytical method are detected.

(German Water Chemistry Society – Non-Target Screening in water analysis)

Non-target screening (NTS): Analytical method for detecting a **broad range** of compounds. Screening in full scan mass chromatograms for masses of interest based on criteria such as signal intensity of frequency of occurrence or other criteria posed by the scientific question in place and subsequent identification using **mass spectrometric information** (e.g. isotope pattern, MS2 fragmentation, RT) and possibly metadata (e.g. environmental context, consumption, commercial relevance). Sometimes also called non-targeted analysis, non-targeted screening or untargeted screening.

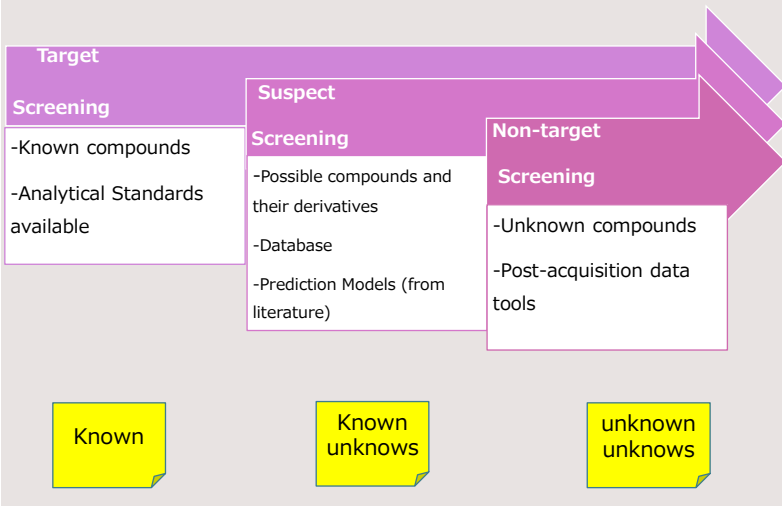
(NORMAN guidance, Hollender et al, doi:

<https://doi.org/10.1186/s12302-023-00779-4>)

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MASS SPECTROMETRY

Workflows



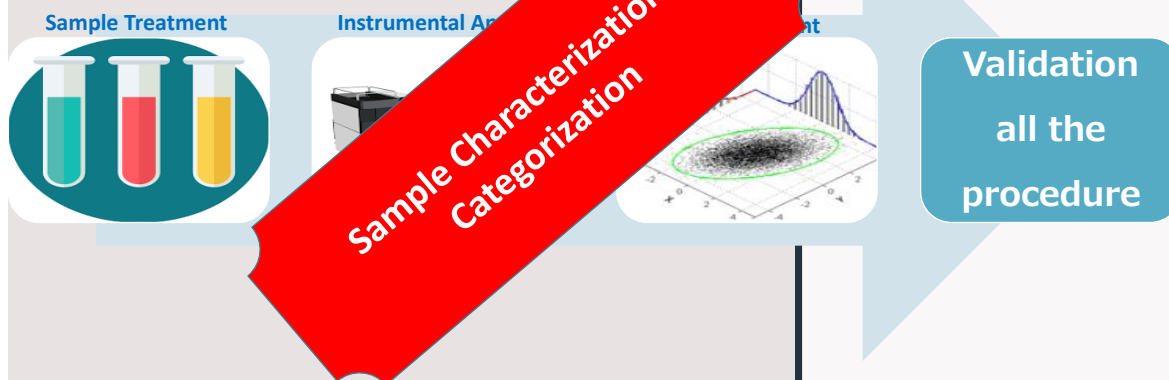
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NON-TARGET METHODS (1ST APPROACH)



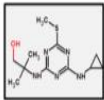
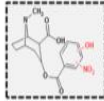
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NON-TARGET METHODS (2ND APPROACH)



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NON-TARGET METHODS

Example	Identification confidence	Minimum data requirements
	Level 1: Confirmed structure by reference standard	MS, MS ² , RT, Reference Std.
	Level 2: Probable structure a) by library spectrum match b) by diagnostic evidence	MS, MS ² , Library MS ² MS, MS ² , Exp. data
	Level 3: Tentative candidate(s) structure, substituent, class	MS, MS ² , Exp. data
$C_6H_5N_3O_4$	Level 4: Unequivocal molecular formula	MS isotope/adduct
192.0757	Level 5: Exact mass of interest	MS

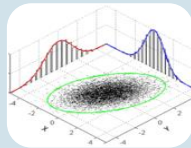
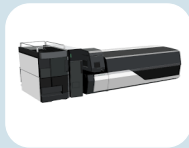
Schymanski, Jeon, Gulde, Fenner, Ruff, Singer & Hollender (2014) ES&T, 48 (4), 2097-2098. DOI: 10.1021/es5002105

1st Approach is a variation of a target method.

The identification criteria are of great importance. They have been already established in the literature, as follows:

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NON-TARGET METHODS

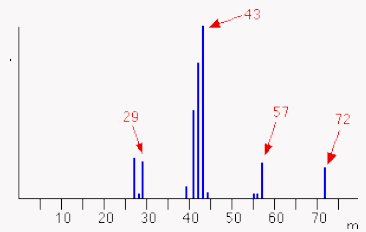


Validation
all the
procedure

2nd Approach is more complicated.



All steps need validation



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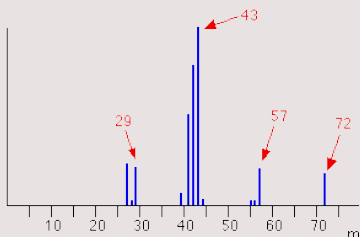
Non-target Methods (2nd approach) – issues to be addressed

Sample Treatment

Generic sample treatment in order to obtain as much information as possible that can be useful.

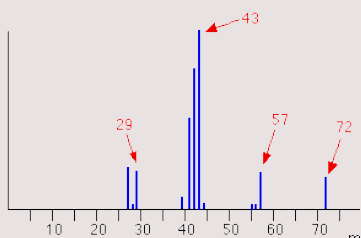
The different procedures are not always comparable due to:

- ❖ Different matrix effect.
- ❖ Different compounds that are finally identified.



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Non-target Methods (2nd approach) – issues to be addressed



Instrument Analysis

Retention time is very important for the identification of compounds.

Changes to column may drastically change the profile of sample.

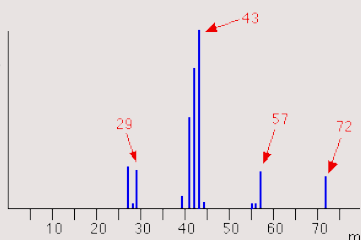
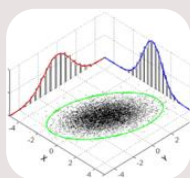
Contamination have to be checked always.

Checking of changes in instrumental sensitivity

Quality Control (QC) samples (pooled) are necessary to address this issue and avoid false positive results.

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Non-target Methods (2nd approach) – issues to be addressed



Data treatment & Statistical analysis

Many software are in-house and based on programming language.

How is an in-house software validated?

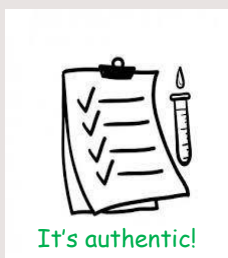
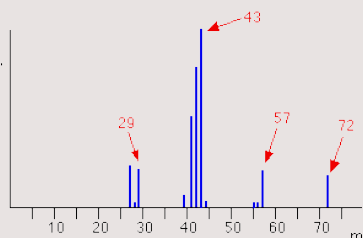
In every case of NTMs one multivariate statistical tool is used, at least.

How is a statistical tool validated?

How to avoid data over-interpretation?

Standardization of statistical and programming procedure is required.

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Non-target Methods (2nd approach) – issues to be addressed

Results interpretation

Most of the NTMs are qualitative (Yes/No) or semi-quantitative (Categorization).

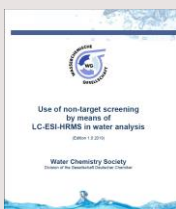
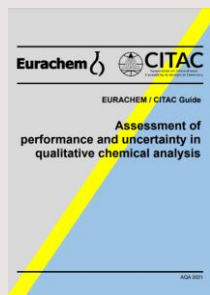
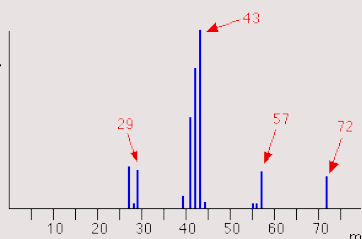
There are not CRMs for Non-target Methods and Proficiency Tests yet.

Reliability of results (uncertainty of qualitative analysis)

In most cases, a NTM was developed for a specific case, at a specific time with specific reagents & samples for **general** purpose.

Robustness of the developed model (What happened a year later?)

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Non-target Methods (2nd approach) – Perspectives



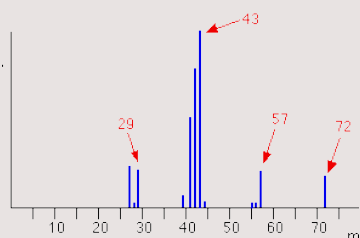
Guidelines of FDA Economically Motivated Adulteration-Dedicated to food fraud.

New Eurachem guide (2021) "Assessment of performance and uncertainty in qualitative chemical analysis" - A start until a dedicated guideline is available.

Water Chemistry Society (2019) "Use of non-target screening by means of LC-ESI-HRMS in water analysis."

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Conclusions

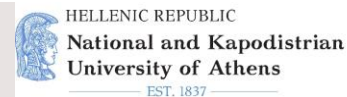


- ✓ High Resolution Mass analyzers are increasingly accessible not only to research labs but also to routine labs.
- ✓ Software and multivariate statistical techniques become more user-friendly.
- ✓ Challenging in analysis can be dealt with more easily than in the past.
- ✗ More information and standardization are necessary in order to implement to official control and help solve complicated analytical problems.
- ✗ Quality assurance of non-target methods is being discussed but has not yet been regulated.
- ➔ Eurachem creates a Task Group for non-target methods as part of Method Validation Working group.
- ➔ Joint Eurachem/AOAC-E webinar series "Trends & Challenges for Non-Targeted Method" (2 webinars organized)

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**Thank you
for your
attention
Questions**



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