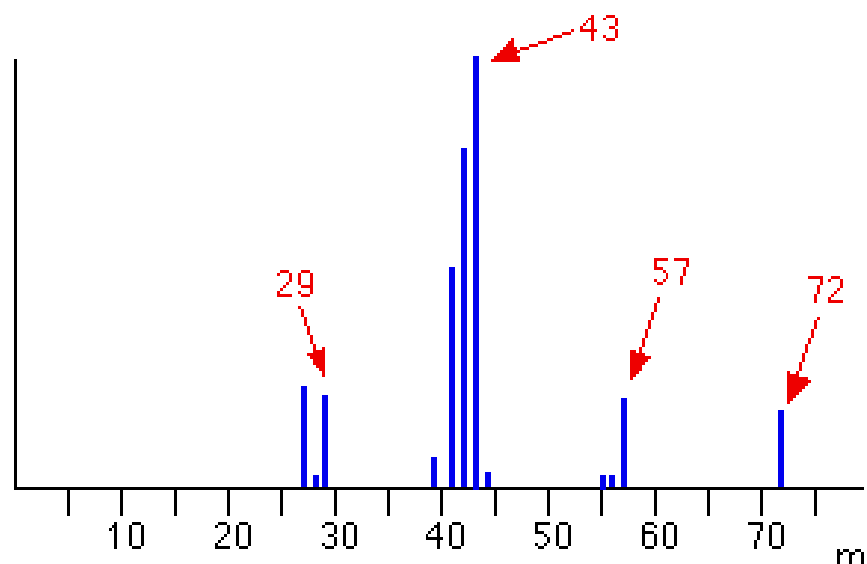




Target Analysis   Non-Target Analysis 



Non-Target Methods: Challenges and Perspectives

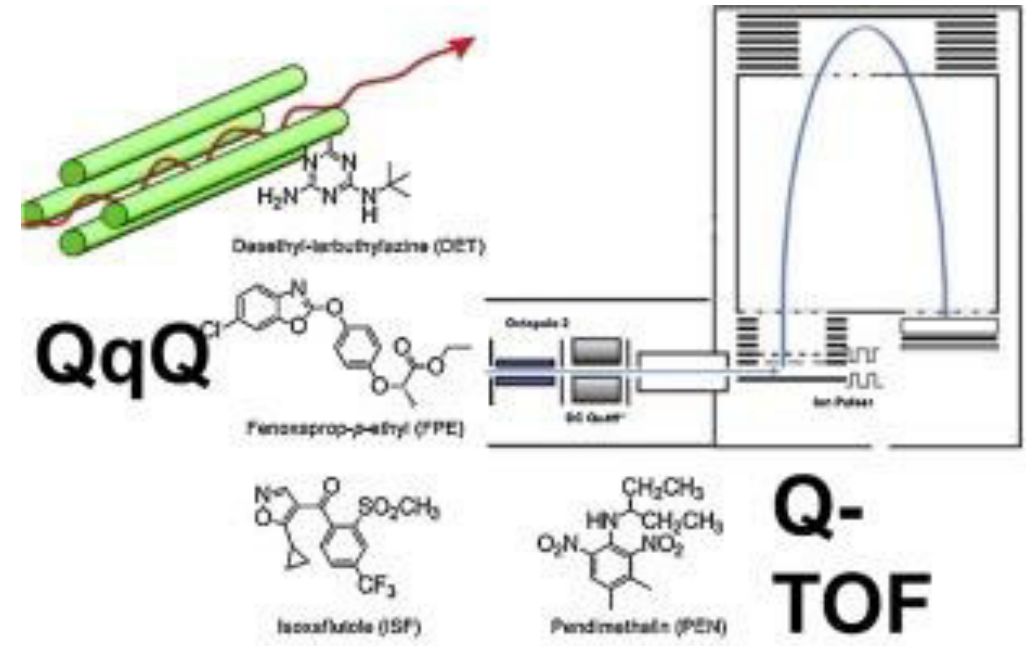
Dr. Marios Kostakis, Prof. Nikolaos Thomaidis

Laboratory of Analytical Chemistry

Department of Chemistry

National and Kapodistrian University of Athens

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

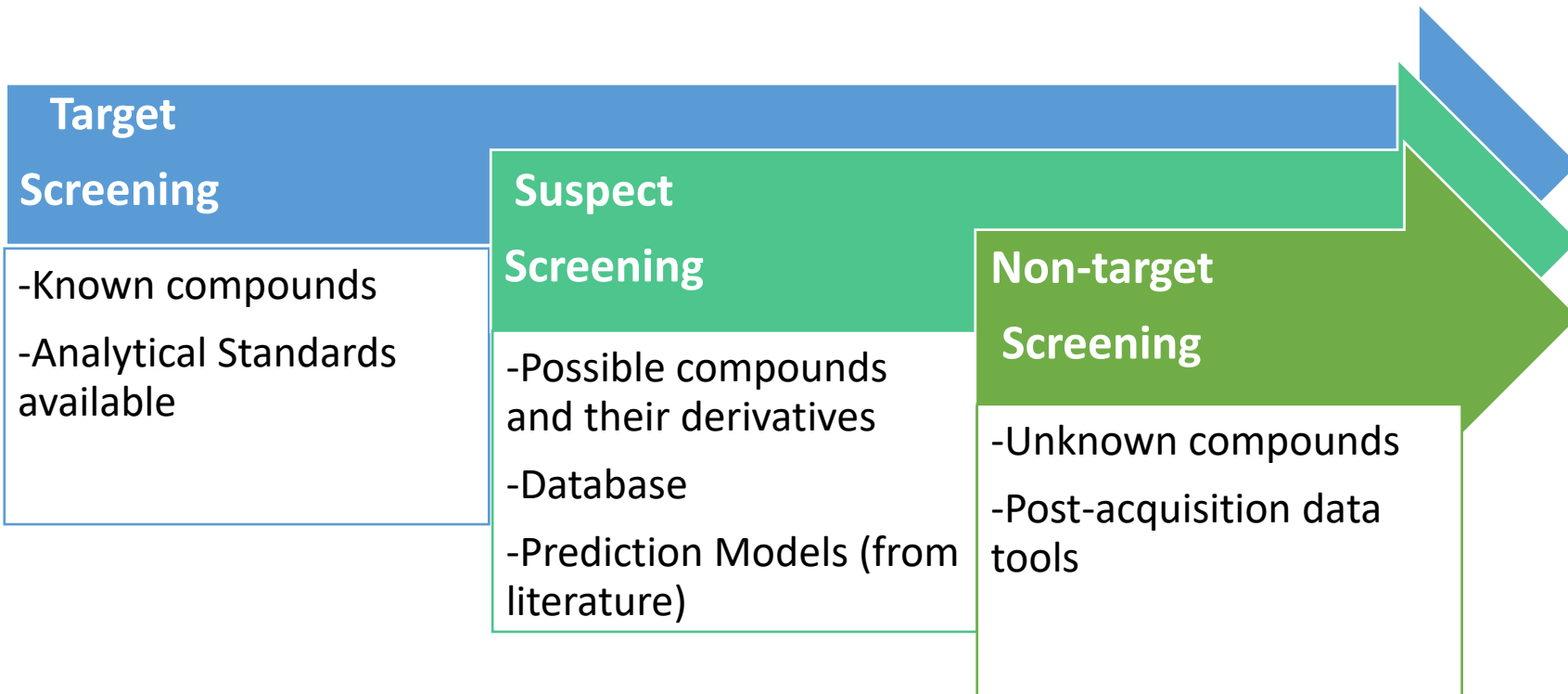


As a result ...

- ✓ 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.



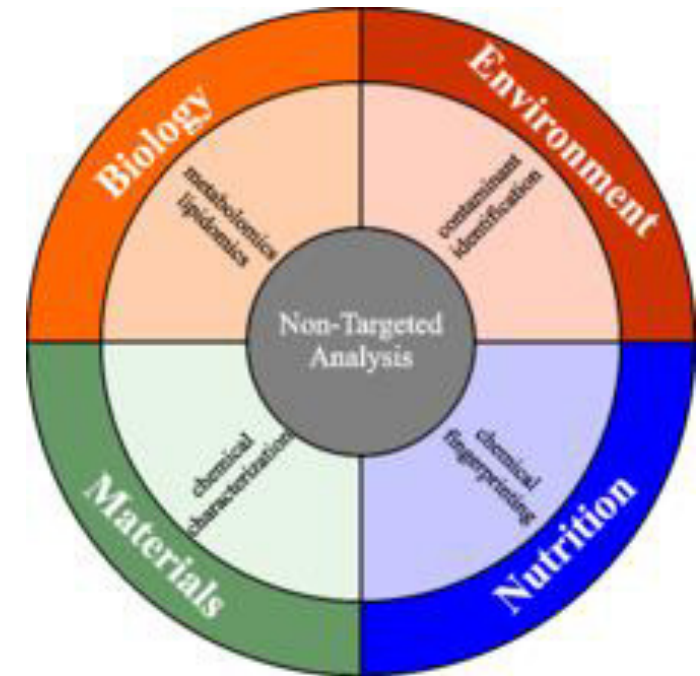
Mass Spectrometry Workflows



Known

Known unknowns

unknown unknowns

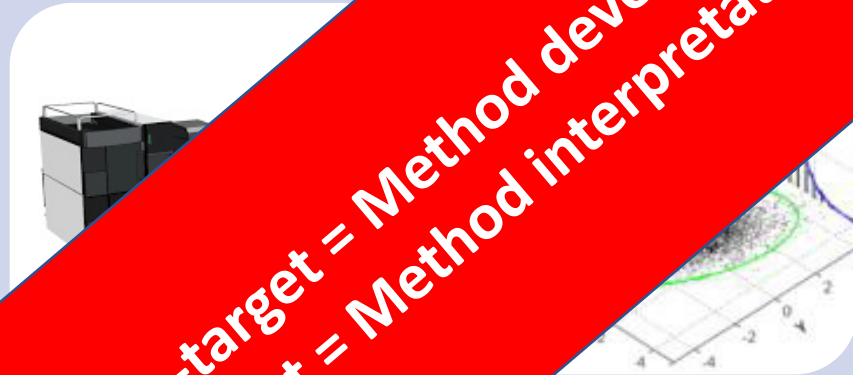


Non-target Methods (1st approach)

Sample Treatment



Instrumental Analysis



Marker Identification



Validation
of Markers

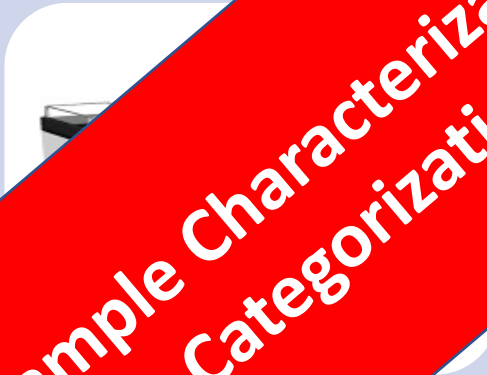
Non-target = Method development
Target = Method interpretation

Non-target Methods (2nd approach)

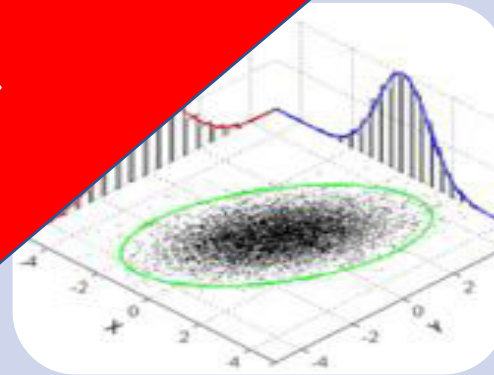
Sample Treatment



Instrumental Analysis



Result

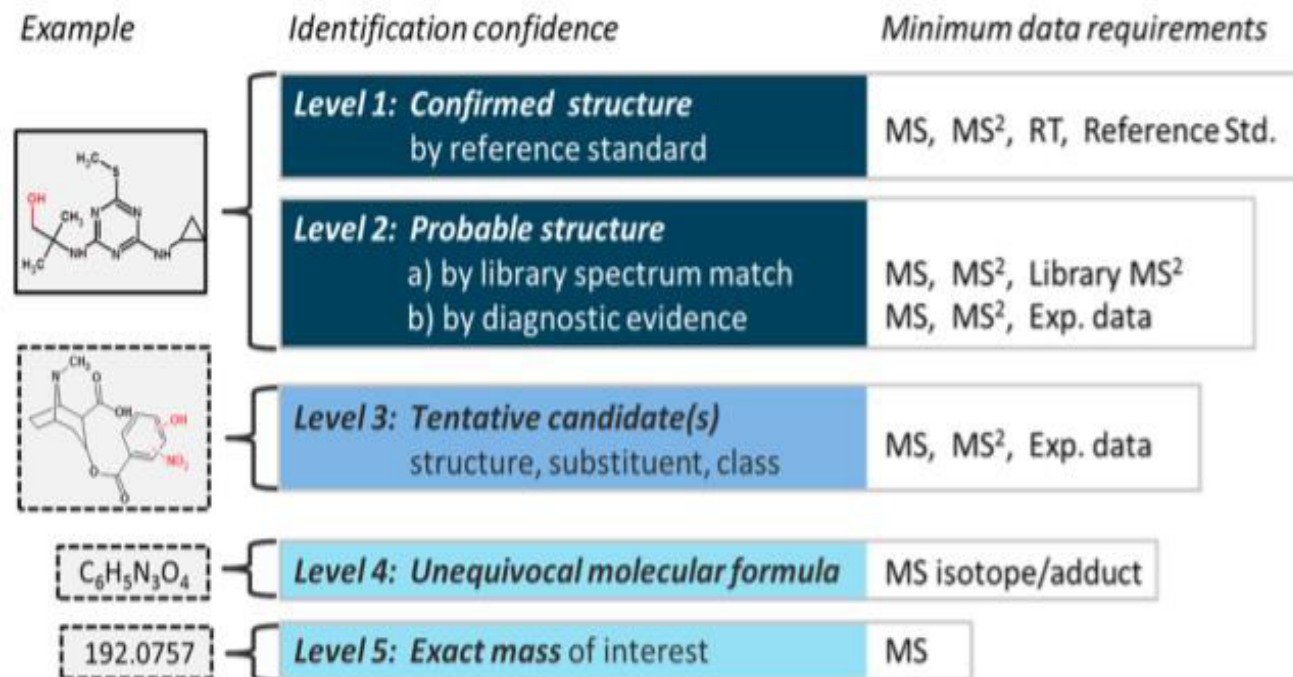


**Sample Characterization -
Categorization**

**Validation all
the procedure**

Non-target Methods

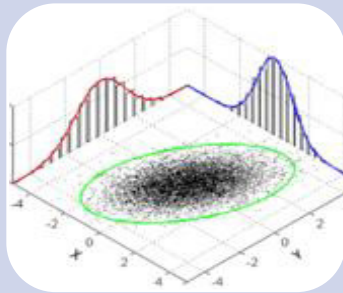
- **1st Approach** is a variation of a target method.
- The identification criteria are of great importance. They have been already established in the literature, as follows:



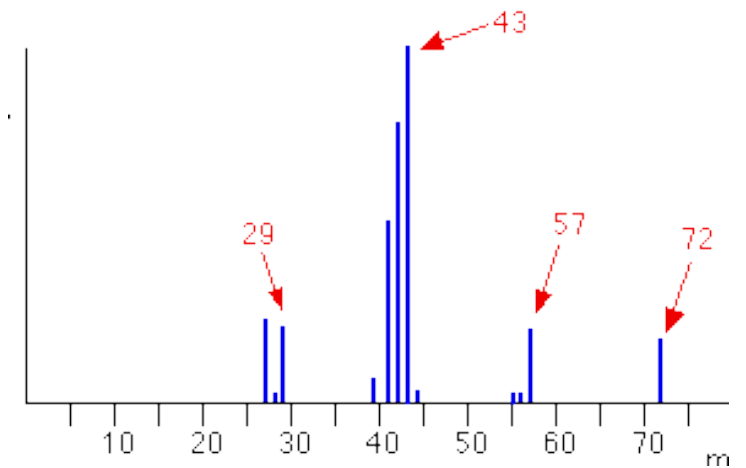
Non-target Methods

- **2nd Approach** is more complicated.

- All steps need validation

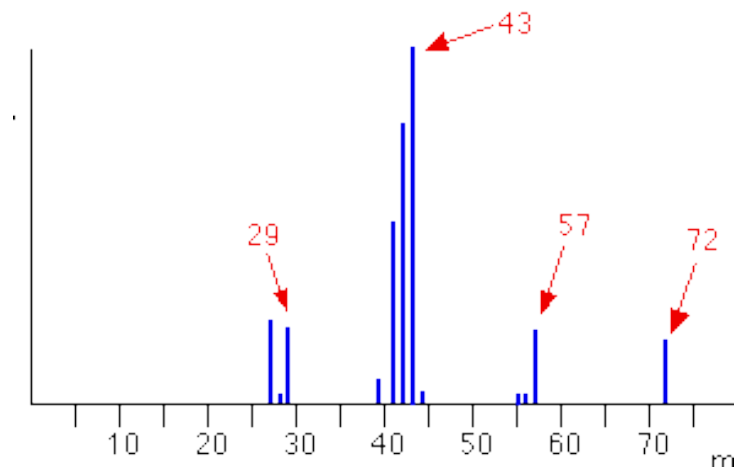


Validation
all the
procedure



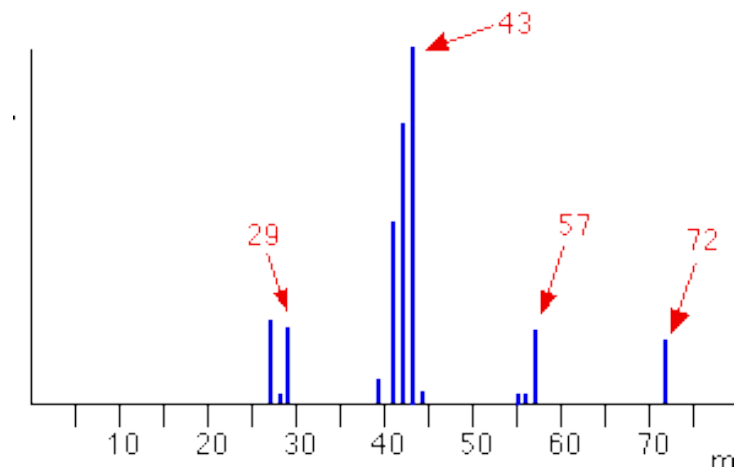
Non-target Methods (2nd approach) – issues to be addressed

- **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.



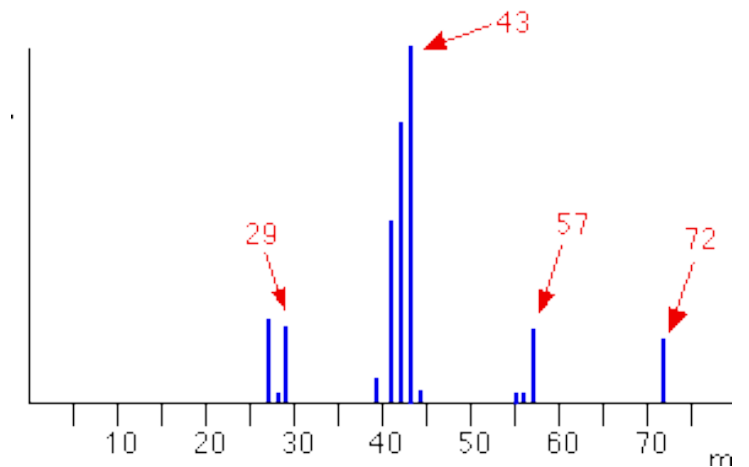
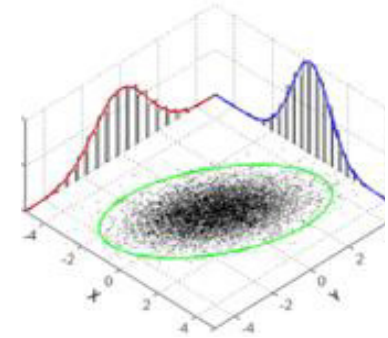
Non-target Methods (2nd approach) – issues to be addressed

- **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.



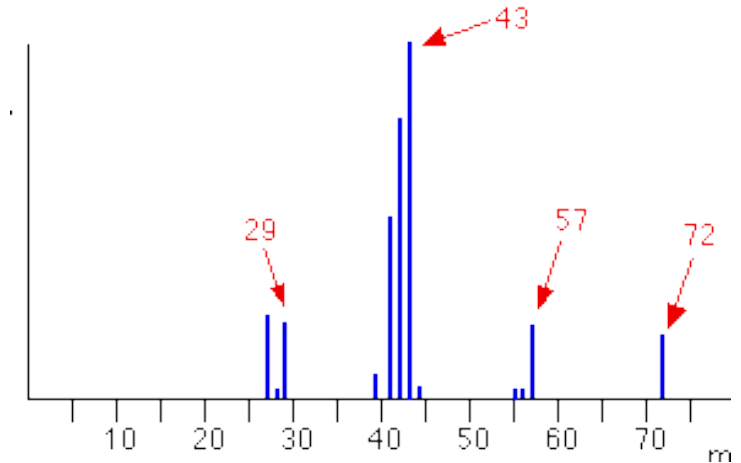
Non-target Methods (2nd approach) – issues to be addressed

- **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.



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?)

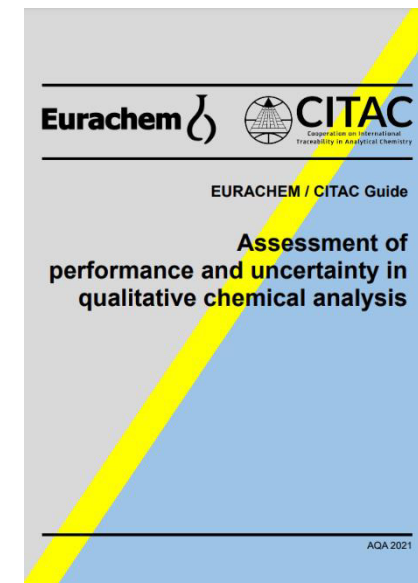
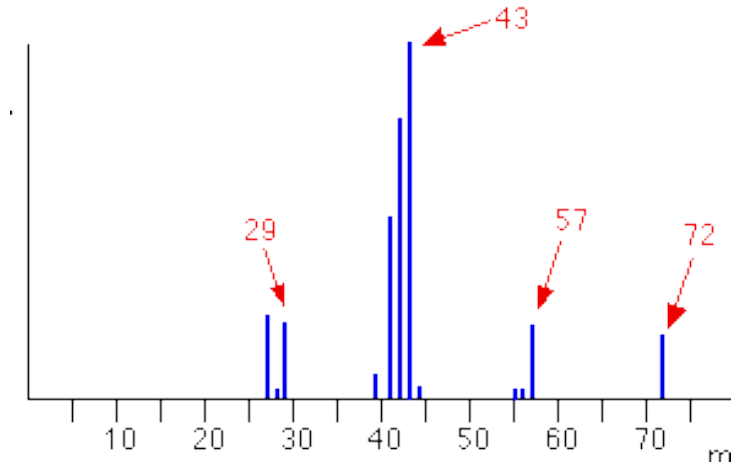


It's authentic!

Non-target Methods (2nd approach) – Perspectives

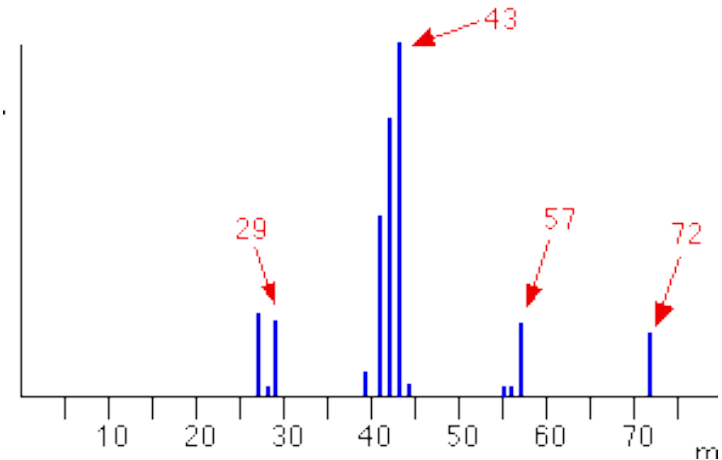


- Guidelines of FDA Economically Motivated Adulteration-*Dedicated to food fraud*.
- New Eurachem guide (2021) “Assessment of performance and uncertainty in qualitative chemical analysis” - *A start until a dedicated guideline is available*.



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”
- 📌 6th of June 2023 is the second webinar.



Joint Eurachem/AOAC-E webinar

Trends & challenges for Non-Targeted Methods

2. “NTMs – qualitative or quantitative?”

June 6th 2023, 13:00-17:00 CET

Thank you for your attention!
Questions?