Mass spectrometry and matrix effect, challenges with complex matrices

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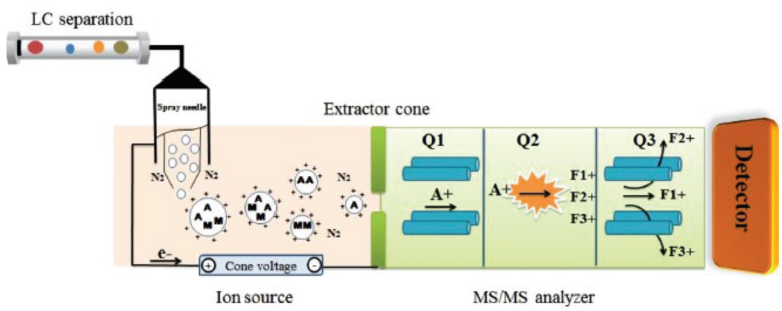
Mass spectrometry - Introduction

- Mass spectrometry has been the dominant analytical technique for the last 20 years.
- It enables the simultaneous detection of a large number of compounds, especially when combined with chromatography.
- Quantification of analytes at low concentration levels
- It is capable of detecting unknown compounds, especially for HRMS.
- It minimises the cost and time of analysis.It offers high selectivity and strong identification criteria, especially in tandem or high-resolution mass analysers.

Mass spectrometry - Introduction

- The signal depends on the structure of the compound.
- Matrix components affect the signal.
- The matrix effect is the main parameter in the development and validation of analytical methods.
- Atmospheric pressure ionization (API) sources are especially affected by the sample components, which are often called 'matrix effects'.



Graph from DOI: 10.1039/d1an01047f

Matrix Effect: Definitions

- What is the Matrix Effect?
- According to the IUPAC *"NOMENCLATURE FOR AUTOMATED AND MECHANISED ANALYSIS"*
- *The combined effect of all components of the sample other than the analyte on the measurement of the quantity*
- Shah et al
- *The direct or indirect alteration or interference in response due to the presence of unintended analytes or other interfering substances in the sample*
- **Is matrix Effect equivalent with interference?**

Matrix effect vs matrix interference

- IUPAC *"NOMENCLATURE FOR AUTOMATED AND MECHANISED ANALYSIS"*
- *"the effect of a specific component that is identified as causing an effect on the measurement"*

- The key aspect of a matrix effect or matrix interference is usually **bias**,
  - matrix interference if you **know** what is causing the bias.
  - matrix effect if you **don't know** what is causing the bias.
- Both terms are the affection to trueness, in validation parameters

Matrix Effect: Definitions

complicated Issue

- In mass spectrometry, it is also used to describe signal affection as:
- **Signal suppression or Signal enhancement.**
- Absolute vs. relative
- **Absolute:** The ratio of the peak areas of the sample matrix to those of a pure solution.
- **Relative:** Variability (RSD) in matrix effects across different lots or sources of the matrix (e.g. different donors or soil types).
- Recovery vs. process efficiency
- **Recovery (or total):** Ratio of peak areas of the sample matrix to the pure solution.
- **Process (or absolute):** Ratio of peak areas of the sample matrix before and after extraction

Matrix Effect-Equations

Terminology Equation References

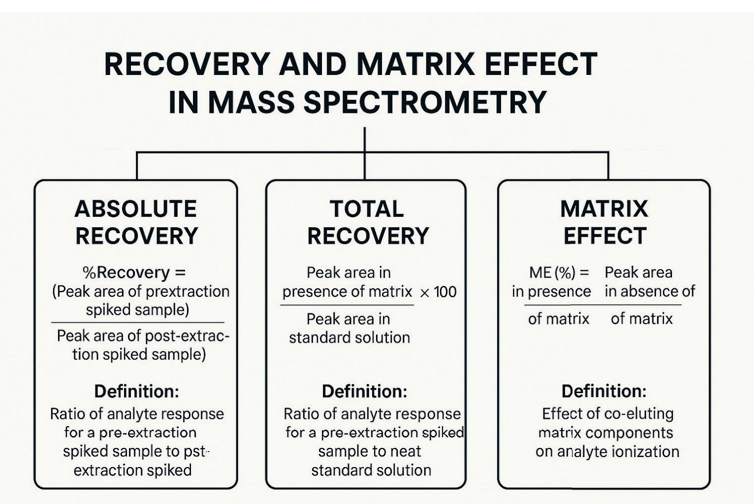
- Buhrman et al.
- Matuszewski et al.
- Chambers et al.
- Kwon et al.

- Also called in some cases Matrix Factor
- Be carefull the units!

Equations

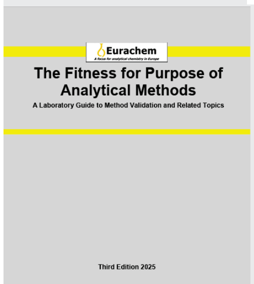
- Ion Suppression (%) 100-[(B/A) * 100] Limit:0
- ME (%)= (B/A) * 100 Limit:100
- ME (%) =[(B/A)-1] * 100 Limit:0
- ME (%)=[(B-A)/A] * 100 Limit:0

- A: Neat Solvent
- B: Sample Extract



Matrix effect

Performance characteristic
Selectivity
Limit of detection (LOD) and limit of quantification (LOQ)
Working range
Analytical sensitivity
Trueness • bias, recovery
Precision • repeatability, intermediate precision and reproducibility
Ruggedness (robustness)
Measurement uncertainty[a]
[a] Strictly, measurement uncertainty is not a performance characteristic of a particular measurement procedure but a property of the results obtained using that measurement procedure. Measurement uncertainty is a crucial part of every measurement result and reflects the effects of the performance characteristics. The calibration function is an integral part of a quantitative method and, as such, is not considered to be a performance characteristic. However, the calibration function is included in section 5 in this Guide, as it is seen as a crucial prerequisite for validation/verification of the various performance characteristics.



Matrix effect & BIAS

- Validation parameters:
- Bias is part of the evaluation of trueness.
- Recovery studies are an approach used to assess trueness.
- According to the literature, the use of the term 'recovery' is unclear as part of matrix effect evaluation.
- Bias is the sum of all effects of the method (positive or negative errors, relative or absolute to concentration levels).
- Recovery is used as an approach to estimate trueness.

Matrix Effect and Regulations

SANTE Guideline for Pesticides:

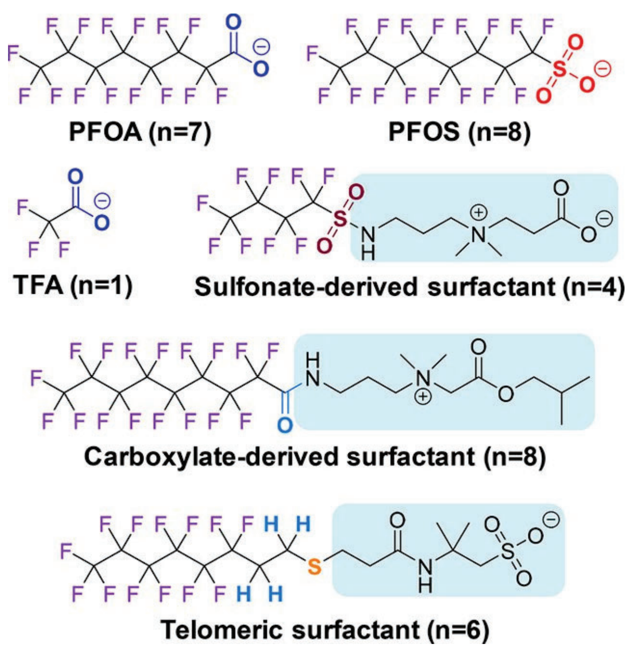
- in case of more than 20 % signal suppression or enhancement, matrix effects need to be addressed in calibration

808/2021/EC: residues of pharmacologically active substances used in food-producing animals

It is used in MS-based guidelines

- Absolute Recovery only if not using internal standard
- Relative Matrix Effect:
- the coefficient of variation (%RSD) shall not be greater than 20 % for the MF

Examples



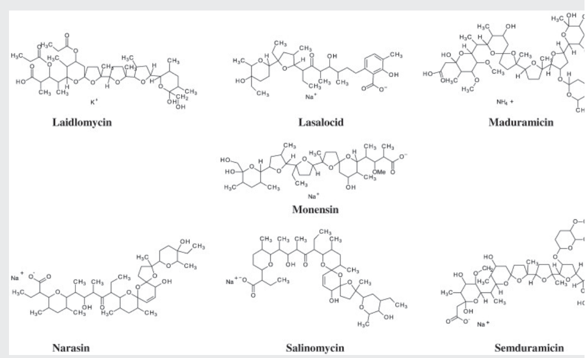
Examples: PFAS in Soils

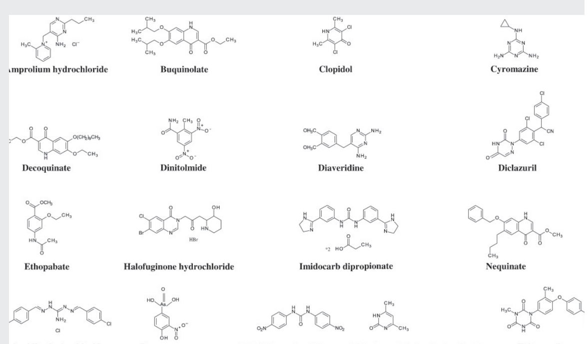
Example: Development of method for PFAS in Soils

		ACN: water: Formic acid	ACN: Formic acid	MeOH: NH_4	MeOH	MeOH: Formic acid
Analyte		**A**	**B**	**C**	**D**	**E**
PFOA	Absolute	26	18	30	31	20
	Matrix Effect	70	89	82	84	108
PFBS	Absolute	80	58	77	70	21
	Matrix Effect	84	90	92	96	168
PFOSA	Absolute	1.7	7.4	16	15	11
	Matrix Effect	86	106	74	98	112
PFHxPA	Absolute	61	0.29	6.9	53	17
	Matrix Effect	47	144	79	101	153
PFOS	Absolute	45	11	34	79	26
	Matrix Effect	104	98	79	112	127

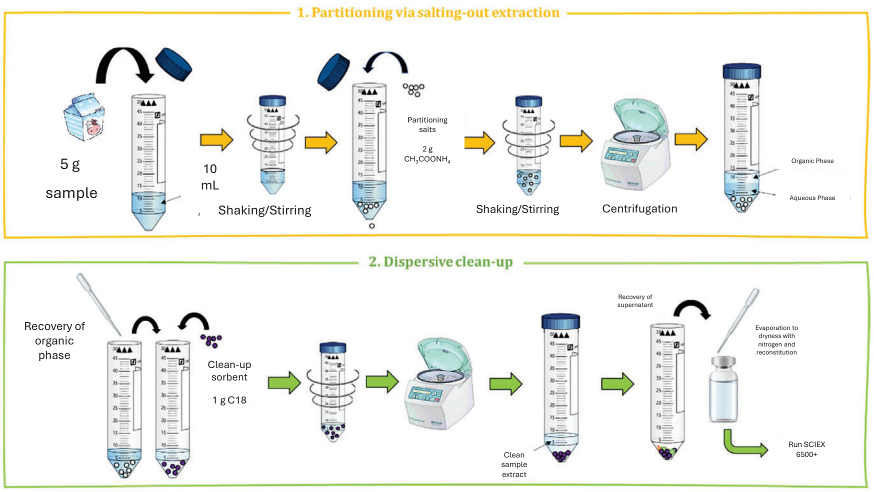
Example:Coccidiostats in Milk

- They used veterinary drugs as anticoccidials.
- Some are prohibited, while others have been approved for use.
- They have diverse structures and molecular weights.
- Many of them are used in chickens.
- Validation of method according to 808/2021 Implementing Regulation
- The relative matrix effect was calculated for three species of milk (bovine, ovine and goat)





Examples:Coccidiostats in Milk Method pretreatment (QuEChERS)



- QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe)
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Examples:Coccidiostats in Milk

N = 21	Cow Milk			Goat Milk			Sheep Milk		
Analyte	%ME	SD	%RSD	%ME	SD	%RSD	%ME	SD	%RSD
Amprolium	57,5	9,5	16,5	28,0	3,0	10,6	27,2	3,7	13,5
Arprinocid	10,9	1,1	10,5	7,29	0,87	11,9	6,8	1,1	16,5
Clopidol	3,24	0,25	7,8	3,89	0,30	7,8	2,88	0,42	14,6
Decoquinate	23,4	1,1	4,6	38,5	6,4	16,7	20,4	2,7	13,2
Diclazuril	10,0	1,0	10,1	2,46	0,40	16,1	0,689	0,085	12,4
Halofuginone	73,5	6,1	8,3	26,6	4,1	15,4	19,2	2,3	11,8
Lasalocid	31,4	3,1	9,8	122	22	18,5	77	14	18,4
Maduramycin	118,3	6,9	5,8	41,8	7,1	16,9	30,8	4,9	15,8
Monensin	29,3	2,9	10,0	76	14	17,9	50,4	7,5	14,8
Narasin	22,5	2,3	10,3	84,2	15,6	18,6	60	11	18,2
Nicarbazin	10,26	0,52	5,1	7,8	1,4	17,3	4,59	0,77	16,8
Robenidine	13,90	0,79	5,7	25,5	3,0	11,8	21,4	1,8	8,6
Salinomycin	23,8	2,7	11,2	84	14	17,1	69	11	16,1
Semduramycin	26,4	3,4	13,0	78,9	8,1	10,3	64,9	5,9	9,1
Toltrazuril	27,0	1,6	5,8	99	17	17,2	31	5,4	17,3

Conclusion

- The Matrix Effect is a parameter that should be applied to every MS method.
- The majority of validation guidelines do not mention ME, and of those that do, there is no consensus.
- On the other hand, all scientific papers are using ME as part of validation process.
- While it does not necessarily need to be eliminated, it must be appropriate for the method.
- It indicates the appropriate calibration curve.
- Caution is needed when choosing the correct equation and applying the appropriate limits.

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