Chemometrics in method validation – why?

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10th May 2016, Gent
Eurachem 2016
Early career scientist – Who am I?

PhD in analytical chemistry in 2013
‘New analytical strategies for the characterization of bioactive compounds’
Early career scientist – Who am I?

Since 2013
Research fellow in the JRC-IRMM

Geel
Outline

• Chemometrics in method validation-why?
• Briefly, what is chemometrics
• Where can we apply chemometrics, how?
• Applicability of chemometrics, some examples
• Conclusions
Chemometrics in method validation – why?

- Sample preparation
- Instrumental setup
- Data treatment

Method validation
- Accuracy
- Precision
- Specificity
- LOD/LOQ
- Linearity
- Etc...
**Where can we apply chemometrics?**

- **DoE for getting the best / optimum analyte disposition**
- **DoE for getting the best working conditions in our method & validate**
- **Multivariate data or image analysis**

**Sample preparation**

**Instrument method**

**Data treatment**

**HOW?**
What is DoE? And what advantages does it offer?

**Design of Experiments = DoE**

Screening designs – Full Factorial Design (FFD)
Information of the statistically significant parameters

Optimisation Design – Central Composite Design (CCD)
Optimum conditions of the system and the interactions among the parameters

**Maximum information - minimum Nº of experiments**

**Interaction between parameters**
Applicability of chemometrics, some examples

1. Characterisation of nanomaterials
2. Extraction of volatiles
3. Quantitative method development in olive oil
4. Deterpenation of cannabis
5. Authentication method for coccidiostats
6. Extraction & digestion of allergens in cookies

Chemometrics useful in many fields/matrixes
Example 1: characterisation of nanomaterial

Optimise the dispersion of TiO$_2$ into minimum dispersible units

Smallest particle size

Focused Ultrasound

Value of interest
Example 1: characterisation of nanomaterials

Optimise an AF4 method that can separate a polydisperse TiO$_2$ material

FFD - Pareto diagram with the significant variables

Variables to be studied in a CCD

Particle size 200nm = Value of interest

Fractogram of TiO$_2$ under optimised conditions
**Example 2: volatiles**

Develop & optimise a **quantitative method** for extracting aromas from plants by means of SFE or FUSE.

1) Screening - FFD
2) Optimisation - CCD

15 volatiles

Highest amount = Value of interest
Example 2: volatiles & antioxidants

Optimise a GCxGC-MS separation method that suits all volatiles

Highest intensity = Value of interest

GCxGC-MS microfluidic modulator

Example 3: aromas in olive oil

Optimise the measuring conditions of a Raman method

We look for the highest signal of the spectra
Without burning the sample
The shortest acquisition time with an acceptable signal/noise ratio

**Example 3: aromas in olive oil**

Develop a **quantitative Raman** method for volatiles in olive oil.

### Spectral windows of interest

<table>
<thead>
<tr>
<th>Wavenumber range</th>
<th>Spectral pre-processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>150-800 cm⁻¹</td>
<td>None</td>
</tr>
<tr>
<td>630-660 cm⁻¹</td>
<td>Normalize, Baseline, 1ˢᵗ derivative, SNV</td>
</tr>
</tbody>
</table>

**Combination of pre-processings**

- 630-660 cm⁻¹
- 150-800 cm⁻¹
Example 3: aromas in olive oil

Develop a quantitative Raman method for volatiles in olive oil

Wavenumber range

- $150-800\,\text{cm}^{-1}$
- $630-660\,\text{cm}^{-1}$

Spectral pre-processing

- None
- Normalize
- Baseline
- $1^{st}$ derivative
- SNV

Number of principal components

- RMSEP
- None: 3, 2
- Normalize: 3, 1
- Baseline: 3, 4
- Baseline + 1st derivative: 2, 1
- 1st derivative: 2, 1
- SNV: 1, 1
- SNV + 1st derivative: 1, 1

Combinations of pre-processings
Example 3: aromas in olive oil

Validate the model

Compare real sample concentrations between two techniques
Example 4: deterpenation of cannabis

Develop & optimise a deterpenation method for extracting aromas & cannabinoids from cannabis by means of SFE or FUSE

1) Screening to see feasibility - FFD
2) Optimisation to get quantitative conditions - CCD

2 fractions: aromas & cannabinoids

Highest amount = Value of interest
Example 4: deterpenation of cannabis

Deconvolute co-eluting sesquiterpenes and cannabinoids in GCxGC-MS

MCR-ALS to deconvolute the co-elutions by means of MS information
**Example 5: authentication of coccidiostats**

**AIM**

Develop a **model for authentication** of coccidiostats in NIR & MIR

Difficult to distinguish with the naked eye, model created by PCA and validated

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**Example 5: authentication of coccidiostats**

Develop a **model for authentication** of coccidiostats in NIR & MIR

Difficult to distinguish with the naked eye, model created by PCA and validated

NIR spectra of coccidiostats

- Monteban
- Maxiban
- Coxidin (CaCO₃)
- Elancoban
- Coxidin (wheat)
- Monimax

Scores

- Nicarbazin
- Narasin
- Monensin

Example 6: allergens in cookies

Develop & optimise an extraction + digestion method for MS based quantification of milk & egg allergens in food products.

Can one method suit all?

DoE 1
Extraction

DoE 2
Digestion

Method validation
(coming soon)

18 peptides to monitor compromise needed
Conclusions

-Time and money saving
-Interactions of parameters visible
-Applicability to many fields / matrixes
-Optimised methods will lead to better figures of merit

Give it a try!
Acknowledgements

University of the Basque Country
Joint Research Centre
Standards for Food Bioscience

Stay in touch

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