# How to address matrix mismatch bias in the uncertainty budget

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## Matrix bias

- In chemical analysis, differences in results are said to be caused by matrix bias when
  - the extraction of analyte is affected by the sample matrix, so that a part of the analyte is not recovered;
  - or when a part of the matrix is extracted along with the analyte and interacts with the measurement's physico-chemical mechanism (e.g. peak suppression, inhibitory effects ... )
- The term *matrix bias* will be used to denote a specific source of variation between results obtained from samples collected from the same material or type.

# One sample, many laboratories

- The basic design for multi-lab method validation studies according to ISO 5725-2 allows the estimation of two random effects: laboratory bias and repeatability errors
- According to this design, all tests are performed with the same test material and the same method.
- Since all laboratories work with the same method, the matrix bias occurring in all laboratories should be the same.
- In addition, due to variation of procedures and different instruments, matrix bias is not constant but varies from laboratory to laboratory.

| | Sample 1 |
|---|---|
| Lab 1 | 93% |
| Lab 2 | 81% |
| Lab 3 | 84% |
| Lab 4 | 92% |
| Lab 5 | 94% |
| Lab 6 | 95% |
| Lab 7 | 98% |
| Lab 8 | 100% |
| Mean of matrix bias | 92% |
| SD of matrix bias | 7% |



# One sample, many laboratories

Results of a multi-lab method validation study (Official Methods, Germany, 2009) for mycotoxins in oat:

| HT2 | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Average across samples | Standard solution |
|---|---|---|---|---|---|---|---|
| Mean [µg/kg] | 9,6 | 26,5 | 8,5 | 21,1 | 15,7 | | 507,6 |
| Relative reproducibility sd | 22,4% | 21,9% | 28,1% | 28,8% | 32,3% | 26,7% | 10,6% |
| Relative repeatability sd | 16,3% | 7,0% | 22,9% | 13,4% | 20,8% | 16,1% | 7,0% |
| Relative laboratory sd | 15,3% | 20,7% | 16,2% | 25,6% | 24,7% | 20,5% | 8,0% |
| Reproducibility sd / Horwitz = HORRAT | 1,02 | 0,99 | 1,28 | 1,31 | 1,47 | 1,21 | 0,60 |
| Repeatability sd / Horwitz | 0,74 | 0,32 | 1,04 | 0,61 | 0,95 | 0,73 | 0,40 |
| Laboratory sd / Horwitz | 0,70 | 0,94 | 0,74 | 1,16 | 1,12 | 0,93 | 0,45 |

Although the concentration differences between the standard solution and matrix samples are too large to compare the corresponding precision data, a comparison of the Horwitz-corrected values suggests that significant matrix effects may be present.

# Many samples, many laboratories

- If the basic design for multi-lab method validation studies according to ISO 5725-2 is performed for several samples/matrices, matrix bias for a specific laboratory is not constant but varies from sample to sample.

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Mean of lab bias across samples | SD of lab bias across samples |
|---|---|---|---|---|---|---|---|---|---|---|
| Lab 1 | 88% | 103% | 68% | 78% | 99% | 107% | 99% | 95% | 92% | 15% |
| Lab 2 | 96% | 93% | 67% | 90% | 94% | 104% | 89% | 93% | 91% | 12% |
| Lab 3 | 80% | 97% | 59% | 74% | 79% | 86% | 80% | 83% | 80% | 12% |
| Lab 4 | 74% | 88% | 56% | 78% | 84% | 87% | 76% | 79% | 78% | 12% |
| Lab 5 | 71% | 89% | 73% | 92% | 84% | 92% | 93% | 81% | 84% | 10% |
| Lab 6 | 88% | 97% | 68% | 81% | 84% | 98% | 77% | 91% | 86% | 11% |
| Lab 7 | 91% | 104% | 69% | 93% | 86% | 102% | 86% | 94% | 91% | 12% |
| Lab 8 | 80% | 82% | 62% | 76% | 82% | 97% | 90% | 83% | 82% | 12% |
| Mean of matrix bias across labs | 84% | 94% | 65% | 83% | 87% | 97% | 86% | 87% | | |
| SD of matrix bias across labs | 10% | 8% | 6% | 9% | 7% | 9% | 9% | 7% | | |



# Many samples, one laboratory

Experimental design for the calculation of the matrix SD within one laboratory

Select **randomly** n=12 **blank** samples (matrices) and spike all of them at a constant level.

Conduct measurements of the 12 samples in duplicate (better: triplicate) under repeatability conditions in **random** order.

Calculate variance between samples by means of ANOVA (or by REML)

## Many samples, one laboratory

- First, compute the overall mean value $\bar{x}$, and the sample-specific mean values $\bar{x}_i$. Then compute the between-sample sum of squares:
- $SSB = n \cdot \sum_{i=1}^{m} (\bar{x}_i - \bar{x})^2$
- and the within-sample sum of squares:
- $SSW = \sum_{i=1}^{m} \sum_{j=1}^{n} \left(x_{ij} - \bar{x}_i\right)^2$
- The repeatability standard deviation $s_r$ is then obtained as
- $s_r = \sqrt{\frac{SSW}{m \cdot (n-1)}}$
- and the between-sample standard deviation $s_M$ is obtained as
- $s_M = \sqrt{\frac{1}{n}\left(\frac{SSB}{m-1} - s_r^2\right)}$.



## Many samples, one laboratory

- Example: **Data from an in-house experiment for the evaluation of matrix bias (spike level = 100 µg/kg)**

| | Replicate 1 | Replicate 2 |
|---|---|---|
| Matrix 1 | 114.51 | 112.24 |
| Matrix 2 | 120.25 | 111.59 |
| Matrix 3 | 88.46 | 86.62 |
| Matrix 4 | 118.93 | 102.35 |
| Matrix 5 | 74.06 | 80.91 |
| Matrix 6 | 117.50 | 102.69 |
| Matrix 7 | 120.96 | 109.35 |
| Matrix 8 | 96.05 | 92.92 |
| Matrix 9 | 98.43 | 87.09 |
| Matrix 10 | 107.99 | 117.42 |
| Matrix 11 | 117.34 | 126.87 |
| Matrix 12 | 76.56 | 109.79 |

## Many samples, one laboratory

- Results of in-house experiment

| $s_r$ | $s_M$ | Recovery across samples |
|---|---|---|
| 9.53 | 12.24 | 103,8 % |

- Here, the sd of in-house matrix bias is larger than the in-house repeatability sd



## Matrix bias versus inhomogeneity

- It is important to distinguish matrix bias from sample inhomogeneity. Test design for sample inhomogeneity looks the same, but the samples are different
  - Test design for matrix bias:
    Identical analyte concentration levels but varying matrix very
  - Test design for sample inhomogeneity:
    Identical matrix but varying analyte concentration levels

# Discussion

- Matrix bias can be a major component of measurement uncertainty.
- Precision data according to ISO 5725 do include the standard deviation of the matrix bias across laboratories. They do not include the standard deviation of matrix bias across samples.
- The matrix standard deviation across samples can be obtained from an in-house study if the true concentration level of samples is known.
- Stratified sampling is often more efficient than random sampling. Procedures for stratified sampling (orthogonal design) have been implemented in the European Commission Decision CD 657/2002, see also Jülicher et al (1998) Analyst, 1998,123, 173-179.
- ISO DTS 23471 provides further experimental designs (draft to be published in 2020).



Many thanks for your attention!

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