Using unbalanced designs to reduce the cost of sampling uncertainty estimation

Overview

1. Example of using the **balanced** experimental design to estimate measurement uncertainty including uncertainty from sampling (example A1 from the Eurachem guide)

2. Reducing the cost of uncertainty estimation using the **unbalanced** design

3. Validation of **robust ANOVA** on the unbalanced design (theoretical)

4. Application of the unbalanced design to real data:
   1. Example A1 from Eurachem guide
   2. Example A2 from Eurachem guide

5. Conclusions
Nitrate concentration in lettuce – Example A1 from Eurachem guide

Nitrate is a potential risk to human health

- Toxicity/benefits unclear
  - Toxicity:
    - ‘blue baby’ syndrome (1981)
    - stomach cancer (1963)
    *Both disputed*
  - Beneficial effects
    - May have antimicrobial effect on gut pathogens (speculative)

- EU threshold 4000 mg kg\(^{-1}\) (summer) - 5000 mg kg\(^{-1}\) (winter)\(^1\)

Scope (from guide): Estimate the measurement uncertainty, and contributions from sampling and analysis, for routine monitoring of glasshouse grown lettuce, using a standard sampling protocol


Nitrate concentration in lettuce – Example A1 from Eurachem guide

- **Sampling target** = 1 bay of lettuce (up to 20,000 heads)
- **Sampling protocol** specifies taking 10 heads to make a single composite sample from each batch *(in ‘W’ or ‘star’ design)*

‘W’ Sampling Design for Lettuce

Duplicate is equally likely interpretation of ‘W’ design
Nitrate concentration in lettuce – Example A1 from Eurachem guide

Measurement uncertainty was estimated using the balanced design.

8 bays selected for duplicate sampling

Recommended sampling duplicates @ 10% of the sampling locations in the whole survey
- Minimum 8
Example 1 - Example A1 from the Eurachem guide – Nitrate in glasshouse grown lettuce

Estimating uncertainty from the sampling/analytical duplicates: **Classical** ANOVA

Visual inspection suggests outlying sampling variance at target C
Use of **Robust** ANOVA down-weights the effect of outlying variances on U estimates
Example 1 - Example A1 from the Eurachem guide – Nitrate in glasshouse grown lettuce

Estimating uncertainty from the sampling/analytical duplicates: **Robust ANOVA**

<table>
<thead>
<tr>
<th>Sample target</th>
<th>S1A1</th>
<th>S1A2</th>
<th>S2A1</th>
<th>S2A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3898</td>
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<tr>
<td>B</td>
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<td>4126</td>
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<tr>
<td>C</td>
<td>5708</td>
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<td>3782</td>
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<td>D</td>
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<td>H</td>
<td>3966</td>
<td>4283</td>
<td>4131</td>
<td>3788</td>
</tr>
</tbody>
</table>

Robust uncertainty more representative of main body of data

NO$_3$ mg kg$^{-1}$

Robust ANOVA

- Robust ANOVA recommended when measurement data includes outlying values (<10%)$^3$

- In practice: Often a small proportion (i.e. <10%) of outlying values exist in the frequency distributions of the analytical, within-sample and between-sample variability$^1$

- Robust ANOVA gives more reliable estimate of the variances of the underlying populations (See example in Appendix A1 of the Eurachem UFS guide$^1$)

- Computer intensive, iterative process

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$^3$Computer intensive, iterative process
Robust ANOVA

- **RANOVA2** - Free download from the AMC Software page on the Analytical Methods Committee section of the website of the Royal Society of Chemistry [https://www.rsc.org/Membership/Networking/InterestGroups/Analytical/AMC/Software](https://www.rsc.org/Membership/Networking/InterestGroups/Analytical/AMC/Software)

- Downloaded as ZIP file, includes help text and examples

- Extract all files to new folder

- Specify location of Ranova2Help.CHM file before the help system can be used. Press “Activate Help” and follow instructions

- See the included installation notes for more information

**Example 1 - Example A1 from the Eurachem guide – Nitrate in glasshouse grown lettuce**

\[ U_{\text{meas}} = 16\% \] from Robust ANOVA

- 1 possible false positive (sampling target D)

- 3 possible false negatives (sampling targets C, E, F)

**Robust ANOVA** enables better decisions on probabilistic classification
Analysing data from the duplicate method

- Balanced experimental design provides empirical estimates of measurement uncertainty, including uncertainty from sampling
- Requires 3 additional measurements (e.g. chemical analyses) at 10% or a minimum of eight sampling targets

Reducing the cost of uncertainty estimation

- Balanced experimental design provides empirical estimates of measurement uncertainty, including uncertainty from sampling
- Requires 3 additional measurements (e.g. chemical analyses) at 10% or a minimum of eight sampling targets

- Alternative unbalanced experimental design provides similar uncertainty estimates but with only 2 additional analyses at each sampling target
- Lower cost alternative
Unbalanced experimental design

- 2 samples required as per balanced design
- 2 analyses are performed on one sample, one analysis on the other sample

  **Reduces analysis cost of U estimation by 33%**
  - Sometimes cost is a reason not to estimate U

  **Equalizes number of duplicates** sampling/analysis.
  - In balanced design there are twice as many analytical duplicates, even though sampling uncertainty is often dominant
  - Unbalanced design treats sampling and analysis with equal importance

  **RANOVA2** now includes Robust ANOVA on the unbalanced design

Method validation of robust ANOVA on the unbalanced experimental design

- Validation by computer simulation
- 1000 simulated normal datasets (balanced design) were generated for each of 3 seed (‘True’) values

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  - 11 different outlier scenarios were applied to each of these

- Each resultant dataset was analysed as a balanced design (S1A1, S1A2, S2A1, S2A2) AND an unbalanced design (S1A1, S1A2, S2A1)

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- Average percentage differences between results of unbalanced ANOVA and balanced ANOVA are shown in the table

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- Average percentage differences between results of unbalanced ANOVA and balanced ANOVA are shown in the table
- Median/Mean differences calculated

Method validation of robust ANOVA on the unbalanced experimental design

- Majority of differences are small (<2%)
- Maximum percentage differences (-6.1%) were found for the sampling standard deviation with high analytical outliers on Seed 2

<table>
<thead>
<tr>
<th>t-test parameter (order in Table 2)</th>
<th>Outlier type</th>
<th>Percent Difference (&gt; ANOVA/ANova)</th>
<th>Robust ANOVA (%)</th>
<th>Methodology (%)</th>
<th>Reference (%)</th>
<th>Robust ANOVA (%)</th>
<th>Methodology (%)</th>
<th>Reference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Difference (%)</td>
<td></td>
<td>Total 10 %</td>
<td>Reference (%)</td>
<td>Sampling (%)</td>
<td>Analytical (%)</td>
<td>Total 10 %</td>
<td>Reference (%)</td>
<td>Sampling (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>0.3</td>
</tr>
</tbody>
</table>

Method validation of robust ANOVA on the unbalanced experimental design

- Majority of differences are small (<2%)
- Maximum percentage differences (-6.1%) were found for the sampling standard deviation with high analytical outliers on Seed 2
- Mean and median of the average percentage differences from each scenario are all < 1%

### Table 1: Percent Difference (%) of Analytical Values to Reference Values

<table>
<thead>
<tr>
<th>Seed</th>
<th>Analytical</th>
<th>Reference</th>
<th>Seed 1</th>
<th>Analytical</th>
<th>Reference</th>
<th>Seed 2</th>
<th>Analytical</th>
<th>Reference</th>
<th>Seed 3</th>
<th>Analytical</th>
<th>Reference</th>
<th>Seed 4</th>
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<tbody>
<tr>
<td>Mean</td>
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<td>0.5</td>
<td>0.5</td>
<td></td>
<td></td>
<td>0.5</td>
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<td>0.5</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Example A1 from the Eurachem guide – UNBALANCED design

- Can ‘simulate’ unbalanced design by removing single columns from the balanced design data
- 4 different unbalanced designs possible
- Compare outputs with balanced design

<table>
<thead>
<tr>
<th>Sample target</th>
<th>Design 1</th>
<th>Design 2</th>
<th>Design 3</th>
<th>Design 4</th>
<th>Average</th>
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<tbody>
<tr>
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<td>4139</td>
<td>4693</td>
<td>3898</td>
<td>4139</td>
</tr>
</tbody>
</table>

Comparison of $U'$ estimates - unbalanced design scenarios vs balanced design.

- In this case, using the unbalanced design saves £320 (33%) on total analysis cost
- Sampling cost remains the same
Example A2 from the Eurachem guide – Lead in topsoil

- Potential housing development site in former landfill (West London) - 9 hectare
- 100 sampling targets in regular grid with soil auger to depth 0.15m
- 10 (10%) designated as duplicate sampling targets

- Same procedure as before applied to evaluate unbalanced design
  - 4 different unbalanced designs analysed from the data
  - Estimated uncertainties compared with balanced design

<table>
<thead>
<tr>
<th>Balanced Design</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Average Unbalanced</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U_{\text{samp}}'$ (%)</td>
<td>83.3</td>
<td>83.3</td>
<td>88.6</td>
<td>77.6</td>
<td>80.2</td>
</tr>
<tr>
<td>$U_{\text{ana}}'$ (%)</td>
<td>7.5</td>
<td>8.5</td>
<td>8.9</td>
<td>6.9</td>
<td>6.9</td>
</tr>
<tr>
<td>$U_{\text{meas}}'$ (%)</td>
<td>82.6</td>
<td>83.8</td>
<td>89.0</td>
<td>77.9</td>
<td>80.5</td>
</tr>
</tbody>
</table>

$U_{\text{samp}}'$ dominant factor. Maximum difference unbalanced/balanced: 7%

Maximum difference in $U_{\text{meas}}'$ unbalanced/balanced also 7%

- Log-normal distribution: Use of Uncertainty factor $^fU$ better representation of positive skew in this case, $^fU = (x/\sigma) 1.3$

- RANOVA2 also calculates Uncertainty Factor $^fU$ for the unbalanced design
Conclusions

• Unbalanced design reduces analysis cost of empirical estimation of sampling and analytical uncertainties by 33%

• Robust ANOVA – more reliable estimates of variances of underlying population when outlying variances

• Method validation by computer simulation shows average robust estimates of uncertainty are not significantly different between balanced and unbalanced designs

• In practice differences may occur depending on the magnitude and distribution of outliers

• Savings made using unbalanced design could be used to obtain more sampling duplicates – Further work needed

Acknowledgements

Financial Support from The Analytical Methods Trust, RSC Analytical Methods Committee
Robust ANOVA

- Robust ANOVA recommended when measurement data includes outlying values (<10%)\(^1\)

- Computer intensive iterative process. To calculate robust mean:
  - Initially estimates:
    - Robust mean \( \mu_r \) = classical mean
    - Robust standard deviation \( \sigma_r \) = median absolute differences
  - Values exceeding \( \mu_r + c \sigma_r \) replaced by \( \mu_r + c \sigma_r \)
  - Values less than \( \mu_r - c \sigma_r \) replaced by \( \mu_r - c \sigma_r \)
    - \( c \) typically set to 1.5

- \( \mu_r \) and \( \sigma_r \) recalculated, and process repeated until \( \mu_r \) converges to an acceptable level of accuracy