ReadMe_SingleAnalyteMU.pdf:-

Outline documentation for MS Excel and Apple Notes Spreadsheets

Purpose

The revised ISO/IEC 17025:2017 standard requires Measurement Uncertainty (MU) of all chemical test results to be available. The Australian National Association of Testing Authorities (NATA) requirements for this standard are described in the NATA General Accreditation Guidance documents {1}.
The spreadsheet may be useful to assist Laboratory Analysts and Assessors to ensure compliance.

Background

Two approaches to establishing a new test method are to use MU “bottom up” evaluations, which are often tedious and difficult to evaluate, or a “top down” approach for a quicker determination of repeatability, reproducibility and trueness of a test, where: “accuracy = trueness (estimates of bias) + precision (estimates of random variability)”.

Volumetric/mass uncertainties can be much smaller that those from sample digestion/extraction/clean-up, or those from even instrument variability. In these cases the bottom up approach is often not suitable - The guidance "simpler is often better" may explain why techniques such as QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) can produce excellent results with a wide range of organic analytes; or ICP-MS with quite "dirty" inorganic sample digests may be better than complex multi-step clean ups.

Suitable reference materials and proficiency tests are not always available to establish methods for new analyte/matrix combinations. An established "top down" technique is to spike “blank” matrices with analytes, and use recovery and statistical information to establish fitness of purpose. A useful indicator is the Horwitz Ratio (HorRat) {2} - The ratio of observed relative standard deviation calculated from actual performance data, to a prediction calculated from the Horwitz equation at a particular concentration. The ratio can be used to define "Recommended", "Acceptable (but requires an explanation)" and "Not Acceptable" ratios {4}. Checking for anomalous test "Outliers" may improve Method Detection Limits. This spreadsheet uses Grubbs’ Test {3} for each set. Techniques such as mass spectrometry with isotopic spiking can produce better "low level" results than expected, these are highlighted for checking.

The spreadsheet has been designed to produce formal "archive" or "customer" MU reports.

The author has used these techniques in spreadsheets over a number of years to evaluate many methods. After development and combination of these basic tools I hope that they may be useful to others.

General

The system uses only "cell formula" and has no "macros", "program code", or external links (e.g. to webpages or email). **It should be "safe" to distribute**. The sheets are [MainTable], [SampleResults], [Distributions] and [Units], the last two are "look-ups". The last sheet [Notes] is a quick users' guide.

There are two versions:-
1: Apple "Numbers" - Can be used with an iPad; a large iPhone (Or a smaller one, connected via the Apple HDMI adapter to an external monitor/TV or the Apple TV and/or a Bluetooth keyboard); an Apple Mac Computer; or, via an iCloud account on any Windows PC with a suitable internet browser. Current versions of the Apple spreadsheet program do not support "cell-level" locking - Some care is needed to avoid deleting data if a field is accidentally selected, and so "locked shapes" are positioned over them.

2: Microsoft Excel - Can be run on any modern PC, or in the Cloud with Office 365. Excel supports cell-level locking, and the spreadsheet's relevant cells are protected . The password to unlock the whole spreadsheet is "**timstrutt**" (no "quotes").

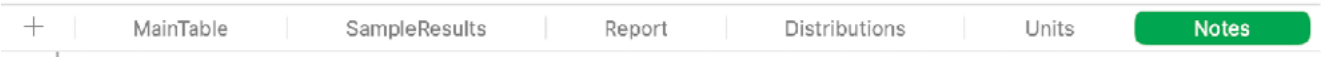
For speed and simplicity, much of the work in creating the original spreadsheet was done using Apple Notes version (Versions 6 to 13 - Often on an iPad). These were then ported to the Excel version and required a small amount of further debugging. The main exception was that cell "Conditional Formatting" colours and backgrounds often varied considerably.

Descriptions and the Use of Individual Sheets Follows Below...

MultiAnalytesMU Spreadsheet - [Notes]

This is the last sheet. It gives an overview of the spreadsheets, and can be used as a reference.

Apple Numbers Spreadsheet Tab



This spreadsheet may be useful to assist Laboratory Analysts and Assessors ensure compliance with ISO/ IEC 17025:2017. The revised standard requires Measurement Uncertainty (MU) of chemical test results to be available. It is designed to help produce necessary information by using sample blanks, spikes, and comparisons with reference materials. Sample "Low Spikes" could be used to determine Recoveries and Method Detection Limits. Spikes establish MUs at different concentrations with different matrices. It may be helpful when developing new methods or for "novel" analytes and matrices. An authorised report for a single analyte can be printed.

Created with Apple "Numbers" Version 13, and Microsoft "Excel" 2013, spreadsheet programs - Using only "Cell Functions". No "Code", Macros, or Hyperlinks are used - **It should be "Safe" to distribute…**

The spreadsheet uses some **conventions**: In [MainTable] cells where the user is expected to input data have blue text : labels, **bold blue** indicates that a selection is made from a list. Default look-ups are **grey**. Checks have [**pale backgrounds**]; Warnings have [**darker backgrounds**]; and Failure/Out-of-range warnings have [**red backgrounds**]; Replaced MDLs or Significances have [blue backgrounds] **Important** data is purple.

Getting Started: The sheets are [MainTable], [SampleResults], [Report], [Distributions] and [Units], and **[Notes]**. The last three are "look-ups" or information. **Data should be added or changed as outlined below…**

[MainTable]: Used to set the parameters of the Test:-
Fill in the Instrument/Test name that was used to create the results.
Fill in the Analyte Name for your set of results
Select Report Type from the drop-down - This will produce different a different [Report] for each type.
Select Units from the drop-down - This will change the Horwitz Ratio (larger concentrations should give higher precision).
Enter 'Spike Value' - Values between 0.00001 and 10,000,000.
MDL (From Mean of Previous Low Spikes) - If a value is entered, it will be used instead of the Results calculation.
Select Number of Significant figures - Usually **2** - 1 may be useful if near Quantitation limits, 3 for special precision.
Select One or two tail Grubbs' Test (α: 0.05) - Normally **1**; **2** is not recommended for <9 **approved** Results.
1 calculates the right and left tails separately, the furthest from the mean is used for the "one outlier" hypothesis.
Now go to the [SampleResults] Sheet…

[SampleResults]: Used to add SampleID and Results. There are 2 columns:-
Used to Add/Edit **SampleID** (or Paste from another spreadsheet).
Used to Add/Edit **Results** (or Paste from another spreadsheet).
Possible **Outliers** can be indicated by adding text e.g. Changing 77 to ?77 or 77!
This will cause the possible Outlier to be ignored by the Horwitz and Grubbs calculations.
The sheet can have many rows, but normally 3-11 are used (5, or more recommended).

[Report]: Used to display or print a formal report:-
The Report Date, Laboratory Name, Address, Contact, and Authorised data can be edited.
Check that the **Bias** and **Horwitz Ratio** are satisfactory - An Outlier can increase these values…

[Distributions]: Used to calculate Method Detection Limit (t-test) and Grubbs' critical value:-
This is not editable. It is recommended that the user consults the NIST and RSC papers, referenced in the text at the bottom of the sheet, for further background information.

[Units]: Used to calculate scale of Horwitz values, and in **[Report]** and **[Calculations - Main Table]**:-
It is normally Locked, but any changes must be reflected in **[MU Calculations]** "Select Units" drop-down.

For further information, please consult these References:-
Planning and Reporting Method Validation Studies; Supplement to EURACHEM Guide on the Fitness for Purpose of Analytical Methods, First edition 2019
Blanks in Method Validation, Supplement to EURACHEM Guide The Fitness for Purpose of Analytical Methods, First Edition 2019

The **password** to unlock individual MS Excel sheets and cells is "timstrutt" (no "quotes"). Apple Sheets use Locked "Shapes".

The **[MainTable]** uses "hidden" rows with a *red font* - Changing or deleting them will cause breakages...

Warning: The calculations are based upon standard literature methods, but some approximations have been used. See the references described above for background information.

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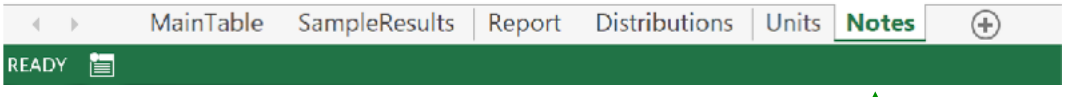

Microsoft Excel Spreadsheet Tab

MultiAnalytesMU Spreadsheet - [MainTable]

This is the first sheet. It shows calculations for the selected Report Type. Ideally, a white background for the Horwitz Ratio field will be seen. Red backgrounds are indications that the spike level is much higher/ lower than the result data, or the result is much higher/lower than expected.

Calculations can be archived by printing, creating a PDF using the software's "print" facility and saving the resulting file in a suitable folder, or archiving the entire (renamed?) spreadsheet in its own folder.

Results Data in [Sample Results] for Test Name :	LC-MS
Analyte :	Acrylamide
Select Report Type (Spike, Ref…) :	**Low Spike**
Select Units (ppm, µg/kg, g/L…) :	**µg/kg**
Enter Expected Analyte Concentration For Analytes :	90
MDL - Optional (From Mean of Previous Low Spikes) :	
Select # of Significant figures :	**2**
Select One or Two tail Grubbs' Test (α: 0.05) :	**1**
Total Number of Results	9
ST DEV σ of Results	10.032
RSD% (CV)	12.5%
bias as %age (100 - Recovery)	10.6%
MDL Factor (n-1 Degrees Freedom)	2.896
Units - 1 Part in This Value	1.00e+09
Predicted Horwitz "Expected Value" as RSD%	22.00
Horwitz Ratio (Ideally 0.3≤ HorRat ≤1)	0.57
Ymax (Maximum Value)	89.84
Ymin (Minimum Value) may be a Grubbs' Outlier	58.97
Result to 2 Significant figures	**80**
MDL = (MDL Factor x SD)	32
PQL (Practical Quantitation limit)	97
% Recovery	**89%**
Corrected for Recovery Check [Report] Sheet >>	**90 ± 32 µg/kg**

For higher level analyses this is normally filled in with the actual MDL obtained from this experiment.

HorRat looks OK... but Grubbs' test indicates an outlier in SampleResults.

If the outlier in [SampleResults] is changed to the non-numeric ?58.97 it has a significant effect:-

Results Data in [Sample Results] for Test Name :	LC-MS
Analyte :	Acrylamide
Select Report Type (Spike, Ref…) :	**Low Spike**
Select Units (ppm, µg/kg, g/L…) :	**µg/kg**
Enter Expected Analyte Concentration For Analytes :	90
MDL - Optional (From Mean of Previous Low Spikes) :	
Select # of Significant figures :	**2**
Select One or Two tail Grubbs' Test (α: 0.05) :	**1**
Total Number of Results	9 (1 Excluded)
ST DEV σ of Results	6.377
RSD% (CV)	7.7%
bias as %age (100 - Recovery)	7.6%
MDL Factor (n-1 Degrees Freedom)	2.998
Units - 1 Part in This Value	1.00e+09
Predicted Horwitz "Expected Value" as RSD%	22.00
Horwitz Ratio (Ideally 0.3≤ HorRat ≤1)	0.35
Ymax (Maximum Value)	89.84
Ymin (Minimum Value)	72.11
Result to 2 Significant figures	**83**
MDL = (MDL Factor x SD)	21
PQL (Practical Quantitation limit)	62
% Recovery	**92%**
Corrected for Recovery Check [Report] Sheet >>	**90 ± 21 µg/kg**

Removing the outlier improves the MU...

This indicates that the hypothesis that "58.97" is an outlier is reasonable. The HorRat has been improved and there appears to be no additional outliers.

The revised MDL and PLQ values can probably be used, but consider using 7 "Spikes" at an even lower level - Say, at 50 ng/L, and at least 7 "Blanks" (See: USEPA 821-R-16_006).

MultiAnalytesMU Spreadsheet - [SampleResults]

This is the second sheet. This is where the Results used by the [MainTable] sheet are entered.

Results can be copied from other spreadsheets or databases into this sheet, but care must be taken not to over-write cell formatting - It is recommended that only "clean", unformatted data is pasted. Additional rows can be added by choosing an empty row towards the bottom and adding additional rows beneath it. Excel can also access data from linked external sheets - Users can unlock this table to implement this.

S1	86.38
S2	87.79
S3	77.94
S4	72.11
S5 Duplicate S3	78.56
S6	89.84
S7	89.30
S8	83.42
S9 (Outlier?)	58.97

HorRat looks OK…, but Grubbs' Test indicates that this may be a low outlier - Adding alpha characters will exclude it : ?58.97

An extreme outlier will show a red Horwitz Ratio background in the [MainTable]:-

Horwitz Ratio (Ideally 0.3≤ HorRat ≤1)	6.53
Ymax (Maximum Value) may be a Grubbs' Outlier	770
Ymin (Minimum Value)	72.11

Typographical Error - Result should be 77.0

S1	86.38
S2	87.79
S3	77.94
S4	72.11
S5 Duplicate S3	78.56
S6	89.84
S7	89.30
S8	83.42
S9 (Outlier?)	58.97
S10 (Typographic Error Outlier)	770

Results with a small standard deviation show a pale Horwitz Ratio background in the [MainTable]:-

Horwitz Ratio (Ideally 0.3≤ HorRat ≤1)	0.19
Ymax (Maximum Value)	99.21
Ymin (Minimum Value)	85.37

HorRat < 0.3 - Requires checking - This may well be expected from well established/highly automated test.

S1	94.21
S2	91.3
S3	91.88
S4	95.31
S5 Duplicate S3	94.69
S6	85.37
S7	99.21
S8	96.33
S9	92.02

It is possible to copy/paste data from the spreadsheet into other files and databases. The default "copy" format is Tab Separated Variable (TSV), which is compatible with most other systems.

MultiAnalytesMU Spreadsheet - [Report]

This is the third sheet, and is used with the [MainTable] and [SampleResults] sheets. It can be edited:-

Summary Report - Low Spike		
Report Date		25 April 2022
Laboratory Name		Tim's Testing & Consulting Laboratory Pty Ltd
Address		15a Bridge Street Some-Town, STATE, X100
Contact		Tim Strutt tim@timstrutt.com
Authorised By		Saga Brondam
Analyte		Acrylamide
Number of Samples in This Report		8
Report Type		Low Spike
Low Spike Level		90 µg/kg
% Recovery		92%
RSD% (CV)		0.1
Bias as a %age		0.1
Is Bias Satisfactory? (Y/N)	:	Yes
Horwitz Ratio (Ideally 0.3≤ HorRat ≤1)		0.35
Is Horwitz Ratio Satisfactory? (Y/N)	:	Yes
Result to 2 Significant figures		83 µg/kg
Method Detection Limit (MDL)		21 µg/kg
Practical Quantitation Limit (PQL)		62 µg/kg
Result Corrected for Recovery Bias		**90 ± 21 µg/kg**

The Laboratory Name, Address, Contact details ,and Authoriser fields can be edited appropriately.
If the Horwitz Ratio and Bias Pop-Ups are not set to "Yes", red warnings will print.

This [Report] can be archived by printing, creating a PDF using the software's "print" facility and saving the resulting file in a suitable folder, or archiving the entire (renamed?) spreadsheet in its own folder.

MultiAnalytesMU Spreadsheet - [Distributions]

This is the fourth sheet, and is used by the [MainTable] sheet look up Horwitz Ratio and Grubbs' test values. It should not be edited.

MultiAnalytesMU Spreadsheet - [Units]

This is the fifth sheet, and is used by the [MainTable] sheet. It contains concentration units covering the range from ~10% to parts per-trillion (ppt). If required additional units may be added; but as noted in the sheet, the "Select Units (ppm, µg/kg, g/L..)" selection list in the [MainTable] must be updated to match.

References & Small Print

{1} NATA, Specific Accreditation Criteria ISO/IEC 17025 Application Document Life Sciences - Appendix, Effective: February 2021

and, Neil Shepherd, NATA WA Meeting May 2018, and NATA Webinars November and December 2017

{2} Anal. Chem. 1982, 54, 1, 67–76 Publication Date:January 1, 1982 https://doi.org/10.1021/ac00238a002

and, Albert R, Horwitz W (1997) A Heuristic derivation of the horwitz curve. Anal Chem 69:789–790

and, M Thompson AMC Technical Brief No.17 July 2004 - Royal Society of Chemistry 2004

and, M Haustein (on behalf of DAPA), FAO & WHO, Specifications for Pesticides CIPAC Symposium Athens, 2015

{3} Frank E. Grubbs. "Sample Criteria for Testing Outlying Observations." Ann. Math. Statist. 21 (1) 27 - 58, March, 1950. https://doi.org/10.1214/aoms/1177729885