Bio-analytical report generator – user guide

Alpha version (25/05/2022)

Step 1: Set up required software and files

Microsoft Excel

- Use excel template file: "**Input-Template_bio-analytical-report-generator_VersionAlpha.xlsx**", can be found in the Github repository (https://github.com/PJDeSutter/Method-Validation)

R studio

- Install **R studio** at: https://rstudio.com/products/rstudio/download/ (R studio desktop, free version is sufficient)
- R calibration **script** **"Rmarkdown-script_Bio-analytical-report-generator_VersionAlpha.Rmd"** , code can be found in the Github repository (https://github.com/PJDeSutter/Method-Validation).
- Required **packages** : tidyverse, readxl, kableExtra, gridExtra, broom, car, plotly (follow notification at user interface when script is opened to install these)

Step 2: Data entry in **Excell**

General

- Use the **template** "**Input-Template_bio-analytical-report-generator_VersionAlpha.xlsx**"
  - Open template and save under new name (with .xlsx filetype)
- Only the **first worksheet** will be analyzed by the R program
  - Enter data in the first worksheet
  - The other worksheets are informative
- When terms are within **" ",** exact spelling is required
- **<Free input>**: can be anything but do no use :
  - spaces between words (use "_ " or "-" instead) or special signs (e.g. µ, /)
  - In practice: only use Latin alphabet, Arabic numerals and _ or – when naming samples or (sub) levels)
- Do not change the **column names**!

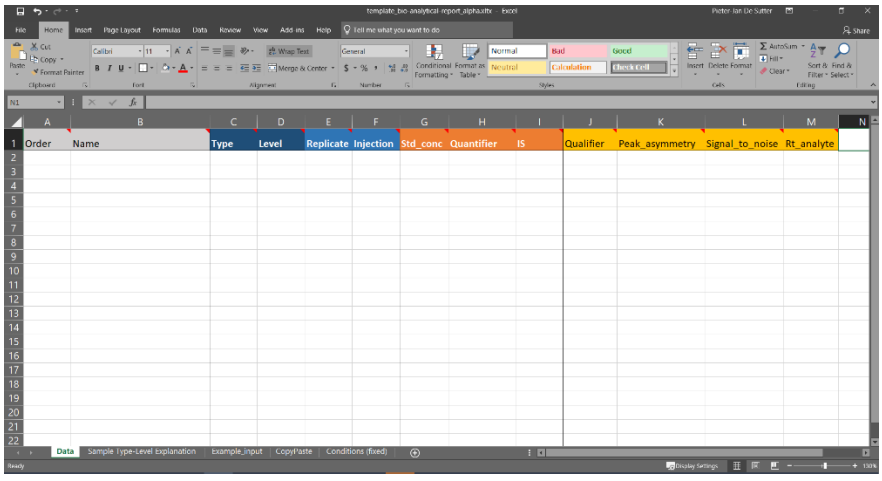


Figure 1 Excell input template

Required input (Column name)

Run order (Order)

- The order in which the samples were run
- Numeric input required

Sample type (Type)

- Type of sample analyzed
- **Only 4 types of sample are allowed!**
    - "Blank" → for blanks (samples without the analyte)
    - "Standard" → for calibration standards (used to construct the calibration curve)
    - "QC" → for quality control (QC) samples (used for accuracy and precision)
    - "Sample" → for all other samples (unknowns which you want to quantify)

Sample subtype (Level)

- This columns identifies the **specific samples within the 4 classes** of sample types (Blank, standard, QC and Sample).
- The **name of the subtype should be unique** for that specific sample, if replicates are used or multiple injections were analyzed, these should be declared in the next two columns, not in the "Level" column
    - E.g. Three LLOQ QC samples were analyzed, then the subtype you need to declare is "LLOQ" for each of these replicates, not "LLOQ1", "LLOQ2", "LLOQ3" for example.
- Denoting a subtype (i.e. **level**) is **always required**
- For **samples** and **standards** the choice of subtype name is free
- For **blanks** and **QC** samples there are certain rules which need to be followed (see table below, also included in sheet 2 of the excel template).
- See also explanation in template, **sheet "Explanation"** and sheet "Example_Input"

Table I Allowed Type-Level combinations

Type	Level	Explanation
Blank	Blank	Processed blank sample without analyte or internal standard
Blank	Zero	Processed blank sample without analyate but with internal standard
Blank	Carry-over	Processed blank sample after highest calibrator
Blank	<free>	Blank which will not be evaluated (e.g. solvent blank)
Standard	<free, Calibrator name>	Calibrator standard sample
QC	LLOQ	Quality control sample at the level of the lowest standard (lower limit of quantification)
QC	LOW	Quality control sample at a low concentration
QC	MID	Quality control sample at a mid concentration
QC	HIGH	Quality control sample at a high concentration
Sample	<free, Sample name>	Samples to be quantified

Replicate number (Replicate)

- Experimental **replication number** of the sample.
    - e.g. QC low made in triplicate: replicate = 1, 2 or 3
- If only one replicate was made, the required input is "1"

Injection number (Injection)

- **Injection cycle** of the sample
    - e.g. sample was injection twice: injection = 1 or 2
- If only one replicate was made, the required input is "1"
- Maximum 3 injections

Nominal concentration (Std_conc)

- **Theoretical concentration** of the sample, only required for type = “Standard” or type = “QC”

Area quantifier ion (Quantifier)

- Area of the **quantifier ion** of the molecule under investigation
- Can also be another response parameter (e.g. peak height) but the graphs in the output will still be labeled with “area quantifier”

Area internal standard ion (IS)

- Area of the quantifier **ion** of the **IS** under investigation
- Can also be another response parameter (e.g. peak height) but the graphs in the output will still be labeled with “area quantifier”

Optional input

Sample name (Name)

- **Name** of the sample
- not used in the analysis (Samples are identified by the Type-Level-replicate-injection combination)

Area qualifier (Qualifier)

- Area of the **qualifier ion** of the molecule under investigation
- Used as a diagnostic measure (ratio quantifier/qualifier) (in function of nominal analyte concentration)

S/N ratio (Signal_to_noise)

- **signal to noise ratio**
- used as diagnostic measure (in function of nominal analyte concentration)

Retention time analyte (Rt_analyte)

- Used as diagnostic measure (in function of nominal analyte concentration and run order)

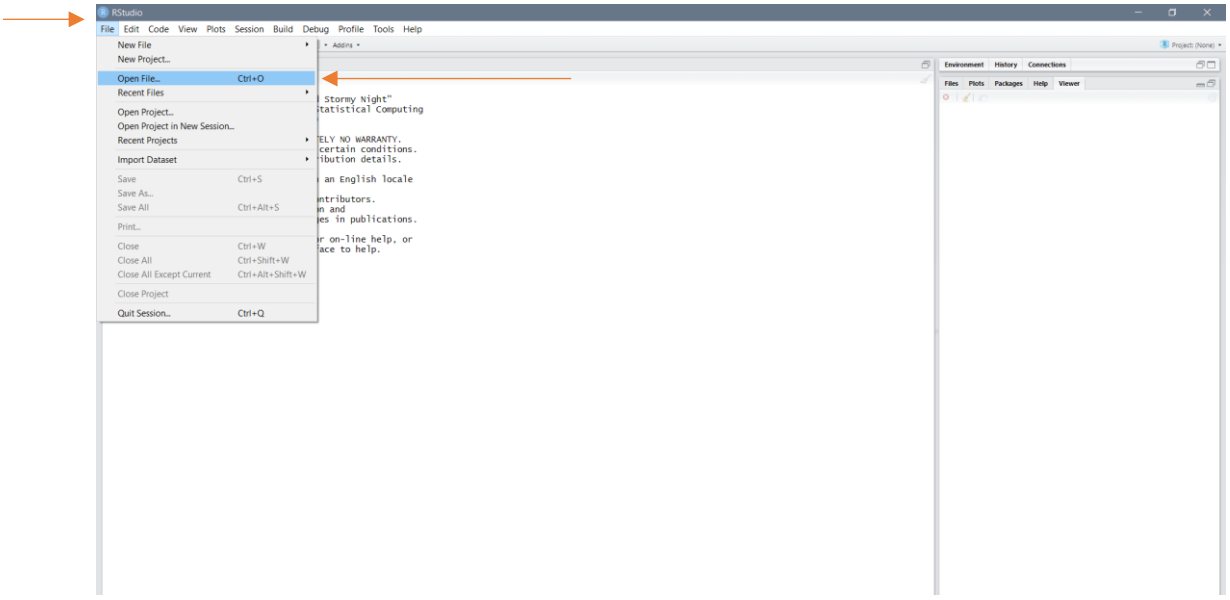
Peak symmetry factor (Peak_assymetry)

- Used as diagnostic measure (in function of nominal analyte concentration)

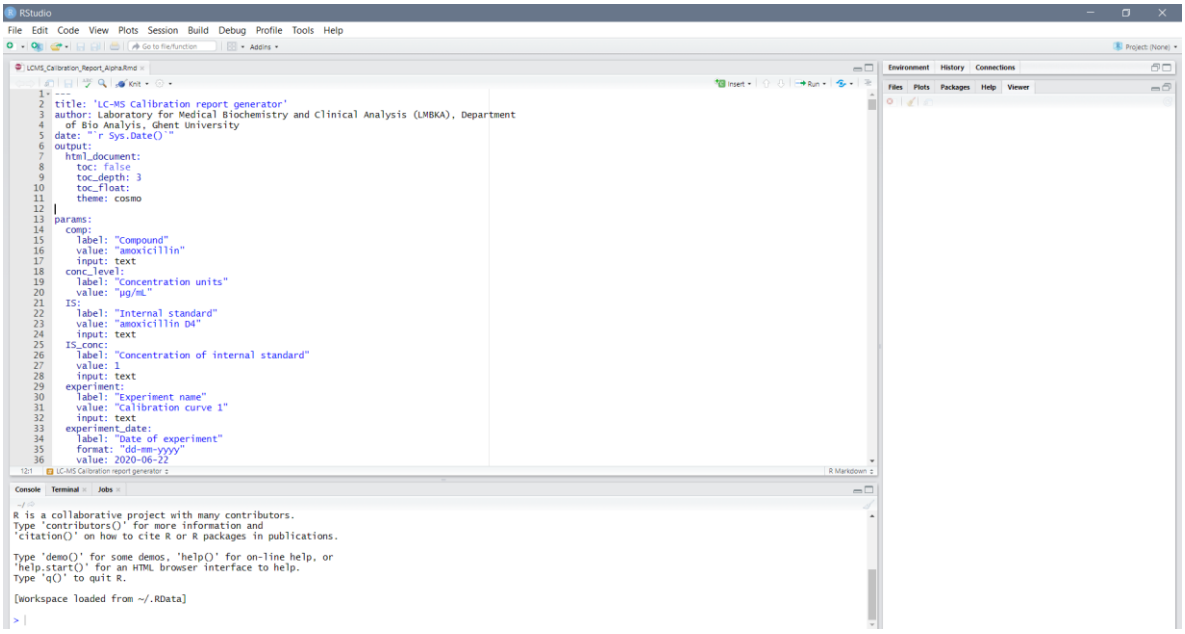
Step 2: run R script in **R studio**

1. Open **Rstudio (installation, see above)**

2. **Open file**
   - Either: Click on “File” > “Open File …” > select file “**Rmarkdown-script_Bio-analytical-report-generator_VersionAlpha.Rmd**”



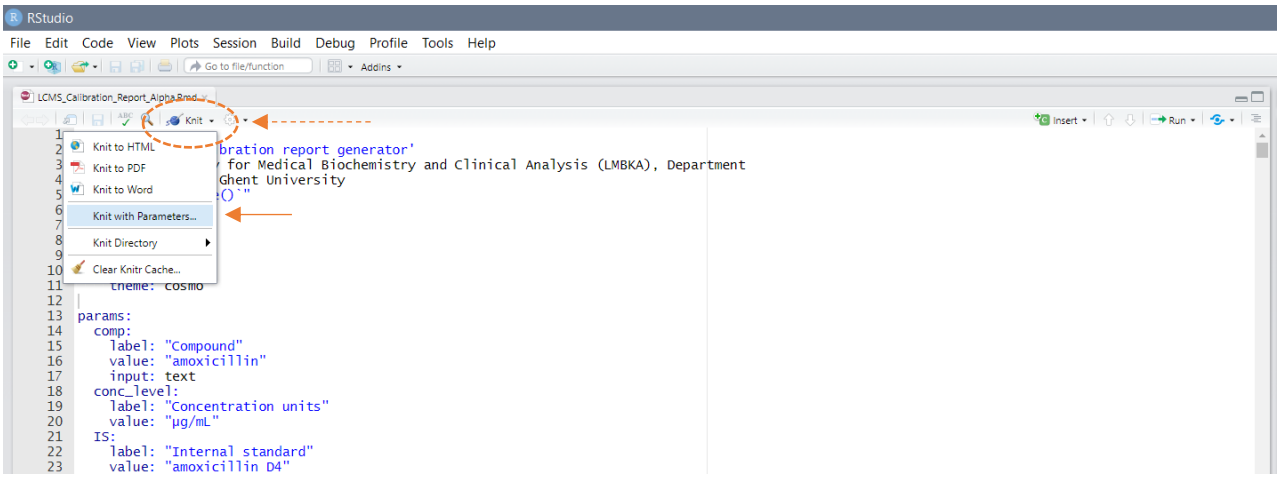
   - The file in Rstudio should look something like this:



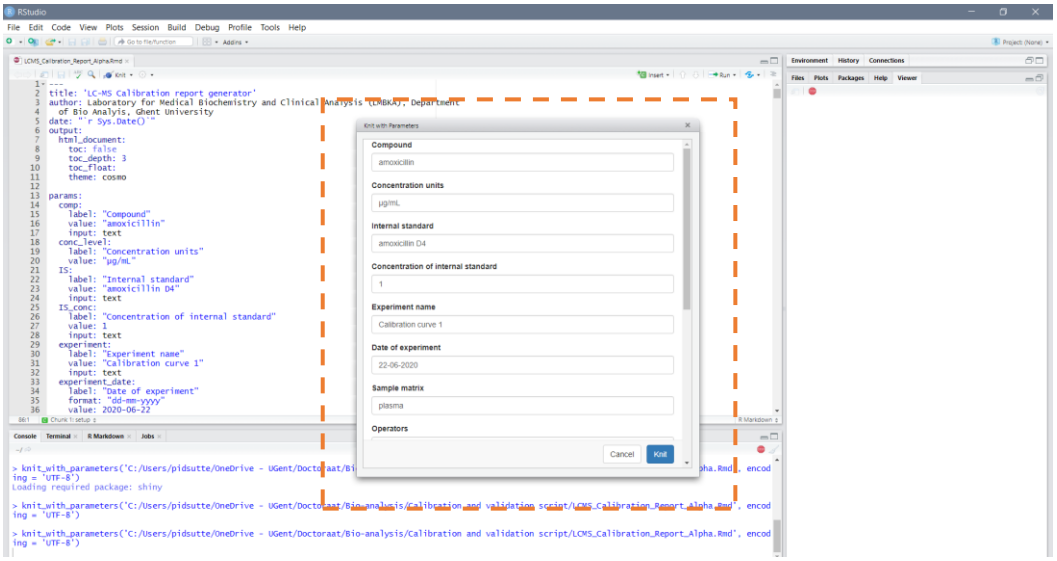
   - Alternative: copy-paste code from github into a new Rmarkdown file (File → New File → Rmarkdown)

3. Open parameter interface (**knit with parameters**)
   - Click the drop-down menu on the “knit” button

- Click on “Knit with Parameters…”



- A pop-up window will appear that looks like this:

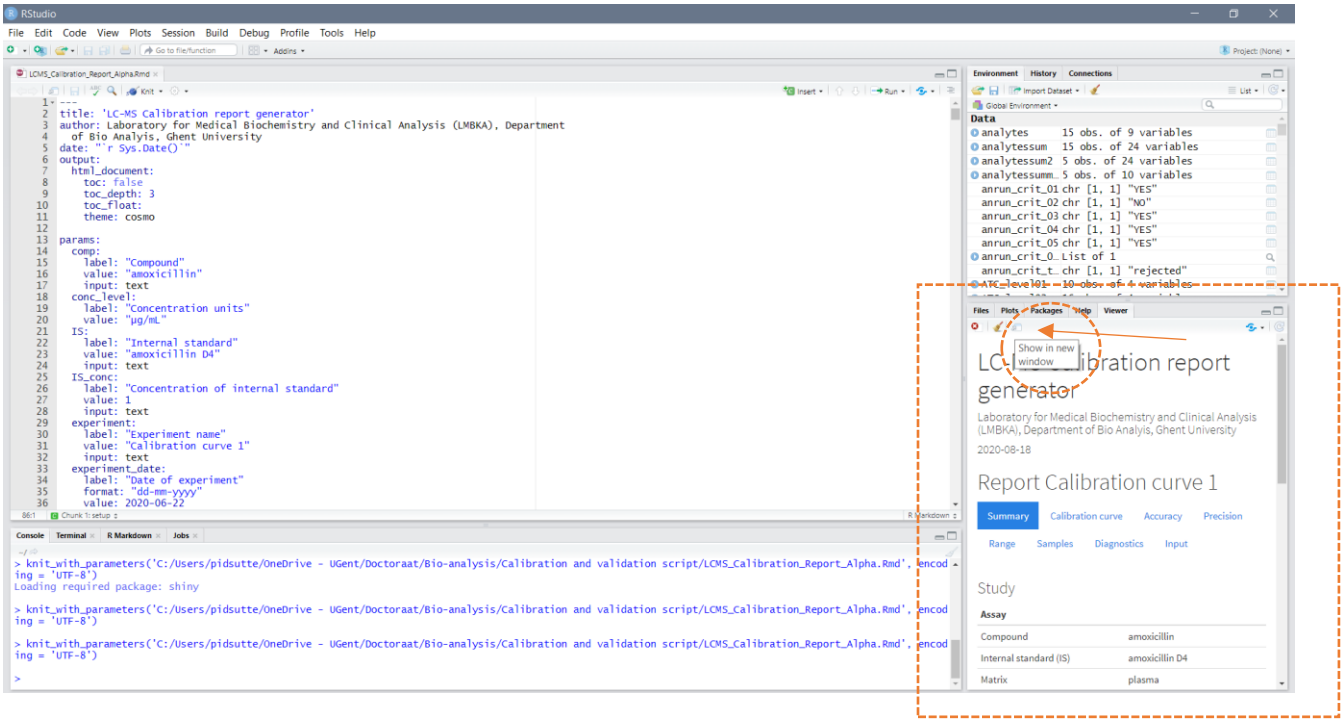


4. Enter parameters

Parameter	Choices	Explanation
Compound	Free (text)	Name of the compound *(e.g. “amoxicillin”)*
Concentration units	Free (text)	Concentration units accompanying the data *(e.g. “µg/mL”)*
Internal standard	Free (text)	Name of the internal standard *(e.g. “Amoxicillin D4”)*
Concentration of internal standard	Free (numerical)	Concentration of the IS, without the concentration units *(e.g. “0.25”)*
Experiment name	Free (text)	Name of the experiment *(e.g. “Calibration curve 1”)*

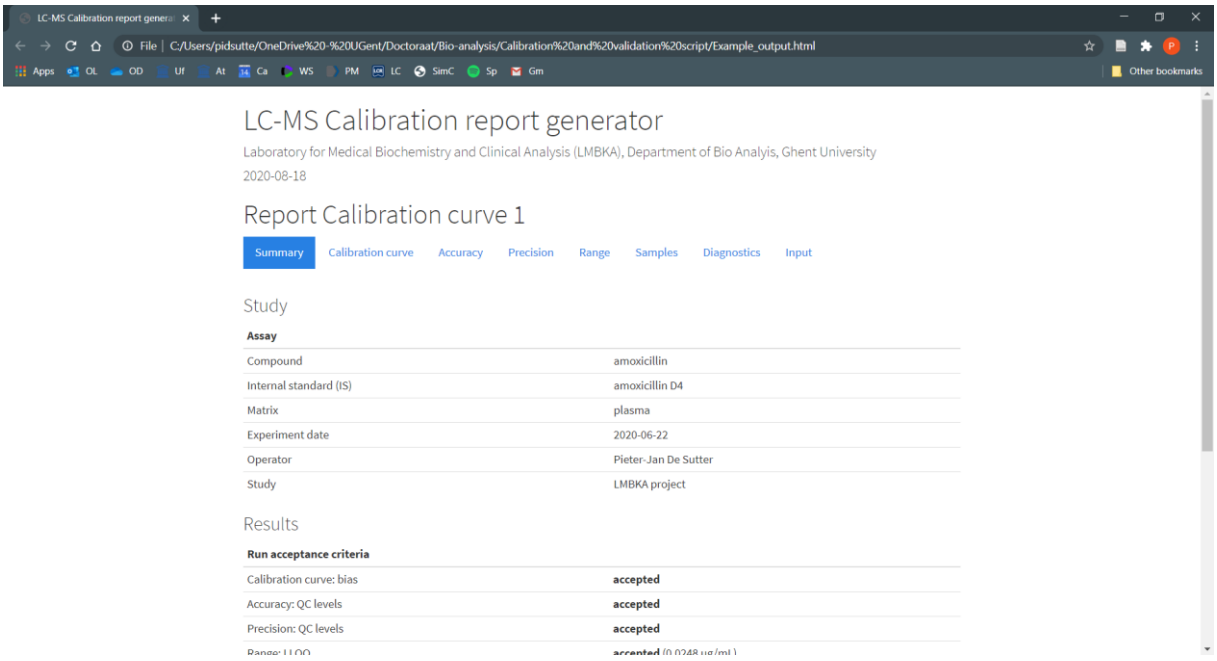
Date of experiment	Free (date)	Date when the experiment was carried out *(e.g. "26/06/20")*
Operators	Free (text)	Name of the operator(s) *(e.g. "Pieter-Jan De Sutter")*
Sample matrix	Free (text)	Matrix of the samples *(e.g. "plasma")*
Study	Free (text)	Name of the study *(e.g. "LMBKA project x")*
Select Excell file	Free (select file)	Select the excel file containing the data (use template as specified at the beginning of this document) (e.g. "*Example Input.xlsx*")
Weighing function	Fixed • No weighing • 1/X • $1/X^2$	Weighing function used when constructing the calibration curve
Injections	Fixed • All injections • 1 & 2, 1 & 3, 2 & 3 • 1, 2 or 3	Injections used for the analysis.

- After entering the parameters: klick "**knit**"
  - The file is now rendering, this can take some time (< 1 minute)
- A **preview** now appears in the lower right hand corner, click "**show in new window**" to enlarge



- The **output** is located in the same **folder** as where the Rmd script "Rmarkdown-script_Bio-analytical-report-generator_VersionAlpha" is kept
- The output (html) can be opened by any **web browser**
  - NB: this does not mean that the content appears online, the content stays *offline*

- click the tabs or scroll down to navigate



Troubleshooting

- Try running the script with the example data found in the github repository, if this works, something went wrong with the data input. If the example data does not work, look if R studio is correctly installed and wheter all required packages are installed.

Contact

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