*DCVtestkit*: a R package for linearity assessment and analysis of quality control dilution curves

## Manuscript Type

Application Note

# Abstract

## Summary

Linearity assessment plays a significant role in the validation of quantitative analytical laboratory procedures. In metabolomic and lipidomic workflows, a linear response in dilution series generated from pooled quality control samples is used to assess the quality of the measurement of molecules measured before further analysis. Most of the currently used software only allow the analyst to repetitively plot, view and analyse the dilution curves one at a time, a tedious and time-consuming process. In addition, summary statistics of dilution curves are limited to the Pearson Correlation Coefficient which is insufficient to fully understand the shape of the dilution curves. *DCVtestkit* aims to provide additional summary statistics for dilution curves, taken from previous publications but which are not implemented in the current software tools. It also helps to reduce the analyst’s workload by analysing many dilution curves automatically, reporting the statistical results in Excel and recording the dilution plots in a pdf file. In addition, it can also create an interactive trellis displayed as a HTML folder for more exploratory analysis.

## Availability and implementation

*DCVtestkit* is available on GitHub <https://github.com/SLINGhub/DCVtestkit>. The documentation and tutorials can be accessed from <https://slinghub.github.io/DCVtestkit/>

## Supplementary information

Supplementary data are available at *Bioinformatics* online.

## Issue Section

Data and text mining

# Introduction

Linearity assessment is one of the criteria used to evaluate the precision of a quantitative measurement procedure as seen in calibration studies Rodríguez *et al.* (1993) and drug analysis Needleman and Romberg (1990). In metabolomics/lipidomics analysis, dilution curves are plotted for each molecule measured from quality control sample, generated by pooling equal aliquots from each study sample. Molecules that exhibits a non-linear relationship are rejected before further statistical analysis, as their quantification in different sample groups might be compromised. Dilution curves are usually plotted using general-purpose software like Excel with their corresponding Pearson Correlation Coefficient value. A threshold value of 0.8 is used to balance the risk of accepting or removing non-linear signals related to relevant molecules.

However, Sonnergaard (2006) warned that the Pearson Correlation Coefficient is not an effective standalone numeric parameter to estimate linearity. While researchers have created other metrics for linearity evaluation, these metrics are rarely implemented in most general-purpose software. Furthermore, today’s metabolomics/lipidomics workflow can measure hundreds of molecules. Having the analyst to individually plot numerous curves to check for linearity is time consuming.

R package, *DCVtestkit* addresses these issues by assisting analysts, to plot dilution curves for many molecules easily with additional metrics, other than the Pearson Correlation Coefficient, that better describe the curve’s shape. It also provides an interactive viewer for analysts to group, filter and sort the plots, allowing them to look at the relevant ones, such as the saturated dilution curves, and identify problematic molecules quickly.

# Approach

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Figure 1: *DCVtestkit* Workflow

The workflow starts with two tables: Transition Signal Data, containing transition signals (y-axis for dilution curve) for each sample and Dilution Annotation, containing dilution curve related information, such as concentration (x-axis for dilution curve) and dilution batches. Using a common column Sample Name, the two tables can be merged into one table (Dilution Table) via create\_dilution\_table.

Next, summary statistics are calculated via summarise\_dilution\_table for each dilution curve. Besides the Pearson Correlation Coefficient, additional parameters introduced are results from Mandel’s Fitting Test Andrade and Gómez-Carracedo (2013) in which a low value gives sufficient evidence that a quadratic model fits better than a linear model, indicating the curve may not be linear. Another parameter is Percent Residual Accuracy Logue and Manandhar (2018) which ranges from to . If the curve is linear, the value should be close to . The software also calculates the concavity of a fitted quadratic model to identify if the curve is dominantly non-linear at high (concavity ) or low (concavity ) concentrations.

Supplementary Figure 1 gives the summary statistics of three manually curated curves: A linear curve and curves with a plateau at higher concentrations (denoted as saturated curves) and lower concentrations (denoted as limit or detection or LOD curves) respectively. The corresponding Pearson Correlation Coefficient values (r\_corr) are (, and respectively), even for the curves that are non-linear. However, both saturated and LOD curves give a much lower Mandel’s Fitting Test values (mandel\_p\_val) ( and respectively vs ). Likewise, the Percent Residual Accuracy values (pra\_linear) are much lower in the saturated and LOD curves compared to the linear curve ( and respectively vs ).

evaluate\_linearity is used to group the curves according to the workflows proposed in Supplementary Figure 2. Workflow 1 uses the Pearson Correlation Coefficient and Percent Residual Accuracy to determine if the curve is linear (labelled as Good Linearity) or not (labelled as Poor Linearity). Workflow 2 goes one step further, using the Mandel’s Fitting Test and the fitted quadratic model’s concavity to check if the non-linear curve plateaus at low (labeled as limit of detection) or high (labelled as saturation) concentrations. Non-linear curves that do not follow these trends are labelled as Poor Linearity.

A benchmark workflow using only Pearson Correlation Coefficient value of is compared with Workflow 2 on simulated data sets of 200 linear curves (labelled as Linear), curves that plateau at low (labelled as Limit of Detection) high (labelled as Saturated) concentrations each. Supplementary Figure 3 showed that Workflow 2 better identifies the saturated and limit of detection curves than the benchmark workflow. While it identifies less linear curves correctly than the benchmark workflow, its score of 181/200 (90.5%) is comparable. See <https://dcvtestkit-simulation.netlify.app> for report details. While the threshold values of Pearson Correlation Coefficient and Percent Residual Accuracy are based on the interpretation of Y. H. Chan (2003) and Logue and Manandhar (2018), respectively, they remain subjective and arbitrary. Nevertheless, *DCVtestkit* allows optimization of these threshold values according to the analyst’s determinants of linearity.

Although *DCVtestkit* can export the results in Excel or pdf, they may be too complex for meaningful interpretation. Supplementary Figure 4 shows a HTML folder, exported by *DCVtestkit*, such that clicking on the index.html file inside the folder will open an interactive trellis plots that analysts can be grouped, filtered and sorted. This allows room for exploratory data analysis, such as identifying molecules with linearity issues or finding out the effects of changing the Pearson Correlation Coefficient threshold to another value. Such information is hard to achieve with the Excel and pdf files. An example of an interactive viewer created by *DCVtestkit* can be viewed at <https://dcvtestkit-interactive-example.netlify.app>

# Conclusion

To verify if a quantitative analytical test method is reliable, it is important to check for linearity. A linear response in quality control dilution curves is required in metabolomics/lipidomics to assess the molecules’ suitability for further analysis. However, there are few software that can analyse dilution curves efficiently. R package, *DCVtestkit*, rectifies this by plotting of many dilution curves quickly by automation and reporting alternative statistics, other than the Pearson Correlation Coefficient, to better describe the shape of dilution curves. It also provides an interactive trellis plot for exploratory data analysis. It is available on GitHub <https://github.com/SLINGhub/DCVtestkit> while the documentation and tutorials can be accessed from <https://slinghub.github.io/DCVtestkit>.

# Acknowledgements

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# References

Andrade,J.M. and Gómez-Carracedo,M.P. (2013) [Notes on the use of Mandel’s test to check for nonlinearity in laboratory calibrations](https://doi.org/10.1039/c2ay26400e). *Analytical Methods*, **5**, 1145.

Logue,B.A. and Manandhar,E. (2018) [Percent residual accuracy for quantifying goodness-of-fit of linear calibration curves](https://doi.org/10.1016/j.talanta.2018.07.046). *Talanta*, **189**, 527–533.

Needleman,S.B. and Romberg,R.W. (1990) [Limits of Linearity and Detection for Some Drugs of Abuse\*](https://doi.org/10.1093/jat/14.1.34). *Journal of Analytical Toxicology*, **14**, 34–38.

Rodríguez,L.C. *et al.* (1993) [Estimation of Performance Characteristics of an Analytical Method Using the Data Set Of The Calibration Experiment](https://doi.org/10.1080/00032719308019900). *Analytical Letters*, **26**, 1243–1258.

Sonnergaard,J.M. (2006) [On the misinterpretation of the correlation coefficient in pharmaceutical sciences](https://doi.org/10.1016/j.ijpharm.2006.06.001). *International Journal of Pharmaceutics*, **321**, 12–17.

Y. H. Chan (2003) [Biostatistics 104: Correlational analysis](http://www.smj.org.sg/article/biostatistics-104-correlational-analysis). *Singapore Medical Journal*, **44**, 614–619.