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Bianaca: A Freely Accessible Tool for Data Analysis of End-Point Biochemical Assay

Abhikumar TT, Sujit RT, Sivakumar G, Venkateswara Rao A, Kulbhushan T, Prabha G and Prasad VB*

Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, Punjab, India

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***Corresponding author:** Prasad V. Bharatam, Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research (NIPER), S.A.S Nagar, Punjab 160062, India, Tel: 91-172-221-4682(2018); Fax: 91-172-221-4692, E-mail: pvbharatam@niper.ac.in.*

Abstract

Currently, available protocols for handling and analyzing the data of an end-point biochemical assay from a microplate reader are insufficient in that more than one tool and time is required. To overcome this problem, we present a novel bio-analytical tool "BiAnaCA" (Biochemical assay Analyzer and CAlculator), for sorting the data sample-wise, interpreting concentration, plotting graphs and statistical analysis of an end point biochemical assay performed in a 96- or more well microplate using a microplate reader. The tool BiAnaCA processes output files of assay data in the form of sample-wise absorbance readings generated by microplate reader. The tool also generates a report containing all the results. BiAnaCA is developed in java language, supported on Windows and Linux systems. It can act as a laboratory information processing and management system. The software is freely available online and open source for all kinds of non-commercial users and academic researchers.

Keywords: BiAnaCA; Biochemical Assay; Microplate Reader; Java

Introduction

In biomedical research, it is very common practice to carry out biochemical assays [1], ranging from simple end-point/kinetic assays to complex enzyme assays that are measured by a simple UV-Visible spectrophotometer or versatile multimode microplate readers [2]. The first step of assay is to measure absorbance (optical density), fluorescence [3], luminescence or fluorescence polarization [4] in various read types like end-point, kinetic [5,6], spectrum, well scan and flex [2,7]. Next step is handling the data obtained from the reader and to interpret it in concentration or activity terms followed by data analysis, graphical visualization and statistical treatments. Current practice inherits several pitfalls including tedious and cumbersome calculations using different available commercial software with a lot of manual interventions which are time-consuming and error-prone. In order to surmount these drawbacks, we engineered a JAVA based, user-friendly, standalone solution called BiAnaCA [8], which can perform all such tasks faster and efficiently on various platforms like Windows and Linux.

Features Implemented in BiAnaCA

Current version of BiAnaCA offers several salient features for data handling, processing, analysis and visualization along with helping interactive message box on screen to guide the user in each step. Figure 1 explains the workflow associated with the tool.

Loading of Data and Output Files of Plate Reader

The Data Handler module processes and organizes the reader output files and retrieves sample codes and 96- or more well microplate absorbance readings from files uploaded by user in .csv or .txt formats.

Create, Update and Save Bioassay Data Matrix

It also gives option to user for manual entry or copying data from system clipboard in case data in file formats is not available or instruments generating data in print format only. Once data is fed, user can save it for further use. It performs outlier detection and reports if any sample found outside limits chosen by user.

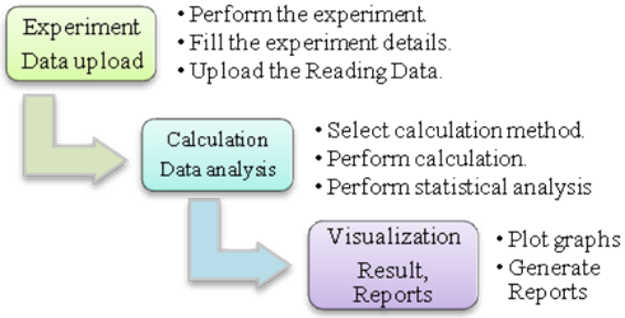

Figure 1: Workflow of BiAnaCA tool.

Calculations and Statistical Data Analysis

The Calculator module interprets the unknown concentration from the assay readings by offering two options to user (i) from single known standard concentration by cross multiplication and (ii) from multiple standard calibration curve points by linear regression analysis. It generates the R-square statistics, plot and equation. From these concentration data, it also calculates Z-factor [9, 10], statistical measures of central tendency and dispersion like Mean, Median, Q1 quartile, Q3 quartile, Standard Error, Standard Deviation, %CV, Minimum and Maximum. BiAnaCA also offers some of most widely used statistical tests like Student's t-test (one-sample, paired, unpaired but equal variance, unpaired unequal variance with Welch correction factor), one-way ANOVA (analysis of variance), and column statistics. Based on selected attributes - alpha value for confidence level and distribution direction as either one-tail or two-tail, BiAnaCA performs these tests yielding the statistical significance (in the scale of * to **** with corresponding P value < = 0.05, 0.01, 0.001, 0.0001), test statistic t-, F-values and suggests whether the null hypothesis should be rejected or not for the group-wise analysis of control and test treatment groups. Figure 2 shows a representative screenshot of GUI containing features of the tool BiAnaCA.

Graphical Visualization and Report Generator

The Plotter module generates the graphical plots for visualization of comparative data analysis. It generates three plots: (i) a group-wise analysis plot, which is bar chart of mean concentration of group ± SEM for comparison of test treatments with control group (ii) a sample-wise data analysis in the form of Bar graph with the value expressed as mean ± SD of the replicates

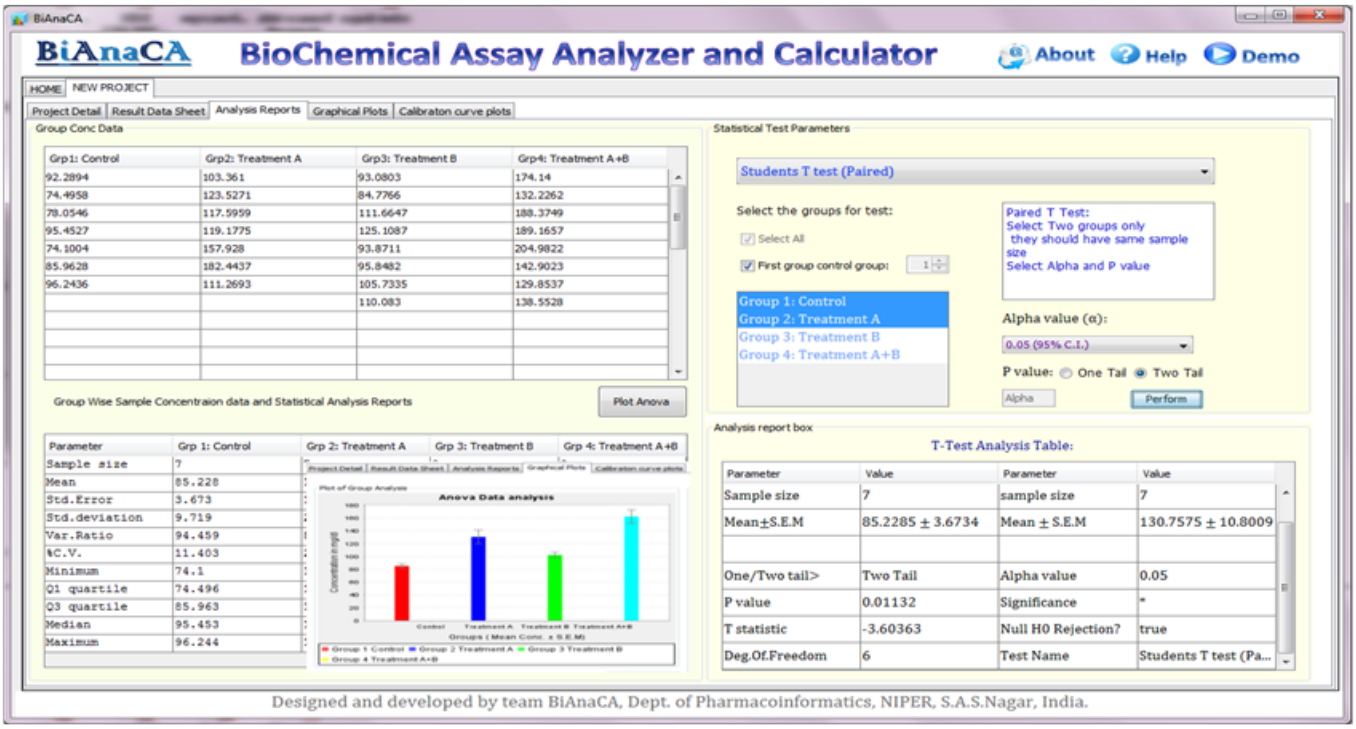

Figure 2: Screenshot of BiAnaCA from analysis report window depicting group-wise concentration analysis, graphical plot and statistical test parameters.

of individual sample (iii) a sample-wise data analysis in the form of graph with the value expressed as mean of the replicates of each sample. User can edit the properties of plot, can print it or have different views and save the plot as different image formats. The PDF Reporter module generates a report in .pdf format containing project details, column statistics table and data analysis plot, which can be printed or produced as tool-generated documentary evidence for regulatory requirement. With each 'New Project' click, it creates a new folder in its dedicated directory and saves input data (by converting manually entered data to a .csv file), generated results and PDF report to a folder of same name as of project name, so that user can easily load the data from same folder and can save time. Java library JFreeChart is used for plotting graphs, Apache commons-math for statistics, and iText for report generation. This repository can serve for laboratory information management system.

Case Study

Consider a simple biochemical assay of glucose estimation in the plasma of 30 rats belonging to 4 groups (7,7,8,8 per group respectively) in a pharmacological intervention study. After performing the assay, the samples were loaded onto a 96-well microplate in triplicate in the order of 30 Samples from 30 animals followed by Blank and Standard (Std) (The concentration of Std is 100 mg/dL as provided by the assay kit manufacturer) and the microplate is read using a microplate reader at a wavelength as per the manufacturer. The output file containing absorbance of each and every well obtained from the microplate reader in the form of .txt file serves as the input file for BiAnaCA. Then following steps are to be performed in BiAnaCA for analyzing the data. Fill the experiment, groups and sample replicate details. Then user has to upload the input data file (.csv/.txt) with the raw or sample-wise sorted absorbance readings. Then user moves to "Result Data Sheet" tab which contains the concentration of each and every sample interpolated from the standard and also the outliers in a group. Upon clicking "next", "Analysis Reports" tab will be opened which contains column statistics like measures of central tendency and dispersion along with t-test. "Graphical Plots" section provides two types of graphs *viz*. group-wise concentration data represented in the form of mean ± SEM and sample-wise concentration data represented in the form of mean ± SD. Finally, upon clicking "Print Report", a report containing experiment details group-wise column statistics along with graph showing group-wise concentration data is generated. For detailed information and steps, please refer BiAnaCA tutorial available at http://www.niper.ac.in/pi_dev_tools/BiAnaCA/tutorial.php. The whole procedure was applied to the data of a pharmacological intervention study in Asthma and the results were found to be exactly same as those returned by GraphPad [11].

Comparison with Other Tools

BiAnaCA allows for calculating the mean of the replicates of absorbance of a sample and/or then interpolation of unknown concentration of the sample from a single-standard by simple cross-multiplication or multiple standards by linear regression which is not possible with GraphPad and Sigma Stat. Though Excel can handle the data initially while calculating the concentration from the absorbance, performing later calculations/analyses like mean, median, standard deviation, standard error of mean, t-test, ANOVA etc. or when insertion of error bars (SD/SEM) in the graphs, it requires the selection of data and may need to insert some formula, which can be error-prone, tedious, time-consuming and laborious. Also, it can sort the pooled data of samples group-wise on its own just with an input of specifying the samples and/or replicates in groups under consideration. BiAnaCA, a freely available standalone tool calculates and analyses assay results in easy, streamlined steps and saves significant time and other computational resources. It can be used to analyze biochemical assays, which are routine in biotechnology, biochemistry and drug discovery & development fields. It is well

customized for the varying purposes of these calculations, graphical visualization and statistical analyses by guiding and directing the naive user in user-friendly and error-free manner.

Conclusion

BiAnaCA has been developed to efficiently process the end-point bioassay data produced by microplate readers and spectrophotometers. It can be used for the analysis of fluorescence, luminescence, and fluorescence polarization data apart from the absorbance data. BiAnaCA is novel in the way of handling and analyzing the biochemical assay data. This tool was not conceived or designed to compete with commercial software(s) having multiple features. Source code is organized and written in a way that can be expanded with more assay types and statistical functionality or can be integrated seamlessly with other tools and analytical instruments in future. Nevertheless, BiAnaCA is well customized, easy to use and integrated as standalone tool having optimal efficiency for bioassay data handling, processing, calculation, analysis, visualization and reporting. Importantly, it is freely available and open source tool running on many platforms and has been proven very useful in several in-house calculations and publications in emerging pharmaceutical and chemical sciences.

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Corresponding author: Prasad V. Bharatam, Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research (NIPER), S.A.S Nagar, Punjab 160062, India, Tel: 91-172-221-4682(2018); Fax: 91-172-221-4692, E-mail: pvbharatam@niper.ac.in.

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