Background

This exercise deals with male rats ttreated with two drugs (Chloroquinone or Amiodarone, both of which are known to induce Phospholipidosis The drugs were administered to two different strains (Sprague-Dawley (s); Fisher (f)). Sprague-Dawley are the standard laboratory rats while Fisher rats are more sensitive strain for treatmet.

Objective

The objective of this study was to investigate whether 1H NMR data collected on rat urine could be used to distinguish control rats from those treated with the drugs.

This data set contains 57 observations and 194 variables (NMR bins). Observations have six different classes.

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| --- | --- | --- |
| **Class** | **Description** | **Number of anmals** |
| S | Sprague-Dawley control rats | 10 |
| sa | Sprague-Dawley rats treated with Amiodarone | 8 |
| Sc | Sprague-Dawley rats treated with Chloroquinone | 10 |
| F | Fisher control rats | 10 |
| Fa | Fisher rats treated with Amiodarone | 10 |
| Fc | Fisher rats treated with Chloroquinone | 9 |

1. Select “New project” from file” and Open the data set “NMR\_Metabolomics.dif”
2. SIMCA will open the data set and look for any missing values or errors.
3. It is necessary to designate a unique ID for the samples and also for the variables. Select a column that contains a unique ID for samples and designate it as “Primary ID”.
4. Select the second column that has ClassID as Secondary ID. Save the file.
5. SIMCA automatically creates the data set using “mean centering” and “unit variance scaling”.
6. Right click on the model M1. Use “Edit model.
7. Go to the “Scale” tab, select all variables (Ctrl-A), and set to “Pareto” from the dropdown menu in the upper right.
8. Select ”Autofit” in the main menu bar.
9. It will perform a Principal Component Analysis (PCA) model. This is a non-supervised modeling, which means that you are just trying to find the patterns in the data set. You are not giving any information on treatment groups (ClassID).
10. Now we are going to build a PLS-DA model. It is a supervised modeling method. Here we are giving information about our treatment groups and asks a different question: “what are the variables that separate study groups?”
11. Right click on M1 and select “New as model 1…”.
12. Select “Observations”
13. Select ClassID in the “Class from Obs ID” dropdown menu and press “set” and select “Ok”..
14. You will see your ClassID under “Class” column.
15. Change the “Model type” to PLS-DA.
16. Now you will see your groups have been separated.
17. Right click on M2 and select “New as model 1…”.
18. Select “Observations”, select “Find in class column” from “Find” dropdown menu. Type “f” and it will highlight all Fisher rats. Click “Exclude”. Select PLS-DA.
19. Create the PLS-DA model for SD rats.
20. Select “Plot/ List”, and select “Scatter 3D”. Check for the observations to see whether they are grouped together. Do you see any subject different from others?.
21. What is the difference of that animal from the rest of the group?
22. Select s9 rat and all other rats separately. Select “Group to point comparison”. Then you will see the NMR bins that separate s9 from the rest.
23. Click on VIP and then you will see the Variable Importance for Projections (VIP) Plot. Variables with VIP => 1.0 are considered as most important for the separation. In this example, you will find the bins that separate all three groups (Control, Amiodarone, Chloroquinone groups).
24. Remove one drug group at a time and create a model to compare threated vs control groups (pairwise). Find the bins important for separation of the groups.
25. Do the same procedure (17 through 24) for Fisher rats.
26. Now we are going to use OPLS-DA modeling. OPLS-DA is an improved modeling method that removes variation within X-data.
27. OPLS-DA is mostly used for pairwise comparisons.
28. Select the model for Control vs Amiodarone treatment for SD rats. Follow steps 11 thorugh 15. Select OPLS-DA instead of PLS-DA.
29. Create a model. Find the bins that separates the groups.
30. Follow the save analysis for other comparisons (s vs sa, s vs sc, f vs fa, f vs, fc).
31. Can you find the difference between the two rat strains?
32. What is the difference in response to drugs between the two rat strains?