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# *Linear Gradient POPLC® Optimizer*

## *Evaluation version V.1.23*

# *Manual & User Guide*


**Dr. Kai Chen and Dr. Frédéric Lynen**

**PARC – Ghent Univeristy**

**Krijgslaan 281-S4 bis**

**B-9000 Gent**

**Belgium**

**Tel: +32 926 49602  Fax: +32 926 44998**

**E-mail: kai.chen@ugent.be**


UNIVERSITEIT GENT

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# *Brief introduction*

**So far the only available software for phase optimizied liquid chromatography (POPLC®) has been comercialized by Bischoff Chromatography. This software is, however, limited to isocratic analysis. Here we introduce the first gradient version of POPLC® software.**

## Motivation:

- **To improve the selectivity and to shorten the analysis time by performing gradient instead of isocratic elution in phase optimized liquid chromatography.**

## Disadvantages:

- **Gradient profile is given before optimization.**
- **Only the optimum combination of segments is shown as a result. No rank list can be obtained.**

## Advantages:

- **Multiple gradient steps (up to 6) can be defined.**
- **Definition of the types of segments is flexible, i.e. from 2 to 6.**
- **Combined optimization restriction on both total column length and analysis time.**
- **Convenient project management.**

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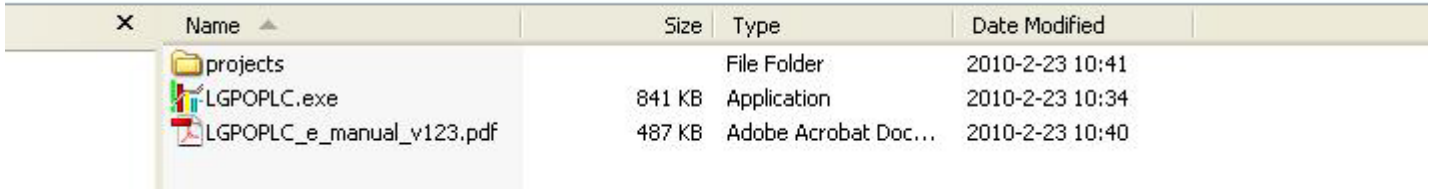
# *Installation & uninstallation*

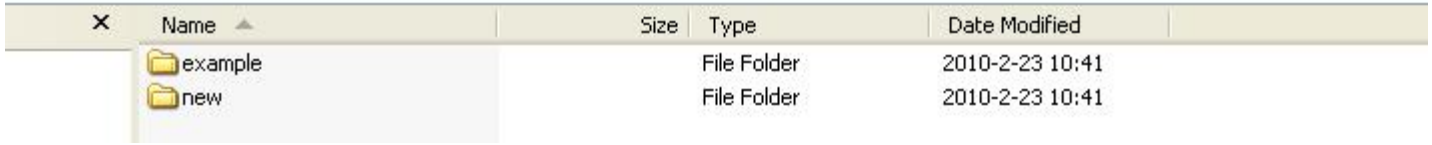
**LGPOPLC is a '*green*' software. It does not write to the Windows registry and instead stores its settings in one or more configuration files (e.g. an INI file) located in its directory.**

**• For installation, unzip the package to your destination folder. After unzipping, the folder contains a executable file 'LGPOPLC.exe', a help file 'LGPOPLC_e_manual_v121.pdf and a sub-folder 'projects' which stores all the working projects.**

**• For uninstallation, just delete the whole folder for this software.**




**Main folder of LGPOPLC**




**Projects folder**

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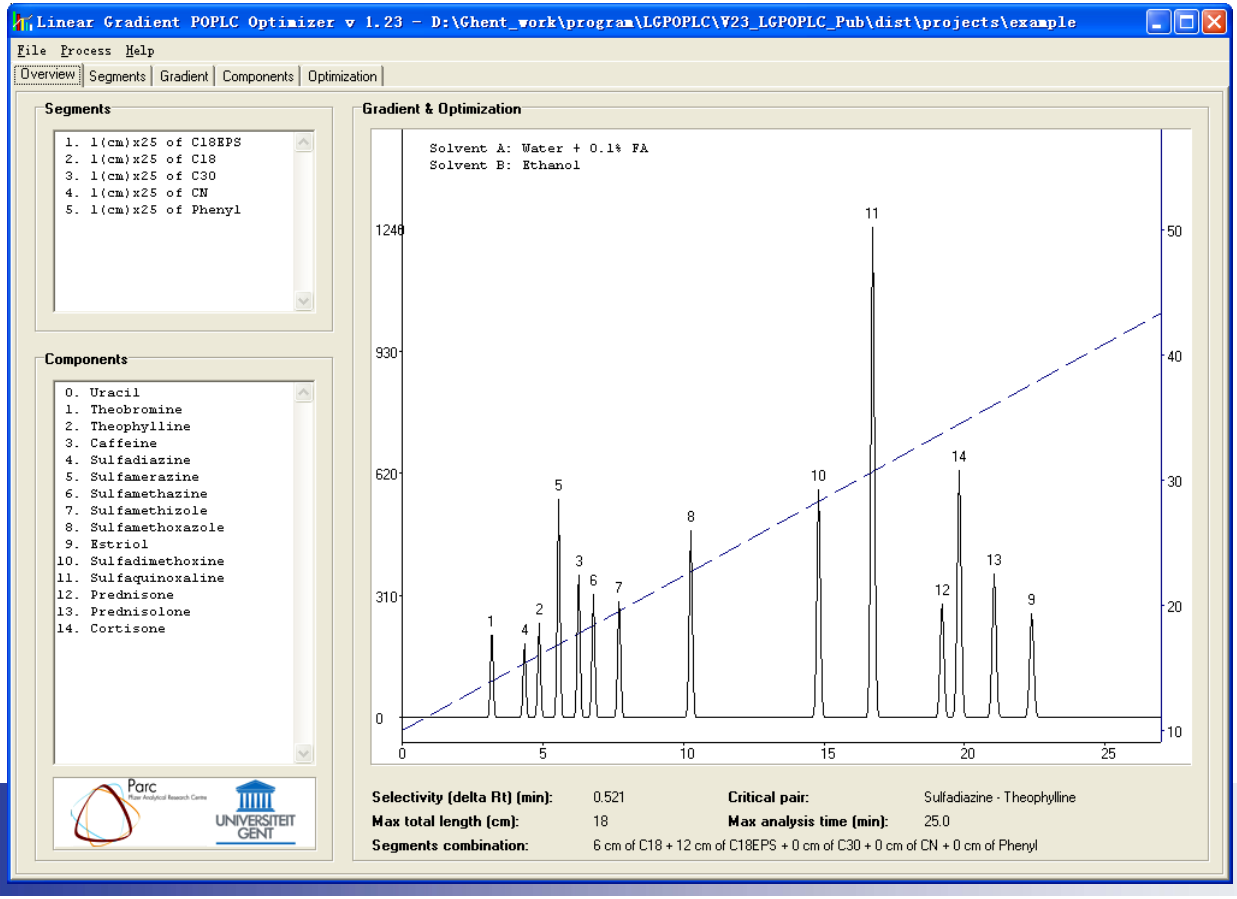
# *Software interface - overview page*

**On the overview page, there is the overview of the project and the results of last optimization.**

- **Segments' definition.**
- **Components' list.**
- **Results of last optimization, including optimization restrictions (max. total length and max. analysis time), segments combination, critical pair and its selectivity.**
- **Predicted chromatogram with gradient profile.**

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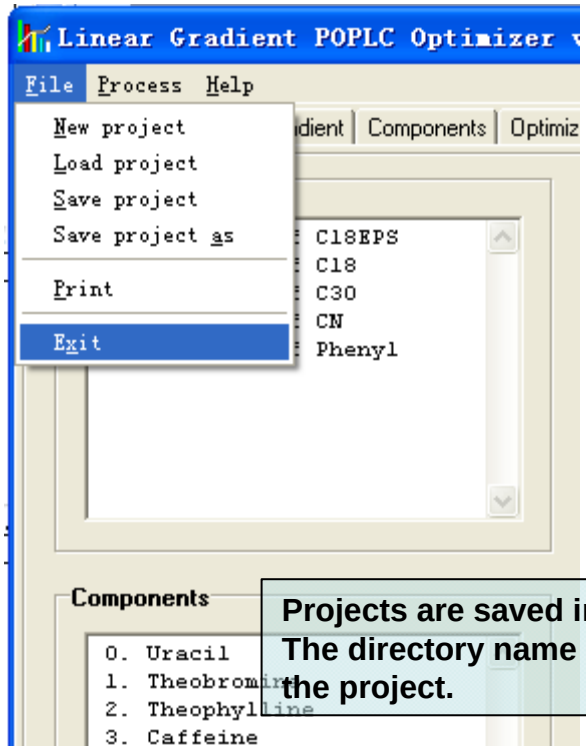
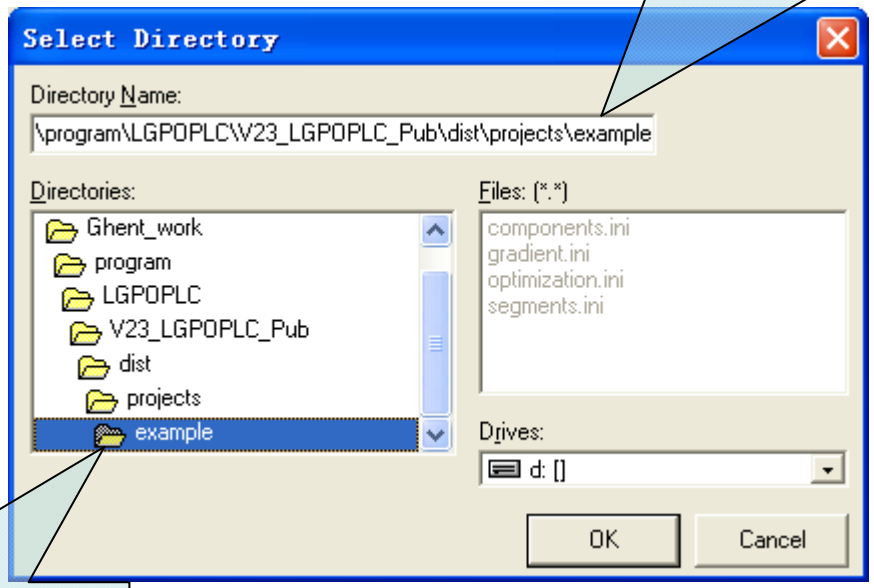
# *Software interface - projects management*

**With project management, you can…**

- **Start a new project from the stored template.**
- **Load data and parameters from a saved project.**
- **Save data and parameters to the current project.**
- **Save data and parameters to a new project.**
- **Print the optimization results.**




**Make sure that the project name appears after the parent directory's name 'projects' and a backslash '\'.**




**Projects are saved in directories. The directory name is the name of the project.**

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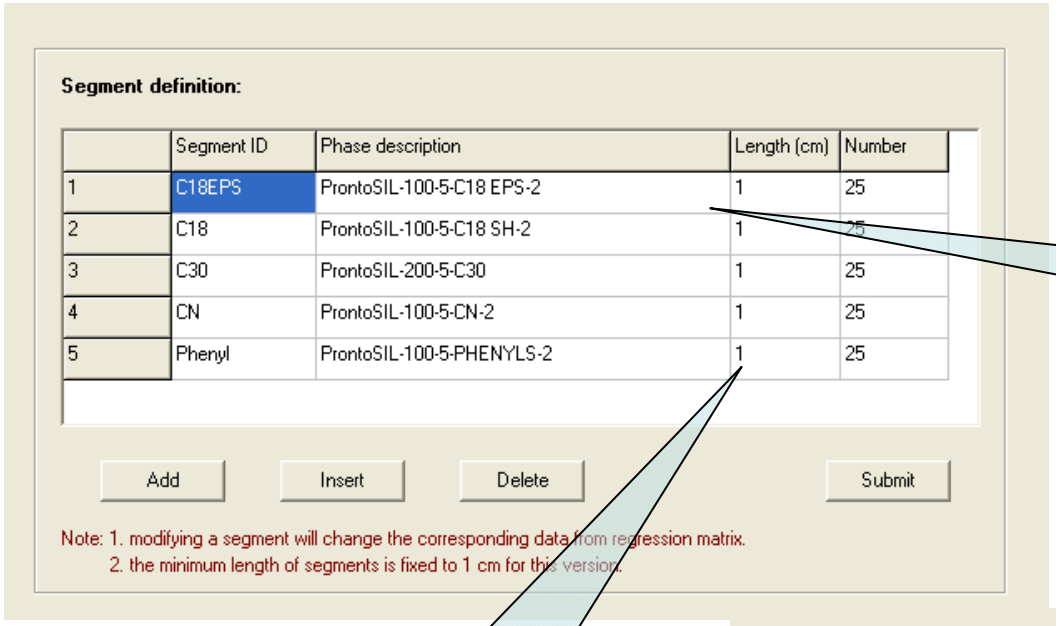
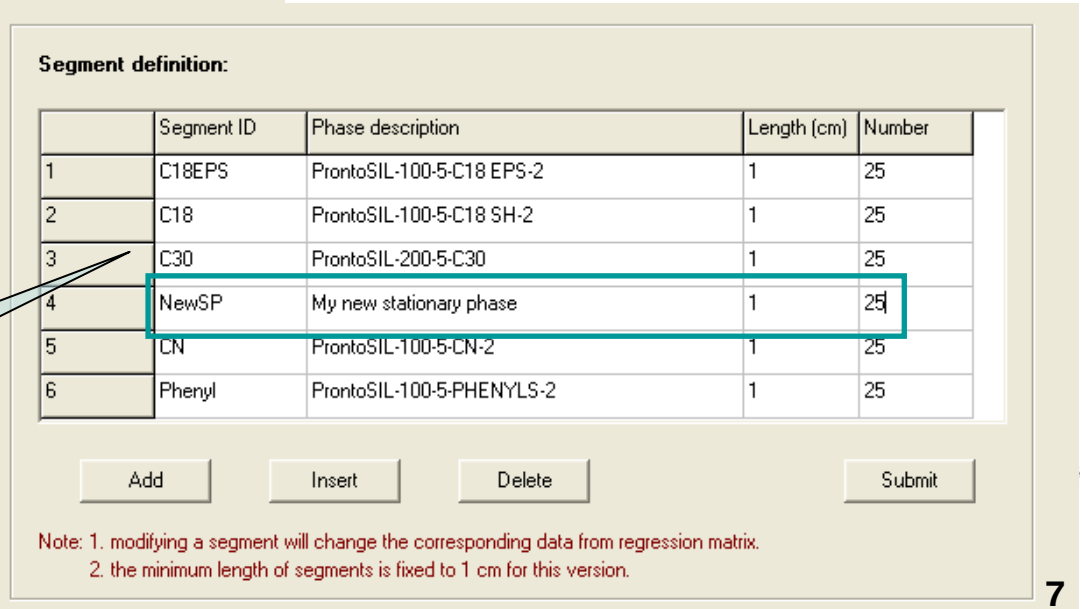
# *Software interface - segments / stationary phase page*



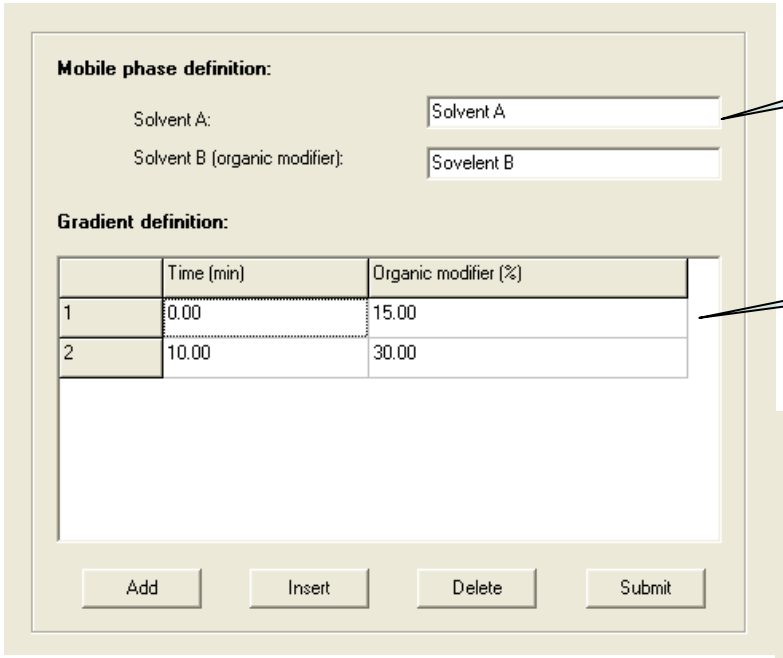

**For default settings, the segments are standard Bischoff POPLink® columns.**

**The minimum segment length is fixed to be 1 cm for this evaluation version.**

**New columns can be added. Max. 6 types of columns can be defined.**

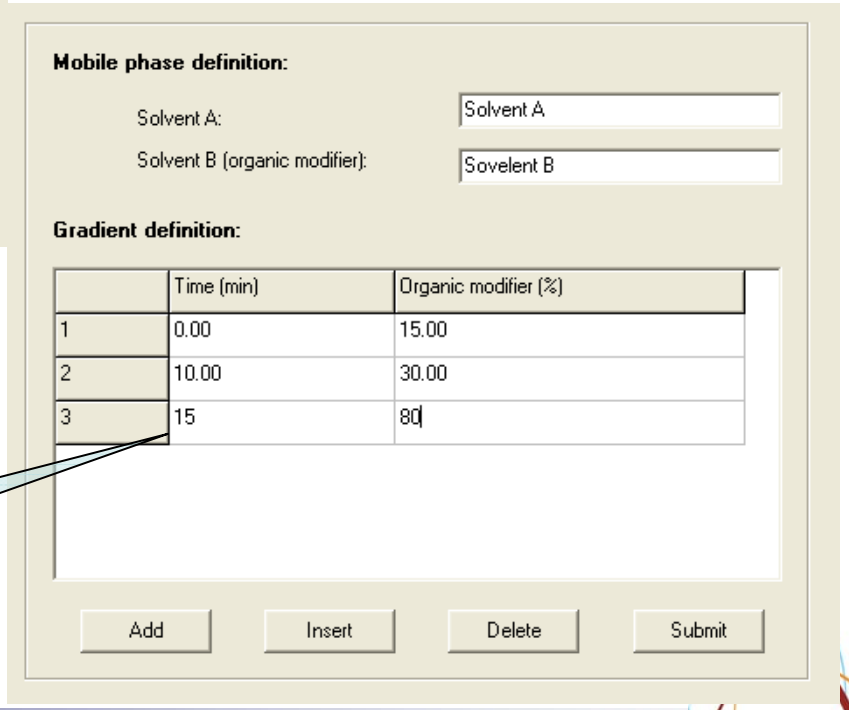


# *Software interface - gradient / mobile phase page*




**Mobile phase name.**

**Multiple gradient steps.**




**New steps can be added. Max. 6 gradient steps (rows) can be defined.**

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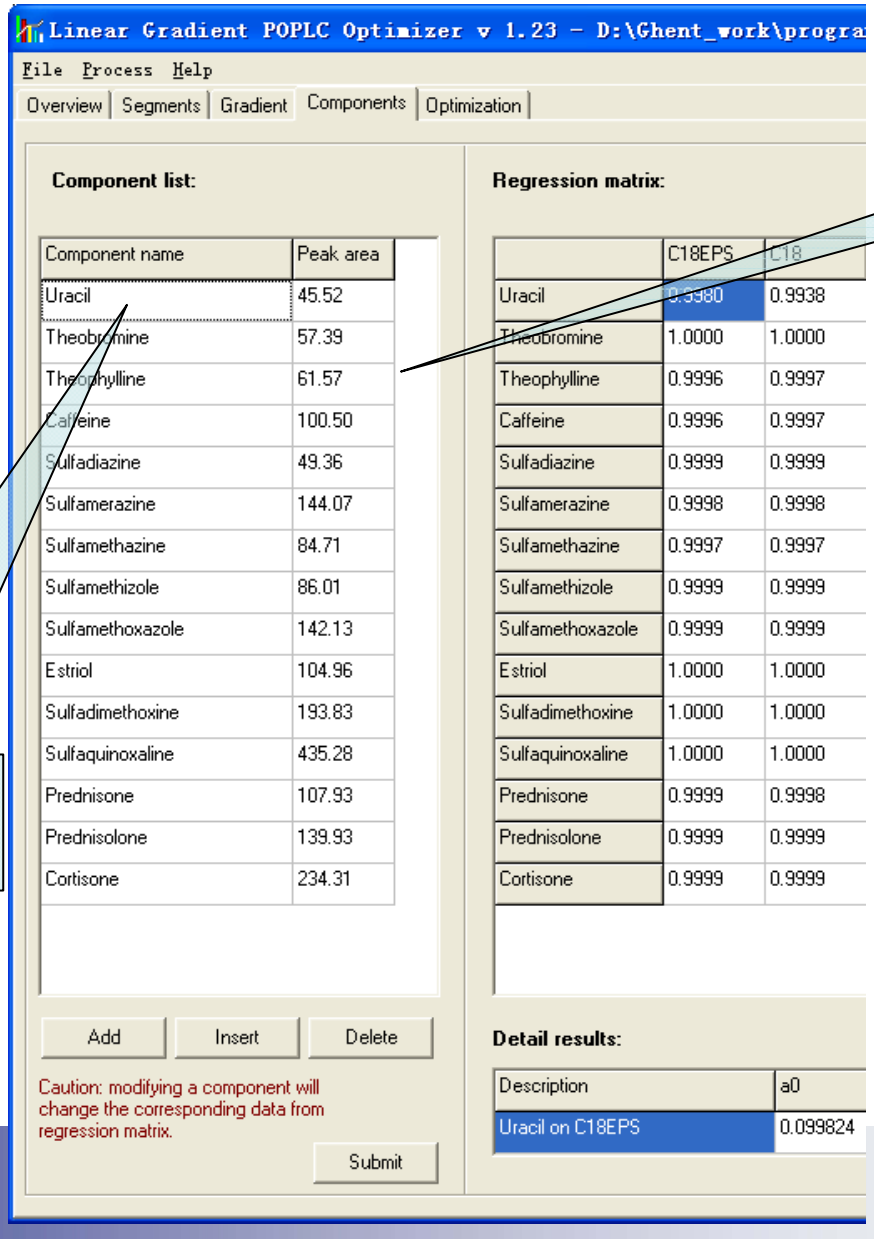
# *Software interface - component / sample page*




**Component list. Max. 20 components can be defined.**

**Void volume marker (unretained marker) is fixed at the top of the list.**

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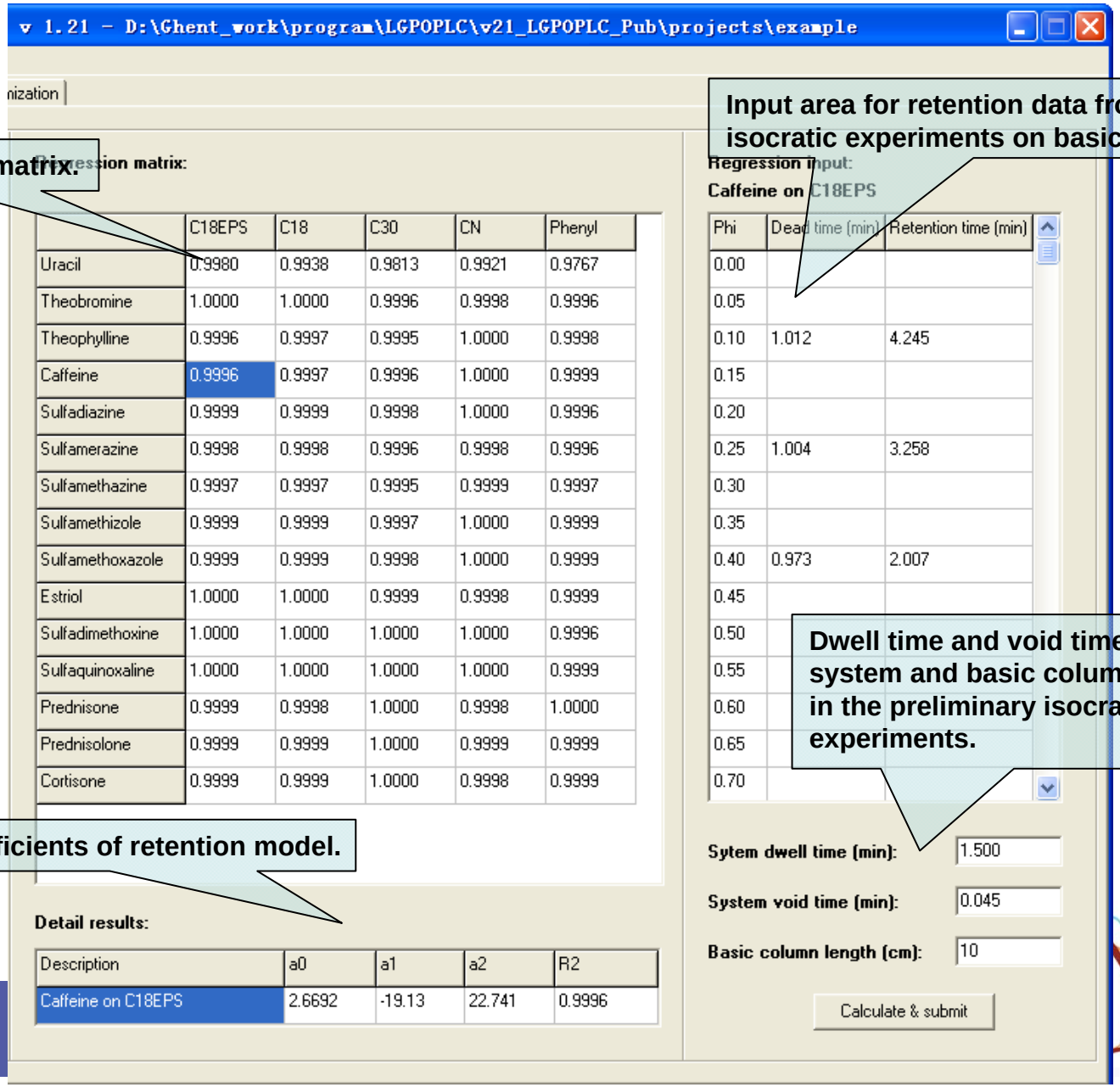
# *Software interface - component / sample page*




**Retention model matrix.**

**Input area for retention data from preliminary isocratic experiments on basic columns.**

**Dwell time and void time of the system and basic column length used in the preliminary isocratic experiments.**

**Regression coefficients of retention model.**

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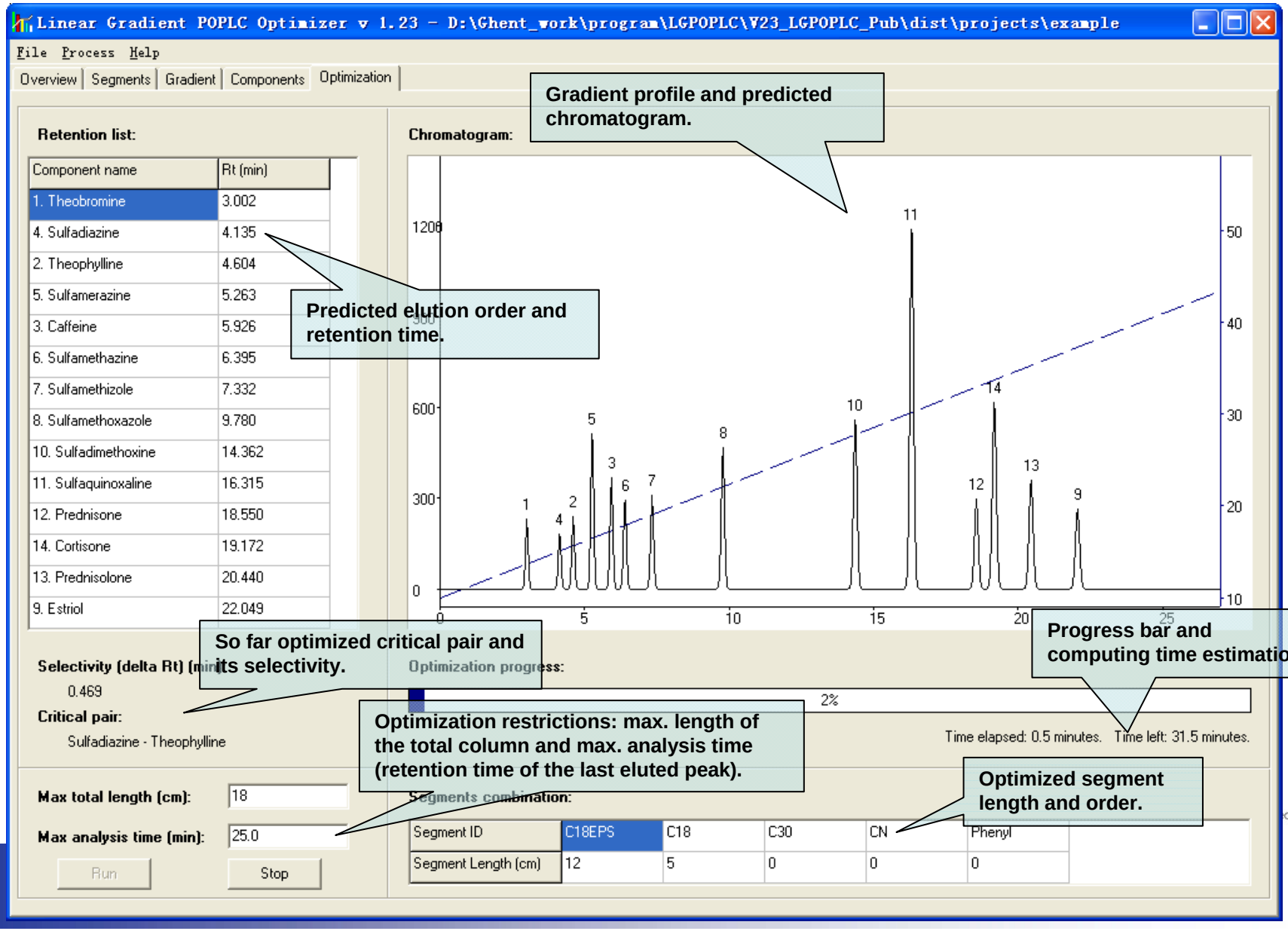
# *Software interface - optimization page*

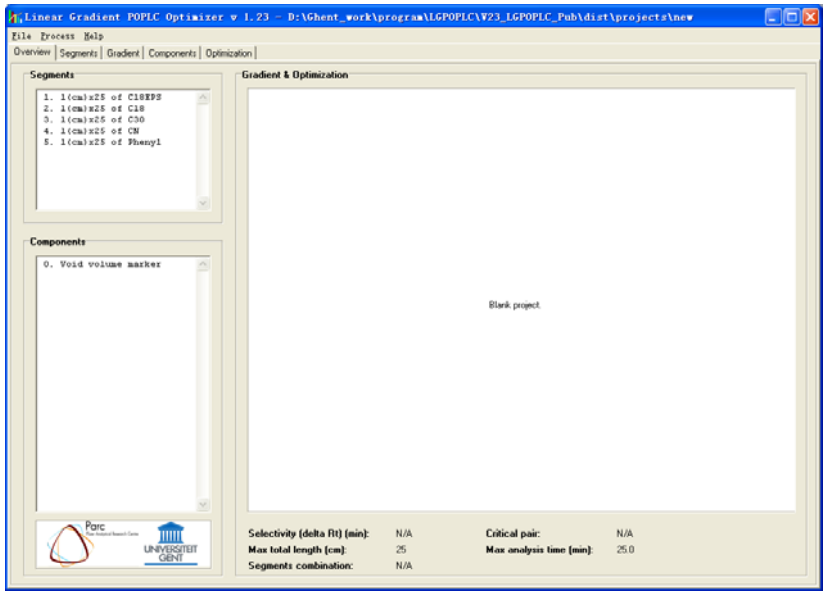


# *Step by step guide – 1. Start a new blank project*

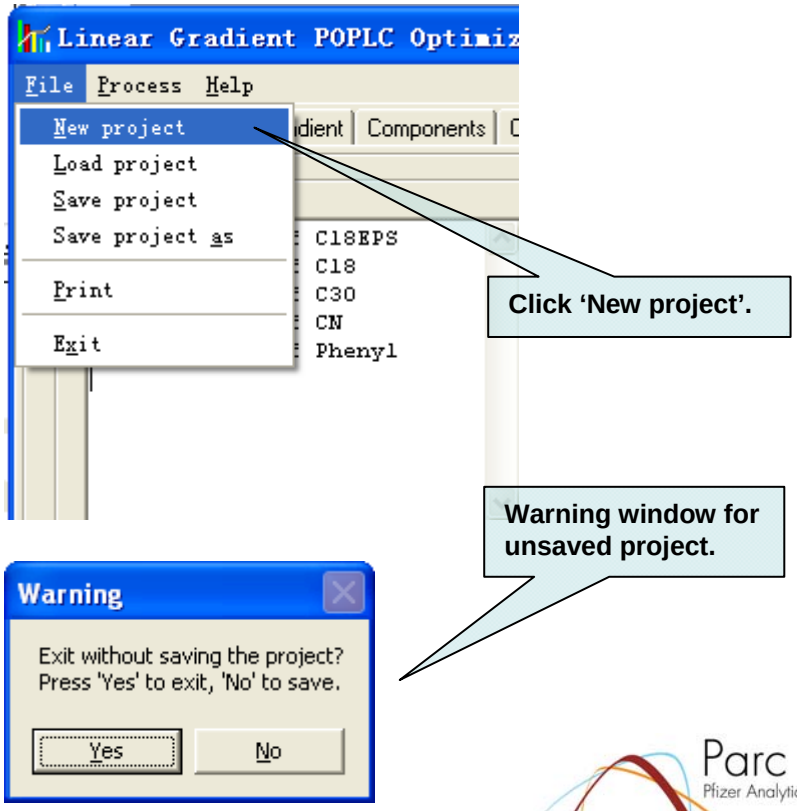
**1.1 When you start the software, the default project is a new blank project.**

**1.2 You can also start a new blank project by**

- **select the ‘File’ menu.**
- **click the ‘New project’ sub menu.**
- **In case the current project is not saved, a warning window will popup.**




**Interface of a blank project**

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# *Step by step guide – 2. Define the segments*

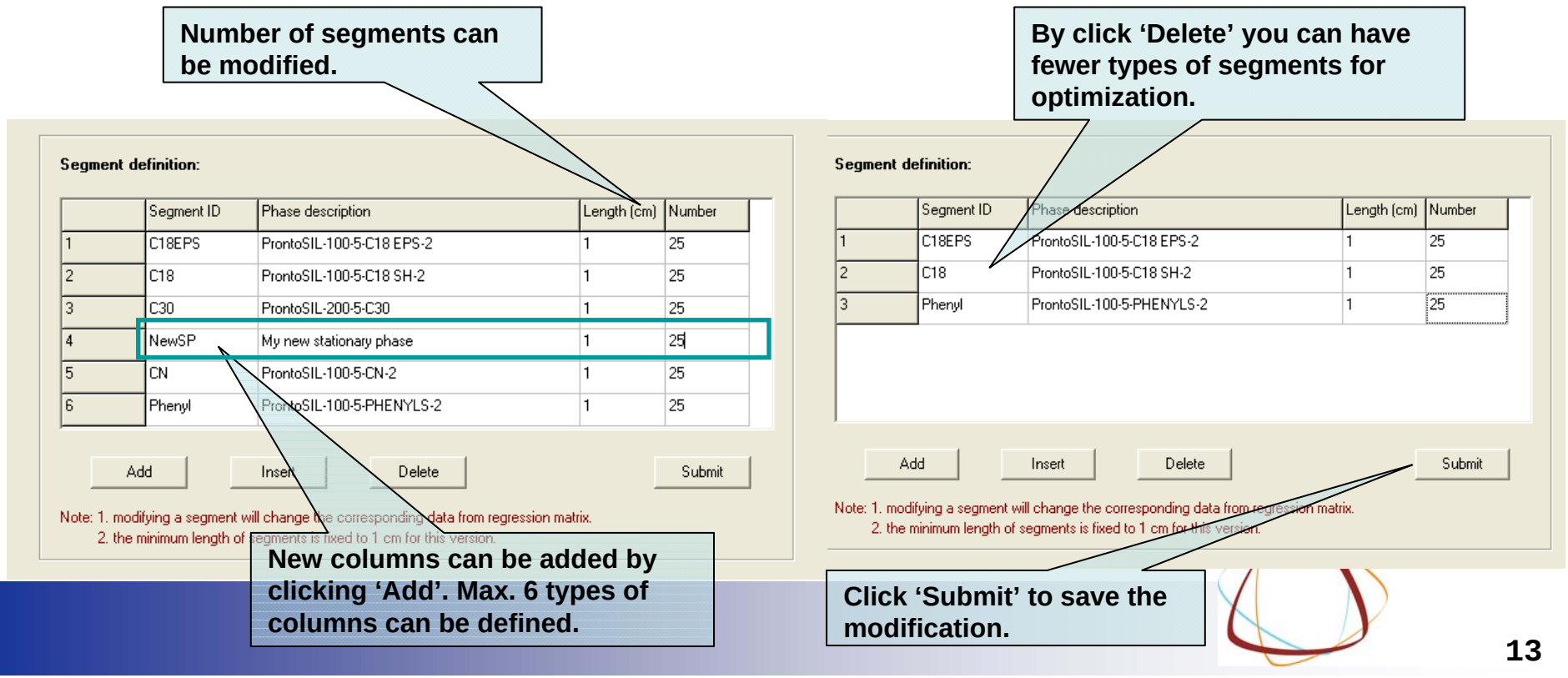
**2.1 The default segments are Bischoff POPLink® columns, which in most cases are sufficient for general purpose analysis.**

- **In case you have a different set of Bischoff columns, you can modify the number of segments.**

**2.2 You can also modify the segment types if…**

- **you have new type segments besides the standard 5 POPLink® columns or**
- **not all types of POPLink® columns is involved in your experiments.**

**2.3 Click 'Submit' to save the modification before you leave this page.**



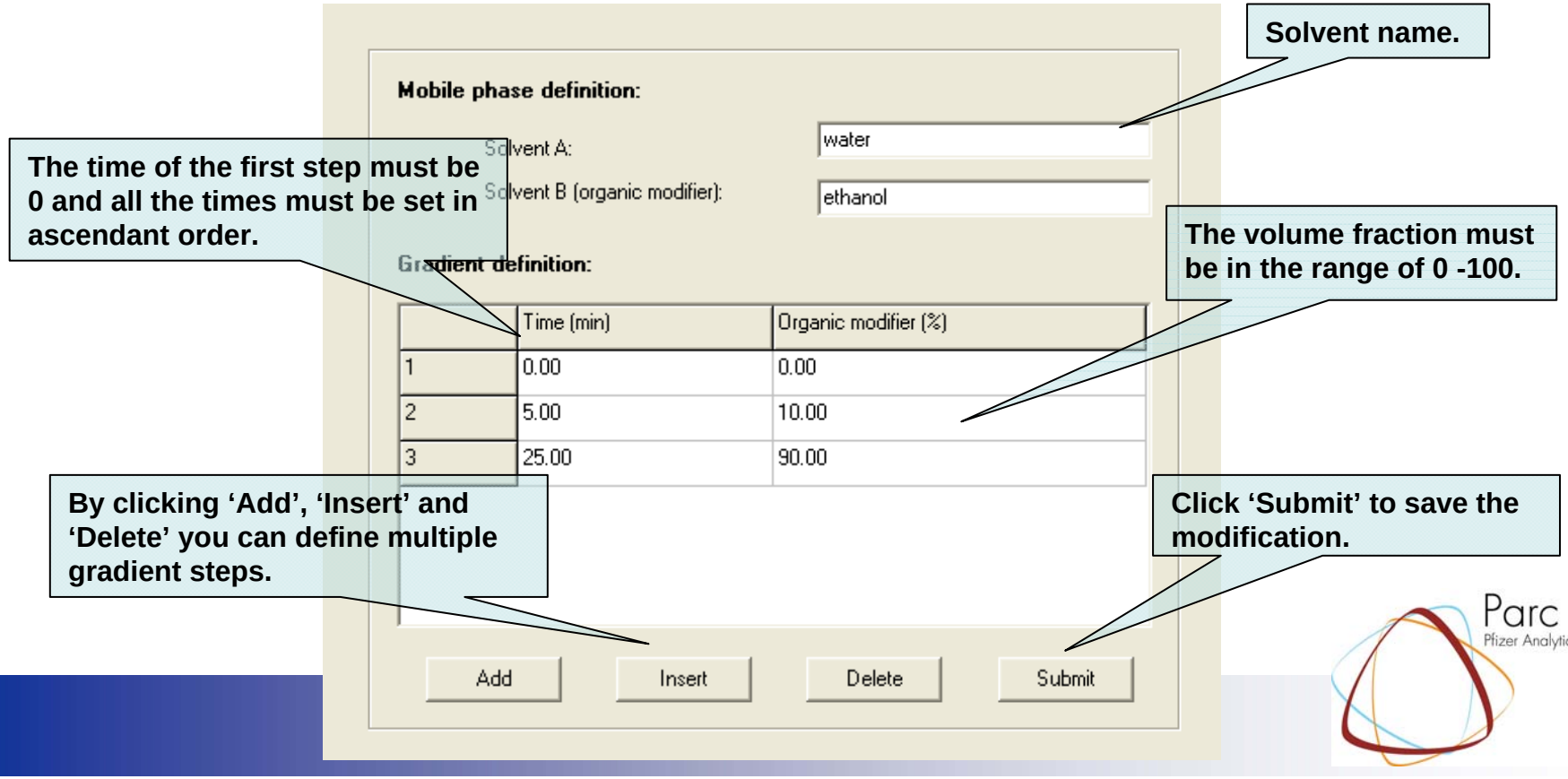
# *Step by step guide – 3. Define the gradient*

**3.1 Input the solvent names into the mobile phase definition blanks.**

**3.2 Modify the gradient profile with up to 6 steps (rows).**

- **The time of the first step must be 0.**
- **The times of defined gradient steps must be set in ascendant order.**
- **The volume fraction of organic modifier must be in the range of 0 – 100.**

**3.3 Click 'Submit' to save the modification before you leave this page.**

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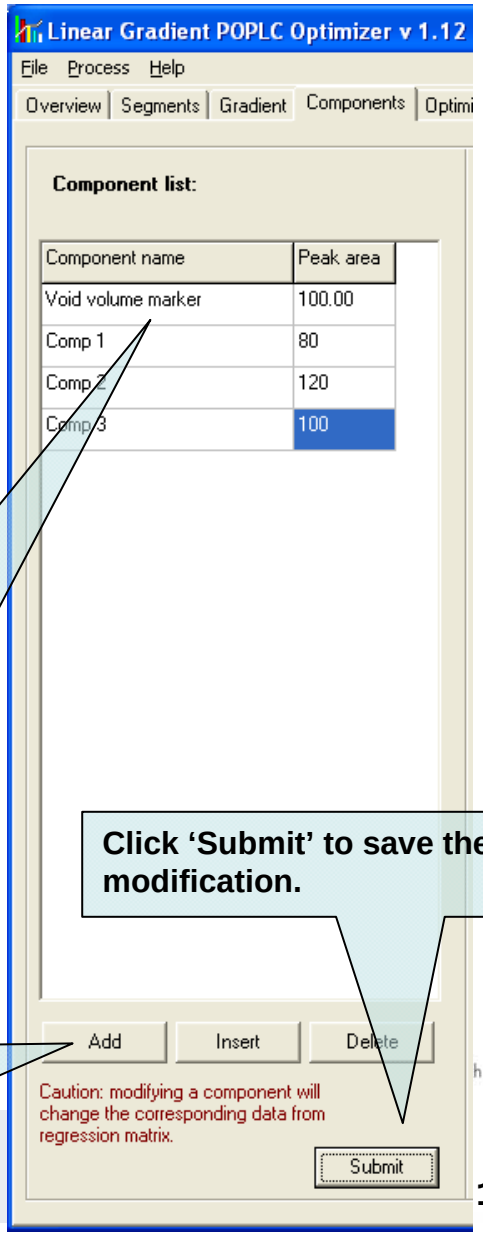
# *Step by step guide – 4. Define the components*

**4.1 Input the component names and peak areas into the component list..**

- **Up to 20 components (void volume marker not included) can be defined in the component list.**
- **The first component is set to void volume marker, which is used to determine the dead time. You can change its name to the compound you use in the real experiment, i.e. uracil or thiourea.**
- **Peak area is only used to regenerate the predicted chromatogram. It does not influence the optimization result.**
- **If you have overlapping peaks in the preliminary isocratic experiments, divide the total peak area into parts and input them as the peak areas of co-eluting components.**

**4.2 Click 'Submit' to save the modification before you leave this page.**




**Void volume marker must be set at the top of the list.**

**Click 'Submit' to save the modification.**

**By clicking 'Add', 'Insert' and 'Delete' you can define the component list.**

# *Step by step guide – 5. Build the retention model*

**5.1 Input the system dwell time and basic column length.**

- **System dwell time is the time of mobile phase migrating from the solvent mixer to the front of column.**
- **System void time is the time of mobile phase migrating through the whole system without column.**
- **Basic column length is the length of columns used in the preliminary isocratic experiments.**

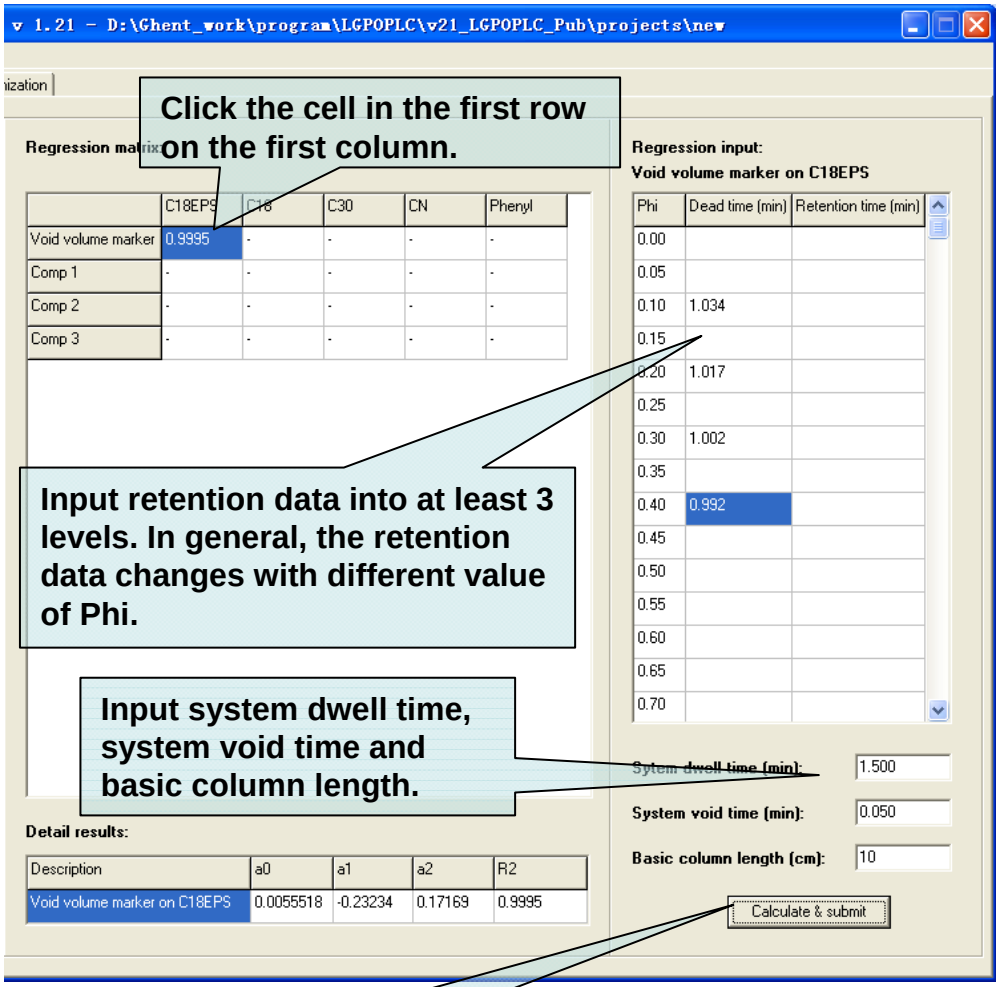
**5.2 Input the dead time determined by void volume marker.**

**5.2.1 In regression matrix, click the cell in the first row (void volume marker) on the first column (C18EPS).**

**5.2.2 In the regression input list, input the retention time of the marker in the column of 'Dead time (min)' at given isocratic levels.**

**5.2.3 Note that at lease 3 isocratic levels are necessary to build the model. You can also change the value of phi (volume fraction of organic modifier) according to your real experiments.**

**5.3 Click 'Calculate & submit', the retention data will be calculated and the regression coefficients will be displayed in the cell of regression matrix.**

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# *Step by step guide – 5. Build the retention model*

**5.4 Input the retention data for the first component.**

**5.4.1 In regression matrix, click the cell in the second row (Comp 1) on the first column (C18EPS).**

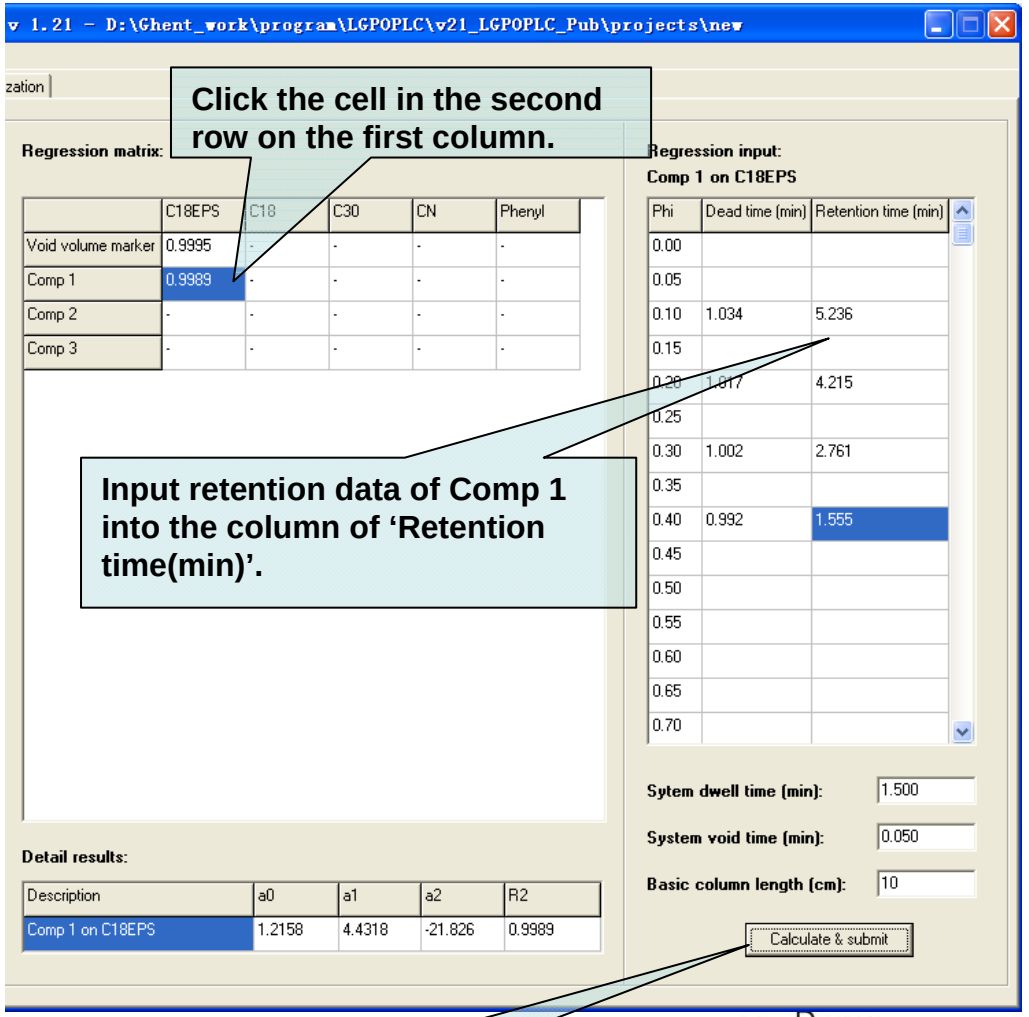
**5.4.2 Keep the dead time data in regression input list unchanged.**

**5.4.3 Input the retention time of Comp 1 in the column of 'Retention time (min)' at corresponding isocratic levels.**

**5.5 Click 'Calculate & submit', the retention data will be calculated and the regression coefficients will be displayed in the cell of regression matrix.**

**5.6 Follow the above-mentioned steps until the whole regression matrix is completely filled.**

**5.7 Check the coefficients displayed in the regression matrix. Verify the original experimental data if the matrix contains any value which is smaller than 0.90.**

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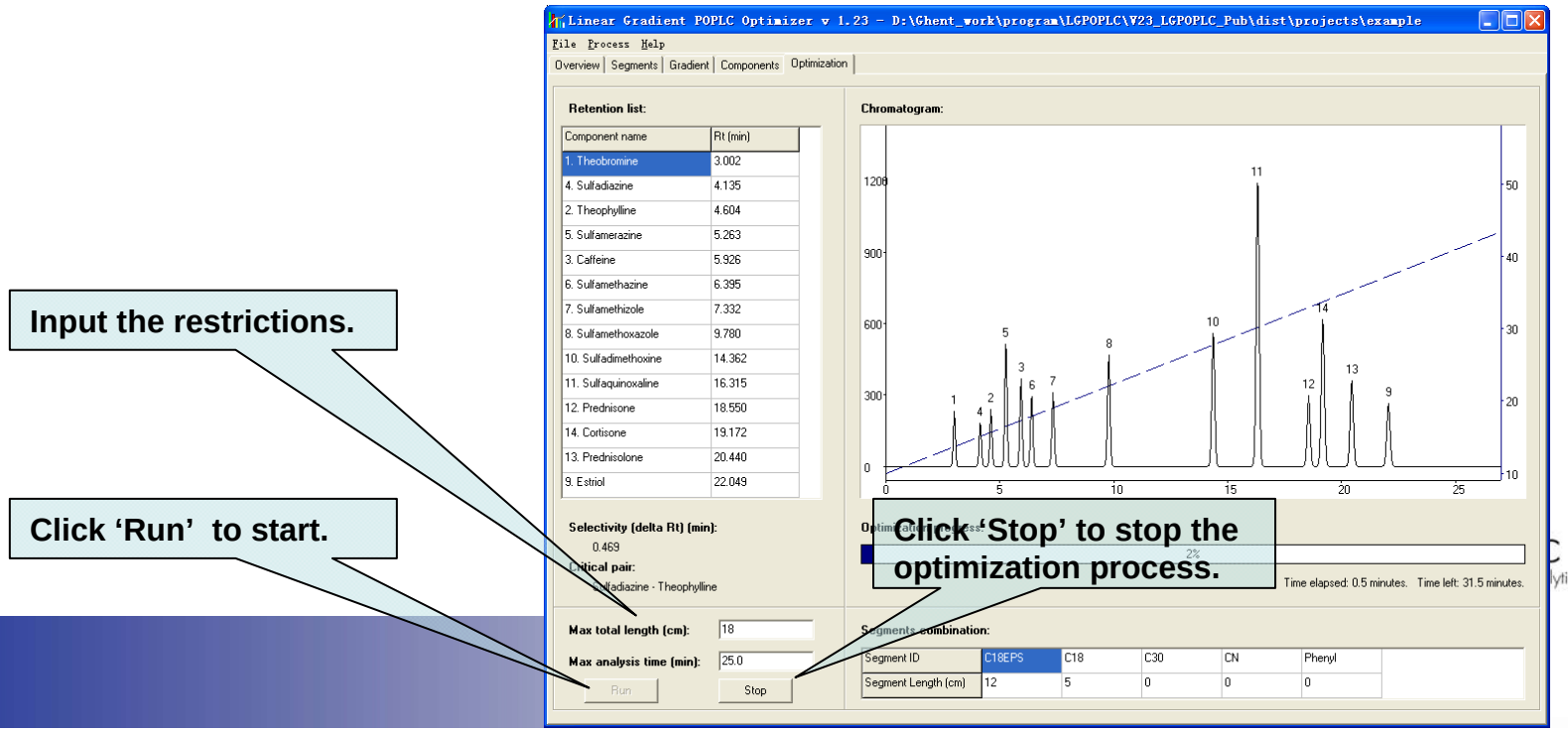
# *Step by step guide – 6. Optimization*

## 6.1 Input the restrictions for optimization.

- Max. total length is the limitation on the total length of segment combination.
- Max. analysis time is the limitation on the retention time of the last eluted component.

## 6.2 Click ‘Run’ to start the optimization.

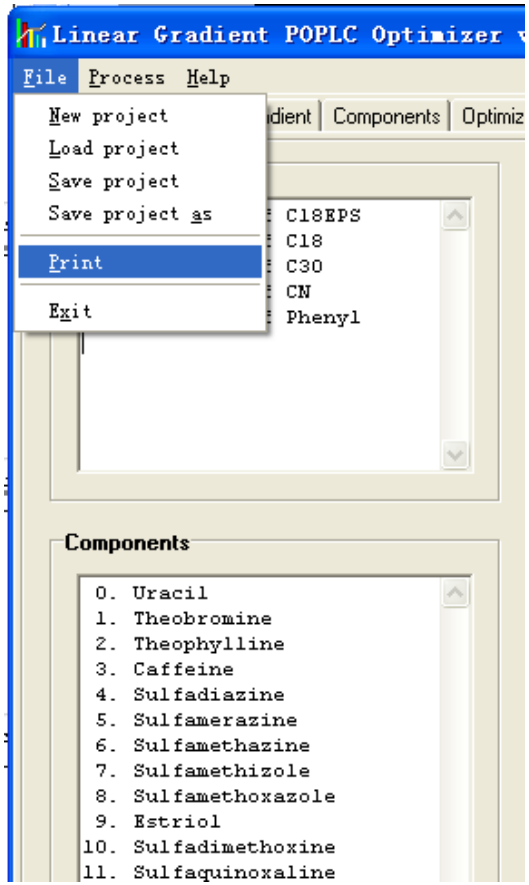
- The computing time for optimization is determined by the number of components, the number of types of segment and the maximum total length. It can varies from a few seconds to a few hours.
- The computing time is estimated and displayed with a progress bar.
- You can stop the optimization process by clicking ‘Stop’.



# *Step by step guide – 7. Print the results*

## 7.1 Optimized results can be printed by clicking the ‘Print’ sub menu.

- **The results consist of the general information of the project, optimal segment combination, predicted retention times and chromatogram, and the corresponding critical pair as well as the retention time difference.**




**OPTIMIZATION REPORT**

Project name: example
generated by Linear Gradient POPLC Optimizer v 1.23
2010-2-23 10:35:14

**General information**

System void time (min): 0.045
System dwell time (min): 1.500

Max total column length (cm): 18
Max analysis time (min): 25.00

**Gradient profile**

Solvent A: Water + 0.1% FA
Solvent B: Ethanol

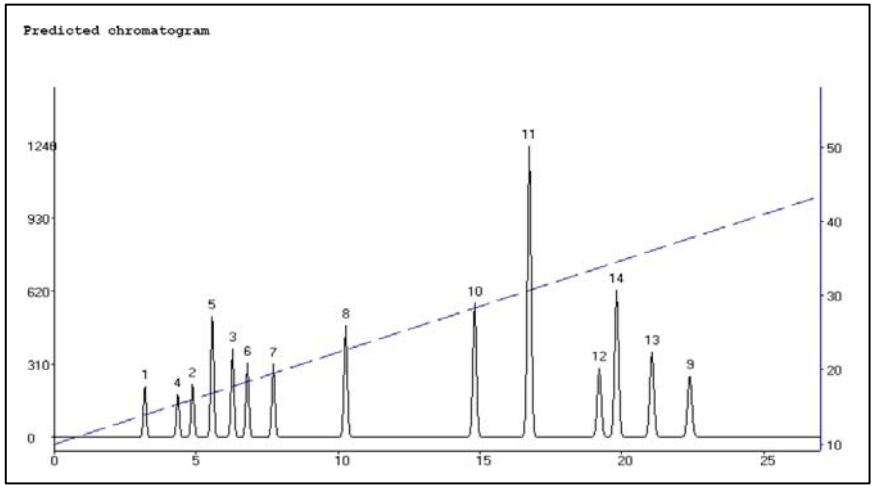
| Time (min) | %B |
|---|---|
| 0.00 | 10.00 |
| 30.00 | 50.00 |

**Optimization results**

Optimal combination: 6 cm C18 + 12 cm C18EPS

Critical pair: Sulfadiazine - Theophylline
Selectivity (delta Rt) (min): 0.521

| No | Component name | Predicted Rt (min) |
|---|---|---|
| 1 | Theobromine | 3.188 |

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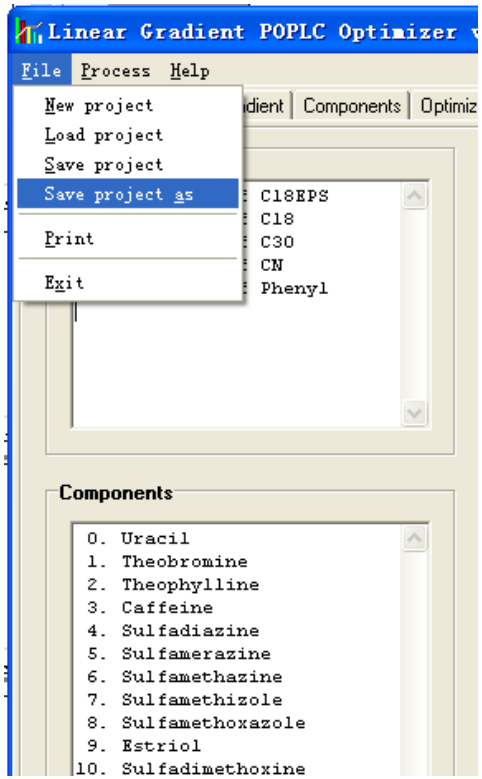
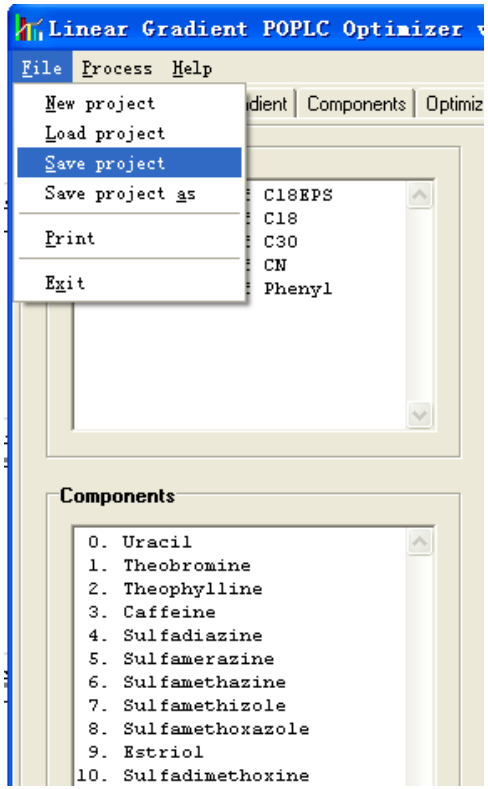
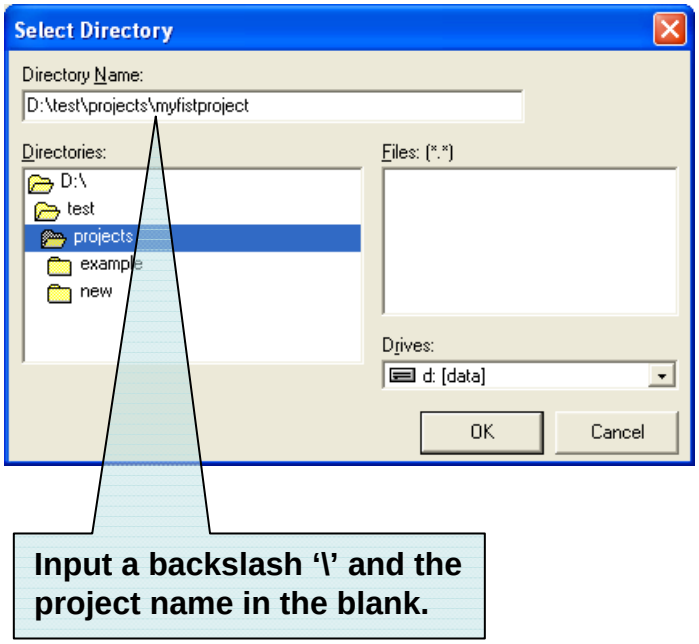
# *Step by step guide – 8. Save project*

**8.1 For a new project, you should click ‘Save project as’ sub menu.**

**8.2 For a loaded project, you can click ‘Save project’ sub menu.**




**Input a backslash ‘\’ and the project name in the blank.**

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# *Frequently asked questions (FAQs)*

**1 Q: When I start the software, why is every page empty?**

**A: This is probably caused by the missing ‘projects’ folder. Please make sure that the ‘projects’ fold exists in the same folder of the executable file. Besides, make sure there is a sub folder named as ‘new’ in the folder of ‘projects’.**

**2 Q: Can I save projects in other directory?**

**A: You can save your working projects in other directory but it is not recommended.**

**3 Q: When I try to load a project, why is there an error message ‘Please double click the name of the project to be loaded’?**

**A: Each project is saved in a directory. When the ‘Select Directory’ window pops up, you should double click the directory with your project name in the directory tree (left part of the window). Then the full directory name with the project name will be displayed in the ‘Directory Name’ blank (top part of the window). And several files with .ini suffix will be displayed in the ‘Files’ list (right part of the window). Then, click ‘OK’.**

**4 Q: When I try to save a new project, why is there an error message ‘Project name missing. Please input the name of project after the directory name "projects\"’?**

**A: For saving a new project or saving the project as a new name, you should input the project name manually into the ‘Directory Name’ blank (top part of the window). Input a backslash ‘\’ first, then input the new project name and click ‘OK’.**

# *Frequently asked questions (FAQs)*

**5 Q: When I try to submit data, why is there an error message 'Invalid numeric data. Possible reasons: 1. character data input. 2. comma "," instead of full stop is used as decimal separator'?**

**A: This is caused by submitting an invalid numeric data. Numeric data consists of only digits and / or full stop ".".**

**6 Q: When I try to change to next page, why is there a warning message 'Exit without submitting data? Press "Yes" to exit, "No" to stay'?**

**A: After any modification, you should click 'Submit' button to submit the modification to the software. If you change to another page without submitting the data, the modification made on this page will be ignored. You can click 'No' to stay at the current page or 'Yes' to change pages without submitting.**

**7 Q: What is the proper set of Phi values for preliminary isocratic experiments?**

**A: In general, it could be better if the range of Phi values covers the start point and end point of gradient profile. The larger the number of isocratic levels is, the more accurate optimization result will be obtained.**

**8 Q: Can I have data sets of different Phi levels for different components?**

**A: Yes.**

**9 Q: When I try to input the retention data, why is there a warning message 'Unqualified data. Retention time must be larger than dead time and the both times must larger than 0'?**

**A: This software can't be used to optimize the separation of sample which has no retention on the given columns. Thus, the retention time of component must be larger than the dead time.**

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# *Frequently asked questions (FAQs)*

**10 Q: When I try to input the retention data, why is there an error message 'Unqualified data. In general, dead time changes at different levels of volume fraction of organic modifier.'?**

**A: This is probably caused by submitting exactly the same dead time value for different isocratic levels. In real experiments, retention times of both void volume marker and components will decrease along with the increment of volume fraction of organic modifier in mobile phase.**

**11 Q: When I try to run optimization, why is there a warning message 'Regression data is not complete or regression coefficient is too smaller.'?**

**A: Before optimization, the regression matrix must be completely filled. In addition, the regression coefficients should be large enough so that the optimization result can be reliable.**

**12 Q: Why does the experimental chromatogram with optimized segment combination have poorer resolution than the predicted chromatogram?**

**A: The current evaluation version of gradient POPLC® software can only optimize the selectivity of the separation under given gradient profile. It can only predict the selectivity but not resolution of the separation. The resolution in experimental chromatogram depends on the efficiency of real columns and the components in the mixture.**

# *Copyright statement*

**Linear Gradient POPLC® Optimizer**

**Evaluation version 1.23**

**Developed by Kai Chen**

**In cooperation with Maarten De Beer, Frederic Lynen, Pat Sandra from Ghent University, Melissa Hanna-Brown, Paul Ferguson, Laure Hitzel from Pfizer Global R&D Labs Sandwich and Bischoff Chromatography.**

**This evaluation version can be downloaded free of charge from the website of Research Institute for Chromatography (RIC) or Bischoff Chromatography.**

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**http://www.poplc.de/**