Overview of the LC×LC method optimisation program MDMO

Introduction

A brief overview of the LC×LC method optimisation program is provided. The program was written in-house in a MATLAB 2019b (The Mathworks, Natick, USA) environment, and works best using this version of MATLAB. Further details on the development, application and capabilities of the program can be found in [1-4].

Definitions

To simplify the program description, some concepts used by the program are first be defined:

Standards:	Compounds for which both the plate height data (reduced Van Deemter parameters and diffusion coefficient/molecular volume) and retention parameters are entered as input.
Analytes:	Sample specific compounds for which only the retention parameters are entered.
Standards set:	A group of standards.
Analytes set:	A group of analytes.
Experimental conditions set:	Contains the values (or ranges of values) of all the fixed and optimisable experimental parameters, as well as system information required to predict performance.
Permutation:	One combination of experimental parameter values.
Method settings set:	Contains settings specifying how certain values should be calculated, user defined restrictions to fine-tune the desirable results, and the optimisation objectives.
Results set:	Represents one optimisation attempt. Contains standard sets (^{1}D and ^{2}D), analyte sets (optional), experimental conditions set, method settings and results (if the results have been calculated already).
Results:	All the points (experimental conditions) remaining in the result set after performance was calculated. This can either be achievable results (if optimisation has not been performed yet), or optimal results.
Points:	Each point refers to one permutation with its corresponding performance values. Represents one set of experimental conditions (one analysis).
Calculate:	Process of calculating the performance for each permutation in the result set.
Optimise:	Removing points that are not optimal in terms of the optimisation objectives from the results set.
Simulate:	Process of predicting elution profiles of compounds using the algorithm designed by Stoll and co-workers [5,6].

Program overview

The main steps of the LC×LC method optimisation program are provided in **Fig 1**. Each step will be further discussed in the following sections.

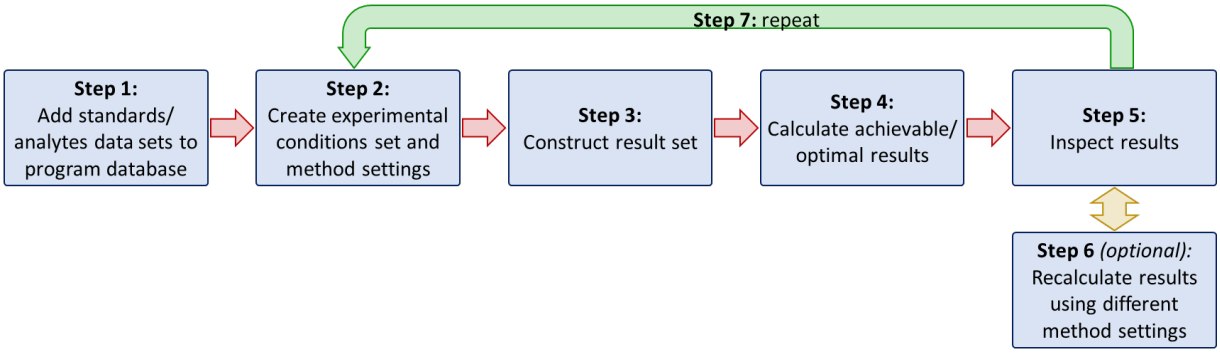


Figure 1. Main steps in the LC×LC method optimisation program.

Step 1: Add standards and analytes data sets to program database

Standards set

Standards are used to predict kinetic performance. In the absence of measured plate height and retention values, generic values can also be entered for the standards, although this will influence the accuracy of the predictions. An example of a sample set is provided in **Fig 2**, with further details provided on the relevant parts indicated. Once created, standards sets are saved to the database of the program for future use or editing (**Fig 3**).

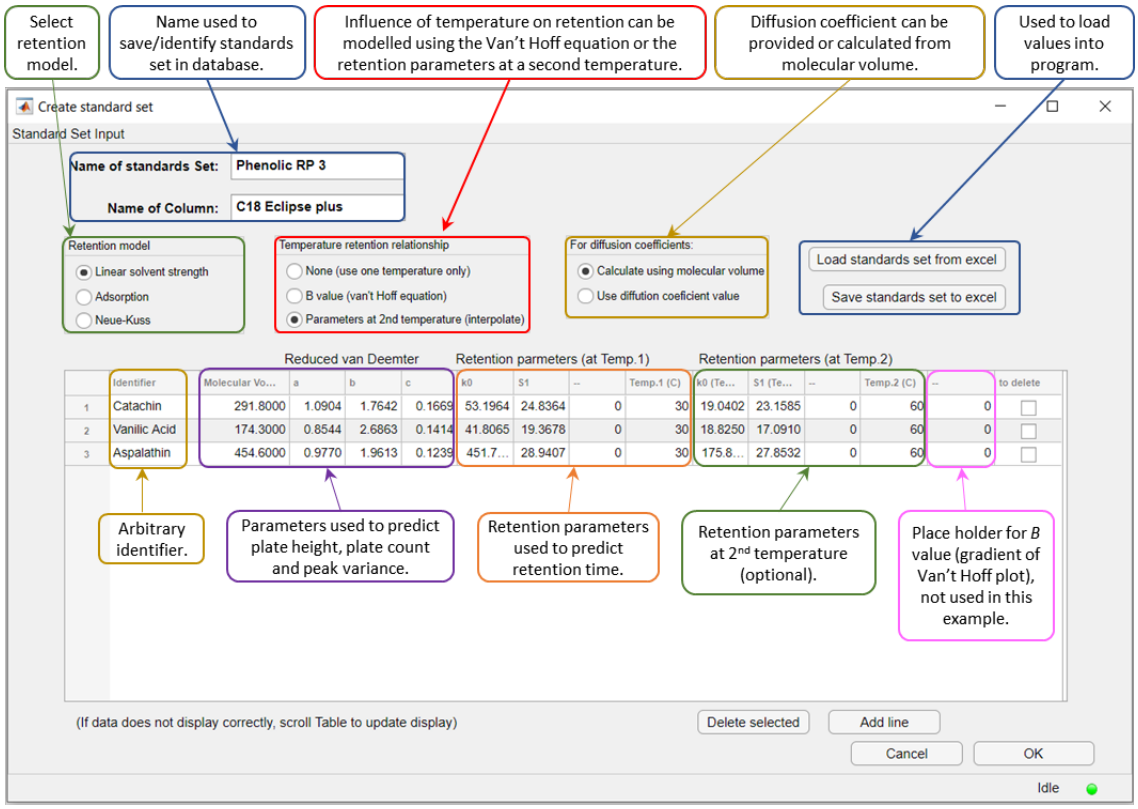
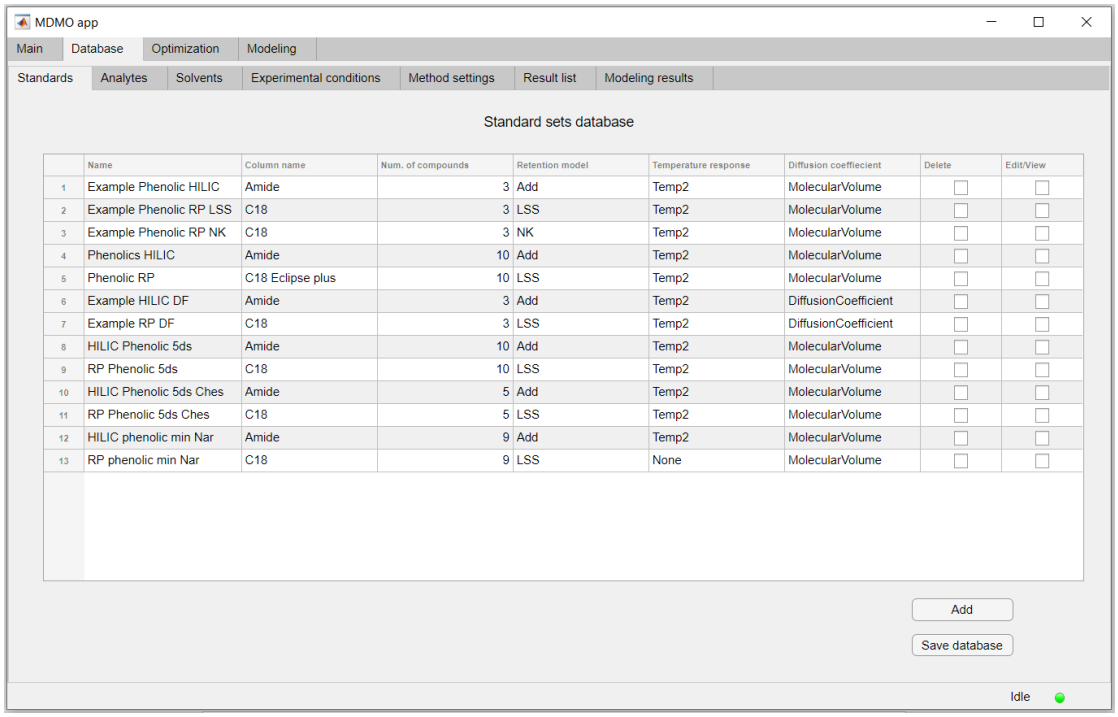


Figure 2. Graphical interface window used to add a standards set.

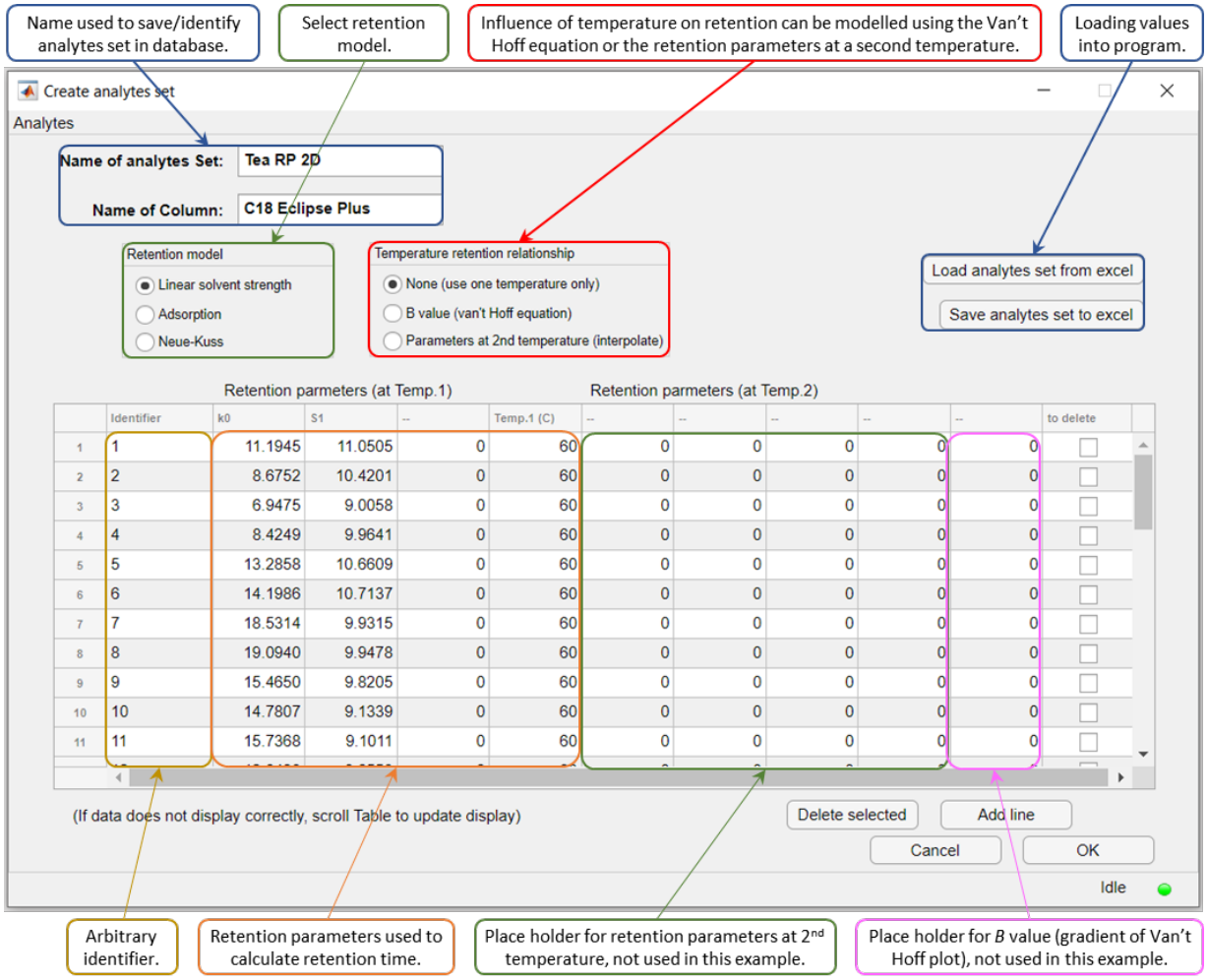


	Name	Column name	Num. of compounds	Retention model	Temperature response	Diffusion coeffiecient	Delete	Edit/View
1	Example Phenolic HILIC	Amide	3	Add	Temp2	MolecularVolume	☐	☐
2	Example Phenolic RP LSS	C18	3	LSS	Temp2	MolecularVolume	☐	☐
3	Example Phenolic RP NK	C18	3	NK	Temp2	MolecularVolume	☐	☐
4	Phenolics HILIC	Amide	10	Add	Temp2	MolecularVolume	☐	☐
5	Phenolic RP	C18 Eclipse plus	10	LSS	Temp2	MolecularVolume	☐	☐
6	Example HILIC DF	Amide	3	Add	Temp2	DiffusionCoefficient	☐	☐
7	Example RP DF	C18	3	LSS	Temp2	DiffusionCoefficient	☐	☐
8	HILIC Phenolic 5ds	Amide	10	Add	Temp2	MolecularVolume	☐	☐
9	RP Phenolic 5ds	C18	10	LSS	Temp2	MolecularVolume	☐	☐
10	HILIC Phenolic 5ds Ches	Amide	5	Add	Temp2	MolecularVolume	☐	☐
11	RP Phenolic 5ds Ches	C18	5	LSS	Temp2	MolecularVolume	☐	☐
12	HILIC phenolic min Nar	Amide	9	Add	Temp2	MolecularVolume	☐	☐
13	RP phenolic min Nar	C18	9	LSS	None	MolecularVolume	☐	☐

Figure 3. Example of standards sets saved in the database.

Analytes

Analytes are used to optimise resolution and orthogonality for a specific sample. Analyte retention parameters must be measured, generalised values cannot be used. This is generally done using scouting gradients. In the absence of analyte sets to use, the standards will be regarded as the sample analytes. An example of an analyte set is provided in **Fig 4**. Analyte sets are saved to the database of the program for future use or editing, in the same way as standard sets.



	Identifier	k0	S1	--	Temp.1 (C)	--	--	--	--	--	to delete
1	1	11.1945	11.0505	0	60	0	0	0	0	0	
2	2	8.6752	10.4201	0	60	0	0	0	0	0	
3	3	6.9475	9.0058	0	60	0	0	0	0	0	
4	4	8.4249	9.9641	0	60	0	0	0	0	0	
5	5	13.2858	10.6609	0	60	0	0	0	0	0	
6	6	14.1986	10.7137	0	60	0	0	0	0	0	
7	7	18.5314	9.9315	0	60	0	0	0	0	0	
8	8	19.0940	9.9478	0	60	0	0	0	0	0	
9	9	15.4650	9.8205	0	60	0	0	0	0	0	
10	10	14.7807	9.1339	0	60	0	0	0	0	0	
11	11	15.7368	9.1011	0	60	0	0	0	0	0	

Figure 4. Graphical interface window used to add an analyte set.

Step 2: Create experimental conditions set and method settings set

Experimental conditions

In the experimental conditions set the values of various optimisable and fixed parameters are specified, as well as some restrictions. Apart from the standards/analytes' properties, these are all the values required to calculate performance. Conditions required include column dimensions, analysis and sampling times, flow rates, modulation parameters and gradients. Most optimisable chromatographic parameters, as well as gradient and column parameters can be varied between minimum and maximum values using user-defined step values. An example of an experimental conditions set is provided in **Fig 5**.

Method settings set

In the method settings set the user can specify how certain values must be calculated and add specific restrictions to control the desired results. The optimisation objectives are also selected in the method settings set. An example of a method settings set is provided in **Fig 6**, along with a short description of the different features.

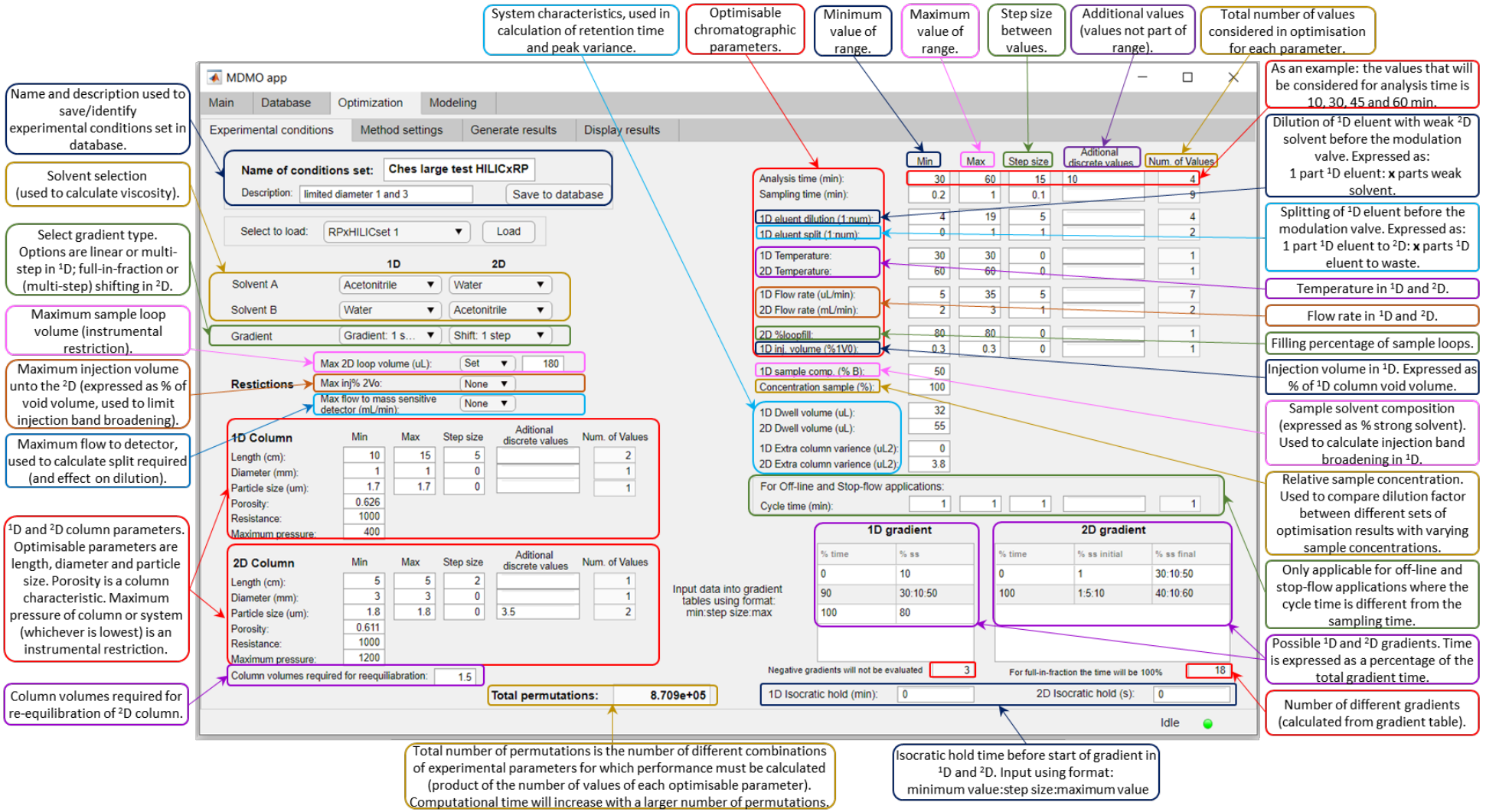


Figure 5. Graphical interface window used to create an experimental conditions set.

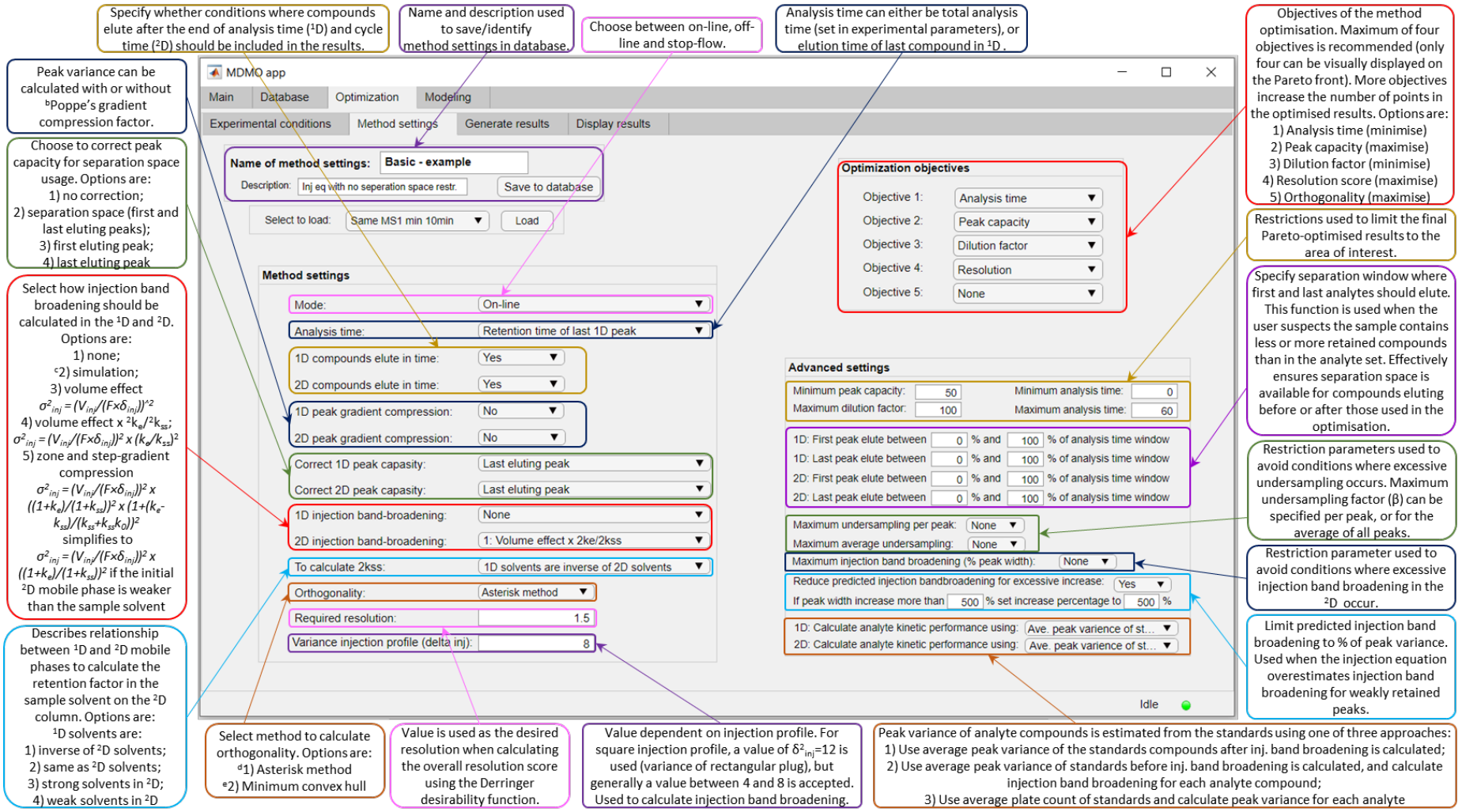


Figure 6. Graphical interface window used to create a method settings set.
[a]Symbols: σ^2_{inj}: peak variance caused by the injection process; V_{inj}: injection volume; F: flow rate; δ_{inj}: standard deviation of injection plug profile; k_e: retention factor at moment of elution; k_{ss}: retention factor in sample solvent; k_0: retention factor in initial mobile phase.; [b][7,8]; [c]Simulated according to [5,6]; [d][9]; [e][10].

Step 3: Construct result set

A result set represents one optimisation attempt. A result set is constructed by selecting the relevant standards sets and analytes sets (optional), along with an experimental conditions set and method settings set (**Fig 7**). A result set can be created, calculated and optimised.

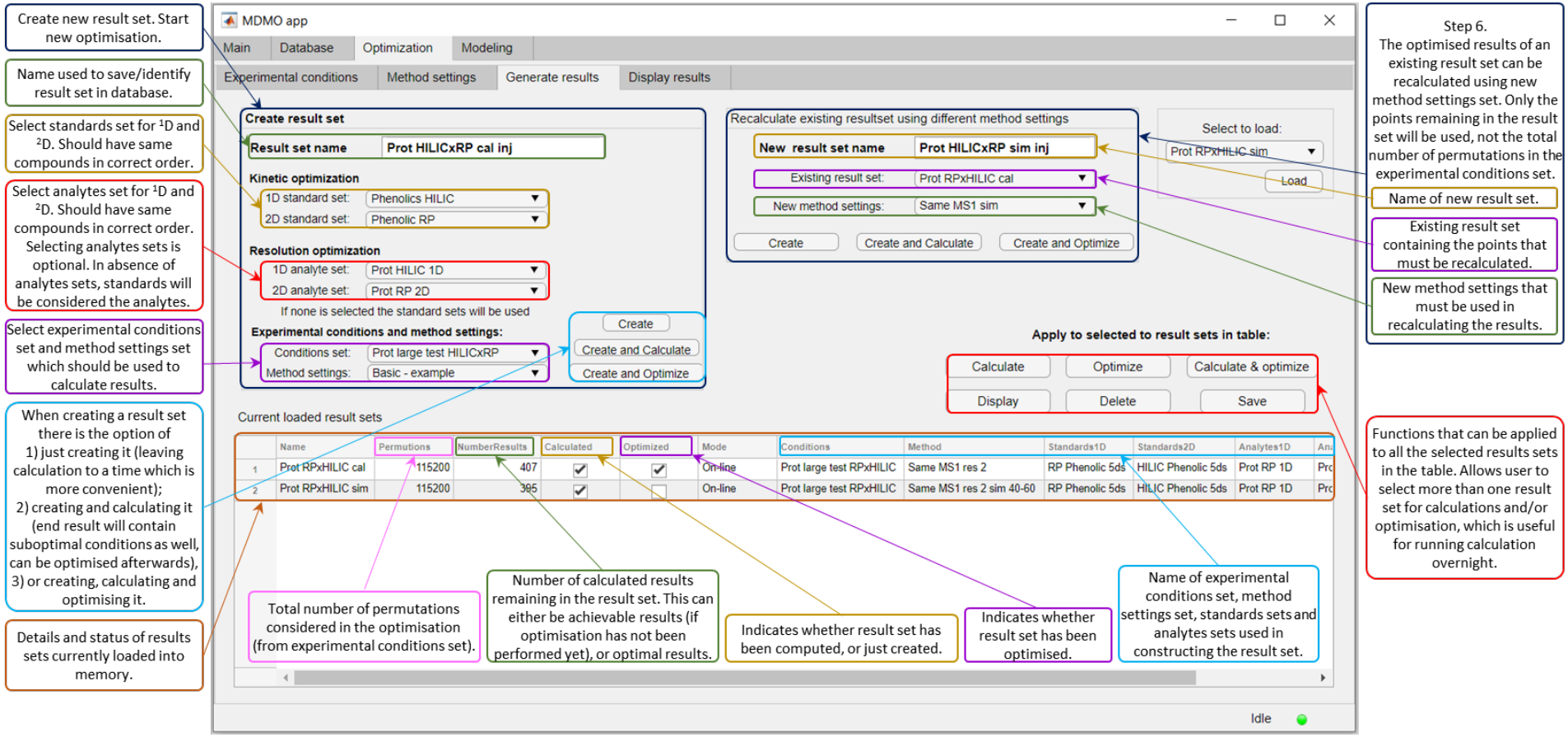


Figure 7. Graphical interface window used to create and manage result sets.

Step 4. Calculate and optimise results

Calculation is performed using an iterative process where performance for each permutation in the experimental conditions set is calculated. The algorithm used to calculate the results is shown in **Fig 8**. After calculation is complete, multi-objective optimisation is performed by comparing the values of the optimisation objectives of each point in the results with each other point in the results. Only points that display the best performance in terms of the optimisation objectives are kept, while the points resulting in sub-optimal performance are removed from the results.

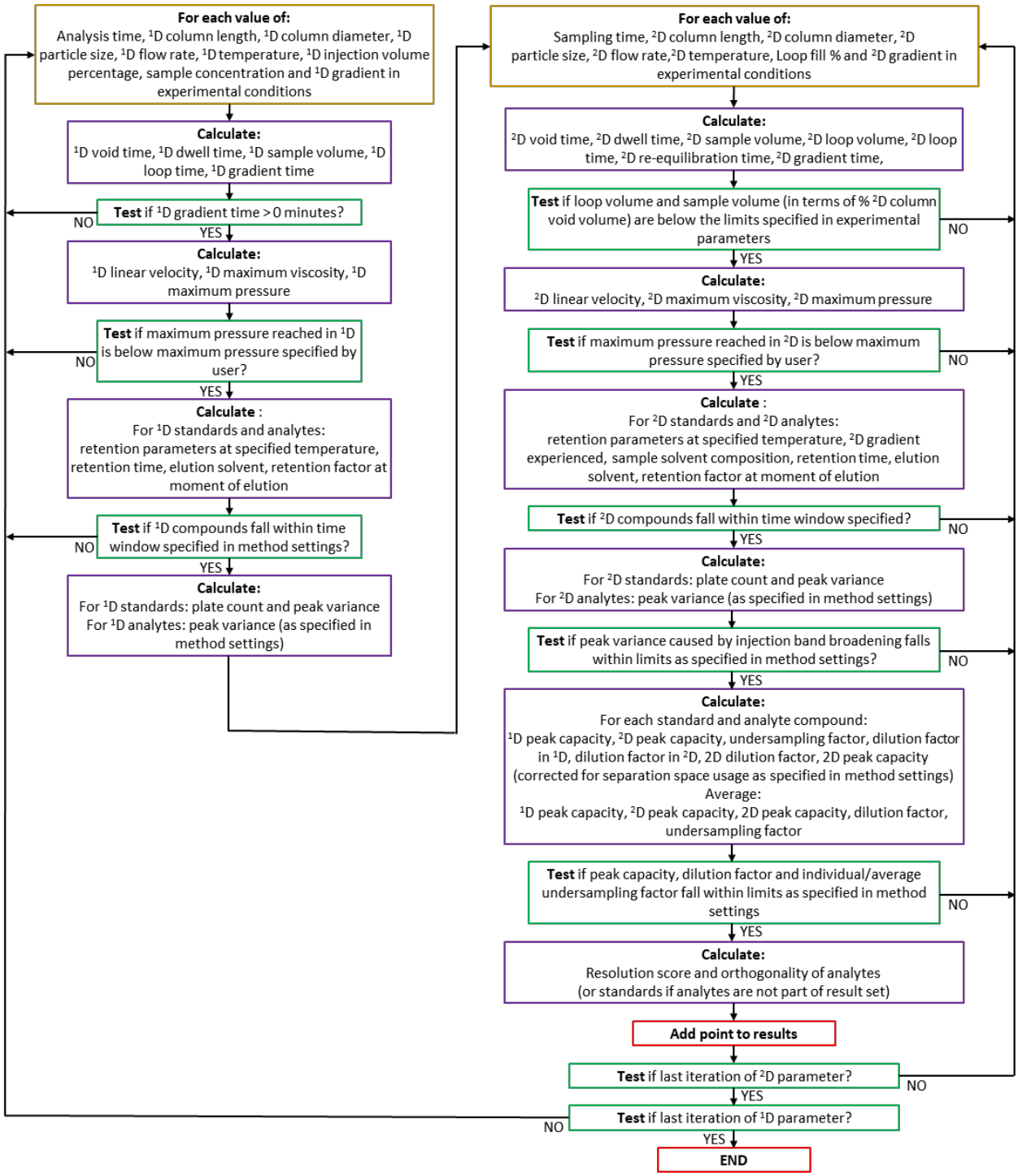


Figure 8. Algorithm used by program to calculate results.

Step 5. Inspect results

After calculating and optimising the results, the points on the Pareto front can be inspected to find suitable conditions. The user can view the experimental parameters associated with each point, as well as the predicted contour plot for the standards and analytes (**Fig 9**). The predicted contour plot can also be viewed as a three-dimensional surface plot, using either the calculated peak variances, or by simulating each peak (for more accurate elution profiles). An example of such a three-dimensional surface plot is provided in **Fig 10**.

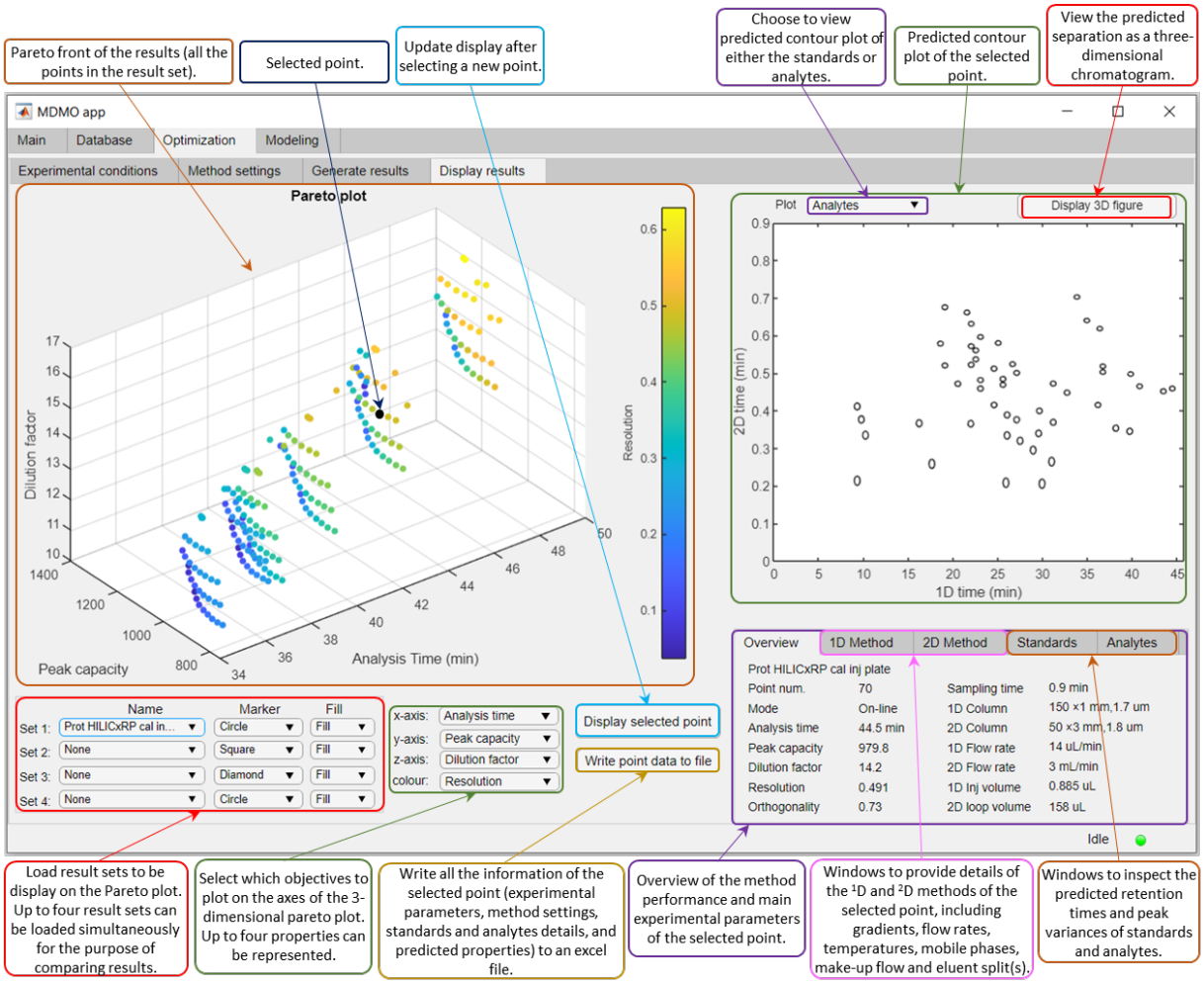


Figure 9. Graphical interface window used to view and inspect points on the Pareto front.

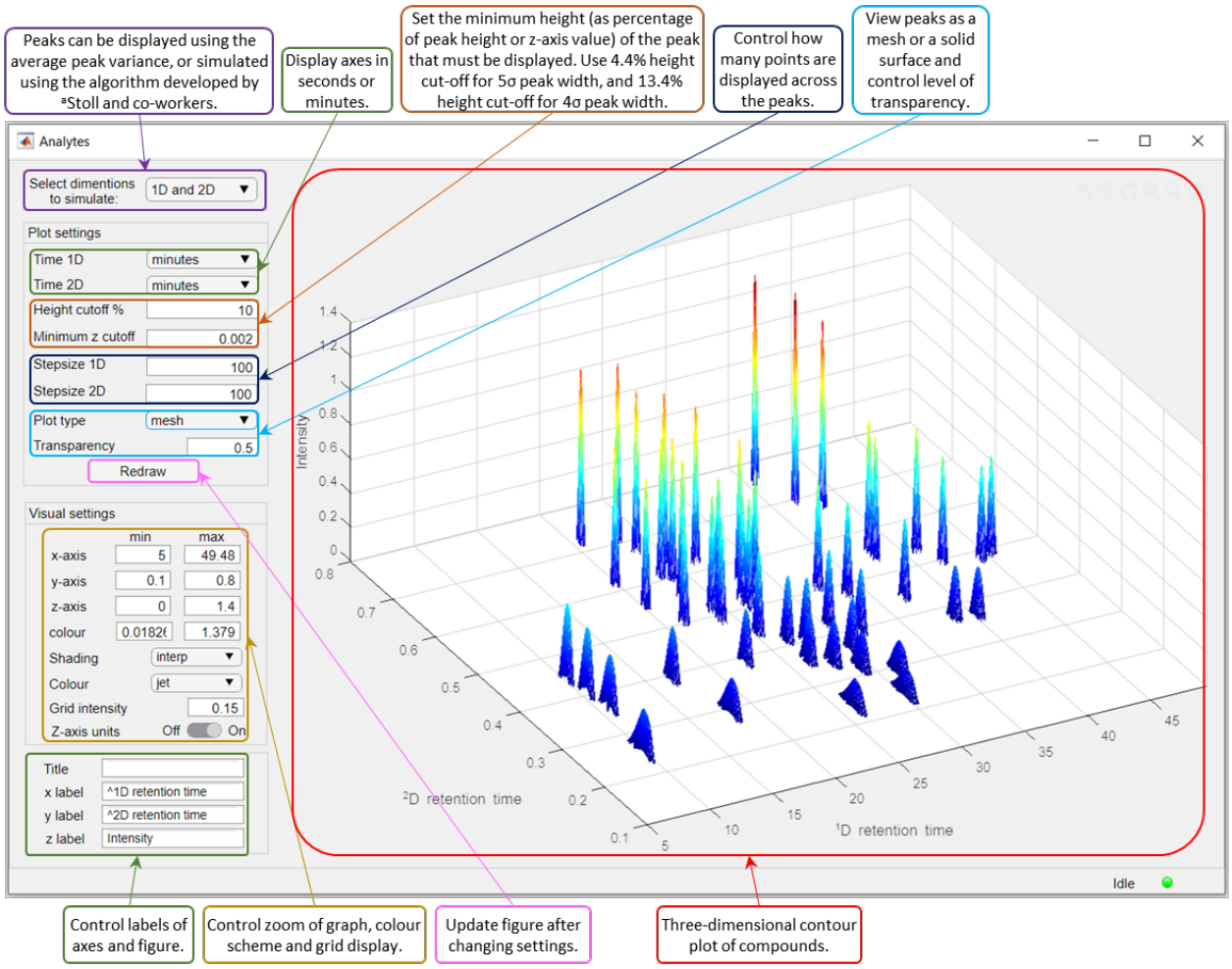


Figure 10. Graphical interface window to view and manage the predicted three-dimensional contour plot of the standards or analytes. [a][5,6].

Step 6. Recalculate results using different method settings set

After calculating and optimising the results of a result set, it is possible to recalculate the performance for each point in the result set using different method settings (**Fig 7**). Only the performance for the limited number of points remaining on the Pareto front are recalculated, not the complete set of permutations in the original experimental conditions set.

Recalculating a result set is primarily used to increase the accuracy of the injection band broadening predictions by using the simulation model (see [4]). Performing a full optimisation using the simulation model is not possible due to the high computational demand, but when recalculating a result set the number of points that must be simulated is reduced to a manageable number.

Step 7. Repeat

Due to the large number of optimisable parameters, the total number of permutations in a result set can easily become excessively large. For this reason, it is advisable to start the initial optimisation attempt using broad ranges of values for the optimisable variables, but with large increments (step-

sizes) between values. This optimisation attempt will not provide the true optimum conditions, but rather an indication of the range of values which will likely result in the optimum conditions. In the next optimisation attempt, this narrower range of values with smaller increments between values can be used to get closer to the true optimal conditions. This process can be repeated until satisfactory results are obtained.

Summary

The LC×LC method optimisation program is a powerful method development tool capable of simultaneously optimising most experimental parameters. The user can control how specific values are calculated and utilise a range of settings to refine the results to suit specific circumstances. The program is however currently still a research tool, and can still be improved significantly. For example, including a peak-detection and peak-tracking algorithm [11] to automatically obtain retention parameters from scouting gradients will be a great addition to the program.

5 References

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