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1 Introduction

We would like to thank you for your interest in our OSIRIS software. This Quick Tour is designed to give you an overview of OSIRIS possibilities and ease of use. For detailed information on the topics described in this tutorial, you can use the index and the contents tabs of the OSIRIS Help. To take full advantage of this Quick Tour, we recommend to go on step by step and to follow the process of the four different optimization projects described in this demonstration guide. For any further information, contact DATALYS or your local reseller.

2 Why use OSIRIS ?

To decrease significantly the time for method development.

HPLC method development are known to be very time consuming. OSIRIS will plan experiments, evaluate results and guide the chromatographer during the optimization process.

To improve analysis method performances.

OSIRIS is able to take into account simultaneously parameters such as the separation quality, the analysis time and the method robustness.

To assist you in the method validation.

OSIRIS can check how a small change in the conditions will influence the separation. Since OSIRIS provides a detailed report for your optimization project, it allows you to document methods.

3 HPLC Method Development

HPLC Method Development can be divided into three schematic steps:

1- Selection of the chromatographic system (reversed phase, normal phase, ion-pair chromatography...).

This selection, done by the chromatographer is based either on his own knowledge or on the literature. It depends on the type of compounds (neutral/weak acid/weak base). The chromatographer selects both the stationary phase and the type of mobile phase (polar or non-polar, buffered or not).

2- Optimization of the analysis conditions (mobile phase composition, pH, temperature).

This step can be driven completely by OSIRIS.

3- Validation of the analysis conditions.

This step is performed by the chromatographer. The robustness is now one of the criteria of the validation step. OSIRIS takes into account the robustness during the previous step and then contributes to avoid a failure of the validation process and is consequently a powerful help for method validation.

4 Why actual separations could be different from predictions ?

The experiments recommended by OSIRIS will usually lead to the most accurate predictions except in few cases where these experiments are inadequate. However, it is important to keep in mind that the success of an optimization process driven by OSIRIS is the result of a sequence of actions. Some of them **exclusively** depend on the user of the software :

- 1- The user **performs** the experiments, **collects** the chromatographic data and **transfers** them into OSIRIS.
- 2- OSIRIS **computes**, from these data, the mathematical models and the response function.
- 3- The user **checks** OSIRIS predictions and **validates** the method

Therefore, the predictions may be mediocre (5% to 10% error on the predicted retention times) or even bad (more than 10% error on the predicted retention times), if one or several of the following situations occurs. Often, they are due to the user although sometimes, they may be caused also by the software :

User :

- 1- Lack of accuracy in measuring the elution conditions (mobile phase composition, pH, temperature...).
- 2- Instrumental troubleshooting (flow rate delivered by the pump, composition delivered by the gradient system...).
- 3- Some essential experimental data entered into OSIRIS are wrong (peak matching, dwell volume, dead volume, plate number, ...)

OSIRIS :

- 4- In some rare cases (in particular for some ionizable compounds) the behavior of the compound is atypical and doesn't follow the mathematical models used by OSIRIS. In this case, it is necessary to reduce the parameter space (see next topic).

5 OSIRIS Optimization Process

The chromatographic behavior of the solutes is modeled as a function of the elution conditions. This behavior is fully dependant on three variables : the retention factor, the column efficiency and the peak asymmetry.

The model is derived from a few preliminary runs. Both their number and type depend on the chosen mobile phase system, on the composition zone explored and eventually on the temperature zone or the pH zone (in case of ionizable compounds). The results (retention times of each compound) and the chromatographic data from these preliminary experiments must be entered in the OSIRIS project file.

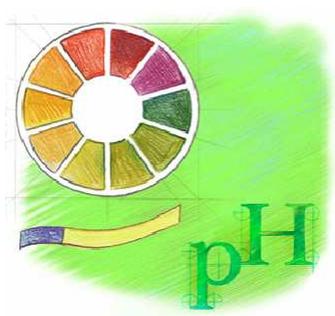
This phase is essential in the optimization process because the optimum validity primarily depends on the model accuracy. The retention model will become more accurate as the parameter space is reduced. That's why to optimize a ternary or quaternary mobile phase, OSIRIS uses a specific technique which searches for the optimum in the most favorable composition zones. These zones are determined by OSIRIS using the CRIT parameter. This limitation of the explored zones reduces the number of necessary preliminary experiments and improves the accuracy of the model.

Five optimization processes (also called project types) are possible :

- pH Optimization (1 parameter, 3 experiments)
- Temperature Optimization (1 parameter, at least 2 experiments)
- Mobile phase Optimization (from 1 to 3 parameters, between 2 and 12 experiments)
- Mobile phase and Temperature Optimization (2 or 3 parameters, between 4 and 10 experiments)
- Mobile phase and pH Optimization (2 parameters, 9 experiments)

OSIRIS guides the user by proposing conditions for the experiments which then are used as the basis of the model. The recommended experiments are those which produce the best accuracy for the retention model. However OSIRIS can also optimize the separation based on calibration runs chosen (within a limited acceptable range) by the user.

5.1 pH Optimization



This project type is dedicated to the separation of ionizable solutes and is performed with a fixed mobile phase composition at a fixed temperature.

It is recommended to select the composition in order to have a retention factors range between [0,5-15] for all the compounds and to maintain the column at an above ambient temperature (30°C for example).

The pH range explored must be less to 2,5 pH units (2 pH units recommended). To increase the selectivity variations and so, maximize the probability of obtaining a good separation, the pH range explored will have to include the pKa of the solutes.

Three (3) preliminary runs are required. They are performed at 3 different pH values distributed within the pH range. For example if the pH range is between 3 and 5, the three experiments will be performed at pH = 3, pH = 4 and pH = 5.

The result of the optimization is represented by the response curve with a maximum corresponding to the optimum pH value.

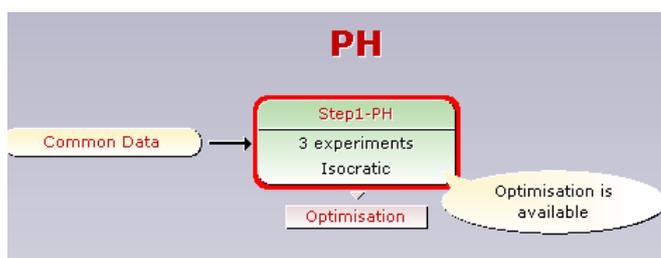
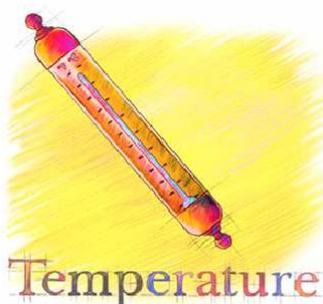


Fig. 1 : The optimization process in pH project type as displayed in the Wizard.

5.2 Temperature Optimization



This project type is intended to resolve the separation of neutral solutes in unbuffered solutions or ionizable compounds in buffered solutions. It is performed with a fixed mobile phase composition at a fixed pH.

It is recommended to select the composition initially to produce a retention factor range between [0,5-15] for all the compounds and, for ionizable solutes only, to set the pH at least 2 units higher or lower than the pKa of the compounds. The temperature range must be between 20 to 150°C ([20°C-70°C] is the recommended range).

At least two (2) preliminary runs are required. They are performed at 2 different temperature values spread within the temperature range. For example, when the temperature range is comprised between 20°C and 60°C, the two experiments will be performed at 20°C and 60°C respectively.

The result of the optimization is represented by a response curve with a maximum corresponding to the optimum temperature value.

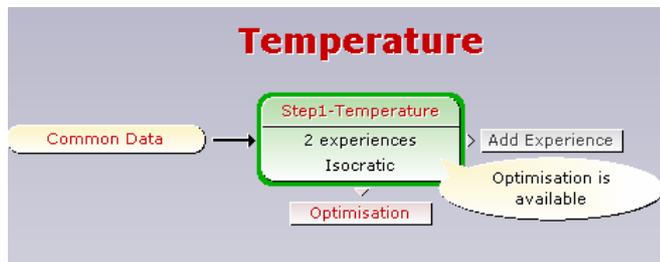


Fig. 2 : The optimization process in Temperature project type as displayed in the Wizard.

5.3 Mobile Phase Optimization



This project type is dedicated to the separation of neutral solutes. It is performed with a fixed temperature at a fixed pH.

It is recommended to maintain the column at an above ambient temperature (30°C for example) and, for ionizable solutes only, to set the pH at least 2 units above or below than the pKa of the compounds.

The solvents of the mobile phase to optimize are those classically used in RP-HPLC : water, methanol, acetonitrile, tetrahydrofuran. However the method may be employed with solvents used for non aqueous reverse phase chromatography (such as acetone, dichloromethane, ethyl acetate, chloroform....). The mobile phase composition can be optimized for 2 solvents (binary system), 3 solvents (ternary system), or 4 solvents (quaternary system).

One to five batches of experiments are required to optimize the mobile phase depending on its nature (binary, ternary or quaternary). These five batches correspond to five steps in the OSIRIS optimization procedure.

At the end of each step, the result is given by either a 2D or 3D response curve (either one or two optimizing parameters). When the separation is possible in isocratic mode, OSIRIS provides the optimum composition(s) of the organic modifier(s). When the separation is impossible in isocratic mode, gradient mode is then required. OSIRIS provides the optimum initial composition (A), the optimum final composition (B), and the optimum gradient time when these optimum conditions exist. If the separation is impossible, the next step must be considered.

First Step: First binary mobile phase

The first step is the optimization of a binary mobile phase (example : water/methanol)

2 linear gradient experiments are recommended. They must be carried out at the **same** initial composition and with **different** slopes. For example :

gradient n°1 : initial composition : 5 % slope : 1 %/min

gradient n°2 : initial composition : 5 % slope : 3 %/min

A third experiment (in isocratic or in gradient mode) can be added in the case of ionizable substances in order to increase calculation accuracy.

At the end of this step, OSIRIS evaluates the separation possibilities with this mobile phase in isocratic or in gradient elution mode. If the separation is impossible, then OSIRIS recommends to study another binary system (for example water/ACN, Step 2).

Second Step: Second binary mobile phase

The second step is the optimization of a second binary mobile phase (example : water/acetonitrile). The process is similar to the first step.

If the separation requires an isocratic elution but is not possible with this binary system, OSIRIS searches for the favorable ternary composition zones using the CRIT parameter, which allows to define the two additional experiments required for the third step. If any favorable zone is found, OSIRIS recommends a third binary system to study (like water/THF, see fourth step).

If the separation requires gradient elution but it is not possible with this binary system, it is necessary to go to the third step.

Third Step: Ternary mobile phase

This step is necessary if none of the two binary systems previously evaluated by OSIRIS (ex : water/MeOH and water/ACN) provides satisfactory separation conditions. Two alternatives are possible depending on the elution mode recommended by OSIRIS:

Isocratic ternary mobile phase :

This optimization is recommended if:

- OSIRIS has shown during the 2 previous steps that the isocratic mode was possible
- OSIRIS has found a ternary favorable composition zone.

In this case, the optimization process requires 2 additional experiments in isocratic elution mode. The 2 compositions are defined from the CRIT curve. They are included in the selected ternary composition zone which is a line of isoelutotropic blends inside the ternary compositions triangle.

Gradient ternary mobile phase :

This optimization is recommended if OSIRIS has shown during the 2 previous steps that the isocratic mode was not possible.

In this case, the optimization process requires 4 additional experiments in gradient elution mode. These experiments are defined by the ternary compositions triangle.

At the end of this step, OSIRIS explores the separation capabilities of this ternary system. If the separation is not possible, OSIRIS recommends the study of a third binary system (like water/THF, see fourth step).

Fourth Step: Third binary mobile phase

The fourth step is the optimization of a third binary mobile phase (for example : water/THF). The process is similar to the first step.

If the separation requires an isocratic elution but is not possible with this binary system, OSIRIS searches for a favorable ternary composition zones using the CRIT parameter, which allows the definition of additional experiments required for the fifth step. If any favorable zone can be found, it will be necessary to change the stationary phase or the chromatographic system.

Fifth Step: Quaternary mobile phase

The fifth step is the optimization of a quaternary mobile phase (example water/MeOH/ACN/THF).

Only isocratic elution mode may be used during this step.

This optimization is necessary if:

- none of the three binary systems (for example : water/MeOH, water/ACN and water/THF) provides acceptable separation conditions.
- OSIRIS has selected a favorable quaternary composition zone.

In this case, the optimization process requires 6 additional experiments in isocratic elution mode. The 6 compositions are defined from the CRIT curve. They are included in the selected quaternary composition zone which is a triangle of isoelutotropic blends inside the quaternary compositions tetrahedral.

At the end of this step, OSIRIS explores the separation capabilities within this quaternary system. If the separation is still impossible, it will be necessary to change the stationary phase or the chromatographic system (see HPLC Method Development).

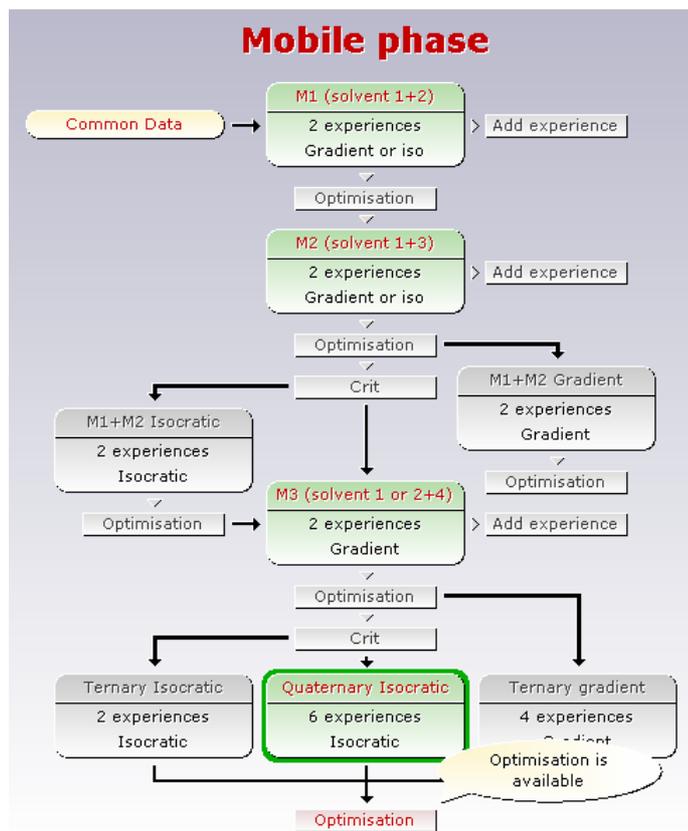
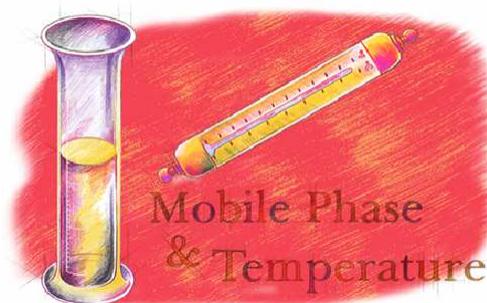


Fig.3 : The optimization process in Mobile Phase project type as displayed in the Wizard.

5.4 Mobile Phase and Temperature Optimization



This project type is dedicated to the separation of neutral solutes in unbuffered solutions or ionizable compounds in buffered solutions. It is performed at a fixed pH in case of ionizable solutes.

Important note : In case of ionizable solutes, it is **necessary** to measure the pH with an accuracy of 0,02 to 0,05 pH units. It is preferable to weight the buffer salts than to adjust the pH using a pHmeter.

The mobile phase solvents to optimize are those classically used in RP-HPLC : water, methanol, acetonitrile, THF. The method also may be extended to optimize solvents used for non aqueous reverse phase chromatography (like acetone, dichloromethane, ethyl acetate, chloroform, etc...). The mobile phase composition can be optimized with 2 solvents (binary system) in case of neutral solutes or ionizable solutes, with 3 solvents (ternary system) in case of neutral solutes only..

The temperature domain [T1, T2] for the optimum search must be chosen by the analyst when you start the project, during the Common data entry.

One to five batches of experiments are required to optimize the mobile phase and the temperature depending on its nature (binary or ternary system). These five batches of experiments correspond to five steps in the OSIRIS optimization procedure.

First Step: First binary mobile phase (M1) / lowest temperature of the temperature domain (T1)

The first step is the optimization of a binary mobile phase (example : water/methanol) at the temperature T1.

2 linear gradient experiments are recommended. They must be carried out at the **same** initial composition and with **different** slopes (a ratio of 3 between the two slopes is recommended). For example :

gradient n°1 : initial composition : 5 % slope : 1 %/min

gradient n°2 : initial composition : 5 % slope : 3 %/min

A third experiment (in isocratic or in gradient mode) **must** be added in the case of ionizable substances in order to increase calculation accuracy. The conditions for this third experiment are suggested by Osiris depending on the first two experiments.

At the end of this step, OSIRIS evaluates the separation possibilities with this mobile phase in isocratic or in gradient elution mode at the temperature T1.

Additional Step for ionizable solutes: First binary mobile phase (M1) / medium temperature of the temperature domain

This step is specific to the ionizable solutes for which it is necessary to increase the calculation accuracy.

The process of this step is similar to the first step.

Second Step: First binary mobile phase (M1) / highest temperature of the temperature domain (T2)

The conditions of the required experiments are similar to those performed within the first step except that they are carried out at a temperature T2.

After these two (or three) steps, OSIRIS evaluates the separation possibilities with this binary mobile phase within the temperature domain [T1, T2] by optimizing simultaneously :

- Either the composition of the first binary mobile phase (M1) and the temperature if the isocratic elution is possible.
- Either the initial composition of the first binary mobile phase (M1) and the gradient slope if the gradient elution is required. In this case, this optimization can be performed for any temperature within the domain [T1, T2].

If the separation is impossible, then OSIRIS recommend to study an other binary system (for example water/ACN, third Step).

Third Step: Second binary mobile phase (M2) / lowest temperature of the temperature domain (T1)

The conditions of the required experiments are similar to those performed within the first step.

Fourth Step: Second binary mobile phase (M2) / highest temperature of the temperature domain (T2)

The conditions of the required experiments are similar to those performed within the second step.

After these last two steps, OSIRIS evaluates the separation possibilities with this second binary mobile phase (M2) within the temperature domain [T1, T2].

Fifth Step: Ternary mobile phase and temperature

The fifth step is the optimization of a ternary mobile phase (example water/MeOH/ACN) and the temperature.

Only isocratic elution mode may be used during this step and it is reserved to neutral solutes.

This optimization is necessary if:

- if none of the two binary systems previously evaluated by OSIRIS (ex : water/MeOH and water/ACN) provides satisfactory separation conditions whatever the temperature.
- OSIRIS has found a temperature and a ternary favorable composition zone. In this case, the optimization process requires 2 additional experiments in isocratic elution mode at the temperature proposed by OSIRIS. The two compositions are defined from the CRIT curve. They are included in the selected ternary composition zone which is a line of isoelutotropic blends inside

the ternary compositions triangle. The most favorable conditions are obtained for the maximum CRIT value.

At the end of this step, OSIRIS explores the separation capabilities of this ternary system for the selected temperature. If the separation is impossible, it will be necessary to change the stationary phase or the chromatographic system (see HPLC Method Development).

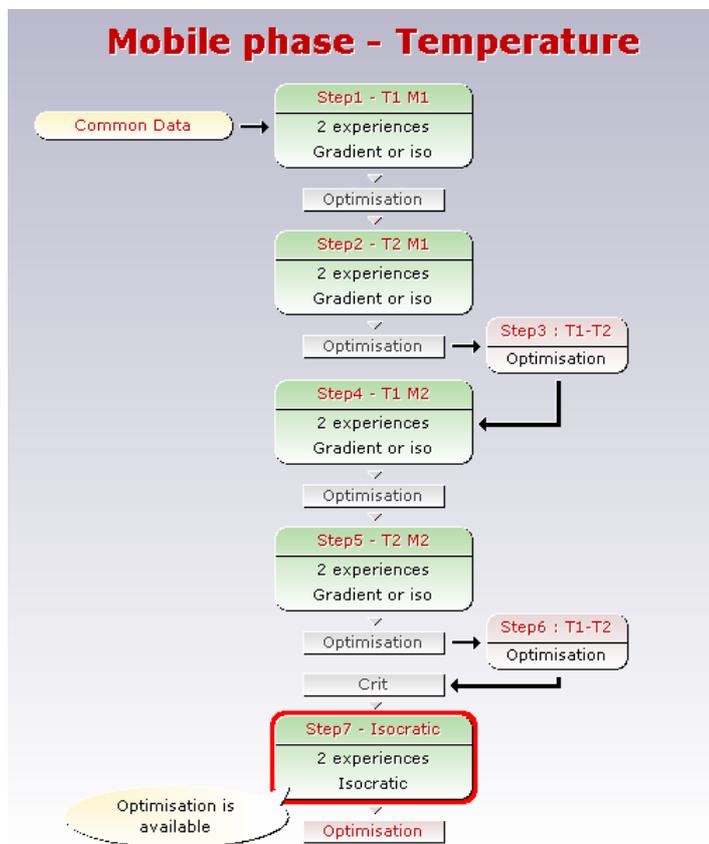


Fig.4 : The optimization process in Mobile Phase/Temperature project type as displayed in the Wizard.

5.5 Mobile Phase and pH Optimization



This project type is dedicated to the separation of ionizable solutes in buffered solutions, for which the ionization rate will vary with the pH.

Important note : It is **necessary** to measure the pH with an accuracy of 0,02 to 0,05 pH units. It is preferable to weight the buffer salts than to adjust the pH using a pHmeter.

The solvents of the mobile phase to optimize are those classically used in RP-HPLC : water, methanol, acetonitrile, tetrahydrofuran. The mobile phase composition is optimized with two solvents (binary system).

The pH domain [pH1, pH3] for the optimum search must be chosen by the analyst when you start the project, based on the pKa of the solutes (to increase the selectivity variations and so, maximize the

probability of obtaining a good separation, the pH range explored will have to include the pKa of the solutes).

Three batches of experiments are required to optimize the mobile phase and the pH. These three batches of experiments correspond to three steps in the OSIRIS optimization procedure.

First Step: Lowest pH (acid solutes) or highest pH (basic solutes) of the pH domain (pH1)

The first step is the optimization of a binary mobile phase (example : water/methanol) at pH1. 2 linear gradient experiments are recommended. They must be carried out at the **same** initial composition and with **different** slopes (a ratio of 3 between the two slopes is recommended). For example :

gradient n°1 : initial composition : 5 % slope : 1 %/min

gradient n°2 : initial composition : 5 % slope : 3 %/min

A third experiment (in isocratic or in gradient mode) **must** be added in order to increase calculation accuracy. The conditions for this third experiment are suggested by Osiris depending on the first two experiments.

At the end of this step, OSIRIS evaluates the separation possibilities with this mobile phase in isocratic or in gradient elution mode at pH1.

Additional Step : Intermediate pH (pH2)

The process of this step is similar to the first step.

Second Step: Highest pH (acid solutes) or lowest pH (basic solutes) of the pH domain (pH3)

The conditions of the required experiments are similar to those performed within the first step.

After these three steps, OSIRIS evaluates the separation possibilities with this binary mobile phase within the pH domain [pH1, pH3] by optimizing simultaneously :

- The mobile phase composition and the pH if the isocratic elution mode is possible.
- The initial composition and the slope of the gradient if the gradient elution mode is required. In this case, this optimization can be performed for any pH within the pH domain [pH1, pH3].

If the separation is impossible, it is recommended to study a second binary mobile phase (for example water / ACN) or to keep the same mobile phase with a different pH domain.

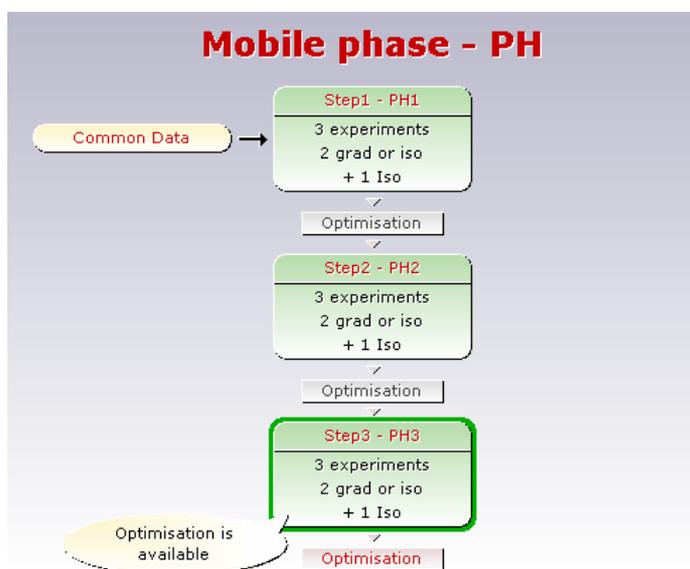


Fig. 5 : The optimization process in Mobile Phase/pH project type as displayed in the Wizard.

6 Installation

To install OSIRIS on your computer :

- 1/ Insert the OSIRIS CD-ROM in your CD-ROM drive.
- 2/ The CD menu will automatically appear
- 3/ Click on **English Version**.
- 4/ The following menu appears :

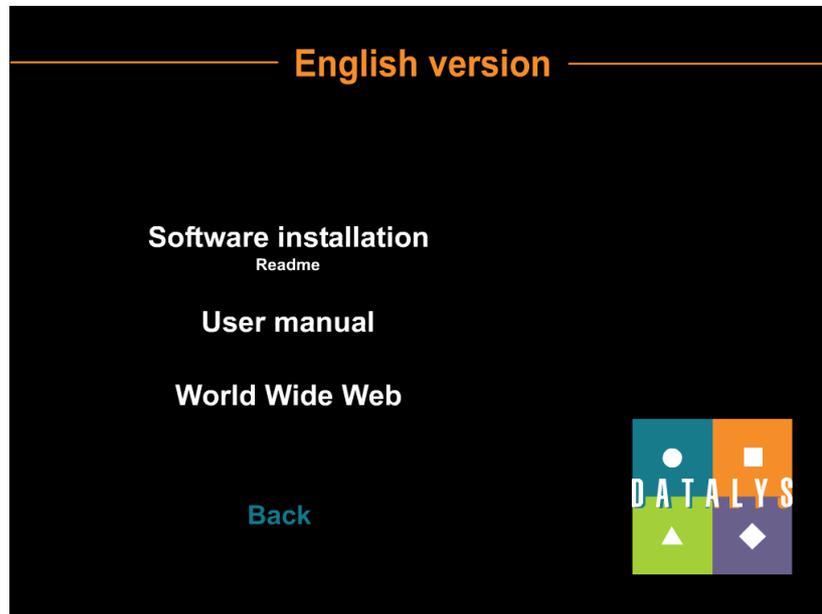


Fig. 6 : The OSIRIS CD-ROM menu

- 5/ Click on **Software installation**
- 6/ Follow the installation instructions

Note : If the installation program does not run, launch Explorer and double-click on setup.exe in the CD or choose Run from the Start menu and type d:\setup.exe (where d:\ is the letter of your CD-ROM drive).

7 Registration

Go to the **Start | Programs | Datalys | Osiris** menu to run OSIRIS. The registration dialog box appears :

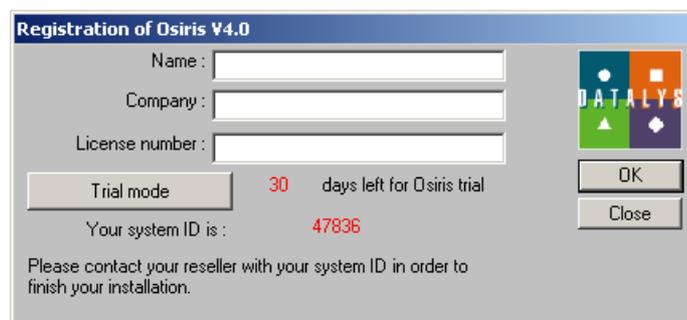


Fig. 7 : The Registration dialog box

Click on the **[Trial mode]** button to launch OSIRIS. You have 30 days of free demonstration use of OSIRIS after you installed it. During this period, you can explore all the capabilities of the software except you can't save or print a project.

8 Examples of optimization projects

In order to guide you through the OSIRIS capabilities, we have presented 4 different optimization process examples :

- **Aminoacids** : Mobile phase project in gradient elution mode
- **Ionizable** : pH project
- **Additives** : Mobile phase project in ternary isocratic elution mode
- **PAH** : Mobile phase & Temperature project

Each time a preliminary run is required, it is possible to import the results from files stored in C:\Program files\Osiris\Quick Tour. It is important to follow the order in which these projects have been listed.

When you start OSIRIS, you access to the Project selection screen where you can choose one of the 5 project types available in OSIRIS:

- Temperature,
- Mobile phase,
- pH,
- Mobile phase /pH,
- Mobile phase /Temperature.

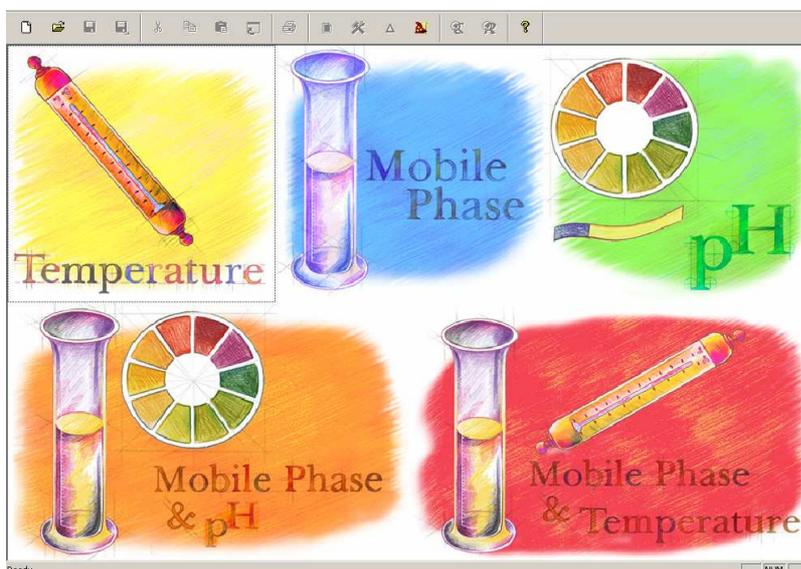


Fig. 8 : The Project Selection screen.

8.1 Mobile Phase Project - Aminoacids

From the project selection screen, select **Mobile Phase**.

In the Common Data section, enter the following conditions :

Column Length : 25,0 cm
Stationary Phase : C18
Diameter : 2,1 mm
Dead volume : 0,602 ml
Flow rate : 0,196 ml/min
Temperature : 40 °C

Click on **[Validate]**. The following tip dialog box appears, describing the first step of the optimization project :

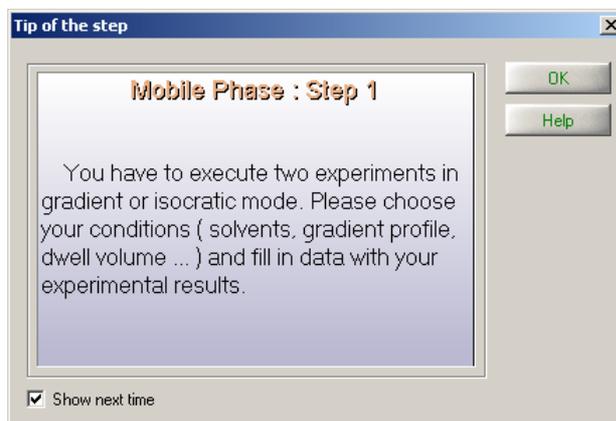


Fig. 9 : The Tip of the first step in Mobile Phase project type.

Click on **[OK]**. As two preliminary gradient runs are required, OSIRIS has created two experiment tabs (1 and 2) like shown in the figure here under :

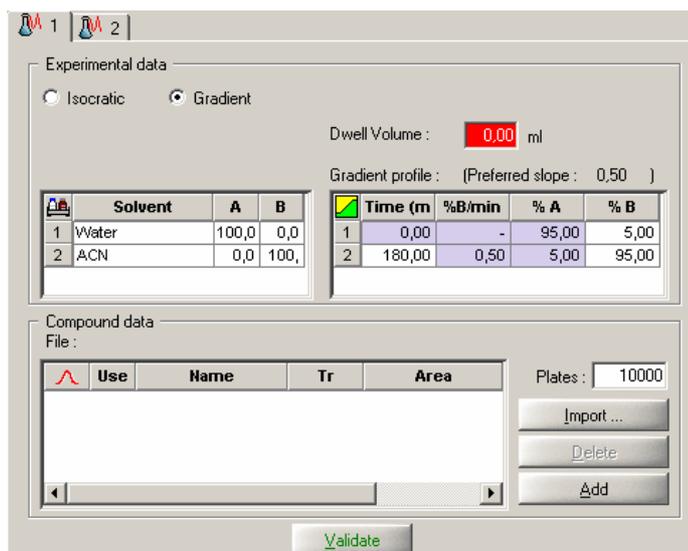


Fig. 10 : Two Experiment tabs have been created after Common Data validation.

First you have to fill the Experimental Data :

- The measured Dwell volume of our chromatographic system is 0,31 ml.
- These runs will be done with a water/ACN mobile phase : the first one, from 0% ACN to 100% in 100 min and the second one in 30 min (the composition range must be the same for both runs).

When you have completed these 2 runs you can fill the Compound data table with your results. The more convenient way is to import the results files obtained from your data acquisition system. OSIRIS can import results data in 3 formats : previous Osiris versions file (*.chr), AIA / ANDI file (*.cdf) and Text file (*.txt).

For this tutorial, we will consider we have made these runs and stored the results in files (*.chr format).

In the tab Experiment 1, click on the button **[Import ...]**. Then select the file *Aminoacids1.chr*. The following dialog box appears :

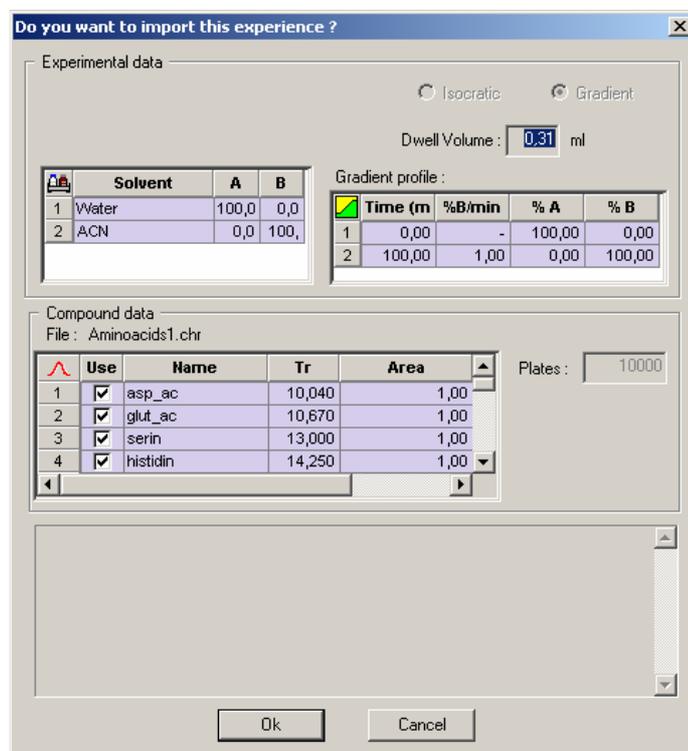


Fig. 11 : This dialog box allows you to check the Experiment files you are going to import.

Click on **[OK]** to confirm import. The Experimental data and the Results data are imported in the Experiment 1 tab. Then proceed to the same operation in the Experiment 2 tab with the *Aminoacids2.chr* file. Click on the button .

OSIRIS checks the coherence of the experiments and displays information in the validation zone in the bottom of the Project Data view. In particular, OSIRIS checks the k' range which determines the elution mode for the optimization. If the k' range remains between 0,5 and 15, then the default recommended elution mode will be isocratic. If k' is outside this range, OSIRIS will calculate the Response Curve in gradient mode, like in the illustration. The two experiments are validated and the button  becomes accessible. Click on this button to switch to the Optimisation view.

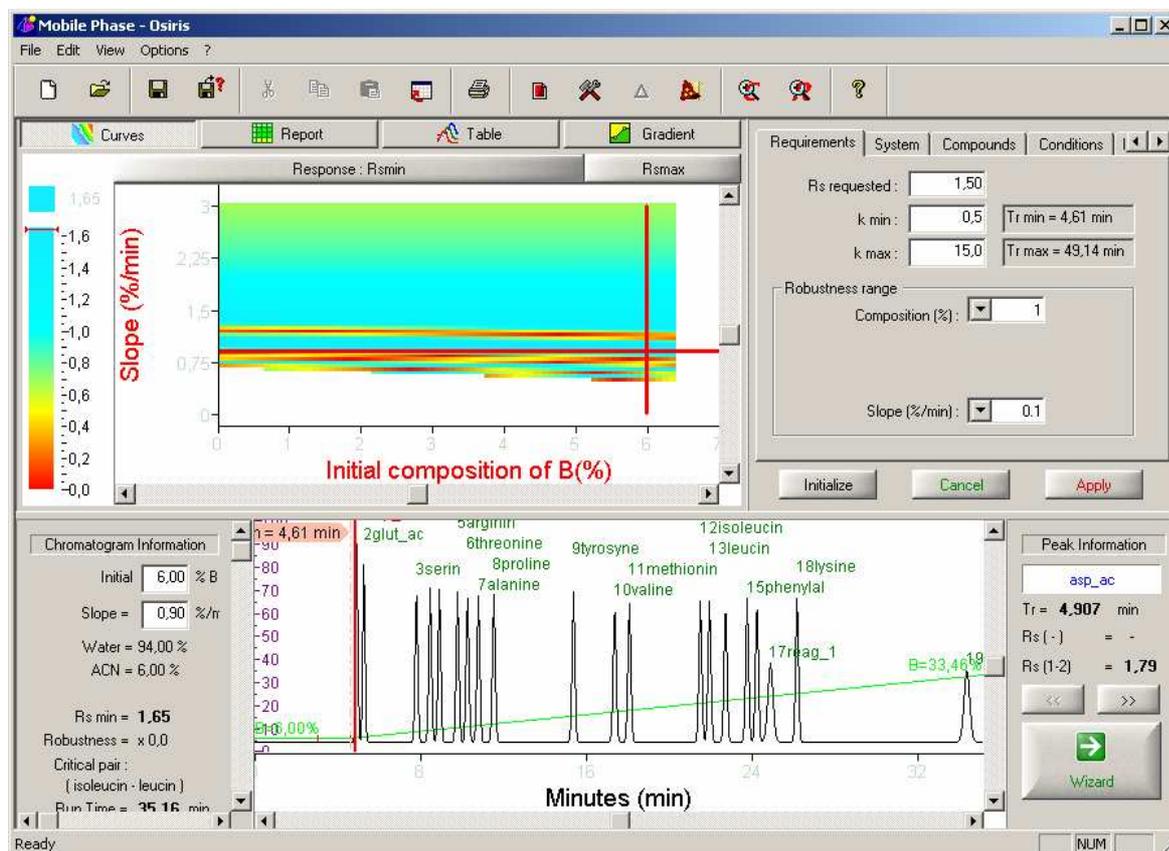


Fig. 12 : The Optimisation view of the "Aminoacids" project.

The Optimisation view is the "heart" of the OSIRIS software. It displays all the results of the optimization process and allows you to perform simulations at different conditions.

The Optimisation view is divided in 3 parts :

- In the upper left, the Response Curve, the Chromato Report, the Simulation Table and the Gradient Editor. You can switch between these tools just by clicking on the appropriate button.
- In the upper right, the Optimization Criteria.
- In the lower part, the simulated chromatogram.

First, we will use the Response Curve. For this project, OSIRIS displays a response diagram showing the resolution of the critical pair depending on the slope of the gradient and the initial composition. The cross-hair lines in this diagram is placed at the point where the resolution is the highest. These cross lines can be dragged to any position in the curve. For each position inside the response diagram, OSIRIS displays the predicted chromatogram and its relative conditions in the lower part of the Optimisation view. Check it and click on  to return to the initial Response Curve

Double-click inside the chromatogram : it is now displayed in full screen mode. You can see the gradient profile which is overlaid on the chromatogram.

You can zoom in the current chromatogram by selecting a zone with your mouse (click with the left button of the mouse, then extend the zoomed zone by holding the left button down then releasing it). You can go back to full scale by clicking in the graph with the mouse right button and choosing the **Full Scale** menu.

Click on the button  to revert to the original Optimisation view.

Click on  Report above the Response Curve. Now the complete report of the simulated chromatogram is displayed. It shows for each peak the value of many variables like Retention Time, Area, Resolution, %B, etc...

Compounds order		Increasing Retention times					
	Compound	Tr	Area	k	Rs	%B	Plates
1	asp_ac	4,907	1,00	0,55	---	6,2	10000
2	glut_ac	5,252	1,00	0,58	1,79	6,5	10000
3	serin	7,787	1,00	0,92	11,55	8,8	10000
4	glycine	8,452	1,00	1,00	2,75	9,4	10000
5	histidin	8,898	1,00	0,99	1,82	9,8	10000
6	arginin	9,772	1,00	1,06	3,49	10,6	10000
7	threonine	10,252	1,00	1,07	1,89	11,0	10000
8	alanine	10,780	1,00	1,12	2,05	11,5	10000
9	proline	11,525	1,00	1,10	2,87	12,2	10000
10	tyrosyne	15,373	1,00	1,07	15,01	15,6	10000
11	valine	17,362	1,00	1,20	7,54	17,4	10000
12	methionin	18,073	1,00	1,21	2,63	18,1	10000
13	isoleucin	21,497	1,00	1,17	12,72	21,2	10000
14	leucin	21,938	1,00	1,18	1,65	21,6	10000
15	norleucin	22,715	1,00	1,17	2,91	22,3	10000
16	phenylal	23,757	1,00	1,17	3,91	23,2	10000
17	tryptopha	24,230	1,00	1,14	1,78	23,6	10000
18	reag_1	24,883	1,00	2,97	1,66	24,2	10000
19	lysine	26,167	1,00	1,08	3,28	25,4	10000
20	reag_2	34,370	1,00	3,29	19,88	32,7	10000

Fig. 13 : The Report of the simulated chromatogram.

On the right upper part of the Optimisation view is the Optimisation criteria dialog box containing four tabs. Select the *System* tab, then the *Isocratic* option.

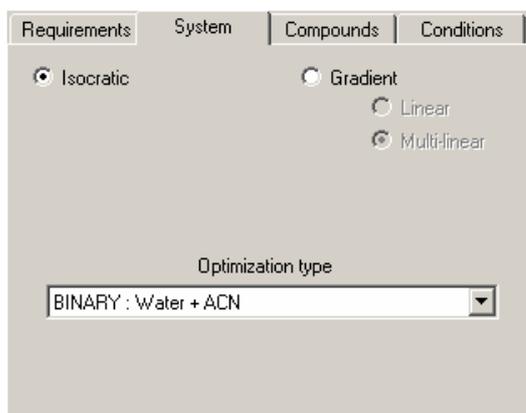


Fig. 14 : The System tab of the Optimisation Criteria.

Then press the button  to recalculate the Response curve in Isocratic mode. As the Isocratic mode is impossible within this k range, the following message appears :

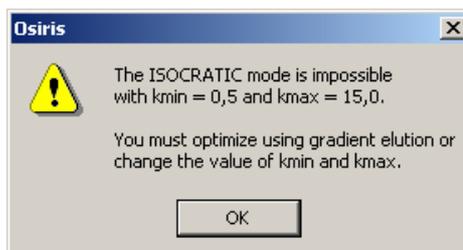


Fig. 15 : OSIRIS informs you the Isocratic mode is impossible.

When you click on **[OK]**, you return to the initial Response curve in Gradient mode. Click now on  Gradient to access to the Gradient Editor. This allows you to define some gradient profiles and simulate the resulting chromatograms. You can change the initial and the final points of the

gradient graphically just by dragging and dropping them with the mouse or by entering values for initial %B and gradient slope in the table.

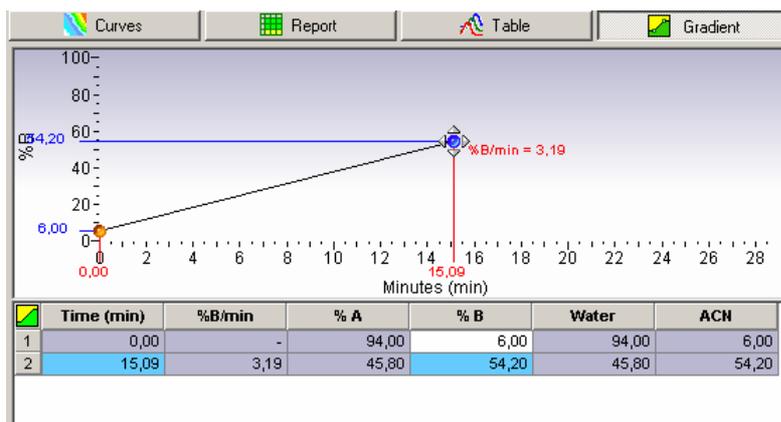


Fig. 16 : The Gradient Editor. Here the final point of the gradient is currently moved by a simple drag & drop.

Modify the gradient profile and observe the simulated chromatogram. Now return in the *System* tab of the Optimisation criteria. Select *Multi-linear* and click on **Apply**. Go to the Gradient Editor and click with the right mouse button : it gives you access to a context menu. The Insert option allows you to create a new point in the gradient profile. You can move this point by drag & drop and add other points to build a multi-linear gradient. The resulting chromatogram is automatically displayed in the lower part of the Optimisation view.

8.2 pH Project - Ionizable

Click on New  to return to the project selection. The following dialog box appears :

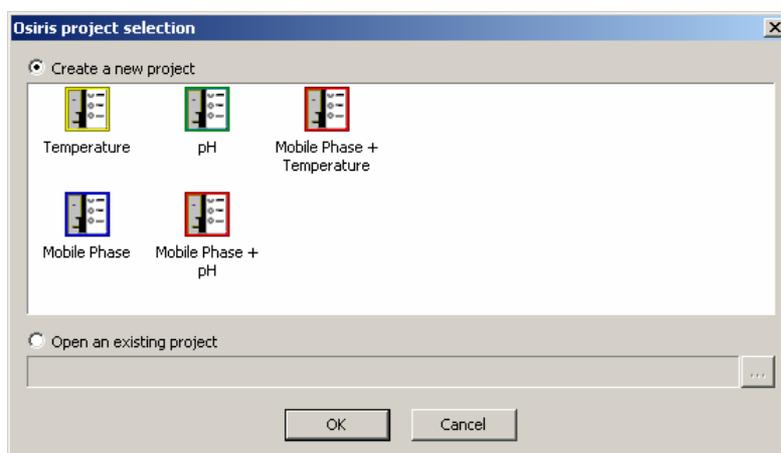


Fig. 17 : The Project Selection dialog box.

Check the box **Create a new project**, select **pH** and click on **[OK]**.

In the Common Data section, enter the following conditions :

Column Length : 15,0 cm
Stationary Phase : C18_Capcell
Diameter : 4,6 mm
Dead volume : 1,500 ml
Flow rate : 1,00 ml/min
Temperature : 30 °C
pH from : 3,00 To 5,00
Compounds acidity : Acid
Mobile Phase : Binary, Citrate Buffer 80% / Acetonitrile 20%

Click on **[Validate]**. The tip dialog box appears, describing the first step of the optimization project :

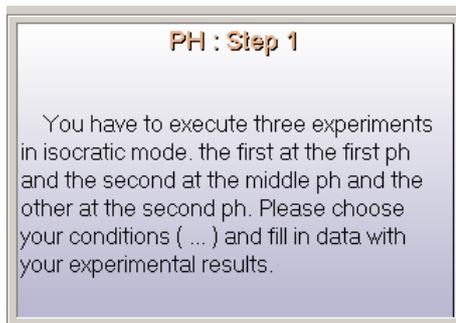


Fig. 18 : The Tip of the first step in pH project type.

Click on **[OK]**. Since three preliminary isocratic runs are required, OSIRIS has created three experiment tabs (1, 2 and 3) like shown in the figure below :

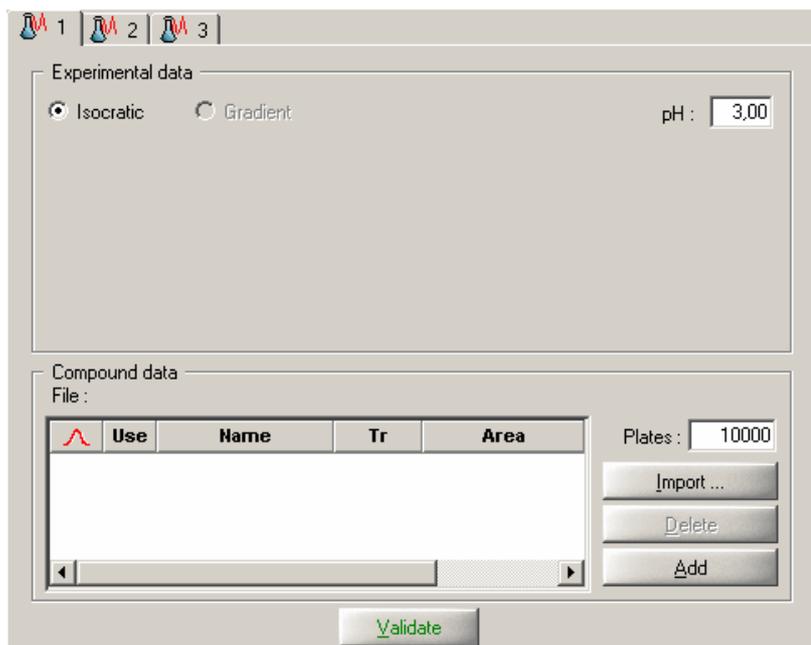


Fig. 19 : Three Experiment tabs have been created after Common Data validation.

For these three experiments, import the files *pH1.chr*, *pH2.chr* and *pH3.chr* corresponding to analyses done at pH 3, 4 and 5. Click on **[Validate]** and then on **[Optimisation]** to go to the Optimization view. A response curve like in the figure below is generated :

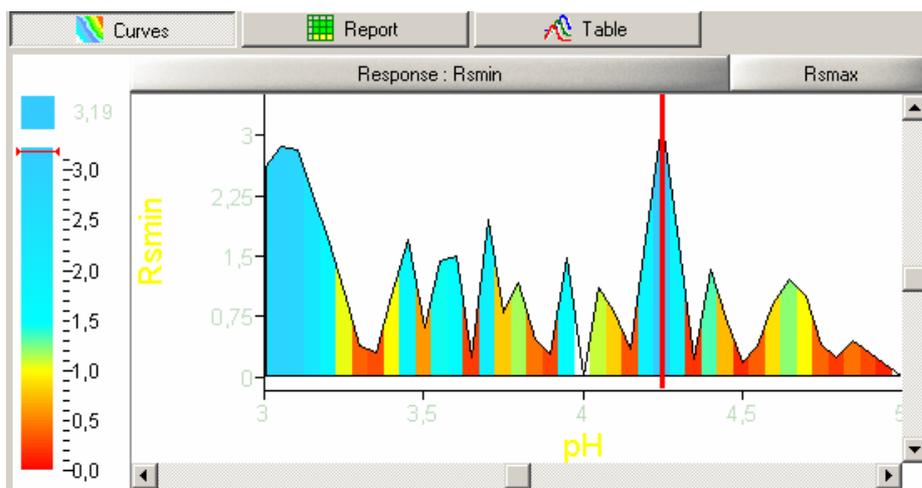


Fig. 20 : The Response Curve obtained after three preliminary runs displays the Resolution versus the pH.

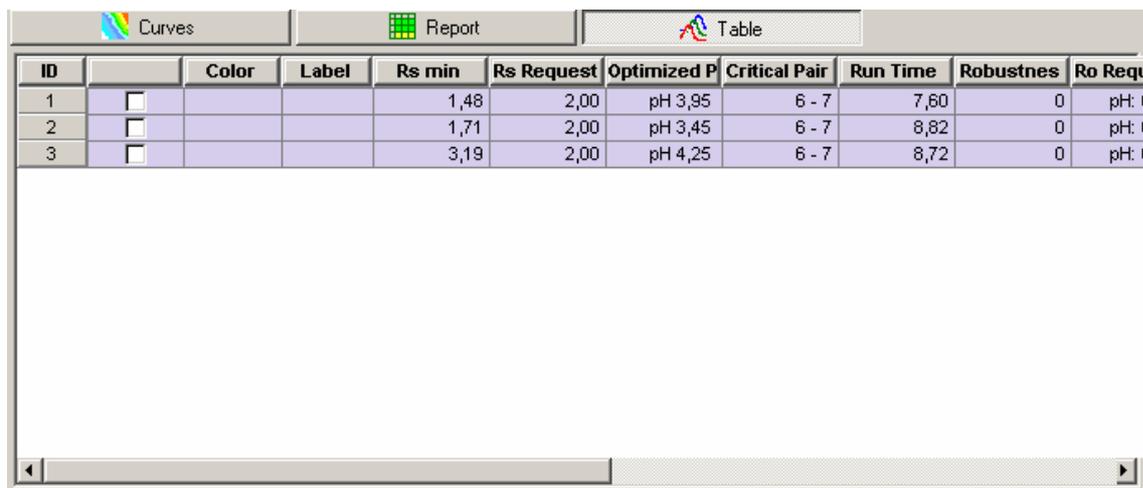
Move the vertical marker inside the Response curve to another position. The predicted chromatogram is automatically updated. Click on the button  in the toolbar. This will allow you to store this simulation.

In the zone on the left of the chromatogram, enter a pH value of 3,45. The vertical marker will move in the Response curve and the simulated chromatogram is updated. You can see that for this pH value the Resolution is equal to 1,71, the critical pair is now p-NitroB / Benzoic and the run time is 8,82 min.

Click again on  to also store the simulation at these conditions.

If now you press the button  above the curve, the vertical marker will return to its initial position in the Response curve : the location where the resolution is the highest. Store also the simulation at the maximum resolution.

Click on the Table button above the curve . Instead of the Response curve, the Simulation table appears :



ID	Color	Label	Rs min	Rs Request	Optimized P	Critical Pair	Run Time	Robustnes	Ro Req
1	<input type="checkbox"/>		1,48	2,00	pH 3,95	6 - 7	7,60	0	pH: 1
2	<input type="checkbox"/>		1,71	2,00	pH 3,45	6 - 7	8,82	0	pH: 1
3	<input type="checkbox"/>		3,19	2,00	pH 4,25	6 - 7	8,72	0	pH: 1

Fig. 21 : Three Simulations appear in the Simulation Table.

Check the box for the lines 1 and 2. The simulated chromatograms are overlaid on the current one in the chromatogram display. You can change the color of the trace by clicking in the Color column (choose blue for 1 and red for 2) :

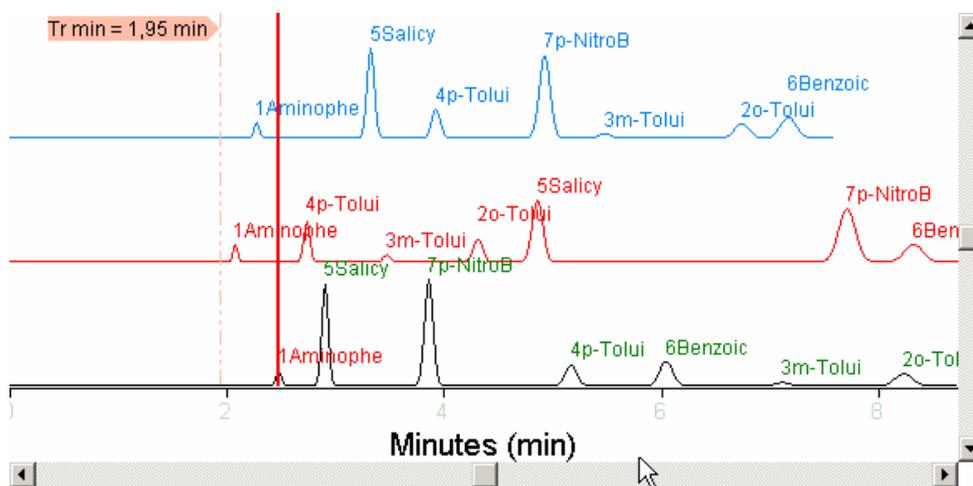


Fig. 22 : You can overlay several predicted chromatograms stored in the Simulation Table.

This allows you to observe the movement of the peaks as the pH changes.

8.3 Mobile Phase Project - Additives

Click on New  to return to the project selection dialog box. Check the box **Create a new project**, select **Mobile Phase** and click on **[OK]**.

In the Common Data section, enter the following conditions :

- Column Length :** 25,0 cm
- Stationary Phase :** C18
- Diameter :** 4,1 mm
- Dead volume :** 2,503 ml
- Flow rate :** 0,80 ml/min
- Temperature :** 31 °C

Click on **[Validate]**. The tip dialog box appears, describing the first step of the optimization project. Click on **[OK]**.

As two preliminary gradient runs are required, OSIRIS has created two experiment tabs (1 and 2) :

Experiment 1 : From 5 to 95% THF in 180 min

Experiment 2 : From 5 to 95% THF in 60 min

The two gradient profiles could be changed (in particular the name of the solvents) but the composition range must be the same for both experiments and it is recommended to have a factor around 3 for the two gradient times.

For these two experiments, import the files *Additives1.chr* and *Additives2.chr*. Then click on  and go to the Optimization view. You see immediately in the Response curve that the highest resolution you can get is 0,58. So there is no satisfactory solution with the ACN/THF mobile phase. What can we do now ?



We will use the Wizard by clicking on the button  which accesses to the following screen :

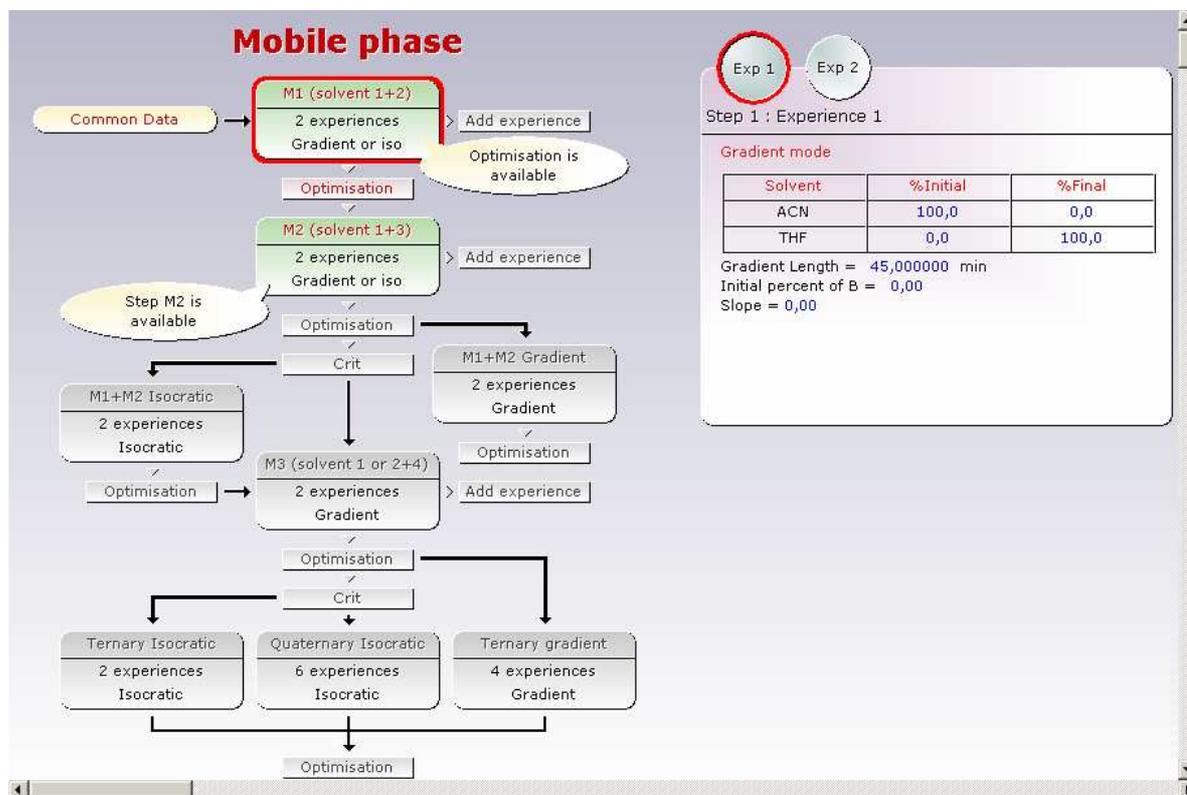


Fig. 23 : The Wizard displays the complete optimization process and the conditions for the already performed experiments. Then it gives you directions to continue the project.

The Wizard displays the complete optimization process and proposes different actions represented by buttons. Each step is represented in the diagram by an icon like below :

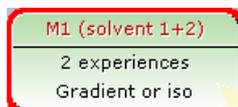


Fig. 24 : The representation of an optimization step in the Wizard.

If this step has been performed or is active, it is displayed with a background color. Click on the icon of the first step we have performed. The Step Summary zone on the right displays the detailed conditions of the experiences. You can switch from one experience to another by clicking on the circle button **Exp**.

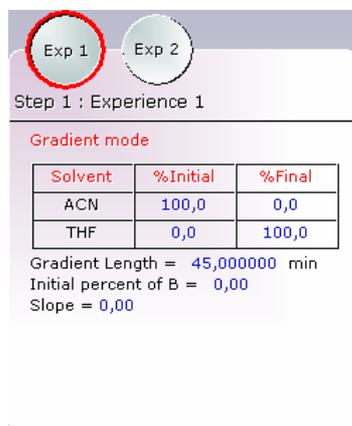


Fig. 25 : The Step Summary box for the first optimization step.

OSIRIS proposes a second step with a different binary mobile phase (M2). Click on this icon with the right button and select the Go to step M2 item in the context menu : you move to the Project Data view where two new experiment tabs have been created. You must now run two gradient experiments, each with a different mobile phase (in this example it will be water/THF). So import the files *Additives3.chr* and *Additives4.chr* from Experiment 3 and Experiment 4, respectively. Then click on **Validate** and go to the Optimization view. Based on the Response curve, it seems there could be a solution using this mobile phase.

However, we have to go further and precisely define our requirements. Go to the Optimization criteria dialog box (right upper part of the Optimization view) and select the *Requirements* tab. Enter the following values :

Rs requested : 1,20
k min : 0,50
k max : 10,0
Robustness range on Composition : 2%

Then press the button **Apply** to recalculate the Response curve. The new curve shows a Rs max value of 2,43. However, this curve doesn't take into account the robustness. To include robustness, we have to change the response function by clicking on **Response : Rsmin** above the curve which provides access to the following menu :

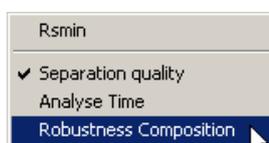


Fig. 26 : The menu allowing to change the Response function.

Select Robustness Composition : the new Response curve is blank indicating that it is not possible to satisfy both requirements (Separation quality and robustness) with this binary mobile phase. Since we want a robust solution, we will continue investigating additional options within this project.

Before continuing, you must check that the CRIT precision (which will be used for the ternary mobile phase) is set to 0,80. The precision can be set anywhere between 0 to 1. However, as the value of the precision is set higher, the time required to calculate the CRIT curve increases. Go to the menu **Options | Setup** to display the following dialog box :

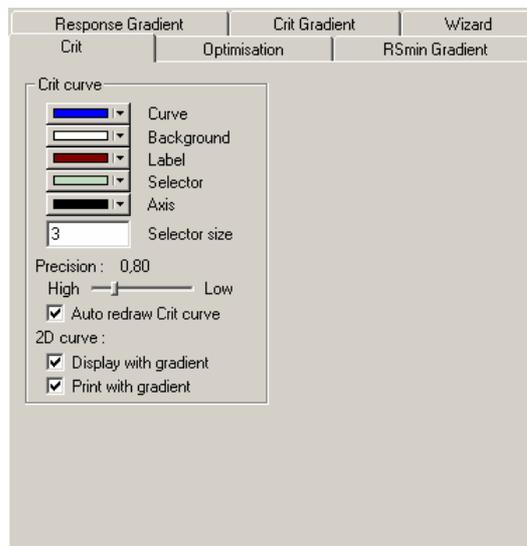


Fig. 27 : The Crit tab of the Setup dialog box. You can also define the color gradient of the different curves in OSIRIS.

Set the **Precision** to 0,80 using the slider and click on **[OK]**.

Once again, we will use the Wizard :

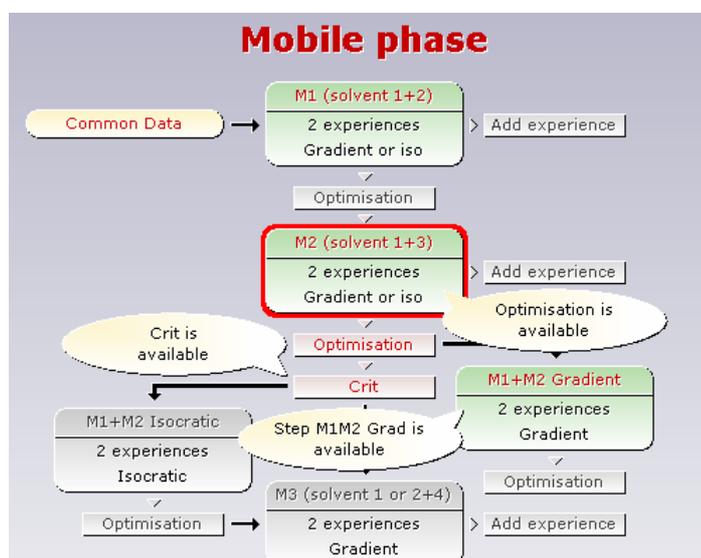


Fig. 28 : After the Step 2, the Wizard proposes to go on in ternary isocratic or gradient.

The second Step has been performed and two possibilities, depending on the elution mode, are offered now : ternary isocratic system (Crit) or ternary gradient system. We will choose the isocratic solution and so click on **Crit**. The CRIT curve displays the favorable zones for ternary and/or quaternary compositions and then, determines which new experiments to run.

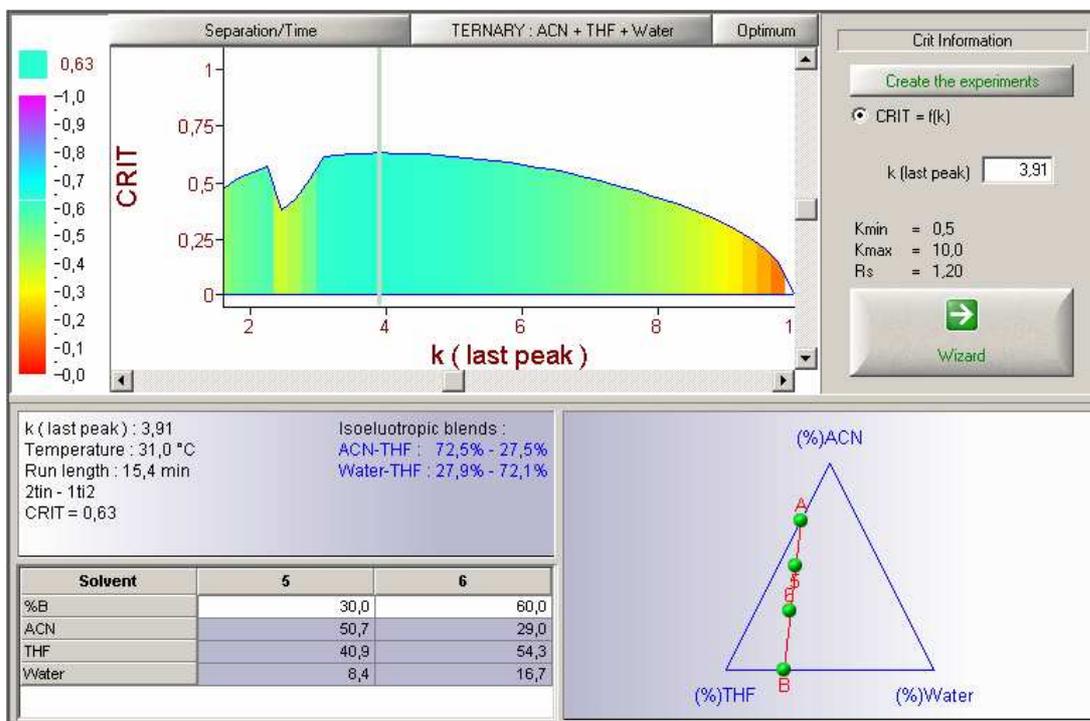


Fig. 29 : The CRIT view shows the favorable composition zone with a ternary mobile phase.

A composition zone exists where the requirements for quality of the separation and/or analysis time can be achieved since the CRIT value is greater than zero. OSIRIS will place the vertical marker at the k value corresponding to the maximum CRIT value within the curve when you click on the **Optimum** button.

In the right lower part, the Isoelutropic blends diagram will take the appearance of a triangle where each corner represents one of the solvents. The diagram is updated when the user move the vertical marker in the CRIT curve. The detailed information about the composition zone is shown on the left : capacity factor of the last peak, estimated run length, the CRIT value and the compositions of the isoelutropic blends. When the selected system is ternary, the compositions zone is represented by a straight line inside the triangle diagram. The ends of the line are the isoelutropic blends of the two binary systems (ACN/THF and water/THF). OSIRIS proposes two new experiments located on this straight line (generally at 30% and 60% of B). The details of these new experiments are described in the table in the left lower part of the CRIT view.

Inside the table, change the %B value for Experiment 6 from 60 to 80. The percentage of each solvent is updated. You are ready to proceed to the next experiments. Click on the **Create the experiments** button to validate the isoelutropic blends selection and create the tab for the new experiments in the Project Data view.

The tip of this step appears as below :

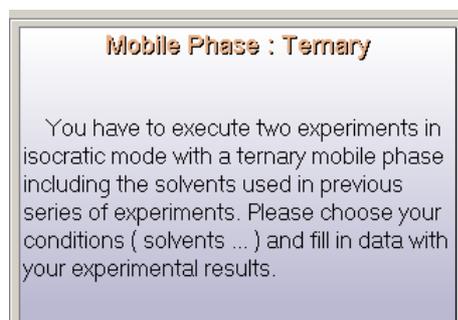


Fig. 30 : The Tip of the third step in Mobile Phase project type.

Import the files *Additives5.chr* and *Additives6.chr* from Experiment 5 and Experiment 6, respectively. Then click on **Validate** and go to the Optimization view. Reselect your requirements : Rs

requested = 1,20 / k min = 0,50 / k max = 10,0 / Robustness range on Composition = 1%. Add the robustness composition in the Response function of the curve using the button

. You will get a Response curve like this :

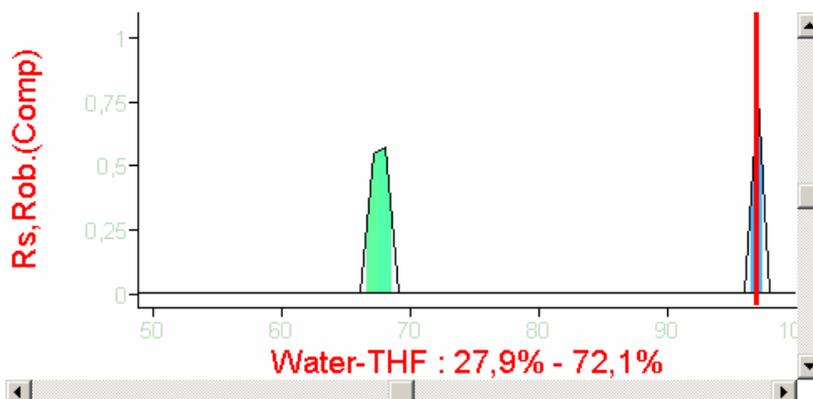


Fig. 31 : The Response Curve taking in account the robustness of the ternary mobile phase.

This means there are some robust solutions in isocratic mode with a ternary mobile phase (ACN/water/THF). The maximum response is obtained with a mobile phase ACN 2,2% / THF 70,7% / Water 27,1%. The predicted analysis time is 17,23 minutes.

8.4 Temperature / Mobile Phase Project - PAH

Click on New to return to the project selection dialog box. Check the box **Create a new project**, select **Mobile Phase & Temperature** and click on **[OK]**. Choose **No** when Osiris asks if you want to use ionic compounds.

In the Common Data section, enter the following conditions :

Column Length : 25,0 cm
Stationary Phase : Pyrene
Diameter : 4,6 mm
Dead volume : 2,500 ml
Flow rate : 1,00 ml/min
Temperature from : 20 To 40 °C

Click on **[Validate]**. The tip dialog box appears, describing the first step of the optimization project. Click on **[OK]**.

Since two preliminary gradient runs are required, OSIRIS has created two experiment tabs (1 and 2).

For these two experiments, import the files *Pah1.chr* and *Pah2.chr*. Then click on and go to the Optimization view. As there is no satisfactory solution with the water/ACN mobile phase at 20°C, go to the Wizard, click on the icon of the Step 2 and select **Go to the step 2** in the context menu.

In the Project Data view, OSIRIS requests two new gradient runs at a different temperature (40°C is recommended). Import the files *Pah3.chr* and *Pah4.chr*. Then click on and go to the Optimization view. Here, you get the following Response Curve based on these last two runs :

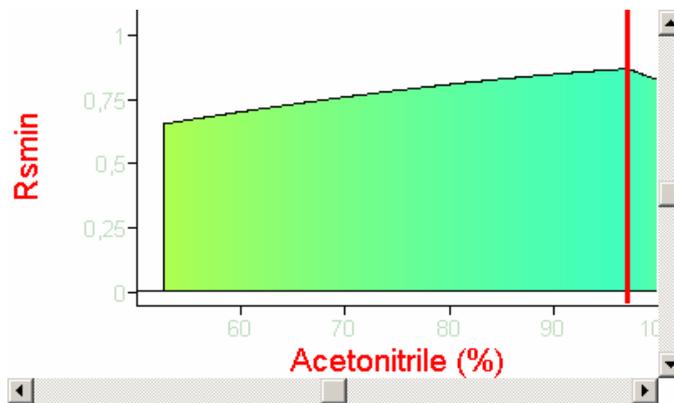


Fig. 32 : The Response curve after the Step 2.

As with the curve for the 20°C runs, there is no solution at 40°C. Go to the Wizard. OSIRIS proposes a third step which is the optimization of the current mobile phase within the Temperature domain. Right

click on the icon of this step  and select **Go to the step 3** in the context menu. You access the Optimization view again where the Response curve is recalculated using the first four runs. Now you get a 3D response curve displaying Resolution versus Temperature and Acetonitrile %.

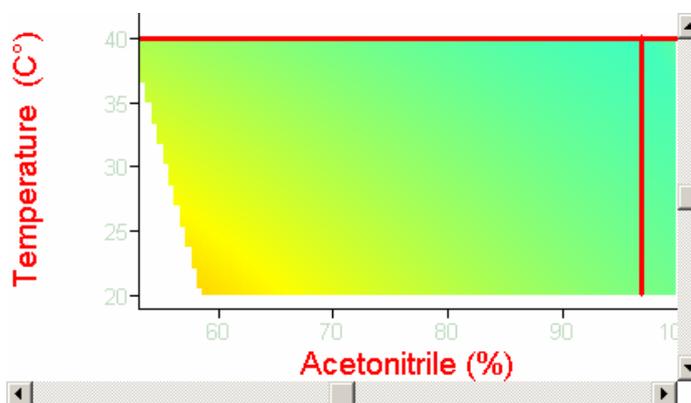


Fig. 33 : Based on the 4 first runs, OSIRIS builds the 3D Response curve.

There is still no solution. Go to the Wizard. Select **Go to step 4**. This time, you return to the Project Data view to include two additional runs with a different solvent blend : water/methanol. These two runs are done at 20°C. Import the files *Pah5.chr* and *Pah6.chr* and click on  to go to the Optimization view.

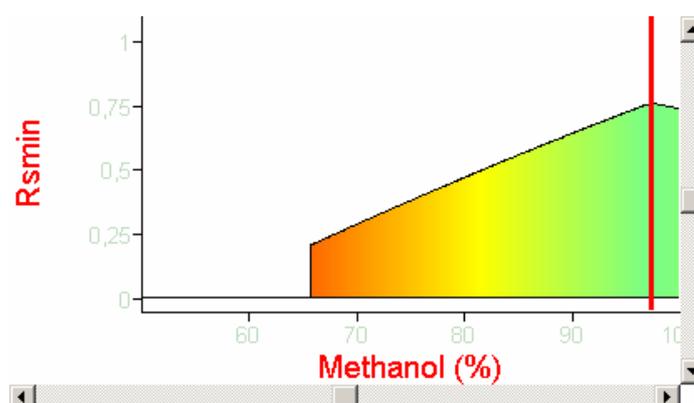


Fig. 34 : The Response curve after 2 runs with water/methanol.

The 2D Response curve shows there is no solution with the water/methanol mobile phase at 20°C either. When you go to the Wizard, this time it proposes an optimization step (step 5) with two new gradient runs at 40° C. Import the files *Pah7.chr* and *Pah8.chr*, click on  and go to the Optimization view. The maximum in the curve does not yield a suitable solution.

Go to the Wizard which proposes to optimize the current mobile phase (M2) within the Temperature domain. You access the Optimization view again where the Response curve is recalculated using the

last four runs (done with the water/methanol mobile phase) to get the Response curve displaying Resolution versus Temperature and Methanol %.

We are going to decrease our requirements slightly by changing the Rs min. In the Optimization Criteria, go to the *Requirements* tab and set the value to 1,30. Click on **Apply** to recalculate the Response Curve. Add the Robustness Composition and the Robustness Temperature to the Response function. There is still no solution.

If you go to the Wizard, it will look like this :

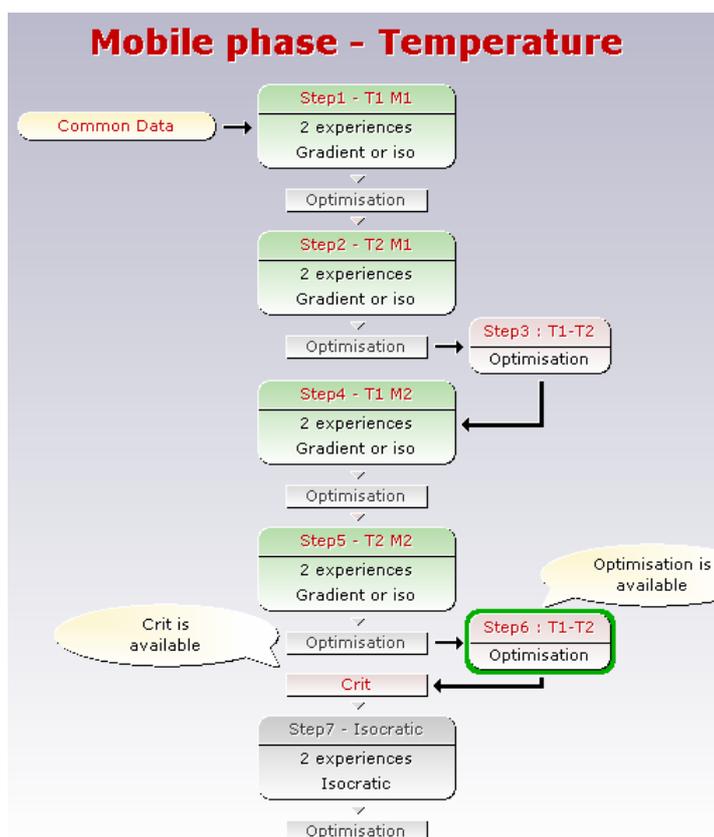


Fig. 35 : After the Step 6, the Wizard proposes to calculate the CRIT curve in ternary mobile phase.

The Wizard suggests calculating the CRIT curve in order to see if there is a favorable ternary composition zone. Click on **Crit**. You access the CRIT view as shown below (click on **Optimum** if the crosshair is not placed in a position similar to that in the figure below) :

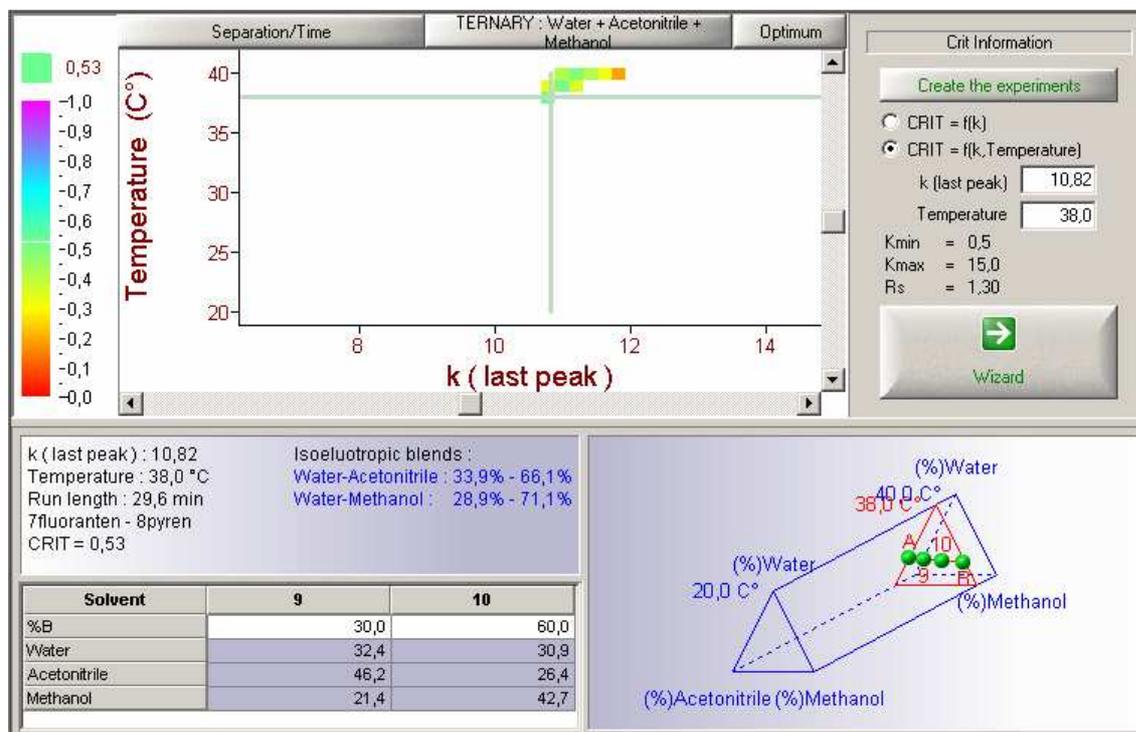


Fig. 36 : The CRIT view shows a favorable composition zone with this ternary mobile phase at 38°C.

The CRIT curve shows there is a favorable zone for a temperature of 38°C. Click on **Create the experiments** to move to the Project Data view in order to import two isocratic ternary runs (files *Pah9.chr* and *Pah10.chr*). Then validate and go to the Optimization view. There is a solution with the following mobile phase : water 29,8%/ acetonitrile 12,6%/ methanol 57,6% at 38°C :

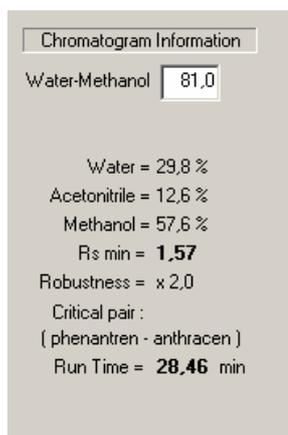


Fig. 37 : The details of the conditions for the optimum solution.

9 Conclusion

The OSIRIS Demo Tour is now finished. It has been designed to allow you to have a preview of the software but it's not describing all the features. You can explore OSIRIS more in details, in particular, by using the help from the menu ? | **Help**.

If you want to purchase OSIRIS, just contact DATALYS or its local distributor to get your license number and receive quickly your complete package.