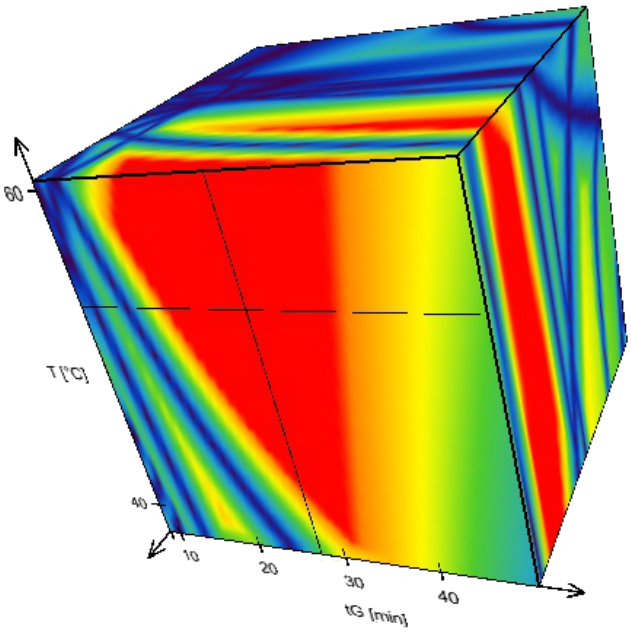
MOLNÁR-INSTITUTE
for applied chromatography

Quality by Design Software for HPLC Method Development in the Design Space for the New Decade with

DryLab® 2010



DryLab® 2010 explores the HPLC Design Space for the most important chromatographic factors in a systematic, straightforward way.

With just a few runs you are able to obtain hundreds of chromatograms and find the best one, to have complete control of resolution, selectivity and robustness. You make your methods reproducible and according to the requests of the authorities.

25 years history of DryLab® software

The increasing demand for Quality by Design (QbD) in analytical sciences is a logical consequence of the often chaotic methods that are developed by trial and error, showing new peaks or disappearing ones in the validation process of HPLC methods. To ensure a higher standard of method quality, the ICH and the FDA have recently demanded a more scientific Design of Experiments (DoE).

The Molnár-Institute has been promoting this type of approach for over 25 years, contributing to the development of the DryLab® software with LC Resources under the leadership of Lloyd R. Snyder. Using DryLab® the accurate preparation of experiments was initiated achieving useful and reproducible results. Additionally, the new addition PeakMatch® is helping the user to ensure safe and precise data entry into DryLab®.

Designed by HPLC experts, DryLab® 2010 supports the user by assuring an organized and systematic modeling of experiments (DoE), supplying an increased consciousness and comprehension of how the substances in use are really behaving and efficiently furthering the success of your chromatographic work.

DryLab® is the world standard for chromatography modelling in both method development and training applications. The program is based on well-documented models of HPLC, but the basic roules are made transparent for the user.

The following time schedule shows the long and well documented development history of the DryLab® software – from the very beginning in 1985 to the essential tool in HPLC method development as we have it today.

1985	DryLab® 1,2,3,4,5	- first HPLC method development software packages programmed in Basic (DOS) for RPC was modeling capacity factors, Rs-values, flowrate and column dimensions, column optimization in ion pair (2) and normal phase (3) HPLC, %B optimization (4) or using gradient input runs (5). Chromatograms plotted with stars ****
1987	DryLab® I	- combination of DryLab® 1,2,3 and 4 with the addition of new graphics in **DOS**
	DryLab® G	- combination of DryLab® 1,2,3 and 5 + gradient modeling
1989	DryLab® I/plus, G/plus	- first versions of DryLab® in "C" programming language, included a number of new features, such as peak name option, zoom and scale of chromatograms, resolution maps for partial peak sets, ASCII files for data storage, and ability to import data system files
1992	DryLab® I/mp	- isocratic multiparameter versions with an entirely graphical interface for the Microsoft® **Windows® 1.0** operating system with mouse control of program functions - many new features, such as automated peak matching (max.8 peaks), customizable data import templates, on-line help, chromatogram comparison, and a number of new modi
1998	DryLab® version 2.0	- first version of two dimensional (2D) DryLab® models to incorporate simultaneous modeling of two separation parameters, e. g. gradient time tG and temperature T or %B vs. T
2000	Drylab® 2000 v. 3.0	- adjusted to **Windows 3.1 and Windows NT** for network applications and rewritten in C++
2002	DryLab® 2000 plus v.3.1	- 2 dimensional modeling any variable vs. any other possible variable
2005	PeakMatch® v.1.0	- the first peak tracking software introduced for DryLab for easier alignment of peaks in 4-6 different chromatograms, running under **Windows XP**
2006	DryLab® 2000 plus v. 3.5	- DryLab acquired by the Molnar-Institute and is developed further in Berlin
2007	DryLab® & PeakMatch® v. 2.0	- automated generation of experiments with Agilent 1100
2009	DryLab® v. 3.9	- introduced at the HPLC 2009, **modelling the "3D Cube"**, **3 factors optimized simultaneously**
2010	DryLab® 2010	- DryLab® 2010, including DryLab® v. 3.9 and PeakMatch® v. 3.5, running 3 tG-T-models automated for the Shimadzu HPLC line and with **Windows Vista**
2011	DryLab® 2010 v. 4.0	- reprogrammed in **C# (C-sharp)** with new user friendly windows control and 3D cube

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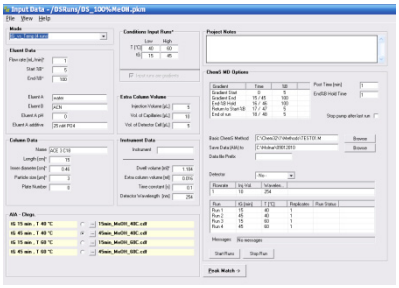
The 3-Dimensional DryLab® 2010 Method Development Suite guides through all steps of systematic QbD method development:

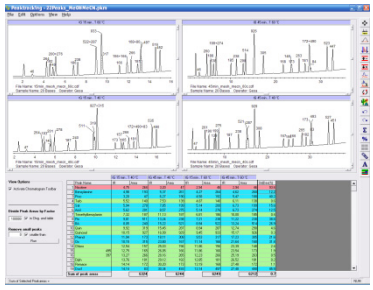
(1) After selecting an excellent column, run input experiments and perform peak tracking with PeakMatch®

Define and run input experiments:

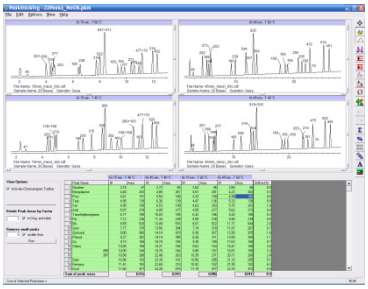
In PeakMatch®, which is part of DryLab®2010, you define the set of experiments needed to optimize the selected parameters (Fig. 1). With most common data systems PeakMatch® is able to *automatically* run the input experiments and import them into PeakMatch® (Fig. 2).



(Fig. 1)



(Fig. 2)



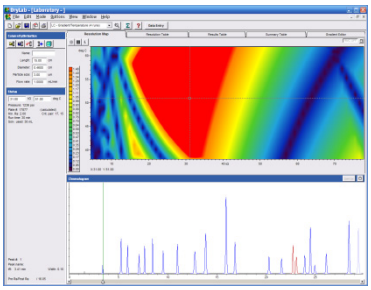
(Fig. 3)

View and evaluate experimental runs:

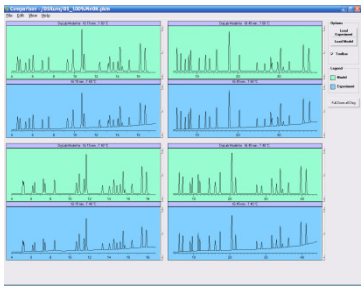
PeakMatch® displays all experimental chromatograms with different selectivities and their corresponding peak tables and with its peak tracking capabilities it is much easier to generate a matched peak table. You have to control your chromatograms before the peak tracking is done to ensure correct modelling (Fig. 3). Red and blue colored lines signalize insufficient peak order; green color corresponds to a reasonably good peak alignment.

(2) Automated data transfer to DryLab®2010, model control and optimum selection of separation conditions

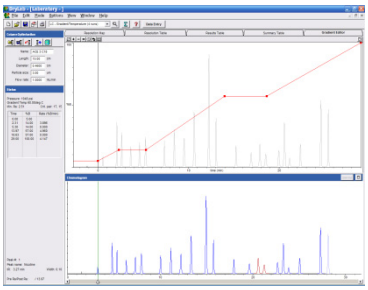
The DryLab®2010 **tG-T-map** of critical resolution represents ca. **10^4** possible separation selectivity's and shows the **best ones** for you in the red region **in seconds** (Fig. 4). Before evaluating the model, we validate how DryLab® calculated the basic input runs by comparison the modeled runs (green) with the original experiments (blue) (Fig. 5).



(Fig. 4)



(Fig. 5)



(Fig. 6)

Fig. 4 - The region in red color is where the method is robust, i.e., $R_{s,crit} > 1.5$. Blue color means peak overlaps. Moving the cursor to a new position, the chromatogram is changing in its time scale and in the separation selectivity.

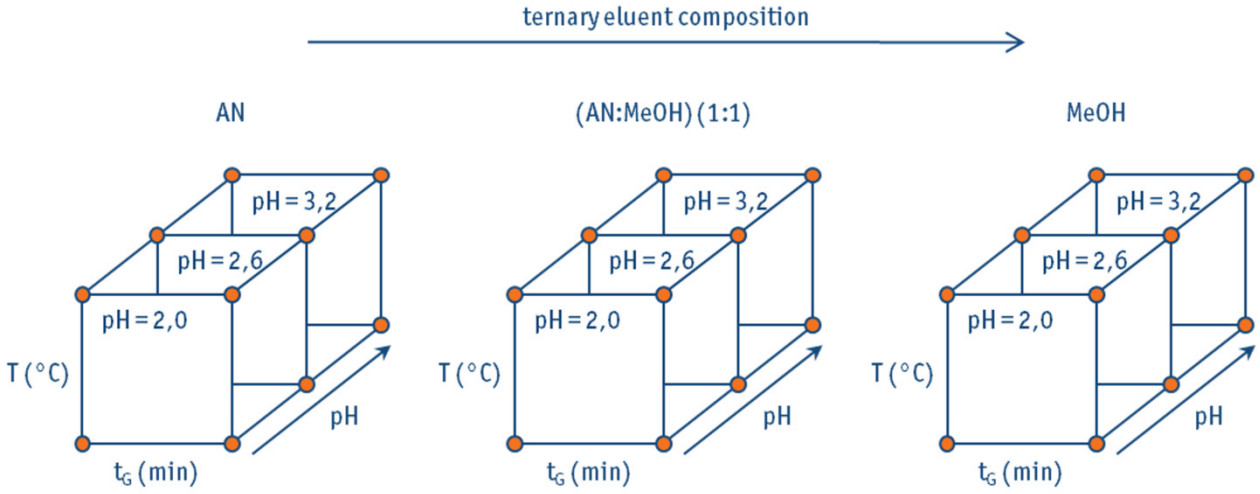
Fig. 6 - By changing the gradient profile, and adding points to the gradient, DryLab® models the separation immediately under consideration instrument settings and the dwell volume V_d to enable better method transfer.

After selecting a robust method (red area in Fig. 4), DryLab® models step gradients of your choice (Fig. 6). DryLab® is modeling the influence of flow rate and column dimensions to optimize your separation in gradient elution for speed, high selectivity and sufficient resolution.

(3) Look for Robust HPLC-methods using 3-Dimensional Resolution Space

The new DryLab® 2010 3D Module extends method development **into the third dimension!**

You can model the combined influence of gradient time, temperature and either pH, ternary eluent composition or additive concentration from one set of 12 experiments. Design and generate your experiments, find the best pH, followed by calculating a 3D-tG-T-ternary eluent composition (or pH) cube (Fig. 7).



(Fig 7)

You have to carry out peak tracking with the 3 x 4 = 12 runs first. Find optimum separation conditions in the 3D tG-T-ternary composition resolution space ("Cube").

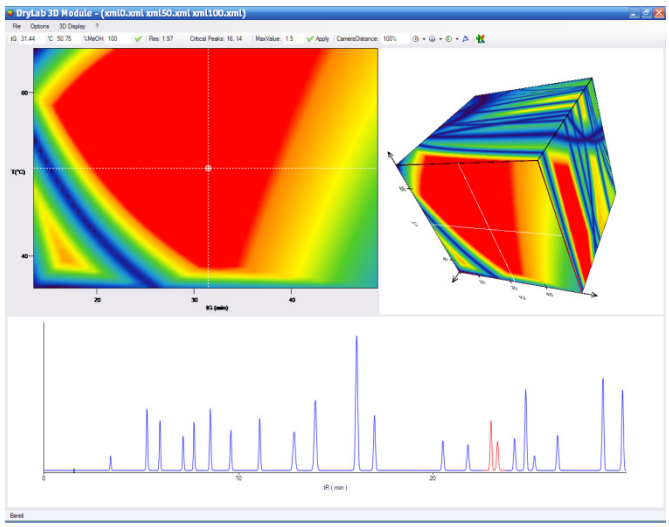


Fig. 8 - Move in the 3D-Space and see selectivity changes. Examine over a million simulated chromatograms.

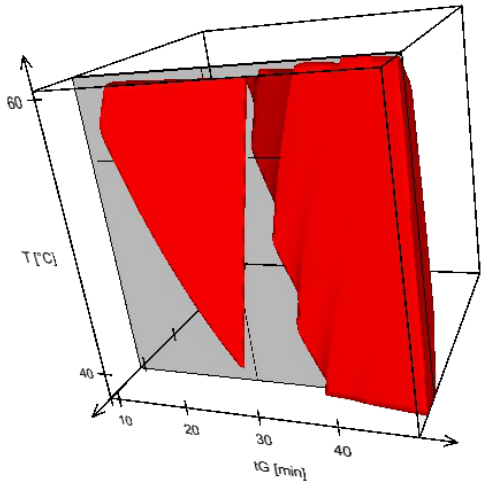


Fig. 9 - Evaluate the robustness conditions for higher flexibility in the control space (in the Version 4.0, in preparation).

See two examples in motion:

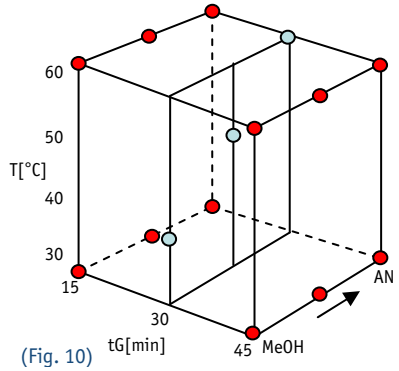
→ ***http://www.molnar-institut.com/3D-Cube-I/3D-Cube-I.html***

→ ***http://www.molnar-institut.com/3D-Cube-II/3D-Cube-II.html***

Application of DryLab® 2010 for a complex mixture

We are thankful to Dr. Melvin Euerby of HiChrom, UK, who tested DryLab®2010 with a mixture of 22 drugs (shown in Fig.1-6 and 8). He looked at the influence of the gradient time tG, the temperature T, and used 3 different ternary eluents (MeOH, AN and 50:50) as eluent B and varied them at the same time.

After preparing the Cube, Dr. Euerby tested the accuracy of the predictions at 3 different positions in the Cube, as is shown in the model here:



(Fig. 10)

Experimental conditions:

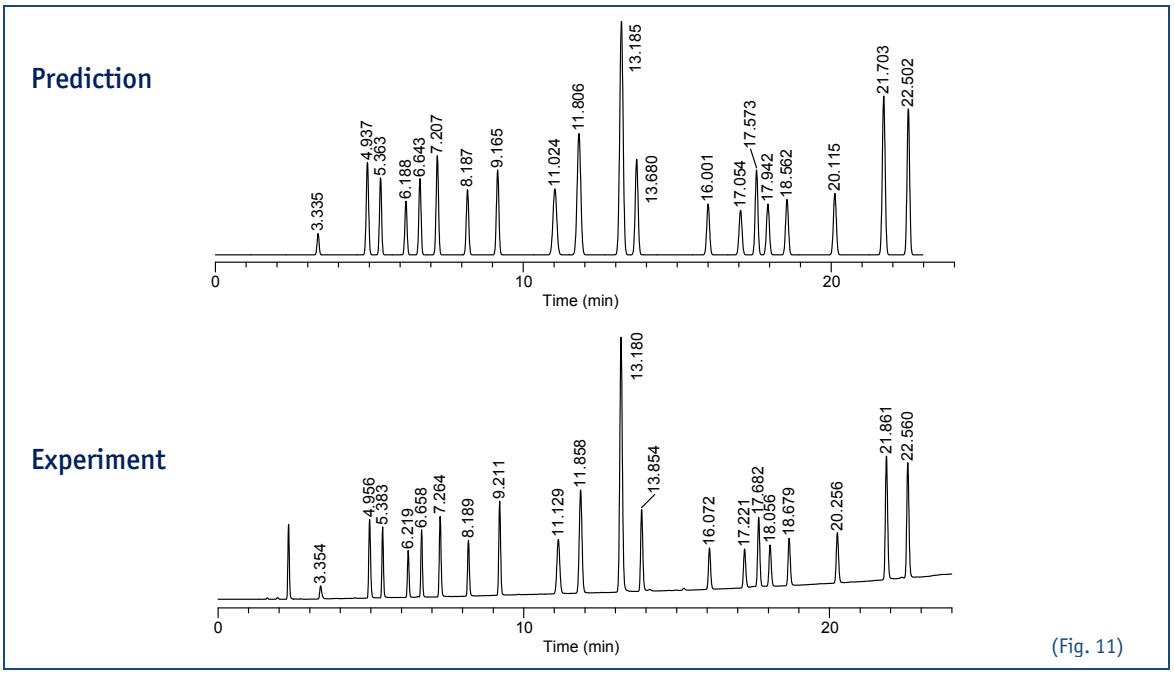
Gradient times:	15 and 45 min
Temperature:	40 and 60°C
Column:	ACE C18, 150x4.6mm, 3µm (HiChrom, Reading, UK)
Eluent A:	5mMol phosphate buffer pH 2.6
Eluent B:	MeOH, AN and 50:50 mix
Gradient range:	5 to 100%B
Flowrate:	1.0 mL/min
Dwell volume:	1.06mL
Modelling software:	DryLab®2010 v. 3.9 (Molnár-Institute, Berlin, Germany)

The results of the tests have shown a high precision of the data for every one of the different test points. Red points are the 12 basic experiments; The light blue points are the positions of the control runs.

The precision of the predicted retention times was excellent, i. e. better than **99.8%** accurate (deviations are in average less than 0.2% as follows: at point 30min 40°C = 0,09%, at point 30min 50°C = 0,17%, at point 30 min 60°C = 0,07%).

DryLab® 2010: Absolute precision of predictions helps in the validation

Comparison between predicted and real chromatograms shows the excellent precision of DryLab® models. Differences in retention times are in the range of a few seconds! Example: tG =22 min, T = 55°C, ternary composition: 100% MeOH



(Fig. 11)

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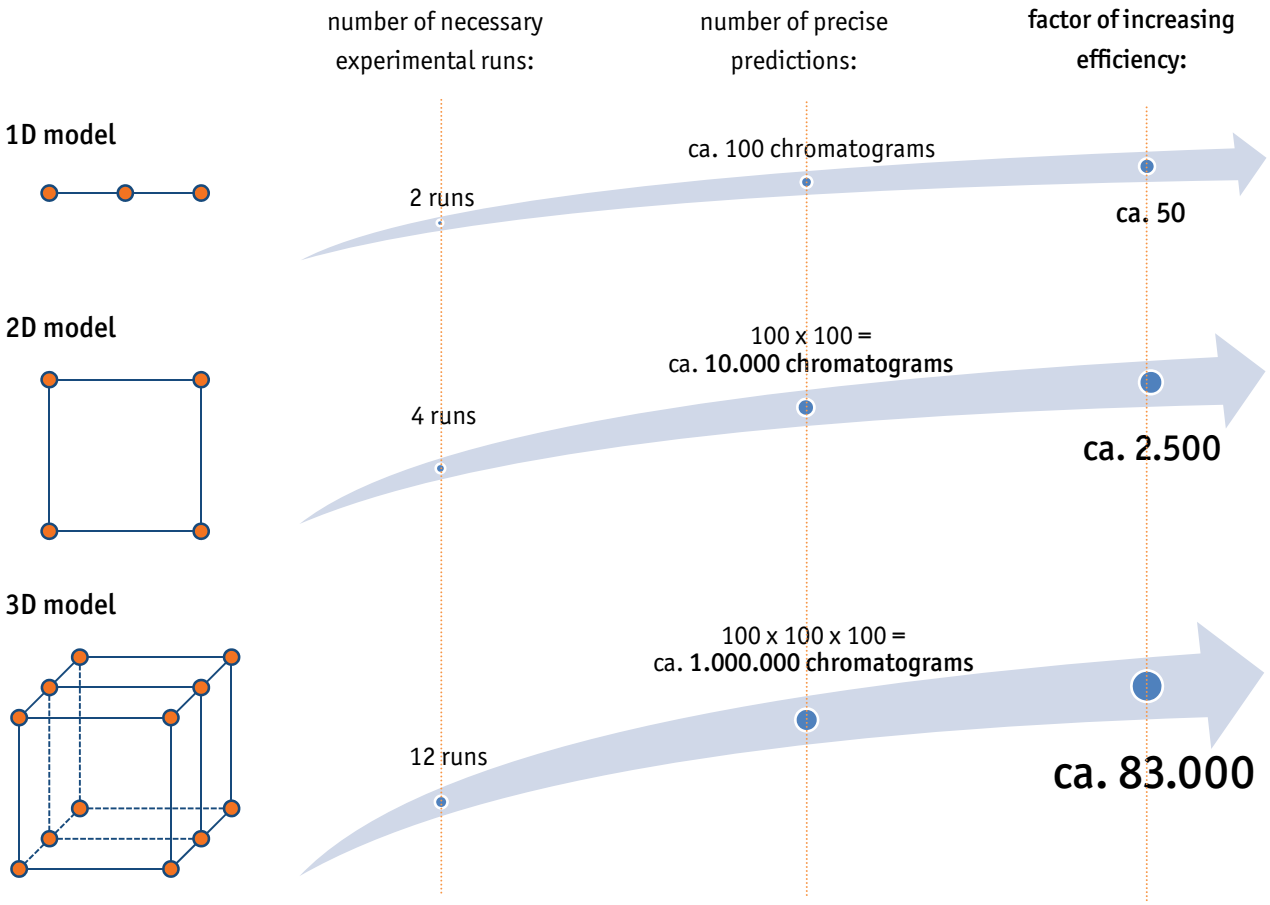
Comparing of increasing efficiency by using DryLab® 2010



The quality of pharmaceutical products is approved mostly by HPLC methods. A safe and robust method is therefore a necessary component of the production process. DryLab® 2010 helps to achieve this aim. It helps to structure and organize your work, save time and material for runs and make the laboratory work more predictable, reliable, transferable, and successful.

Saving potential on costs, time and resources by using DryLab® 2010

Save time! develop your methods in a fraction of the time previously needed.

Save money! substitute acetonitrile by the less expensive methanol.

Save materials! your columns live longer, and use less solvents, etc., per method.

Protect your equipment! your HPLC instrument are running with less trouble.

And last but not least:

Protect the environment! consume less energy and chemicals, produce less waste and make **green HPLC**!

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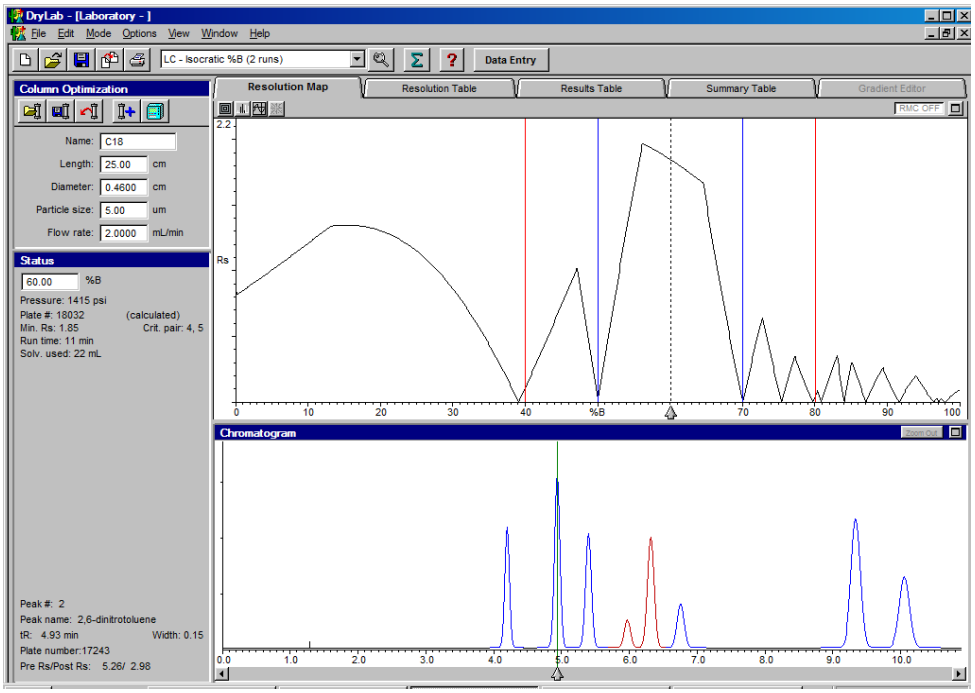
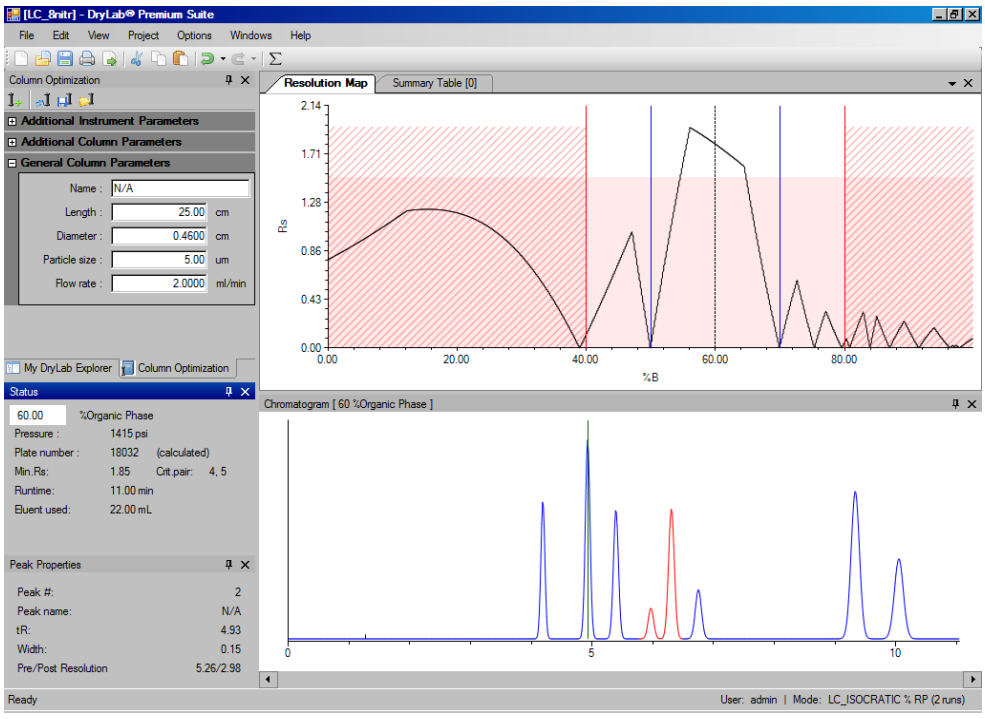
New generation of DryLab® 2010 v. 4.0
program language C-sharp (C#) is in preparation (release is planned in January 2011)

Comparison between the version 3.9 (top) and 4.0 are showing a seemless link between both generations of DryLab®.



The LC_8nitr.dlb example file in DryLab® v.3.9 ↑

↓ The example file LC_8nitr.dlproj in DryLab® v. 4.0



The comparison shows the high precision in the version 4.0.

New generation of DryLab® 2010, version 4.0

The comparison between DryLab® v.3.9 and 4.0 shows an excellent correlation of all important values.

Resolution Map | Resolution Table | **Results Table** | Summary Table | Gradient Editor

#	Name	tR (min)	Area	Tail	k	Width	Sel.	Rs
1	nitrobenzene	4.19	140.5	1.00	2.3	0.13	1.25	5.26
2	2,6-dinitrotoluene	4.93	233.5	1.00	2.9	0.15	1.13	2.98
3	benzene	5.39	167.0	1.00	3.2	0.16	1.14	3.38
4	2-nitrotoluene	5.97	44.0	1.00	3.7	0.18	1.07	1.85
5	4-nitrotoluene	6.31	185.5	1.00	3.9	0.19	1.09	2.30
6	3-nitrotoluene	6.75	78.5	1.00	4.3	0.20	1.47	10.98
7	2-nitro-1,3-xylene	9.33	313.0	1.00	6.3	0.27	1.09	2.55
8	4-nitro-1,2-xylene	10.05	185.0	1.00	6.9	0.29		

LC_8nitr.dlb Results table DryLab v.3.9 ↑

↓ LC_8nitr.dlb Results table DryLab v.4.0

Resolution Map | Summary Table [0] | **Results Table**

#	Name	tR(min)	Area	Tail	k	Width	Rs
1	N/A	4.19	140.00	1.00	2.28	0.13	5.26
2	N/A	4.93	235.00	1.00	2.85	0.15	2.98
3	N/A	5.39	164.00	1.00	3.22	0.16	3.38
4	N/A	5.97	43.00	1.00	3.67	0.18	1.85
5	N/A	6.31	191.00	1.00	3.93	0.19	2.30
6	N/A	6.75	77.00	1.00	4.28	0.20	10.98
7	N/A	9.33	314.00	1.00	6.30	0.27	2.55
8	N/A	10.05	183.00	1.00	6.86	0.29	

The great advantage of the precise prediction of retention values as DryLab® is offering them, is in the validation process. Here we normally have to carry out many experiments to find the highest point of $R_{s,crit}$. Than we have to investigate the environment around of that "working" point, hoping to find meaningful tolerance regions for a robust routine application. If we selected the working point at a wrong place, we have to start to move to different, but better location to establish a higher robustness of the method as before. This process is time consuming and tedious and with DryLab® we can reduce this work considerably, due to the fact, that to make an experiment in DryLab® takes one second, in reality in 10-20 min (600-1200 times slower).

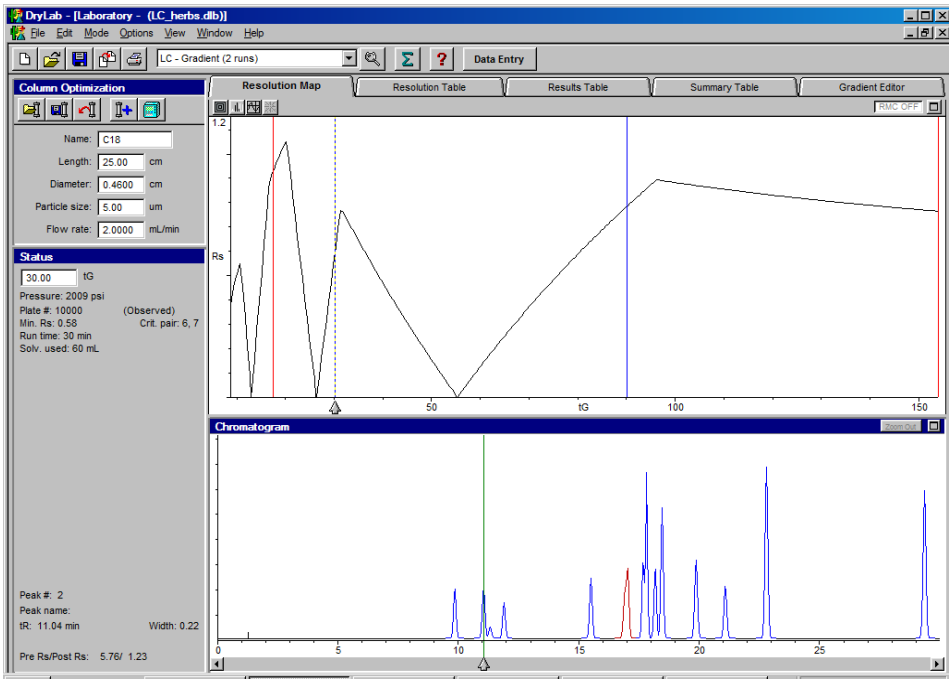
We are showing the region of robustness, where the $R_{s,crit} > 1.5$ (baseline resolution) visually in form of irregularly shaped bodies. We can walk through the cube, see the cross-sectional size of the **Design Space** and get a feeling for the deepness of such a region also. For a precise definition of the size of each tolerance windows we are developing visual tools and can use in addition available statistic software packages with special interfaces to DryLab®.

The advantage of this type of visual robustness check is to be able to react quickly in case of selectivity problems, as we don't have to repeat a large number of experiments to find out what went wrong. In this way we even can automatically correct the method using DryLab® to achieve the previously defined system performance level according to the system suitability test (SST).

In case you still have unresolved peaks, DryLab® has a special column database with ca. 500 different columns, in which your columns can be compared in their selectivity with other available columns. The software "Column Match" is based on the **Snyder-Dolan hydrophobic subtraction model** and allow you to find "equivalent" (= similar) columns for a necessary replacement of a column. The other application of the database is, if you need columns, which is different in their selectivity from your reference column. This is normally in the case if you need to separate difficult peak pair.

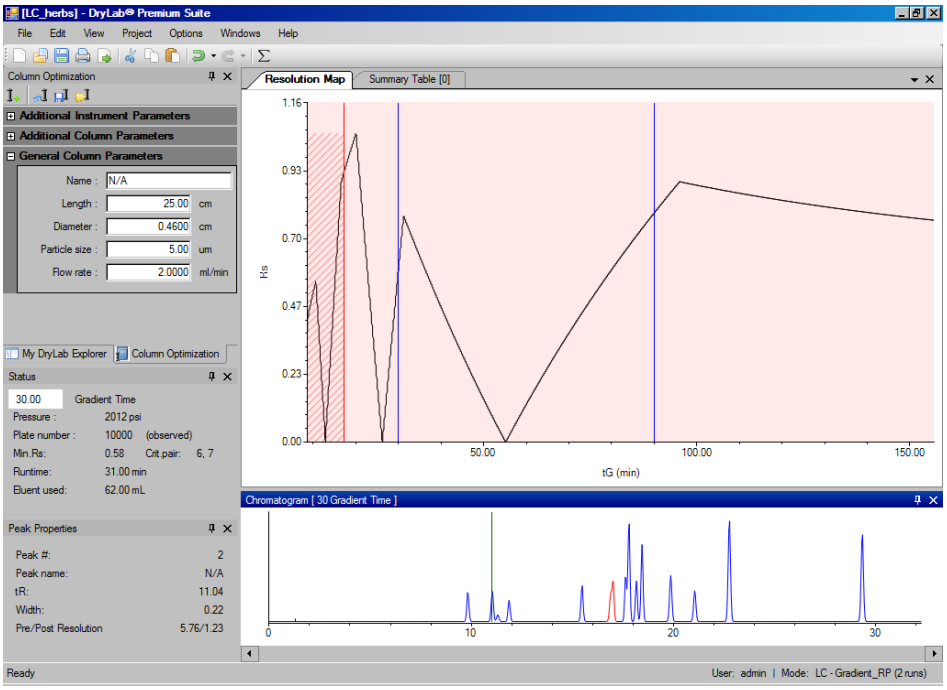
New generation of DryLab® 2010, version 4.0

Gradient elution calculations are especially complex. Therefore it is important to be extremely precise.



The example file LC_herbs.dlb in DryLab® v. 3.9 ↑

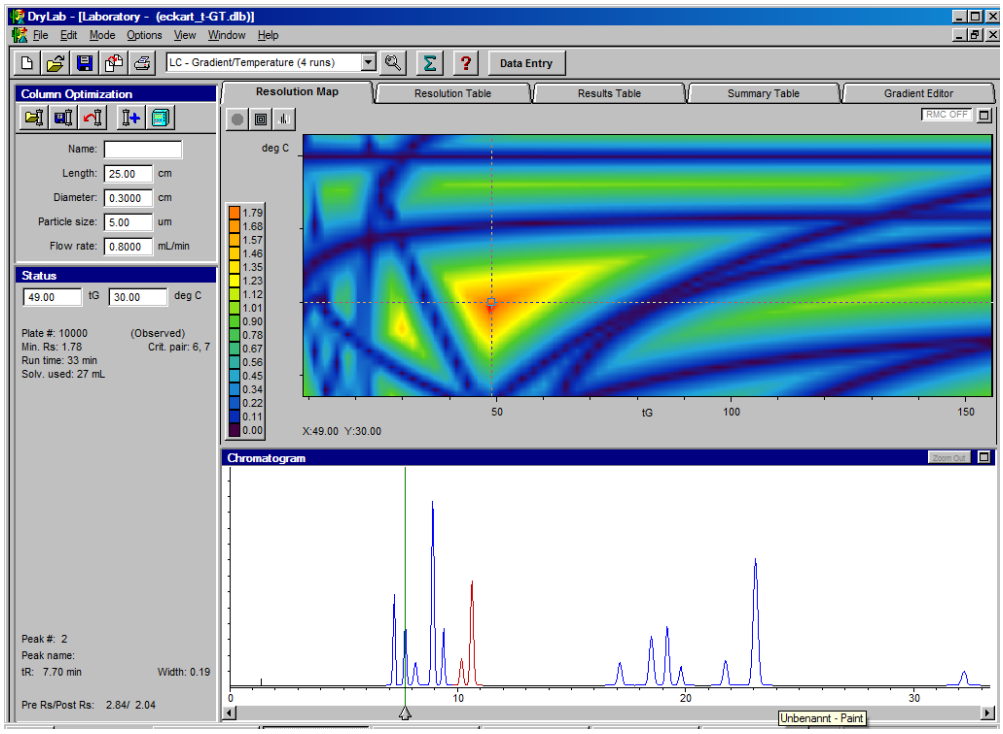
↓ The example file LC_herbs.dlb in DryLab® v. 4.0



The comparison of both screens show the same information for all peaks. Each window is scalable and movable to any position. In this way the user can design his own DryLab® desktop configuration. The windows can also be blended out and are placed at the frame and can in case they are needed, with a single click on them.

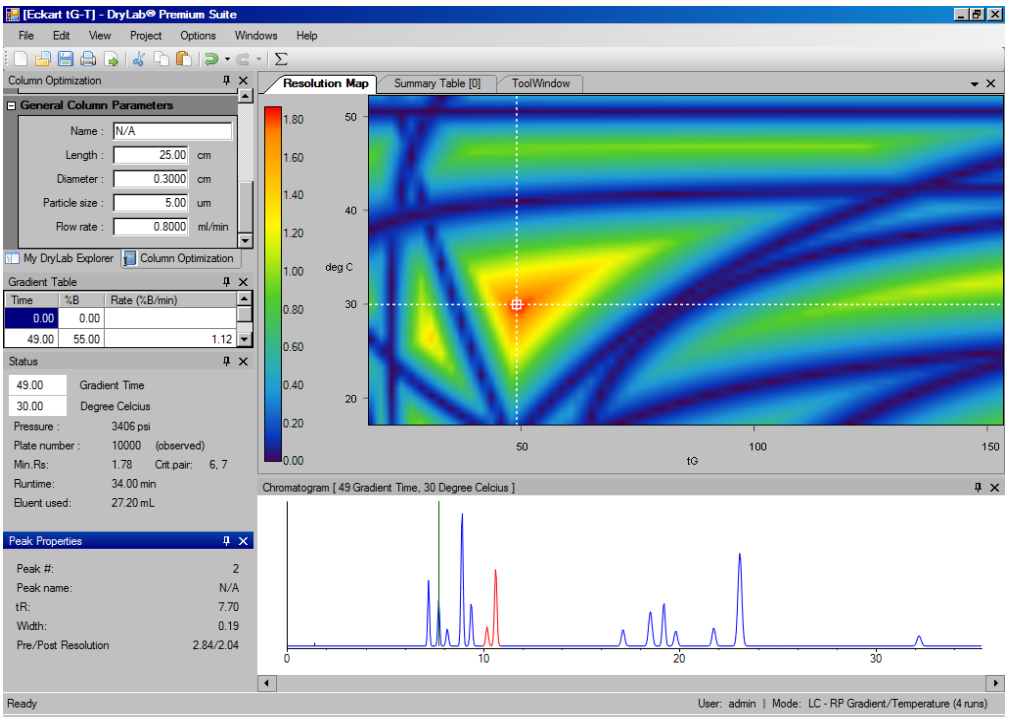
New generation of DryLab® 2010, version 4.0

Comparison between the version 3.9 (top) and 4.0 in 2D-modes are showing the same screen details between both versions.



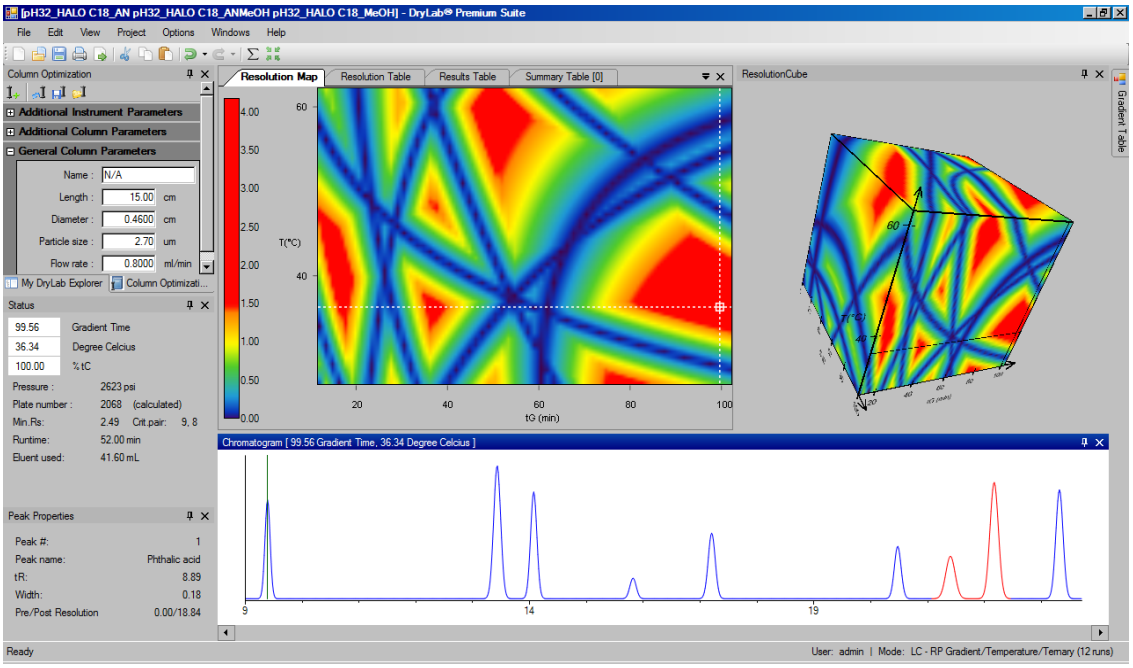
Example file tG-T.dlb, DryLab® v. 3.9 ↑

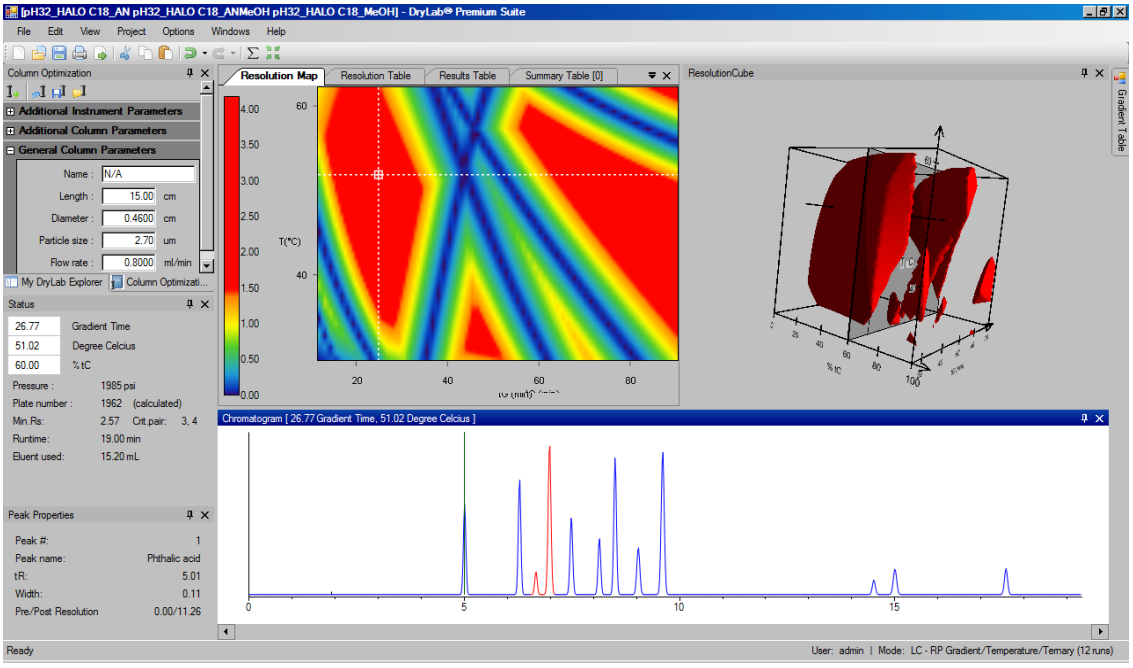
↓ Example file tG-T.dlb, DryLab v. 3.9



You can find the best working point (red spot in the middle) in just a second. These screens show > 10,000 experiments with a high accuracy and reliability.

New generation of DryLab® 2010, version 4.0





The robust region is shown in the empty cube as red irregular geometric bodies, having a critical resolution of $R_{s,crit} > 1.5$ which corresponds with baseline resolution. The critical peak pair is shown in red color.

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HPLC Trainings in Berlin, Germany

Once learned, the use of DryLab® software becomes routine very fast. In order to prevent that mistakes at the beginning become a habit, we recommend to all users to participate in a training in front of their method development activity with DryLab® 2010.

Only a precise handling will lead to correct results!

(A) HPLC User Training: DryLab® & PeakMatch® (2 days in Berlin)

This course teaches basic principles and technologies for the development of robust and transferable HPLC methods with the help of the leading method development software products DryLab® and PeakMatch® (together = DryLab® 2010). Topics covered include the development of new methods, adjustment of old ones, improvement of problematic methods as well as reduction of run time while preserving the desired selectivity.

We encourage you to bring some of your own data with you for discussion. We are sure to impress you by demonstrating the chromatographic modeling power possible with only a few experiments. One can expect a significant portion of time spent with hands-on, practical instruction using DryLab®.

(B) HPLC Course for advanced users: "Quality by Design in HPLC Method Development" (1 day in Berlin)

This course covers the modern approach to HPLC method development, which is based upon the principles of "Quality by Design (QbD)". Typically QbD is attained by rigorous scientific testing to find the best combination of parameters/factors, such that various features of a process can be optimized.

With DryLab HPLC method development, you can better grasp the complex relations between parameters and their influence on experimental outcome. This means we are independent from random tests because we can model changes in retention time and the resolution of peaks in a chromatogram. Using DryLab® and PeakMatch® we will learn how to fulfill the Quality by Design conditions by excellent chromatographic knowledge.

DryLab®'s resolution maps permit users to visualize the most robust working regions and to plan subsequent tests without running experiments again. In this way only the optimal separations need to be verified by experiment.

In-house Trainings (2 days at your site)

As an alternative to our workshops in Berlin, we can also come to your company and offer in-house training workshops. These courses can be arranged individually according to the requests from the users of HPLC systems.

Contact us for more information, dates and prices.

For more information, literature, quotation, course registration, etc.
call or mail the Molnár-Institute directly or visit our homepage.

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„Home of DryLab® Software"

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