CHROMuLAN
basic instruction

1. Introduction

This freeware is designed for the control system of various sets of apparatuses and subsequent assessment of results.

The project is initialised and sponsored by the PiKRON company whose products support communication and control through the communication protocol uLAN.

At present the system is developed in the DELPHI environment for WINDOWS NT or WINDOWS 2000 and an extension for LINUX is expected.

2. Installation

Install the program by starting the installation program **SetupChromulan**. Follow the instructions of the installation software.

The new tested version is obtainable from the address www.pikron.com, the development version is available from www.jindrich.com.

2.1 Data file types

The CHROMuLAN program works with these basic types of files.

.ulf Data file containing the analysis data, method and other information on the analysis.

.ulm The method file. It contains a description of files, parameters for searching for the peaks and the zero lines. It forms a part of the data file (.ulf) but it is possible to save it separately and read it into a different analysis.

.ult Template. This file contains a template for a certain type of analysis. A part of this file is formed by the analysis description, method and by the description of apparatuses.

The program is able to work with other types of files as well (asc, dat, txt). These types of files serve for data transmission to other systems.

3. Controls

3.1 Menu

The following menu options are available:

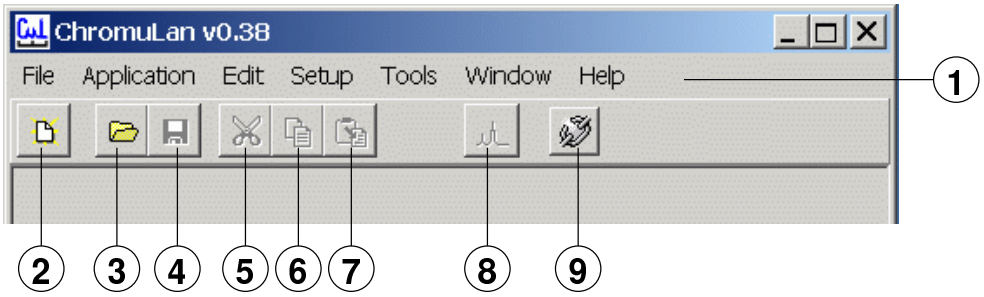
File - work with files
Application - Application selection
Edit - copying and inserting
Setup - setting up
Window - window selection
Help - menu

Besides the above options also the chromatogram menu is available. It can be called out by the right button in the chromatogram window or button, see obr. 1

Setup - setting of the analysis parameters
Method - Method submenu
Baseline - Zero lines submenu
Peaks - Peaks submenu
Math - Conversions submenu, to be used during the overlapping of analyses, see 7
View - setting of visible items (axes, peak description, zero line and others)
Scale - basic scale
Copy to clipboard - copying of a graph to other applications
Print - print

3.2 Buttons

Basic menu functions can be called out directly by means of buttons under the main menu. see obr. 1.



1. Main menu
2. New chromatogram
3. Open a chromatogram
4. Save a chromatogram
5. Cut out
6. Copy
7. Insert
8. Chromatogram menu
9. Setting

Obr. 1. Buttons

Further buttons are situated in the lower part of the analysis window. Their meaning is described in obr. 2.

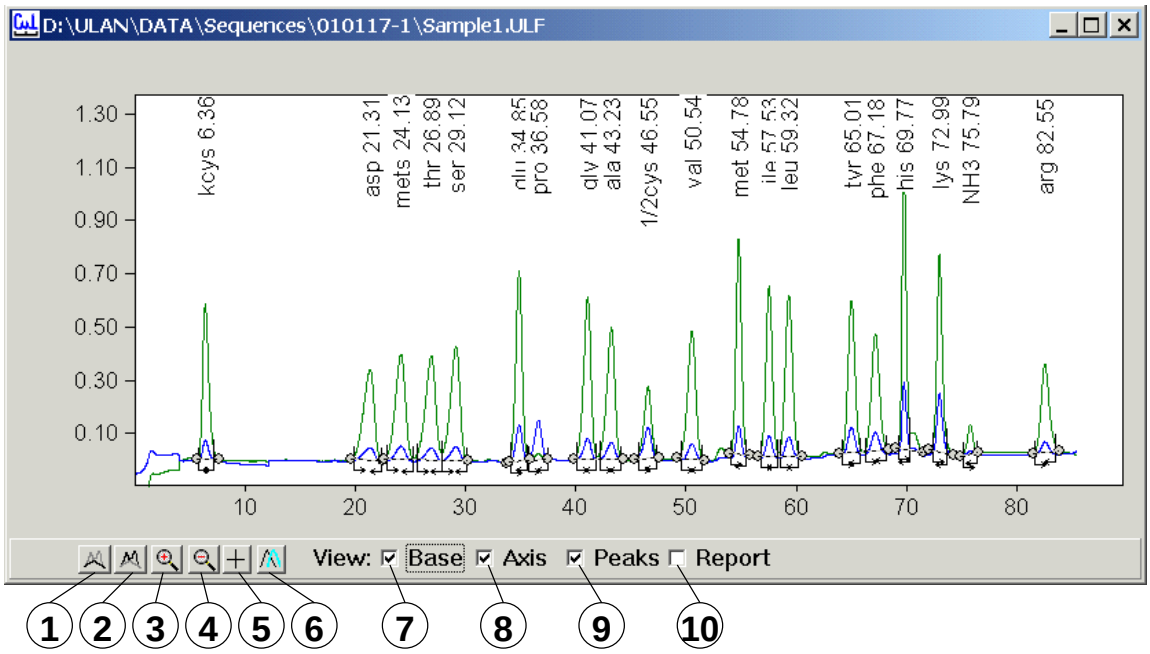
By pressing the button it is possible to use the function once, the repeated use will be activated by pressing the button simultaneously with the SHIFT key.

3.3 Selection

The peaks displayed and the zero line can be selected individually by mouse clicking. Should you want to select more peaks (zero line sections), mark them by pressing simultaneously the CONTROL key. The entire section can be selected by clicking on the first element, and then on the last one while the SHIFT key is held pressed.

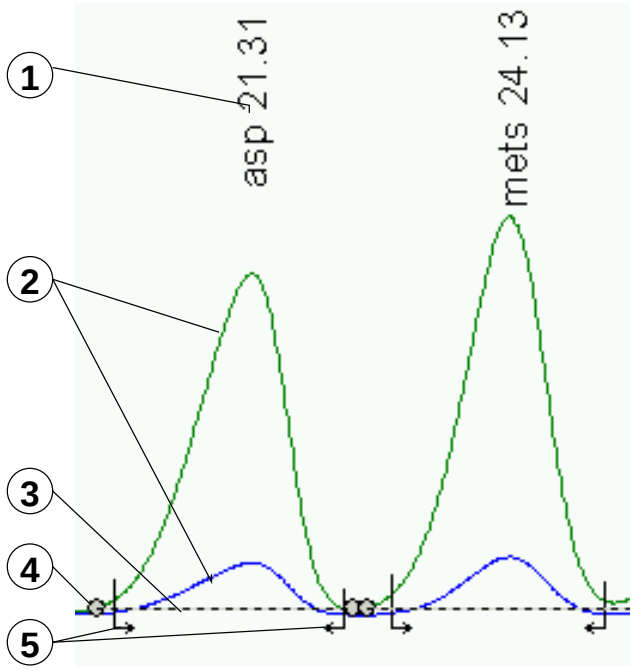
3.4 Editing of peaks

It is possible to edit peak parameters directly in the graph, or in the peak table. In the graph it is also possible to edit the peak line and integration marks of peaks, see obr. 3.



1. Manual creation of a zero line (3.4)
2. Manual creation of peaks (3.4)
3. Setting of a cut-out
4. Back to the previous cut-out
5. Cursor position display mode
6. Comparison of analyses, see 7
7. Zero line display ON
8. Axes display ON
9. Peak description display ON
10. Report display ON

Obr. 2. Chromatogram window



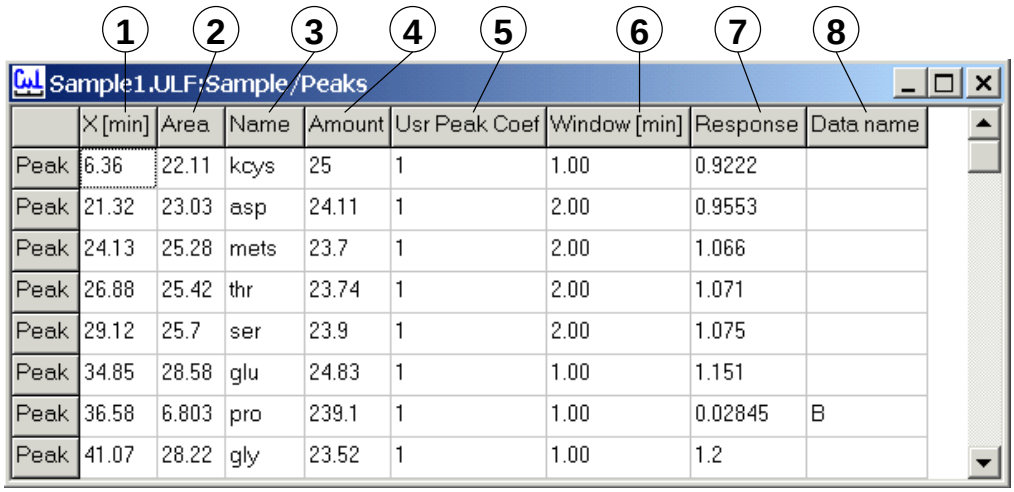
1. Peak description
2. Data measured
3. Zero line
4. Zero line end point
5. Integration marks of a peak

Obr. 3. Editing of a peak

Peak parameters in the graph are edited in such a manner that you mark the peak by clicking on the description, and by repeated clicking you will call out a dialogue for the editing of peak parameters. The position of the end points of the zero line and integration marks can be changed directly with mouse.

Addition of other peaks and zero line section shall be carried out with the help of buttons in the lower part of the chromatogram window (3.2).

The peak table is called out by using the function **Peak** ⇒ **Browse** see obr. 4



	X [min]	Area	Name	Amount	Usr Peak Coef	Window [min]	Response	Data name
Peak	6.36	22.11	kcys	25	1	1.00	0.9222	
Peak	21.32	23.03	asp	24.11	1	2.00	0.9553	
Peak	24.13	25.28	mets	23.7	1	2.00	1.066	
Peak	26.88	25.42	thr	23.74	1	2.00	1.071	
Peak	29.12	25.7	ser	23.9	1	2.00	1.075	
Peak	34.85	28.58	glu	24.83	1	1.00	1.151	
Peak	36.58	6.803	pro	239.1	1	1.00	0.02845	B
Peak	41.07	28.22	gly	23.52	1	1.00	1.2	

1. Retention time
2. Peak surface area
3. Peak name
4. Amino acid quantity
5. Coefficient for the computation of quantity, see 6
6. Windows for the assignment of method peaks, see 5.1
7. Response, see 6
8. Name of the line from which the peak is assessed. It is used at a multi-channel record, e.g. for an amino acid analyser.

Obr. 4. Peak table

4. New analysis

1. Call out the command **File** ⇒ **New**.
2. Select the template for the analysis type in question. If the template is not created , use *Default_ulan*
3. Fill in the name and description of the sample.
4. If you are carrying out a quantitative computation, fill in the factors *Multiply Factor* a *DivideFactor*
5. Confirm the form filled in by using the OK button.
6. Start the analysis, mainly by rotating the dispensing valve, possibly by pressing the key RUN on the computer or thr key MARK on the detector.
7. If the automatic setting is not set in the method, stop reading data after completion of the analysis. Use the chromatogram menu function **Stop**. Once the analysis has been completed, the system makes automatically an assessment. The analysis record can be abbreviated by using the function **Truncate**.

8. If the analysis is O.K., save it **File** ⇒ **SaveAs**, otherwise it is possible to repeat the analysis by repeated activation of the command **RUN**.

5. Assessment preparation

If you want to assess repeatedly samples of the same type, it is quite advantageous to prepare a template for the type of analysis in question, according to which the assessment is carried out automatically.

5.1 Method

Method is a data structure containing parameters for an automatic detection of peaks, peak list with names, retention time and other parameters. At the moment of a peak creation the corresponding parameters are copied from the method to the peak description.

The method peaks are assigned to the peaks measured with the help of the retention time and a window. If the peak is not assigned in a correct manner, change the retention time in the method peak table or enlarge the window. In the case of a window change it is necessary to avoid any overlapping of windows. The method peak table can be called out by using the function **Method** ⇒ **Peaks**Browse: This table is the same as the table of peak description obr. 4.

The method forms a part of the analysis but it can be saved separately as well (**Method** ⇒ **Save To**). The method saved in this manner can be read into an arbitrary analysis (**Method** ⇒ **Load From**). The method can be read into the analysis also from another analysis or template.

The procedure of the method creation is as follows:

1. Call out the function **Method** ⇒ **Edit** and set the following parameters:
   1.1. *Base min. interval* This parameter says how long a section must be so that it can be deemed as a zero line.
   1.2. *Base max. diff* This parameter states a maximum noise which can appear on a section deemed as a zero line.
   1.3. *Min. peak height* Minimum height of a peak. The peaks which are smaller are ignored.
   1.4. *Min. peak width* Minimum width of a peak. The peaks which are narrower are ignored.
   1.5. *Use negative peaks* Mark this field if you want to assess negative peaks.
   1.6. *Calc Amounts* Mark this field if you want to compute quantity on an automatic basis, see 6.2
   1.7. *Use Calibration File* Mark this field if you want to use an external standard, see 6.3
   1.8. *Use Internal Standard* Mark this field if you want to use an internal standard, see 6.4
   1.9. *Factor* Conversion factor, see 6.2
2. Call out the function **Peak** ⇒ **Autodetect**.
3. Assign names to those peaks which are interesting for you. It is also possible to set other parameters as well (Window, Amount, Response and others, see Chapter 6.2)

4. Mark the peaks (3.3), which you want to have in the method and call out the function **Peaks** ⇒ **Copy Selected To Method**.
5. Call out the function **Method** ⇒ **Peaks** ⇒ **Browse** and check the method peaks in the table.
6. If you have, in your method, any peaks in excess, you can delete them by using the command **Delete**
7. Save the method **Method** ⇒ **Save To**. It is also possible to save the method as a part of a template, see 5.2.

5.2 Template

The template parameter serves as a template for a new analysis. A part of the template is also formed by a method, which means that for the actual operation it is not necessary to save the method, it is sufficient to save the template.

The template creation procedure is as follows:

8. Open the initial file. It can be another template, or an analysis.
9. By using the function **Setup** set the heading of the file.
10. Create a method, see 5.1.
11. Save the template by using the command **File** ⇒ **Save As** where you can select a type of the file **.ult** in such a manner that the template will be available during the creation of a new analysis, it must be saved in a directory **Templates**.

6. Assessment

6.1 Assessment without computation

1. If the method is correctly set in the template, the assessment of peaks is automatically carried out after the analysis has been completed. If you want to change the method, use the following procedure.
   1.1. By using the function **Method** ⇒ **Load From** read the method.
   1.2. For finding the peaks use the function **Peak** ⇒ **Autodetect**.
2. After the automatic assessment has been completed, there will be a possibility of editing manually the zero line and the peaks, see 3.2.
3. You can look at the peak parameters in the table which is called out by using the function **Peaks** ⇒ **Browse**. These parameters can be edited in this table by using the edit button, possibly by double clicking on the peak name in the chromatogram window.

6.2 Assessment without any standard

This assessment is used if you know the response of individual peaks from the tables. The computation is carried out according to the formula

$$Amount = \frac{Area}{Response} * UsrPeakCoef * Factor$$

By using the following procedure you will prepare a method, and the computation is carried out on an automatic basis.

1. Select the table values of the response in the method peak table (**Method** ⇒ **Peaks** ⇒ **Browse**) into the item *Response*

2. In the window **Method** ⇒ **Edit** check off the item *Calc Amount*
3. Set the method prepared in this manner as the initial method for the type of analysis in question.

6.3 External standard

Assessment with the external standard is used if you have a standard sample with known content of individual components. The computation is carried out according to the following formulas

$$Amount = \frac{Area}{Response} * UsrPeakCoef * Factor * \frac{MutiplyFactor}{DivideFactor}$$

where the Response is computed from the standard

$$Response = \frac{Area_{std}}{Amount_{std}} * UsrPeakCoef_{std} * Factor_{std} * \frac{MutiplyFactor_{std}}{DivideFactor_{std}}$$

By using the following procedure prepare the method, and the computation will be carried out on an automatic basis.

1. Carry out the analysis of the standard. Mark this analysis as a standard, in the windows **Header** activate the item *Cal. Standard*
2. In the peak table (**Peaks** ⇒ **Browse**) select the quantities of individual components, contained in the standard, into the item *Amount*.
3. In the window **Method** ⇒ **Edit** activate the items *Calc Amount* and *Use Calibration File*
4. Then, by using the command **Method** ⇒ **Load Calibration File** read the standard into the method.
5. Set the method prepared in this manner as an initial method for the type of analysis in question.

6.4 Internal standard

The computation with an internal standard is carried out according to the following formulas

$$Amount = \frac{Area}{Response} * UsrPeakCoef * Factor * \frac{MutiplyFactor}{DivideFactor}$$

where Factor is computed as

$$Factor = \frac{Amount_{is} * Response_{is}}{Area_{is} * UsrPeakCoef_{is}} * \frac{DivideFactor}{MutiplyFactor}$$

By using the following procedure prepare the method, and the computation will be carried out on an automatic basis.

1. Select the table values of response in the method peak table (**Method** ⇒ **Peaks** ⇒ **Browse**) into the item *Response*
2. In the window **Method** ⇒ **Edit** check off the item *Calc Amount* and *Use Internal Standard*
3. For the peak which you are using as an internal standard fill in the items *Amount* and *Internal standard* in the table **Method** ⇒ **Peaks** ⇒ **Browse**

4. Set the method prepared in this manner as an initial method for the type of analysis in question.

6.5 External and internal standard

The computation with an internal standard is carried out according to the following formulas

$$Amount = \frac{Area}{Response} * UsrPeakCoef * Factor * \frac{MutiplyFactor}{DivideFactor}$$

where Factor is computed as

$$Factor = \frac{Amount_{is} * Response_{is}}{Area_{is} * UsrPeakCoef_{is}} * \frac{DivideFactor}{MutiplyFactor}$$

and Response is computed from the standard

$$Response = \frac{Area_{std}}{Amount_{std}} * UsrPeakCoef_{std} * Factor_{std} * \frac{MutiplyFactor_{std}}{DivideFactor_{std}}$$

By using the following procedure prepare the method, and the computation will be carried out on an automatic basis.

1. Carry out the analysis of the standard.
2. In the window **Method** ⇒ **Edit** check off the item *Calc Response*
3. In the peak table (**Peaks** ⇒ **Browse**) select the quantity of individual components in the standard into the item *Amount*.
4. For the purpose of analysis of a sample modify the method in such a manner that in the window **Method** ⇒ **Edit** you will enable the items *Calc Amount*, *External Calibration* and *Internal standard*, and disable *Calc Response*
5. Then, by using the command **Method** ⇒ **Load Calibration File** read the analysis of the standard into the method.
6. For the peak which you are using as internal standard fill in the items *Amount* and *Internal standard* in the table **Method** ⇒ **Peaks** ⇒ **Browse**
7. Set the method prepared in this manner as the initial method for the type of analysis in question.

7. Comparison of analyses

The program makes it possible to insert several analyses into a single graph. This can be carried out in such a way that you press the button for comparison of analyses, see obr. 2 and then open the next analysis of functions **File** ⇒ **Open**. Individual analyses can be shifted and enlarged by using the function from the submenu **Math** in the chromatogram menu.

It is possible to switch the active analyses by using the function **Overlay** from the chromatogram menu.

8. Content