

CHROM_uLAN

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 μ LAN.

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 CHROM_uLAN 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.
- .uls** Sequence. This file is used for sets with an auto-sampler.

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.

2.2 Setup

2.2.1 Communication

This software communicates with apparatuses on a serial line RS485, communication protocol μ LAN. In terms of hardware the serial line can be implemented in three ways:

1. through a converter from RS232 to RS485. This is a special connector which is to be connected to a standard line RS232. This connector contains the converter electronic

equipment. For this converter the number of connected apparatuses and cable length are limited.

2. ISA card RS485. This card is to be mounted into an ISA slot of the computer. It cannot be used for new computers which do not have any ISA slot.
3. PCI card RS485. This card is to be mounted into a PCI slot of the computer.

In all of the three cases the μ LAN communication is software-served by a special controller. The controller works in the operating systems Linux and Windows NT, 98 and 2000. For a proper performance of the Chromulan program it is necessary to ensure that this controller can be properly installed. For a description of the installation see the Controller Manual.

Chromulan makes it possible to set the communication parameters. They are set in a special dialogue invoked by buttons **UL_DRV setup** in the setup dialogue (**Setup** \Rightarrow **Options**). This setting is important only for Win NT. In the case of Win 98 and 2000 setup forms a part of Windows.

Default setting of the baud rate is BaudRate=19200. Default setting of the address and port is as follows:

- Converter RS232/RS485 connected to COM1. Port Adres=0x3F8, IRQ=4.
- Converter RS232/RS485 connected to COM2. Port Adres=0x2F8, IRQ=3.
- ISA card RS485. Port Address=0x2E8, IRQ=5.
- PCI card RS485. The setting does not matter.

Apparatuses are connected to computers in a serial manner, every apparatus has two connectors for connection of communication facilities. The communication cable from the computer is connected to a connector of the first apparatus. Another cable continues from the second connector of the first apparatus to the second apparatus and still another cable continues from the second apparatus to the third apparatus, and so on up to the last apparatus at which one connector remains free.

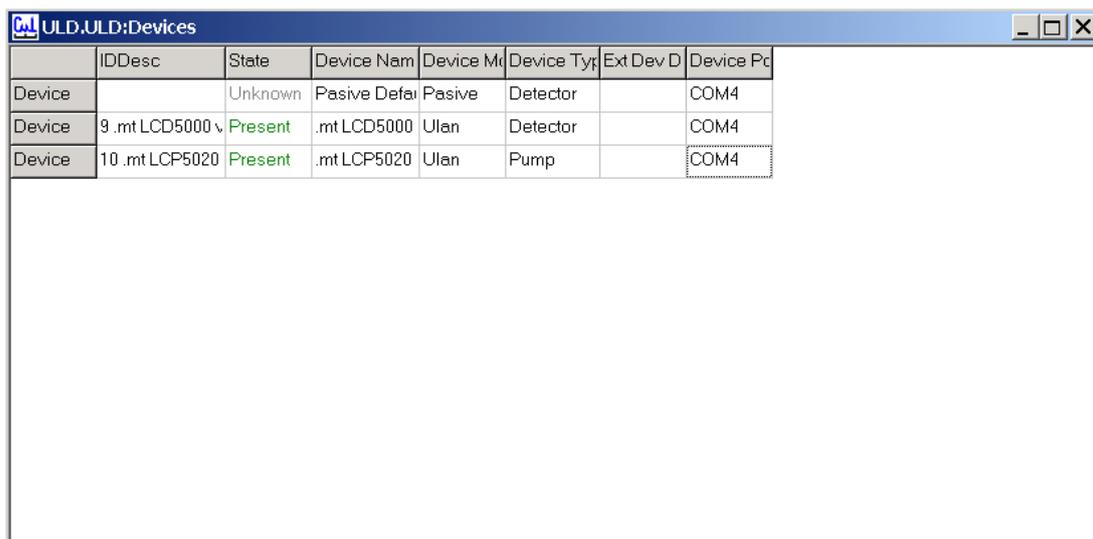
Every apparatus has a communication address which is pre-set. Apparatuses having the same address cannot be connected to a μ LAN network. A setup procedure of the apparatus address is described in the Manual to the apparatus in question.

Communication with apparatuses can be checked by using the command **Setup** \Rightarrow **Devices** \Rightarrow **Autodetect** see obr. 1. After entering this command the program will search for all connected apparatuses and will display them. Display of a list of the apparatuses found can be invoked at any time later on by using the command **Setup** \Rightarrow **Devices** \Rightarrow **Browse** .

2.2.2 Channels

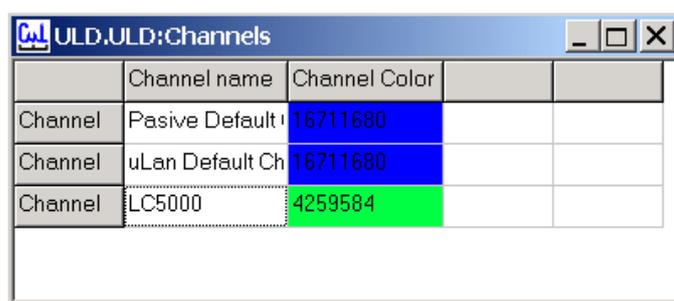
The program makes it possible to operate simultaneously multiple sets of apparatuses. If you have more sets connected together, it is necessary to create a channel for each set. The list of all channels can be invoked by using the command **Setup** \Rightarrow **Channels** see obr. 2. For adding a new channel use the key **Insert**.

If you mark a channel and by using the right mouse button invoke the function **Channel** \Rightarrow **Browse**, you will open a list of apparatuses in the given channel, see obr. 3. Apparatuses can be added and removed by using the keys **Insert** and **Delete**. It is necessary to set the first two columns. The first column includes the apparatus name which is selected from the apparatus list (**Setup** \Rightarrow **Devices** \Rightarrow **Browse**). The second is an alias name, through which the program refers to the apparatus in question. For transferability



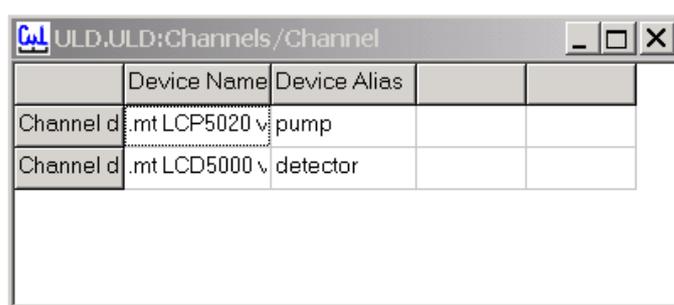
	IDDesc	State	Device Nam	Device Md	Device Typ	Ext Dev D	Device Pc
Device		Unknown	Passive Defai	Passive	Detector		COM4
Device	9 .mt LCD5000 \	Present	.mt LCD5000	Ulan	Detector		COM4
Device	10 .mt LCP5020	Present	.mt LCP5020	Ulan	Pump		COM4

Obr. 1. List of connected apparatuses



	Channel name	Channel Color		
Channel	Passive Default	16711680		
Channel	uLan Default Ch	16711680		
Channel	LC5000	4259584		

Obr. 2. Channels



	Device Name	Device Alias		
Channel d	.mt LCP5020 \	pump		
Channel d	.mt LCD5000 \	detector		

Obr. 3. Apparatus setting in the channel

of programs between individual channels it is necessary to have the same alias name for the same apparatus in all the channels (e.g. pump, detector, etc.).

Selection of the channel which is to be used for the analysis in question will be carried out in the dialogue **Setup** in the section *Instrument*. It is advantageous to create a different

template for each channel. Then the channel is selected by the template selected during the initialisation of the new analysis (5).

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. 4

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 8

View - setting of visible items (axes, peak description, zero line and others)

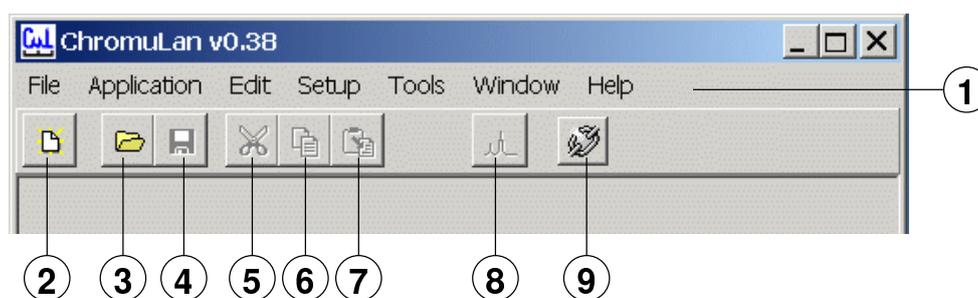
Scale - basic scale

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. 4.

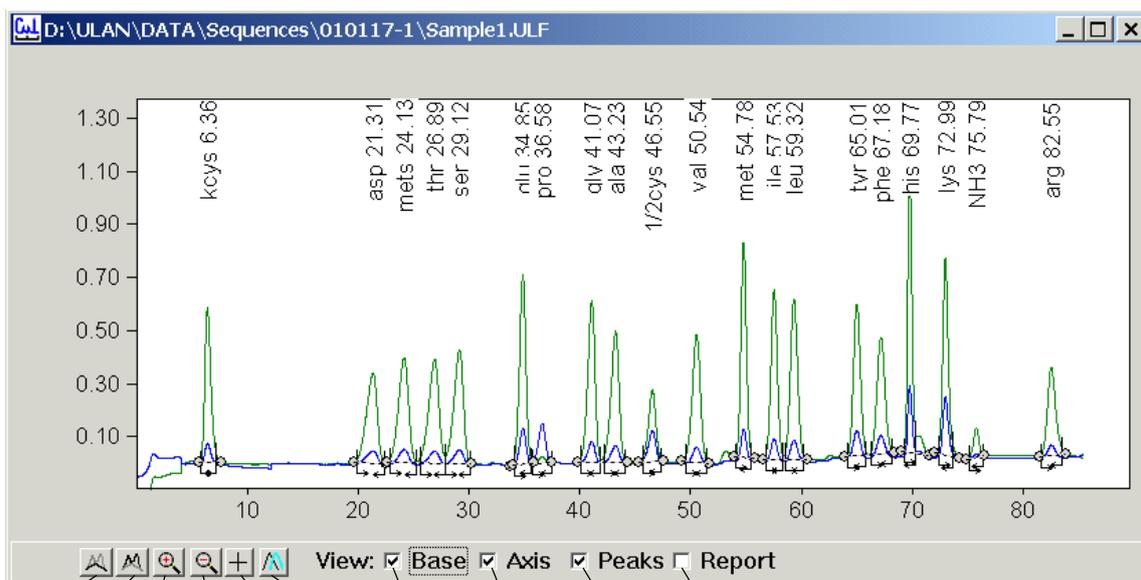


- | | |
|------------------------|----------------------|
| 1. Main menu | 6. Copy |
| 2. New chromatogram | 7. Insert |
| 3. Open a chromatogram | 8. Chromatogram menu |
| 4. Save a chromatogram | 9. Setting |
| 5. Cut out | |

Obr. 4. Buttons

Further buttons are situated in the lower part of the analysis window. Their meaning is described in obr. 5.

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.



- | | | | | | | | | | |
|---|-----------------------------------|-------------------------|---------------------------------|---------------------------------|----------------------------------|-------------------------|--------------------|--------------------------------|-----------------------|
| ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ | ⑨ | ⑩ |
| 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 8 | 7. Zero line display ON | 8. Axes display ON | 9. Peak description display ON | 10. Report display ON |

Obr. 5. Chromatogram window

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.

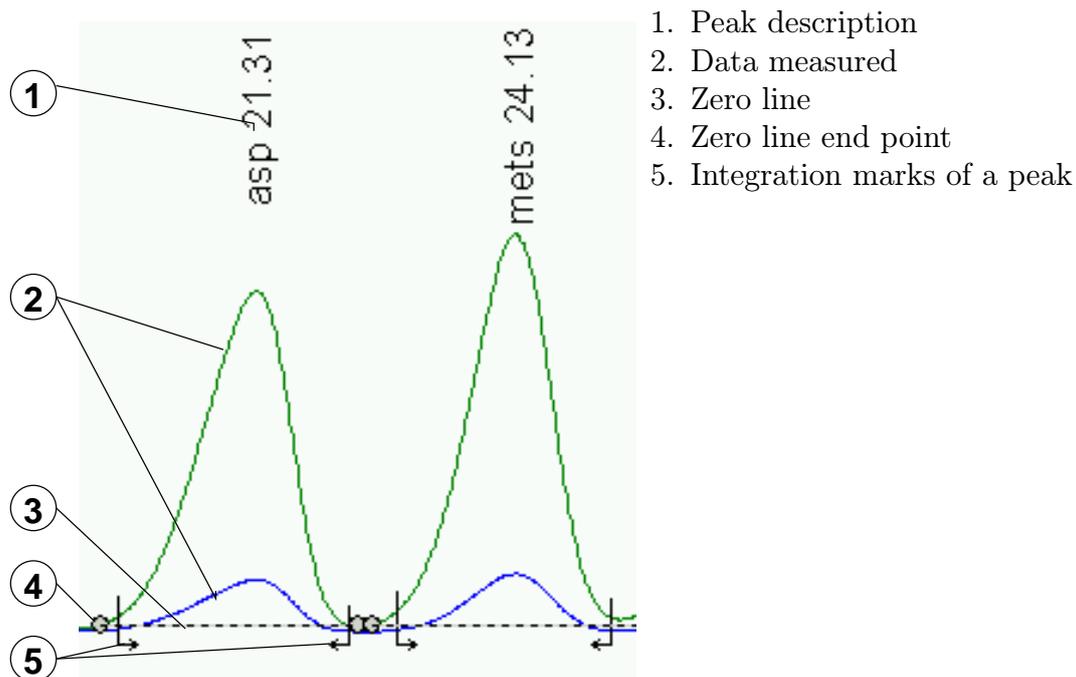
3.4 Editing of peaks

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. 6.

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. 7



Obr. 6. Editing of a peak

	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 7
6. Windows for the assignment of method peaks, see 6.1
7. Response, see 7
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. 7. Peak table

4. Program

In addition to the assessment of analyses, the Chromulan program makes it also possible to control the apparatuses. This control is implemented by means of a program.

The program forms a part of each analysis file. During initialisation of a new analysis the program is transferred from the template selected. It may be modified before the start-up of the analysis.

The program window (obr. 8) is called by using the button **Program** in the section *Instrument* of the dialogue Setup (chromatograph function menu **Setup**). The program can include three types of lines:

- Prg.EventLine** These program lines shall be carried out at the moment when there occurs an event which is pre-set in the line in question. For systems without auto-sampler only the lines *peBeforeStart* are important and they are performed at the moment when the button **Prepare** is pressed. It serves for the setting of the initial conditions of the analysis.
- Dev.Prg.Line** These program lines are transferred to the apparatus which subsequently executes these lines on a real time basis. They are used for programming the pump gradient.
- Seq.Prg.Line** These program lines are executed in the extra time.

	Event	Condition	Script	1st param	2nd param	3rd param	Note	State
Prg.Event	peBeforeStart		PrgAndRunLCP					None
Prg.Event	peBeforeStart		WLen	254				None
Prg.Event	peBeforeStart		MotorStart					None
Prg.Event	peBeforeStart		LampLow					None
Prg.Event	peBeforeStart		Zero	detector				None
Prg.Event	peBeforeStart		CoTemp	20				None
Dev.Prg.L	0.00	pump	GRADBC	40	60			None
Dev.Prg.L	0.00	pump	FLOW	1.000				None
Dev.Prg.L	0.00	pump	WAITSW					None
Dev.Prg.L	0.01	pump	SENDMARK	2				None
Dev.Prg.L	2.50	pump	GRADBC	0	100			None
Dev.Prg.L	3.50	pump	END					None

Obr. 8. Program

Lines of the types Prg.EventLine and Seq.Prg.Line can contain two types of commands. The first type is formed by internal chromulan commands and the second one is formed by scripts.

4.1 Scripts

Scripts are short programs in the *pascal* language. These programs are interpreted by a chromulan program and can perform various operations. In the program line it is possible to enter parameters which are passed to the script. A part of the program installation is formed by basic scripts:

- WLen** Sets wavelength on the detector. The first parameter is wavelength. As the second parameter it is possible to enter the apparatus name, if it is omitted a default value *detector* is used.

LampHigh	Turns on the lamp for a high current value. As a parameter it is possible to enter the apparatus name, if it is omitted a default value <i>detector</i> is used.
LampLow	Turns on the lamp for a low current value. As a parameter it is possible to enter the apparatus name, if it is omitted a default value <i>detector</i> is used.
Zero	Resets the absorbance value on the detector to zero. As a parameter it is possible to enter the apparatus name, if it is omitted a default value <i>detector</i> is used.
MotorStart	Turns on the pump motor. As a parameter it is possible to enter the apparatus name, if it is omitted a default value <i>pump</i> is used.
PrgAndRunLCP	Transfers the program to the pump and runs it. The program is defined by lines of the type Dev.Prg.Line.
Temp	Sets the column temperature. The first parameter is temperature. As the second parameter it is possible to enter the apparatus name, if it is omitted, a default value <i>tempper</i> is used.

Users can create other scripts by themselves. For testing the scripts use the window to be invoked by using the command **Setup** ⇒ **Script**. If you at the same time open the channel window (2.2.2), you can specify, by marking a channel, with which channel the script under tests is working.

5. 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. Press the button PREPARE. Chromulan will set the initial apparatus parameters, depending on how they are set in the program (4).
7. 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.
8. 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**.
9. 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**.

6. 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.

6.1 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** \Rightarrow **PeaksBrowse**: This table is the same as the table of peak description obr. 7.

The method forms a part of the analysis but it can be saved separately as well (**Method** \Rightarrow **Save To**). The method saved in this manner can be read into an arbitrary analysis (**Method** \Rightarrow **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** \Rightarrow **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 7.2
 - 1.7. *Use Calibration File* Mark this field if you want to use an external standard, see 7.3
 - 1.8. *Use Internal Standard* Mark this field if you want to use an internal standard, see 7.4
 - 1.9. *Factor Conversion* factor, see 7.2
2. Call out the function **Peak** \Rightarrow **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 7.2)
4. Mark the peaks (3.3), which you want to have in the method and call out the function **Peaks** \Rightarrow **Copy Selected To Method**.
5. Call out the function **Method** \Rightarrow **Peaks** \Rightarrow **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** \Rightarrow **Save To**. It is also possible to save the method as a part of a template, see 6.2.

6.2 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 6.1.
11. Save the template by using the command **File** \Rightarrow **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**.

7. Assessment

7.1 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** \Rightarrow **Load From** read the method.
 - 1.2. For finding the peaks use the function **Peak** \Rightarrow **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** \Rightarrow **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.

7.2 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

$$Amount = \frac{Area}{Response} * U_{sr}PeakCoef * Factor$$

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** \Rightarrow **Peaks** \Rightarrow **Browse**) into the item *Response*
2. In the window **Method** \Rightarrow **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.

7.3 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

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

where the Response is computed from the standard

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

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.

7.4 Internal standard

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

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

where Factor is computed as

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

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.

7.5 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** \Rightarrow **Edit** check off the item *Calc Response*
3. In the peak table (**Peaks** \Rightarrow **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** \Rightarrow **Edit** you will enable the items *Calc Amount*, *External Calibration* and *Internal standard*, and disable *Calc Response*
5. Then, by using the command **Method** \Rightarrow **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** \Rightarrow **Peaks** \Rightarrow **Browse**
7. Set the method prepared in this manner as the initial method for the type of analysis in question.

8. 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. 5 and then open the next analysis of functions **File** \Rightarrow **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.

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