AUTOMATIC AMINO ACID ANALYSER

AAA 400

Manual for ChromuLan

Producer	:	Laboratory Instruments division of INGOS Ltd.
Development	:	PiKRON Ltd.
Chemical system	:	V. Havlíček - ZMBD chemik

Supplier and service	:	INGOS Ltd. K Nouzovu 2090 14316 PRAGUE 4 CZECH REP.	Tel: +420 296 781 683 +420 296 781 692 Fax: +420 244 403 051 e-mail:instruments@ingos.cz

CE

1. INTRODUCTION

This is a freely disseminated software serving for controlling the sets of apparatuses and subsequent assessment of results. The project is initialized and subsidized by PiKRON whose instruments support communication and control through the uLAN communication protocol.

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

This manual deals with the use of the ChromuLan program for the Amino Acid Analyzer AAA 400. In this case the program uses standard assessment and a special program module for the control of the Amino Acid Analyzer.

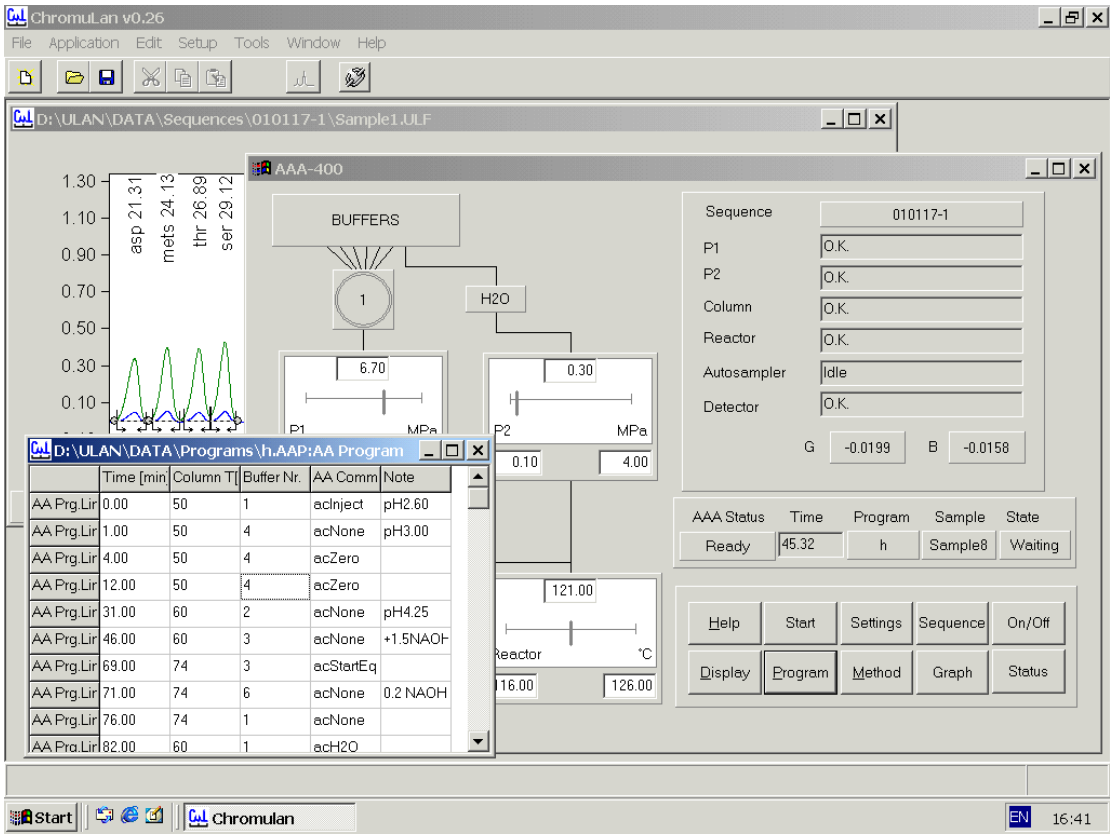
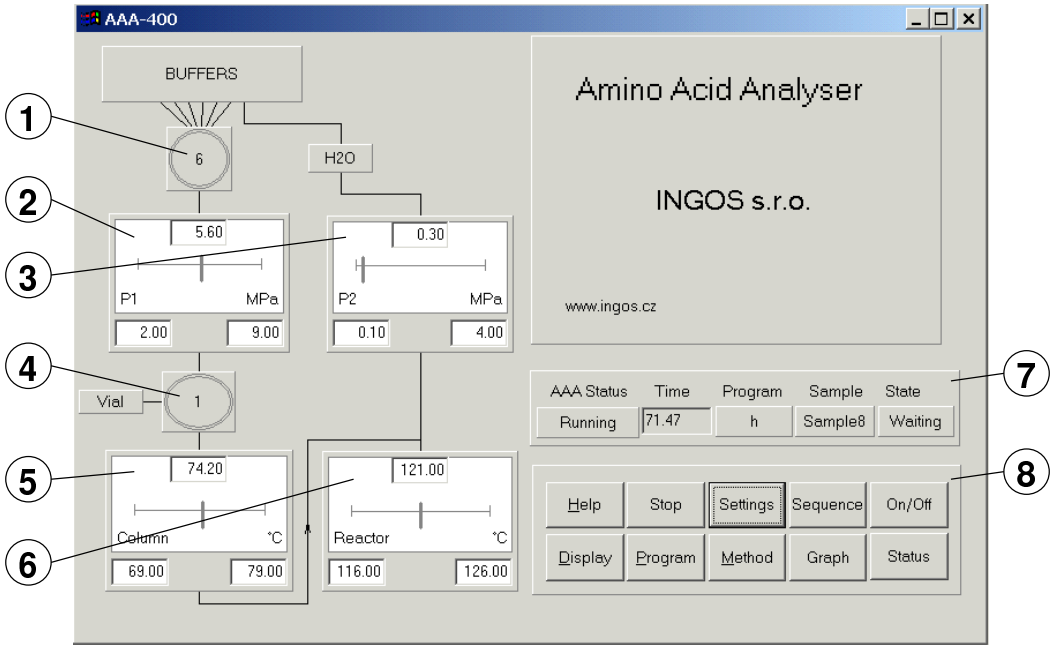


Fig. 1. ChromuLan

The program is structured in such a manner that the entire system can operate in a fully automatic mode. Set a sequence in the program, within the framework of which each sample is assigned an analytic program and assessment method. The apparatus will process samples in an automatic manner, ensures the equilibration of the column during the passage between analytical programs and will automatically assess the results according to the method pre-set.

2. CONTROL

The control module for AAA 400 is activated through the function **Aplication** ⇒ **Amino Acid Analyzer**. At the same time when the control module is activated, also the technological window of the Analyzer will open (fig. 2).



1. Current puffer number display
2. Pump 1 pressure display
3. Pump 2 pressure display
4. Current sample test tube number display
5. Column temperature display
6. Reactor temperature display
7. State line, see Chapter 2.5
8. Control button panel

Fig. 2. Technological window

The left part of the technological window contains a chart of the analyzer in which the status of individual apparatuses is displayed. The lower right part contains buttons serving for controlling individual functions.

2.1 Turning on of the Analyzer

1. Check the apparatus and operation chemicals (see the User's Manual for AAA 400 and its chapter on the PUTTING INTO OPERATION)
2. Check the setting of parameters (2.2).
3. Turn on the apparatus by using the button **On/Off** situated in the technological window.
4. Insert samples into the dispensing disk and prepare the sequence (2.4).
5. Wait until the apparatus is ready (2.5).
6. Turn on the sequence by using the **Start** button.

2.2 Setting

By pressing the button **Setting** you will call out the dialog serving for the setting of basic parameters of the program and starting parameters of the apparatus.

Sequences directory	Directory which contains all sequences.
Programs directory	Directory which contains analytical programs.
Methods directory	Directory which contains assessment methods.
Current Sequence	Current sequence name (2.4).
Default Prog. Name	Name of the program to be set in the heading during the creation of a new sequence (2.4.1).
Default method for new seq	Name of the method to be set in the heading during the creation of a new sequence (2.4.1).
P1 Flow [ml/min]	Pump 1 flow rate [0.3].
P1 Press Min [MPa]	Lower limit of the pump 1 pressure [2.0].
P1 Press Max [MPa]	Upper limit of the pump 1 pressure [9.0].
P1 Deviation [%]	Permitted oscillation of the pump 1 pressure [50].
P2 Flow [ml/min]	Pump 2 flow rate [0.2].
P2 Press Min [MPa]	Lower limit of the pump 2 pressure [0.1].
P2 Press Max [MPa]	Upper limit of the pump 2 pressure [3.0].
P2 Deviation [%]	Permitted oscillation of the pump 2 pressure [80].
Column Temp. [C]	Column temperature [60].
Col. Temp. Min Dif [C]	Maximum permitted column temperature drop [5].
Col. Temp. Max Dif [C]	Maximum permitted column temperature increase [5].
Colum Temp. Dev. [%]	Permitted oscillation of the column temperature [10].
React. Temp. [C]	Reactor temperature [121].
React. Temp. Min Dif [C]	Maximum permitted reactor temperature drop [5].
React. Temp. Max Dif [C]	Maximum permitted reactor temperature increase [5].
React. Temp. Dev. [%]	Permitted oscillation of the reactor temperature [10].

2.3 Analytical program

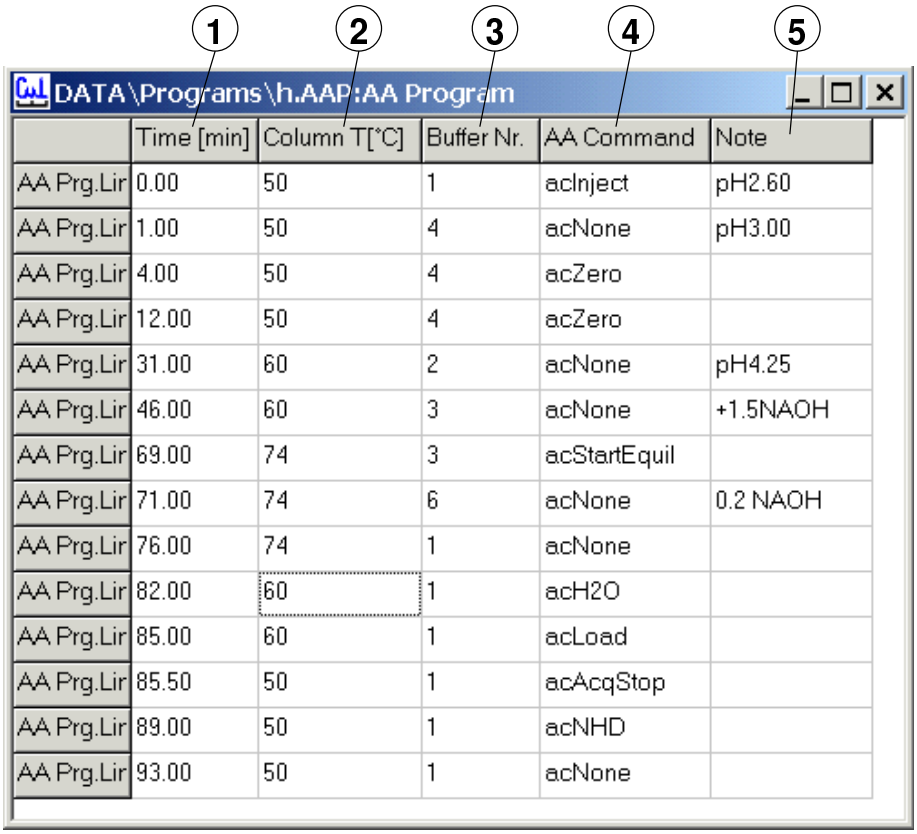
The analytical program serves for controlling the apparatus during the sample analysis. Each line of the program defines activities of the apparatus at a given time.

By pressing the **Program** button in the technological window call out the menu which has three items:

New	Calls out the window for editing a new program.
Open	Calls out the menu for opening a program.
Edit	Calls out the window for editing the program which was edited during the last session.

The program editing window is in fig. 3. The program lines are automatically arranged according to the time. Inserting new lines and deleting old lines can be carried out by using the **Insert** and **Delete**keys or from the menu which can be called out by using the right mouse button.

With regard to the automatic arrangement of the lines it is possible that at a change of time the line will move to another position. During modifications being made in a



	Time [min]	Column T[°C]	Buffer Nr.	AA Command	Note
AA Prg.Lir	0.00	50	1	acInject	pH2.60
AA Prg.Lir	1.00	50	4	acNone	pH3.00
AA Prg.Lir	4.00	50	4	acZero	
AA Prg.Lir	12.00	50	4	acZero	
AA Prg.Lir	31.00	60	2	acNone	pH4.25
AA Prg.Lir	46.00	60	3	acNone	+1.5NAOH
AA Prg.Lir	69.00	74	3	acStartEquil	
AA Prg.Lir	71.00	74	6	acNone	0.2 NAOH
AA Prg.Lir	76.00	74	1	acNone	
AA Prg.Lir	82.00	60	1	acH2O	
AA Prg.Lir	85.00	60	1	acLoad	
AA Prg.Lir	85.50	50	1	acAcqStop	
AA Prg.Lir	89.00	50	1	acNHD	
AA Prg.Lir	93.00	50	1	acNone	

1. Time
2. Column temperature
3. Puffer number
4. Command
5. Note

Fig. 3. Program editing window

program it is necessary to work with this function carefully and always, after a change in time, to check whether the right line is being edited.

The following commands may appear in the AA Command column:

Inject	Dispense the sample from the loop. This command should always be in the time 0.
Zero	Zero the detector. This command should be addressed to the place for which it is sure that there is a baseline.
StartEquil	From this command the equilibration analysis starts (2.4.2).
H2O	Switch the pump 2 entry to water.
NHD	Switch the pump 2 entry to ninhydrin.
StopAcq	terminates the data record.
Load	Prepares another sample to the loop.
None	No action is being made.

After modification it is necessary to save the program. Saving will be carried out by using the **Save** command from the menu which is called out with the help of the right button. If you are correcting a program while an analysis is running, the changes will only be applied during the next sample.

2.4 Sequence

The sequence specifies an arranged set of samples during processing. Each line of the sequence corresponds to one sample. The sequence is automatically named according to the date of creation. At the same time when the sequence is created, a directory of the same name is created on the disk and individual analyses are stored into it.

By pressing the **Sequence** button in the technological window call out the menu which has three items:

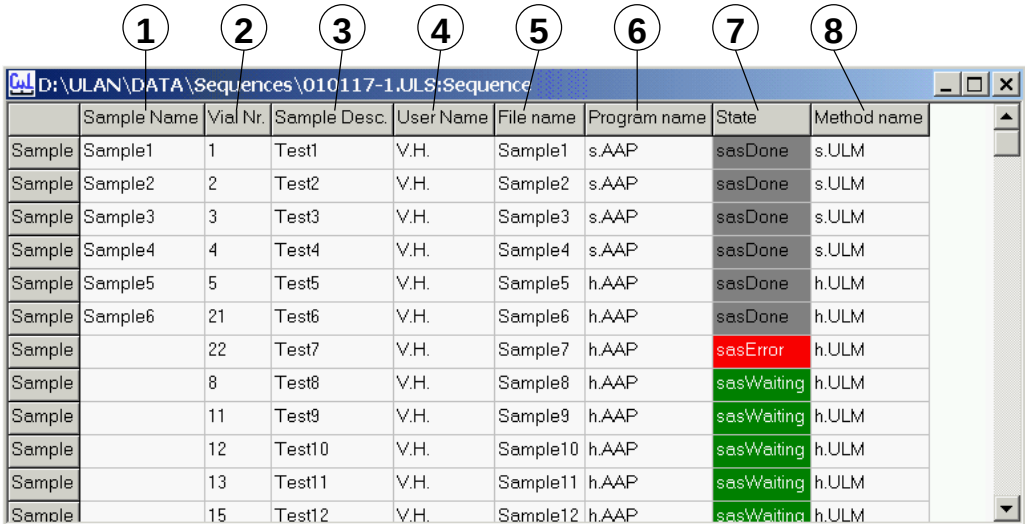
New — Makes it possible to create a new sequence. Before actual editing it is necessary to fill the heading of the sequence first (2.4.1).

Open — Calls out the menu for the opening and editing of a sequence which has already been saved. At the same time it will set this sequence as the current one.

Edit — Calls out the window for editing the current sequence.

If the sequence is being executed, you will call out directly the editing of the current sequence. In the sequence being executed it is only possible to change the samples which are in the "Waiting" state.

The window for editing the sequence is in fig. 4. Individual columns have the following meaning:



	Sample Name	Vial Nr.	Sample Desc.	User Name	File name	Program name	State	Method name
Sample	Sample1	1	Test1	V.H.	Sample1	s.AAP	sasDone	s.ULM
Sample	Sample2	2	Test2	V.H.	Sample2	s.AAP	sasDone	s.ULM
Sample	Sample3	3	Test3	V.H.	Sample3	s.AAP	sasDone	s.ULM
Sample	Sample4	4	Test4	V.H.	Sample4	s.AAP	sasDone	s.ULM
Sample	Sample5	5	Test5	V.H.	Sample5	h.AAP	sasDone	h.ULM
Sample	Sample6	21	Test6	V.H.	Sample6	h.AAP	sasDone	h.ULM
Sample		22	Test7	V.H.	Sample7	h.AAP	sasError	h.ULM
Sample		8	Test8	V.H.	Sample8	h.AAP	sasWaiting	h.ULM
Sample		11	Test9	V.H.	Sample9	h.AAP	sasWaiting	h.ULM
Sample		12	Test10	V.H.	Sample10	h.AAP	sasWaiting	h.ULM
Sample		13	Test11	V.H.	Sample11	h.AAP	sasWaiting	h.ULM
Sample		15	Test12	V.H.	Sample12	h.AAP	sasWaiting	h.ULM

1. Name of sample
2. Number of test tube
3. Sample description
4. Name of the user who has been analyzing the sample
5. File name
6. Analytical program name
7. Sample processing state
8. Method name

Fig. 4. Window for editing the sequence

Sample name — Sample name. If it remains undefined, the file name will be filled in automatically.

Vial Nr. — Number of test tubes from which the sample in question will be taken. It must be filled in. if a sample is added, the number which

	follows by the highest number used will be filled in automatically (greater by 1).
Sample Desc.	Sample description. Not obligatory.
User name	Name of the user who has been analyzing the sample
File name	Name of the file on the disk. It is obligatory, if a sample is added, it will automatically be filled in as "Sample", together with the ordinal number.
Program name	Analytical program name. It is obligatory. If a sample is added, the program name from the sequence heading is automatically added (2.4.1).
State	Sample processing state. Individual states are marked in red for the purpose of fast orientation.
Error	Error state. There was an error during the sample analysis.
Done	The sample was processed in order (OK).
Running	The sample is currently being processed.
InLoop	The sample is ready in the loop.
Waiting	The sample is waiting for processing.
Method name	The name of the method which will be used for the sample assessment (3.3). It is obligatory, but during assessment it is possible to change the method on an additional basis (3.3). When the sample is added, the method name is filled in automatically from the sequence heading (2.4.1).

Inserting new lines and deleting lines can be carried out by using the **Insert** and **Delete**keys or from the menu called out by means of the right mouse button.

2.4.1 Sequence heading

The sequence heading serves for the entering of parameters common for the entire sequence. The dialog for its editing will be called out automatically during the creation of a new sequence, alternatively it can be opened by using the command **Header** in the menu, which is called out by using the right button in the sequence window.

In the case of the Amino Acid Analyzer only the Program and Method items are used from the sequence heading.

2.4.2 Equilibration analysis

During the sequence processing and on any change in the analytical program an equilibration analysis is automatically put on the beginning. This analysis serves for the cleaning and stabilization of the column.

The equilibration analysis runs according to the program of the following analysis. It does not begin at zero time, but at the place where the program contains the StartEquil (2.3) command.

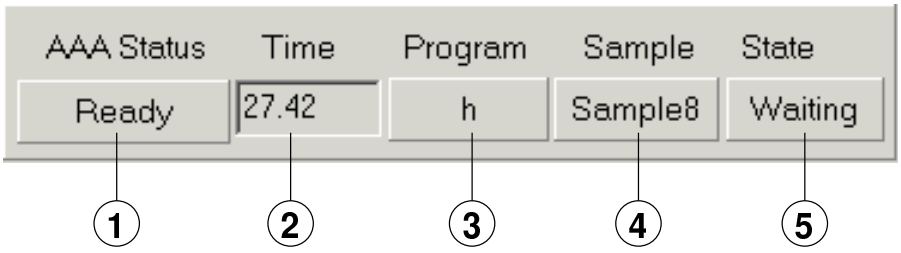
2.4.3 mass assessment

In the sequence window it is possible to carry out mass assessment of several samples. The samples which we want to assess are marked, and by using the function **Show result for sel.** from the menu under the right mouse button the result window is called out.

Samples for mass assessment must have the correct method assigned to them (3.3) and a standard. The standard can be assigned to selected samples by means of a function **Assign calib. file**.

2.5 State information

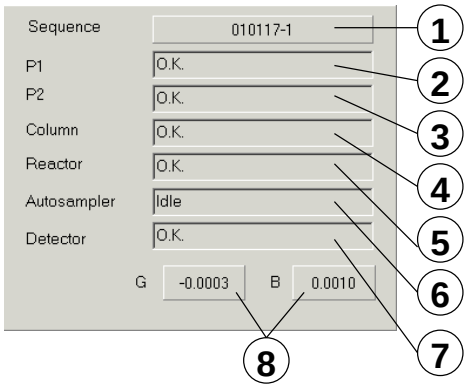
The right part of the technological window contains a state line, see fig. 5.



1. Apparatus state
2. Analysis time
3. Current program name
4. Current sample name
5. Current sample state

Fig. 5. State line

Other information concerning the state of the apparatus can be called out by pressing the button **State** see fig. 6.



1. Current sequence
2. Pump 1 state
3. Pump 2 state
4. Column state
5. Reactor state
6. Dispenser state
7. Detector state
8. Absorbance values

Fig. 6. State information

3. ASSESSMENT

For AAA400 the assessment with the help of a standard is used. With regard to the fact that chemistry used in the Analyzer changes in the time (NHD is getting old), it is necessary to arrange a standard after each 5 to 10 samples.

For the purpose of elimination it is possible to use also an internal standard. It is mainly used for the elimination of an error arising during the preparation of the sample.

3.1 Assessment procedure

1. Open the analysis of the standard. Press the button **Graph** in the technological standard and select a standard.
2. If you did not mark the standard in the sequence, mark it additionally, in the headings (function **Setup**) mark the field *Cal. Standard*.
3. If you did not have in the sequence (2.4) a correctly set method, use the function **Method** ⇒ **Load From** for reading the right method for the standard, see 3.3.3 and activate the function **Peak** ⇒ **Autodetect**.
4. Check whether all peaks have been assessed. If not, correct the method, see 3.3.2 and activate the function **Peak** ⇒ **Autodetect**.
5. Save the standard **File** ⇒ **Save**.
6. Press the button **Graph** in the technological window and select the analysis which you want to assess.
7. If you did not have in the sequence (2.4) a correctly set method, use the function **Method** ⇒ **Load From** for reading the right method for the analysis, see 3.3.4 and activate the function **Peak** ⇒ **Autodetect**.
8. Check whether all peaks have been assessed. If not, correct the method, see 3.3.2 and activate the function **Peak** ⇒ **Autodetect**.
9. Read the standard of functions **Method** ⇒ **Load Calibration File** and activate the function **Peak** ⇒ **Calculate Amounts**.
10. Call out the peak table (fig. 9) **Peak** ⇒ **Browse**, where it is possible to check the results, possibly to print them by using the function **Print** ⇒ **Report**.

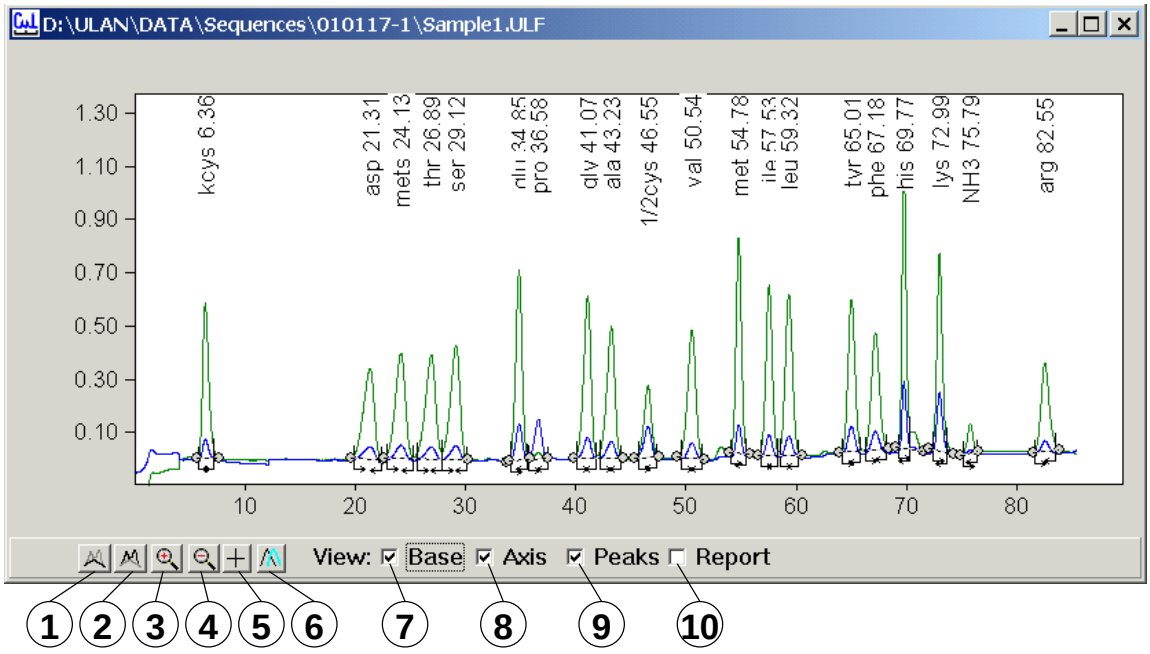
3.2 Chromatogram window

It is possible to open the chromatogram window in two ways. By pressing the button **Graph** in the technological window or from the menu by using the command **File** ⇒ **Open**.

You can carry out basic operations with the graph with the help of buttons in the lower part of the window, see fig. 7. By pressing the button at the same time with the **Shift** key you may use the function in question on a repeated basis.

By pressing the right mouse button in this window call out the chromatogram menu which would have the following items:

Setup - setting the analysis parameters
Method - method submenu
Baseline - baseline submenu
Peaks - peak submenu



1. Button for manual creation of a baseline (3.2.1)
2. Button for manual creation of peaks (3.2.1)
3. Button for the setting of a cut-out
4. Button for the returning of the previous cut-out
5. Button for the cursor position display mode
6. Button for the comparison of analyses, see 3.5
7. Activation of the baseline display
8. Activation of the axes display
9. Activation of the peak description display
10. Activation of the report display

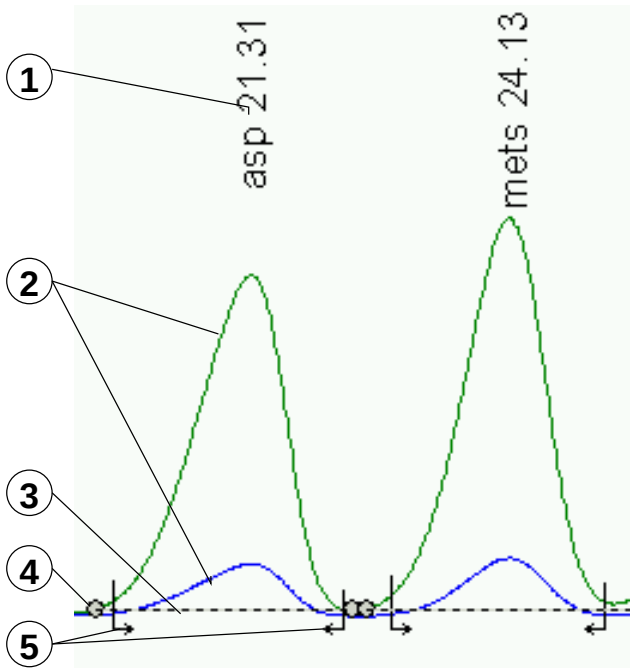
Fig. 7. Chromatogram window

Math - conversion submenu, to be used in the case of overlapping of analyses, see 3.5
View - setting of visible items (axis, peak description, baseline and others)
Scale - basic scale
Copy to clipboard - graph copying to other applications
Print - printing

3.2.1 Editing peaks

You can edit peak parameters directly in the graph or in the peak table. In the graph you can edit also the baseline and the integration marks of the peaks, see fig. 8.

If you want to edit peak parameters in the graph, mark the peak by clicking on the description and by further clicking on the description call out the dialog for editing peak parameters. You can change the position of the end points of the baseline and integration marks directly by using the mouse.



1. Peak description
2. Data measured
3. Baseline
4. Baseline end point
5. Peak integration marks

Fig. 8. Editing peaks

Addition of further peaks and sections of the baseline is carried out with the help of buttons in the lower part of the chromatogram window (3.2).

Call out the peak table by using the function **Peak** ⇒ **Browse** see fig. 9

3.3 Method

The method contains information for the assessment of the analysis. The method forms a part of any analysis. But it can also be saved in a separate file and to be read again from this file. This can also be made by using the function **Method** ⇒ **Save To** and **Method** ⇒ **Load From**.

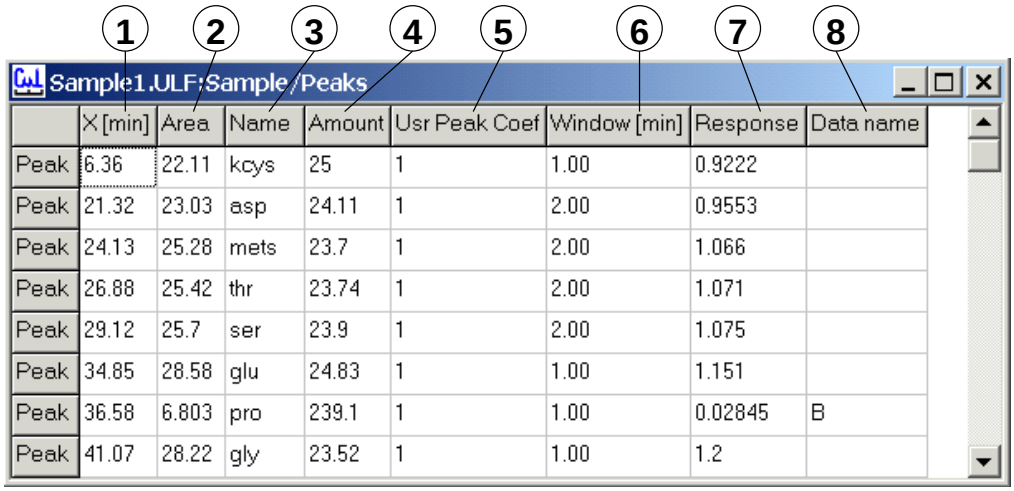
In the case of the Amino Acid Analyzer the method set in the sequence is automatically inserted into the analysis, see 2.4.

The data in the method can be divided into three parts: method heading, peak description ad baseline description.

3.3.1 Method heading

Call out the method heading by using the function **Method** ⇒ **Edit** see fig. 10. The meaning of individual items is as follows.

Method Template	Name of the file from which the method was created
Calibration File	Name of the standard
Calibration File	Method file name
Duration	This parameter is not used for the time being
Base min. interval	To be used during automatic detection of the baseline, it says how long the straight section must be so that it can be considered as a baseline.



	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 area
3. peak name
4. amino acid quantity
5. Coefficient for the computation of quantity, see 3.3.4 and 3.4
6. Window for the assignment of the method peaks, see 3.3.2
7. Response, see 3.4
8. Name of the line from which the peak is assessed. If the field is empty, it is assessed according to a green color, while the "B" letter means that it is assessed from a blue color.

Fig. 9. Peaks table

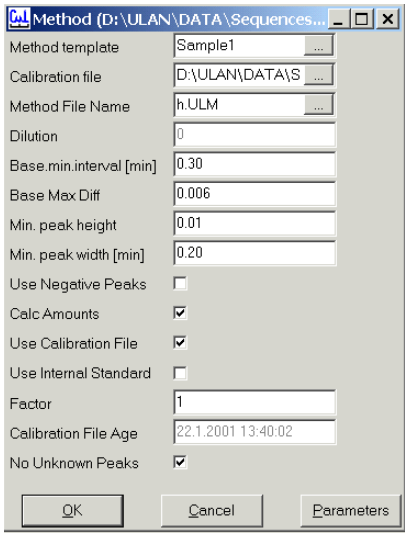


Fig. 10. Method heading

Base max. diff Specifies maximum noise which could appear on the section which is considered to be the baseline.

Min. peak height	Minimum peak height. The peaks which are lower are ignored during the self-detection.
Min. peak width	Minimum peak width The peaks which are narrower are ignored during the self-detection.
Use negative peaks	Mark the field if you want to assess negative peaks. It is not used in the case of the Amino Acid Analyzer.
Calc Amounts	Mark the field if you want to compute the quantity automatically. In the case of the Amino Acid Analyzer it is always marked.
Use Calibration File	Mark the field if you want to use the standard. In the case of the Amino Acid Analyzer it is always marked.
Use Internal Standard	Mark the field if you want to use the internal standard.
Factor	Conversion factor, see 3.4 .
Calibration File Age	Date and time for the reading of the calibration file. During computation it is necessary to check whether the calibration file was not modified after this date. In the case that it was, it is necessary to carry out its new reading.
No Unknown Peak	If you mark this field, only those peaks are assessed which are defined in the method in question.

3.3.2 Method peaks

By using the function **Method ⇒ Peaks ⇒ Browse** you will call out the method peak table. This table is the same as the peak table. You can add peaks into this table by using the **Insert**key, alternatively you can copy the peaks marked from the analysis by using the command **Peaks ⇒ Copy Selected To Method**.

The peaks of the method are assigned to the peaks measured with the help of the retention time and window. If the peak is not assigned correctly, change the retention time in the method peak table, or enlarge the window. If the window is changed, be careful that the windows should not overlap.

3.3.3 Method for the standard

The method for the standard must have in the heading (3.3.1) the following setting: *Faktor*=1. Moreover, the method peak table must have in the column *Amount* the settings of quantities of individual amino acids in the standard. The column *UsrPeakCoef* must be 1.

Also *Multiply Factor* and *DivideFactor* in the sequence must be set to 1.

3.3.4 Method for the sample

If you want to know the results in grams, you must set molar weights of individual amino acids in the method peak table in the column *UsrPeakCoef*.

If you are using a constant base and dilution, include it into *Faktoru* in the method heading, if you do not use *Multiply Factor* and *DivideFactor* in a sequence.

The name of the peak in the method for the sample and in the method for the standard must be the same, otherwise the peak in question will not be assessed.

3.4 Computation

In the case of the Amino Acid Analyzer it is necessary to use always a computation with the standard. This computation is carried out according to the following formula:

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

where: Area is the peak area, UsrPeakCoef is the coefficient from the peak table. Th factor is set in the method heading and is the same for all peaks and for all samples assessed by using the method in question. MutiplyFactor and DivideFactor are set for each sample separately in a sequence or in the heading of the sample, they are all for all peaks. Response is computed from the standard according to the formula:

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

The meaning of individual members is the same, they are only taken from the standard.

3.5 Comparison of analyses

The program makes it possible to enter several analyses into a single graph. This can be made by pressing the button serving for comparison of analyses, see fig. 7 and then open a subsequent analysis of functions **File** ⇒ **Open**. Individual analyses can be moved and enlarged by using the functions from the submenu **Math** in the chromatogram menu.

Another possibility of displaying several analyses into a single graph is by using the function **Open selected in one window** in the menu which will be called out by using the right button in the sequence window.

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

4. TABLE OF CONTENTS

4.1 List of picturtes and tables