

SST EXTENSION

Clarity Extension

ENG

Code/Rev.: M020/80C
Date: 4/1/2019

Phone: +420 251 013 400
Fax: +420 251 013 401
clarity@dataapex.com
www.dataapex.com

DataApex Ltd.
Petrzilkova 2583/13
158 00 Prague 5
The Czech Republic

Clarity[®], DataApex[®] and ▲[®] are trademarks of DataApex Ltd. Microsoft[®] and Windows[™] are trademarks of Microsoft Corporation.

DataApex reserves the right to make changes to manuals without prior notice. Updated manuals can be downloaded from www.dataapex.com.

Author: DM

Contents

1 SST - System Suitability Test	1
1.1 System Suitability Testing	1
2 Specification	2
3 Installation	3
4 SST Extension Description	4
4.1 Chromatogram - SST Results	4
4.1.1 Component Table	5
4.1.2 Validating multi-detector chromatograms	6
4.1.3 Parameters Table	6
4.1.4 Validation results hierarchy	9
4.1.5 SST menu	11
4.2 SST Properties	13
4.2.1 SST Properties - General	13
4.2.1.1 DBF file structure	15
4.2.2 SST Properties - Parameters	16
4.2.3 SST Properties - Subparameters	17
4.2.4 SST Properties - Calculate By	20
4.2.5 SST Properties - Events	21
4.3 Report Setup - SST	22
5 How to set the SST	23
5.1 Conditions for SST use	23
5.2 Creating SST method	24
5.3 Step-by-step validation	25
5.3.1 Online - Sequence (or Single Analysis)	26
5.3.2 Offline - Manual	27
5.3.3 Offline - Batch reprocess	28
5.3.4 Validation of the chromatographic system	29
5.3.5 Summary table of validated chromatograms	30
5.3.6 Storing results in DBF	31
5.3.7 Chromatogram validation	32
6 Workarounds	33
6.1 Stopping a running sequence	33
6.2 Sequence summary table	33
6.3 Using DBF in MS Excel	34
7 Appendix - Mathematical Formulas	36

To facilitate the orientation in the **SST Extension** manual and **Clarity** chromatography station, different fonts are used throughout the manual. Meanings of these fonts are:

Instrument (blue text) marks the name of the window to which the text refers.

Open File (italics) describes the commands and names of fields in **Clarity**, parameters that can be entered into them or a window or dialog name (when you already are in the topic describing the window).

WORK1 (capitals) indicates the name of the file and/or directory.

ACTIVE (capital italics) marks the state of the station or its part.

The bold text is sometimes also used for important parts of the text and the name of the **Clarity** station. Moreover, some sections are written in format other than normal text. These sections are formatted as follows:

Note: Notifies the reader of relevant information.

Caution: Warns the user of possibly dangerous or very important information.

Marks the problem statement or trouble question.

Description: Presents more detailed information on the problem, describes its causes, etc.

Solution: Marks the response to the question, presents a procedure how to remove it.

1 SST - System Suitability Test

The **System Suitability Test (SST)** is an optional **Extension** for the **Clarity Chromatography Station**. **SST** module is designed to validate a chromatography system based on the evaluation of chromatograms that have been acquired from the **Clarity** station. Any **Clarity** Instrument can use **SST** Extension.

Validation is a process that determines whether certain components (peaks or groups of peaks) from a chromatogram, comply with selected parameters (calibrated quantity, retention time, asymmetry, etc.).

Clarity permits both individual and batch validations of either individual chromatograms or whole chromatogram sets. The user can easily change the number of validated components, set the validation parameters, values of validation limits and the type of calculations. It is also possible to choose between the testing of mean of all values or individual results within a group of chromatograms.

Even though validation primarily focuses on the testing of system reliability, it can also be used in many other ways. Validation can be run during measurements or additionally using batch processing. Validated data can be automatically saved to a database file for further processing.

In connection with a sequence, the **SST Extension** can even pause a sequence when a specified value has been exceeded or to perform predefined functions.

SST Extension is also compatible with **Clarity Offline** software.

1.1 System Suitability Testing

Validation is a key **GLP/GMP** requirement in analytical laboratories nowadays. This requirement is divided into the validation of equipment (chromatograph and PC) and the validation of methods. **SST** verifies the parameters achieved by measuring on a validated instrument using a validated method.

The most frequent parameter monitored is *Analysis Reproducibility*, where the standard deviation of monitored parameters must not exceed the specified range.

SST Extension provides tools for the *Performance Qualification (PQ)* and, together with the **Validator**, also for the *Operational Qualification (OQ)*.

2 Specification

The **SST Extension** is an optional, fully integrated part of **Clarity** software. It can be ordered as a part of new datastation or as an Extension to existing datastation (p/n A22).

3 Installation

The **SST Extension** is enabled by appropriate user code entered during installation or later by using the *Help - User Code* command from the [Clarity](#) main window.

4 SST Extension Description

The **SST Extension** is represented in the **Clarity** station as the **SST Results** tab in the bottom part of the **Chromatogram** window.

4.1 Chromatogram - SST Results

The **SST** tab in the **Chromatogram** window contains two tables used for setting the validation parameters - **Component Table** (on the left) and **Parameters Table** (on the right) of a selected component.

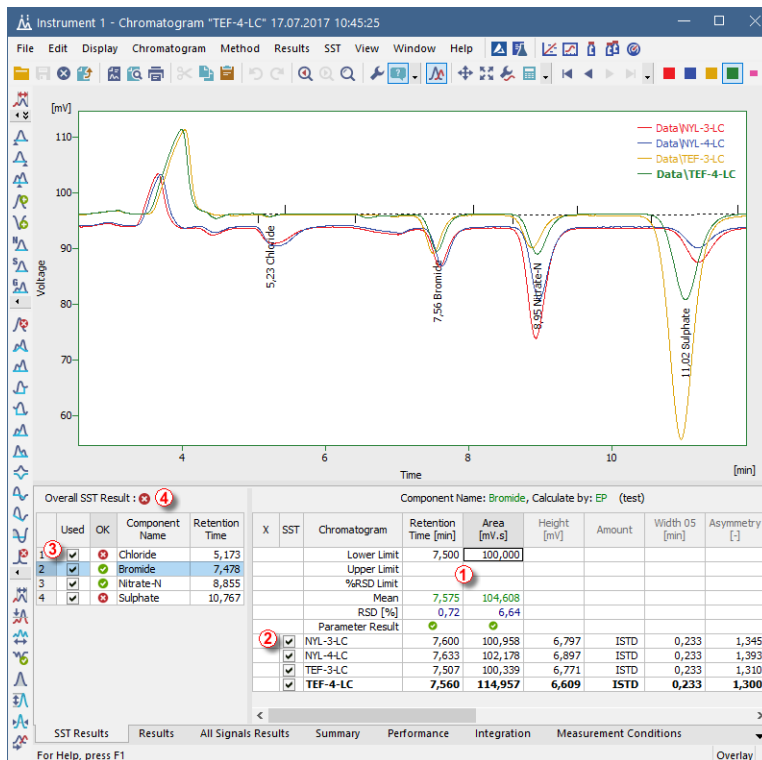


Fig 1: Chromatogram - SST Results

Tables in the **SST Extension** use graphical symbols depicting the validation result at four different levels: on the level of individual parameters ①, chromatograms ②, components ③, or the overall ④ success of the validation. Symbol meanings are as follows:

Tab 1: Validation result symbols

Symbol	Meaning
✔	Validation was successful.
?	Validation could not be run (e.g. no parameters specified) - validation result unknown.
✘	Validation was unsuccessful.

4.1.1 Component Table

The left section of the [SST Results](#) tab contains the **Component Table**, a table of results for all components included in a validation. By selecting or deselecting a component in the *Used* column you determine whether or not a component will be included in the overall validation.

Note: The overall validation result (*Overall SST Result*) is displayed above the **Component Table**. The validation results for individual components are shown in the **Component Table** (in the *OK* column).

Overall SST Result : ✘				
	Used	OK	Component Name	Retention Time
1	<input checked="" type="checkbox"/>	✘	Chloride	5,173
2	<input checked="" type="checkbox"/>	✔	Bromide	7,478
3	<input checked="" type="checkbox"/>	✔	Nitrate-N	8,855
4	<input checked="" type="checkbox"/>	✘	Sulphate	10,767

Fig 2: Chromatogram - SST Results - Component Table

Overall SST Result:

The overall validation result in the **Component Table** will be marked as successful (✔) only if all components were validated successfully in all monitored parameters.

Used

By selecting/deselecting the checkbox on particular row you include or exclude the component from a validation.

OK

The symbol in the *OK* column marks the validation result for a specific component. A component is validated successfully if all monitored parameters are complied with.

Component Name

Shows the name of a monitored component taken from the calibration.

Retention Time

Shows the retention time of a monitored component taken from the calibration.

Caution: In validated chromatograms the components are identified by the compound name and not by retention time.

4.1.2 Validating multi-detector chromatograms

It is possible to select which signal has to be used for **SST** evaluation when using multi-detector chromatograms.

Evaluate

In the header of the **Parameters Table** on the **SST Results** tab will appear the *Evaluate* combobox with following options: *Active*, *Signal 1*, ..., *Signal n*.

The default *Active* option will evaluate the signal that is currently active in the **Chromatogram** window.

Note: The name of the active signal is indicated in the window header and its description in the graph legend is highlighted in bold letters.

4.1.3 Parameters Table

The table on the right side of the **SST Results** tab holds the **Parameters Table**, which shows a detailed breakdown of the individual parameters validation for the component which has been selected in the **Component Table** on the left side of the **SST Results** tab.

Note: You can hide or show unused (shaded) columns and rows using the *Show All Columns/Rows* commands in the context menu, which is accessible after right clicking on the **Parameters Table**.

Component Name: Bromide, Calculate by: EP (test)								
X	SST	Chromatogram	Retention Time [min]	Area [mV.s]	Height [mV]	Amount	Width 05 [min]	Asymmetry [-]
		Lower Limit	7,500	100,000	6,700		0,230	
		Upper Limit	7,700	100,500	6,800		0,250	
		%RSD Limit						
		Mean	7,600	100,958	6,797		0,233	
		RSD [%]	0,00	0,00	0,00		0,00	
		Parameter Result	○	○	○		○	
<input checked="" type="checkbox"/>		NVL-3-LC	7,600	100,958	6,797	ISTD	0,233	1,345

Fig 3: Chromatogram - SST Results - Parameters Table

The name of the current component (*Component Name*), parameter calculation method (*Calculate by*) and the name of the current **SST** method (in parenthesis) are displayed above the **Parameters Table**.

Note: If you have changed the **SST** method and not saved it yet, it will be indicated by the *MODIFIED inscription.

Individual validation parameters are set in the top section of the **Parameters Table** (down to the bold line). The mean values for all loaded chromatograms are displayed below them. A list of validated chromatograms is below the bold line.

1. Limiting parameters - Lower, Upper, %RSD Limit

A detailed description of limiting parameters is provided in the chapter "**SST Properties - Subparameters**" on pg 17.

Note: If more limits are entered for one parameter, then all conditions must be complied with for a successful validation to occur. Limit values are entered directly into the **Parameters Table** and can differ from component to component.

2. Summary values - Mean, RSD[%]

Mean - arithmetic mean of monitored parameters from all loaded chromatograms.

RSD[%] - the percentage standard deviation (variation coefficient) of the parameter for the displayed component calculated from all loaded chromatograms. A formula is shown in the chapter "**Appendix - Mathematical Formulas**" on pg 36.

Note: These parameters are useful only when you validate more chromatograms simultaneously. The *Overlay* mode must be enabled.

The validation procedure is described in detail in the chapter "**Validation of the chromatographic system**" on pg 29.

3. Parameter Result

The result of the specific parameter validation. If more chromatograms are validated then the validation result will relate to the mean parameter value of all validated chromatograms.




X	SST	Chromatogram	Retention Time [min]	Area [miv.s]	Height [miv]
		Lower Limit	7,500	100,000	
		Upper Limit	7,700	100,500	
		%RSD Limit			
		Mean	7,600	100,958	6,797
		RSD [%]	0,90	0,00	0,00
		Parameter Result	●	●	●
<input checked="" type="checkbox"/>		NYL-3-LC	7,600	100,958	6,797

Fig 4: Parameter validation result

4. List of validated chromatograms

A bold line, followed by a list of validated chromatograms completes the **Parameters Table**. The first column (*OK*) of this section indicates the validation result for a specific chromatogram.

Tab 2: Symbol meanings in the Parameters Table

Symbol	Meaning
	Chromatogram validation was successful (i.e. all values met the defined criteria).
	The validation result could not be determined (i.e. at least one parameter could not be determined - e.g. parameter was not found in the chromatogram or at least one parameter limit value was left un-entered). For more details see the chapter SST Properties - Subparameters on pg 17.
	Chromatogram validation was unsuccessful (i.e. at least one parameter did not meet the defined criteria).

The other columns hold the chromatogram name and the summary of values for individual parameters. Successfully validated values are displayed in green color, values not complying with the criteria are in red and blue color indicates the value whose result cannot be determined.






	Parameter Result			
 	NVL-3-LC	7,600	100,958	6,797

Fig 5: Explanation of colors in the Parameter Table

4.1.4 Validation results hierarchy

Validation results are created hierarchically, their importance is arranged in ascending order in the sequence:

parameter -> **component** -> **overall** evaluation.

Note: Chromatogram validation is not subject to this hierarchy when the system validation does not involve individual chromatograms, that is when the *Mean of all values* option is selected on the [SST Properties - Subparameters](#) tab.

Parameter

Validation results for individual parameters are found in the **Parameters Table** on the **Parameter Result** line. For successful validation, the mean value (or standard deviation) must lie within specified limits.

X	SST	Chromatogram	Retention Time [min]	Area [mV.s]	Height [mV]
		Lower Limit	7,500	100,000	
		Upper Limit	7,700	100,500	
		%RSD Limit			
		Mean	7,600	100,958	6,797
		RSD [%]	0,00	0,00	0,00
		Parameter Result	✔	✘	?
<input checked="" type="checkbox"/>		HVL-3-LC	7,600	100,958	6,797

Component

The validation result for a component is found in the **Component Table** in the **OK** column. For successful component validation, all parameters for the given component must be successfully validated.

Overall SST Result : ✘

	Used	OK	Component Name	Retention Time
1	<input checked="" type="checkbox"/>	✘	Chloride	5,173
2	<input checked="" type="checkbox"/>	✔	Bromide	7,478
3	<input type="checkbox"/>		Nitrate-N	8,855
4	<input checked="" type="checkbox"/>	?	Sulphate	10,767

Overall validation

The overall validation result (*Overall SST Result*) is found above the **Component Table**. For successful overall validation all components in all monitored parameters must be successfully validated.

Overall SST Result : ✔

	Used	OK	Component Name
	<input checked="" type="checkbox"/>	✔	

One unsuccessfully validated **parameter** will cause an unsuccessful **component** validation and therefore, an unsuccessful **overall validation**. If at least one parameter is validated with an unknown result, then the component validation and overall validation results will also be unknown. An **unsuccessful validation** is of a higher priority than an **unknown validation result**. Consequently, if one parameter is validated as unsuccessful and another as unknown, the component validation result will be unsuccessful.

To perform a validation, the **SST** method must be opened (defined) on the instrument.

As soon as you open a chromatogram in the [Chromatogram](#) window, validation calculations will automatically be made.


4.1.5 SST menu

You can access all the **SST Extension** functions and settings in the main **SST** menu of the **Chromatogram** window.

Note: Most commands from the main **SST** menu can be also accessed through the context menus which are opened by right clicking on the **SST Results** tab.

SST Result	Switches to the SST Results tab - the same operation is performed by clicking on the tab name at the bottom of the Chromatogram window.
New	Creates a new SST method. During the initial file saving you will be prompted to enter a filename. The filename NONAME is reserved and cannot be used.
Open...	Opens the saved SST method.
Close	Closes an open SST method. If the file contains any unsaved changes, you will be prompted to save them.
Save	Saves the SST method.
Save As	Saves a copy of the current SST method under a new filename.
Update from Calib	Transfers the data on components (known as the Peak Table) from the current chromatogram, which must be calibrated. Components are selected by the name, not the retention time. All components will be transferred from the calibration, even those not identified in the current chromatogram.
Show All Columns	Shows / hides the unused (shaded) columns in tables.
Show All Rows	Shows / hides the unused (shaded) rows in tables.

X	SST	Chromatogram	Retention Time [min]	Area [mV.s]	Height [mV]
		Lower Limit	7,400		
		Upper Limit			
		%RSD Limit			
		Mean	7,556		6,726
		RSD [%]	0,62		1,51
		Parameter Result			



X	SST	Chromatogram	Retention Time [min]	Area [mV.s]	Height [mV]
		Lower Limit	7,400		
		Mean	7,556		6,726
		RSD [%]	0,62		1,51
		Parameter Result			

General...

Opens the [SST Properties - General](#) tab with the description of the **SST** method, which sets the saving of results in the database file (for more details see also chapter **SST Properties - General** on pg 13).

Parameters...

Opens the [SST Properties - Parameters](#) tab with the selection of validation parameters (e.g. *Retention Time*, *Area*, ...) (for more details see also chapter **SST Properties - Parameters** on pg 16).

Subparameters...

Opens the [SST Properties - Subparameters](#) tab with the selection of limits according to which parameters will be evaluated (*Lower Limit*, *Upper Limit*, *%RSD Limit*) (for more details see also chapter **SST Properties - Subparameters** on pg 17).

Calculate By...

Opens the [SST Properties - Calculate By](#) tab with the selection of the calculation method according to which the parameters will be calculated (*EP*, *USP*, *JP*) (for more details see also chapter **SST Properties - Calculate By** on pg 20).

Events...

Opens the [SST Properties - Events](#) tab with the special events settings depending on validation results (e.g. *Pause Sequence*) (for more details see also chapter **SST Properties - Events** on pg 21).

Clear Parameters

Deletes all limiting parameters in the **Parameters Table**. Applies only to the currently displayed component.

4.2 SST Properties

4.2.1 SST Properties - General

In the **General** tab of the **SST Properties** dialog the information about the **SST** method are displayed or edited and the logging of validation results into the database file is set.

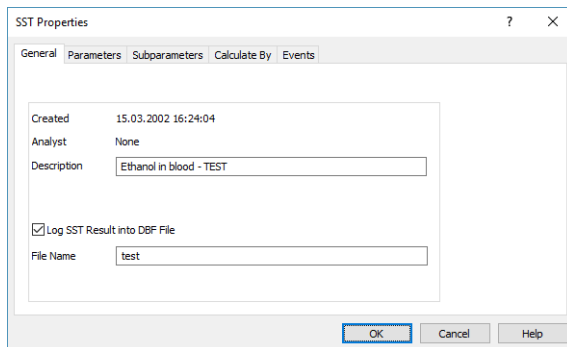


Fig 6: SST Properties - General

Created

Shows the date of the **SST** method file creation. This date cannot be edited.

Analyst

Displays the name of the user who created the **SST** method. The name that the user enters when logging on to the Instrument will be used. This field cannot be edited.

Description

Allows to enter a personal description of the **SST** method.

Log SST Result into DBF file

The **Clarity** station can save all validation results into an independent database file. This field, together with the *File Name* field, sets the logging of validation results into the specified file.

The database file will automatically be created in the current project directory. It can be then processed further in any arbitrary database program or in **Excel**, e.g. using contingency tables and graphs (for more details see the chapter **Using DBF in MS Excel** on pg 34).

File Name

Serves for specifying the file name for a DBF file, which can be entered without suffix.

Caution: Validation results for a chromatogram are saved when the chromatogram is closed using the *Close Window* command or after opening another chromatogram in the **Chromatogram** window. The same applies in the *Overlay mode* - files are saved gradually after closing.

Caution: Make sure that the database file is not open at the same time in another program. In such case it would not be possible to save validation results in it!

4.2.1.1 DBF file structure

Each validated chromatogram is saved separately, a separate row is created for each monitored parameter. Columns irrelevant to a given parameter remain empty.

Note: The database file is automatically created in the current project directory.

Tab 3: Description of individual columns (category):

Column	Description
CHROM_NAME	Chromatogram name (including path, if applicable). The CHROM_NAME field is designed for 256 characters, so the entire name will fit. It can, however, happen that in MS Excel, for example, only the first column of an open *.DBF will be displayed - other columns being beyond the screen size.
DATE	Date of the validation.
COMPONENT	Component name.
PARAM_NAME	Parameter name.
LO_LIMIT	Lower limit.
HI_LIMIT	Upper limit.
VALUE	Parameter value.
RESULT	Validation result (in the selection <i>PASS</i> , <i>FAIL</i> , <i>UNKNOWN</i>).

Note: Working with *.DBF files in **MS Excel** is described in detail in the chapter "**Using DBF in MS Excel**" on pg 34.

4.2.2 SST Properties - Parameters

The parameters to be displayed in the **Parameters Table** and used in a validation are defined by selecting individual items in the **SST Properties - Parameters** dialog.

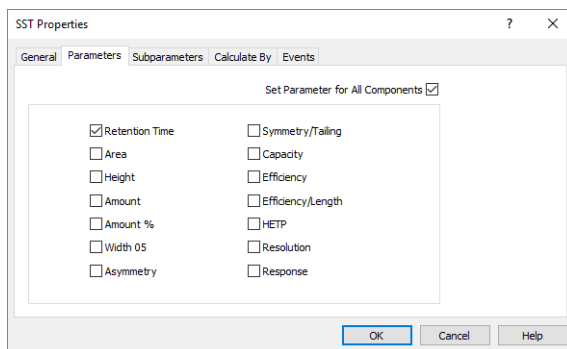


Fig 7: SST Properties - Parameters

Parameters are included or excluded from a validation by checking/unchecking the corresponding checkbox. The list of parameters used in the validation is a part of the **SST** method file.

Note: Selecting or deselecting a parameter in the **SST Properties - Parameters** tab can be replaced by double-clicking on the header of the respective column in the **Parameters Table**. *Show All Columns* option must be enabled to switch the parameter on and off this way.

Note: Mathematical formulas for parameter calculation according to selected methods can be found in the chapter "**Appendix - Mathematical Formulas**" on pg 36.

Set Parameter for All Components

This checkbox governs whether the list of used parameters will be applied to all validated components or only to the currently selected component (= component highlighted in the **Component Table**).

Retention Time

Validates the retention time of the component.

Area

Validates the peak area of the component.

Height

Validates the peak height of the component.

Amount

Validates the amount of the component.

Width 05

Validates the peak width of the component peak at half its height.

Asymmetry

Validates the asymmetry of the component peak.

Symmetry/Tailing

Validates the symmetry of the component peak.

Capacity

Validates the capacity ratio of the component.

Efficiency

Validates the column efficiency counted from the component.

Efficiency/Length

Validates the column efficiency related to the column length counted from the component.

HETP

Validates the height equivalent to a theoretical plate (HETP) of the component.

Resolution

Validates the resolution relating to the previous integrated peak (even non-calibrated one) of the component peak.

Note: The *Efficiency*, *Efficiency/Length*, *HETP* and *Resolution* parameter values depend on the selected calculation method that is set in the [Calculate By](#) tab. The formulas for these parameters/calculation methods are described in the chapter "**Appendix - Mathematical Formulas**" on pg 36.

Response

Validates the response of the component peak, as set in the [Calibration](#) using the *Response Base (RB)* column.

4.2.3 SST Properties - Subparameters

In the [Subparameters](#) tab of the [SST Properties](#) dialog you select which limit values can be entered in the **Parameters Table**, i.e. whether to check the lower or upper limits or the variation coefficient.

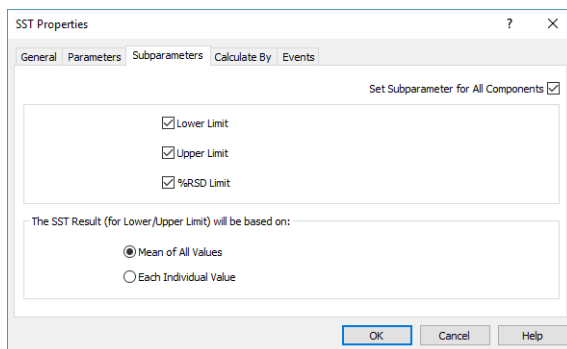


Fig 8: SST Properties - Subparameters

For a successful validation, at least one of the three offered limit items must be selected.

Note: You can also enable/disable items directly in the **Parameters Table** by clicking on their names. *Show All Rows* option must be enabled to switch the parameter on and off this way.

Set Subparameter for All Components

This checkbox governs whether the list of used subparameters will be applied to all validated components or only to the currently selected component (= component highlighted in the **Component Table**).

Lower Limit

The validation will involve the minimum arithmetic mean value of all loaded chromatograms.

Upper Limit

The validation will involve the maximum arithmetic mean value of all loaded chromatograms.

Note: If a single chromatogram is validated, the current value will be equal to the mean value. It follows that exceeding the parameter limit value will immediately be visible in the overall validation result (*Overall SST Result*).

%RSD Limit

The validation will involve the maximum value of the percentage standard deviation (variation coefficient) of the displayed component parameter for all loaded chromatograms.

The formula for *%RSD Limit* is available in the chapter "**Appendix - Mathematical Formulas**" on pg 36.

The SST Result (for Lower/Upper Limit) will be based on:

Mean of All Values

The **SST Extension** will compare the mean of all evaluated values with the *Upper Limit* or *Lower Limit*.

It is not necessary to have all values within the limits to receive successful validation result.

Each Individual Value

The **SST Extension** will compare each evaluated value with the *Upper Limit* or *Lower Limit*.

Every single value must be within the limits to receive successful validation result.

4.2.4 SST Properties - Calculate By

The method of an individual parameter calculation is selected on the [SST Properties - Calculate By](#) tab. The selection of the calculation method effects the following parameters: efficiency (*Efficiency*), length efficiency (*Efficiency/Length*), height equivalent of theoretical plate (*HETP*) and resolution (*Resolution*).

Note: A mathematical formula for parameter calculation according to selected calculation methods are found in the chapter "**Appendix - Mathematical Formulas**" on pg 36.

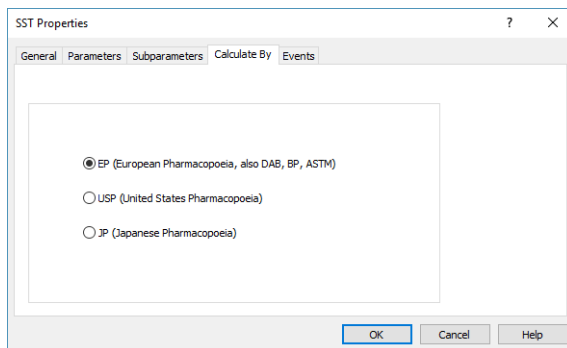


Fig 9: SST Properties - Calculate By

EP (European Pharmacopoeia)

Calculation of efficiency and related parameters based on the *width at half height*.

USP (United States Pharmacopoeia)

Calculation of efficiency and related parameters based on *width given by intersection of tangents with baselines*.

JP (Japanese Pharmacopoeia)

Calculation of efficiency and related parameters based on the *width at half height*.

4.2.5 SST Properties - Events

The **SST Properties - Events** tab is used to set the running of other events (like sequence stop or running an external program) in dependence on the **SST** validation results .

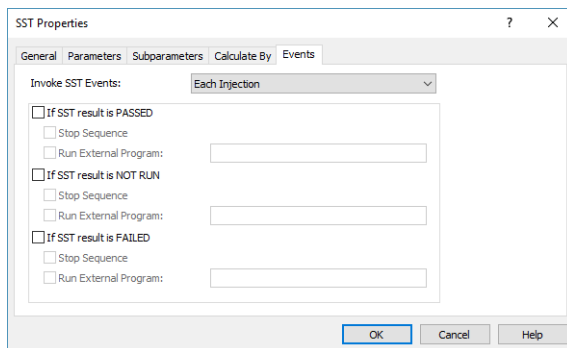


Fig 10: SST Properties - Events

Invoke SST Events:

This listbox allows user to appoint exactly when SST events will be invoked. Options are : Each Injection, Vial Finished and Row Finished. In the simplest form of sequence (and also most often), every row of sequence contains one injection from one vial, thus in this case all the options are equal.

If SST result is PASSED (V), If SST result is NOT RUN (?), If SST result is FAILED (X)

Each section will perform the action on the specific result of the validation - on a successful validation result (✔ - PASSED), validation not performed (? - NOT RUN) or failed validation result (✘ - FAILED). The same set of two possible actions is available for each category.

Stop Sequence

Stops the running sequence.

Run External Program

Runs the specified external program. If the executable file of the program is not placed in the **Clarity** root directory, it must have the full (absolute) path included.

4.3 Report Setup - SST

The printed output properties are set in the [Report Setup](#) dialog. Settings for the print of the **SST** validation results are placed on a separate tab. The *Print* and *On New Page* checkboxes are described in the chapter "Report Setup".

Note: To print validation results only disable all other tabs.

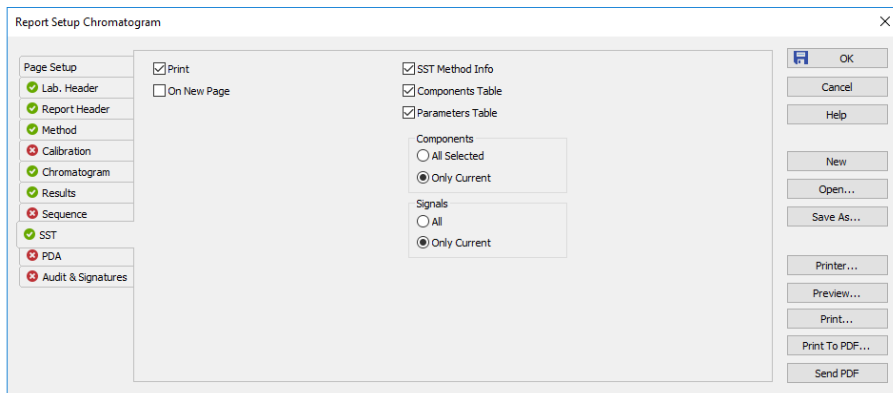


Fig 11: Report Setup - SST

SST Method Info

Adds the current SST method description to the printout - that means its name, date of last save, username of the author and a description.

Components Table

Adds the **Component Table** into the printout.

Parameters table

Adds the **Parameters Table** into the printout.

Components

This section serves for the decision whether the print should contain *All Selected* components or *Only Current* component. The selected component is highlighted in the **Component Table**.

Signals

This section serves for the decision whether the print should contain *All* or *Only Current* signals of the validated chromatogram. The selected signal is highlighted in the header of the **Component Table**, in the *Evaluate* box.

5 How to set the SST

This chapter includes practical procedures for setting the **SST Extension** to suit your individual requirements.

5.1 Conditions for SST use

The following requirements must be fulfilled in order to start a validation:

Setting the SST method on an Instrument

You can either open an existing **SST** method using the *SST - Open...* command in the [Chromatogram](#) window, or create a new **SST** method using the *SST - New* command.

Calibrated chromatograms

Components in validated chromatograms are identified according to the component name (i.e. you can only validate identified components).

Opening the chromatogram(s) in the Chromatogram window

Validation calculations will automatically run after a chromatogram has been opened in the [Chromatogram](#) window (it is not necessary to press any button).

Remember that when recording validation results in a database file, they will only be recorded when the chromatogram closes.

Note: Chromatograms are closed using the *File - Close* and *File - Close All* commands or by closing the [Chromatogram](#) window. If the **Overlay** mode has been disabled, loading a new chromatogram will close the previous one.

5.2 Creating SST method

- Open the **Chromatogram** window.
- Click on the **SST Results** tab.
- Open any calibrated chromatogram.
- Use the *Update from Calib* command in the **SST** menu (for more details see the chapter **SST menu** on pg 11). This will transfer the list of all components from the linked calibration into the **Component Table** on the left of the **SST Results** tab, regardless of whether they are identified in the current chromatogram or not.

Note: The *Update From Calib* command is also accessible in the local menu after clicking the right mouse button on the **SST Results** tab.

- Select the components to be included in the validation using the checkboxes in the *Used* column of the **Component Table**.
- On the **SST Properties - Parameters** tab specify which parameters are to be monitored. The dialog is opened using the *SST - Parameters...* command.
- Switch to the next **SST Properties - Subparameters** tab. Specify the type of limit values according to which will the parameters be evaluated.
- Select the method for calculating parameters on the **SST Properties - Calculate By** tab, set the user-defined events for exceeded limits on the **SST Properties - Events** tab and whether the validation results should be saved into the database file on the **SST Properties - General** tab.
- Close the **SST Properties** dialog by clicking the **OK** button.
- Mark the component for which you want to enter a limit value in the **Component Table**.
- Fill-in the limit values in the **Parameters Table** on the right side of the **SST Results** tab.

Note: Each component may be validated according to different parameters. For example; one may be validated according to the amount of a component (*Amount*), while another is validated according to the peak height (*Height*).

- Repeat this procedure for all components that you want to include in the validation - select them in the **Component Table** and fill in their validation parameters in the **Parameters Table**.

Note: Parameters can be easily added or removed from a validation process for the given compound by double-clicking on the header of the relevant parameter column in the **Parameters Table**.

- Save the **SST** method using the *SST - Save* command.

Note: You can make the **Parameters Table** more synoptic by hiding unused (grayed) columns and rows using the *Show All Columns* and *Show All Rows* commands in the *SST* main menu. In this case, columns/rows cannot be activated or deactivated by double-clicking on them.

5.3 Step-by-step validation

Prior to running the validation, the following points must be made clear:

What do I want to validate?

- Individual chromatograms - monitor whether the values of individual parameters meet specified criteria or not - the *Overlay* mode must be disabled. For more details see the chapter **Chromatogram validation** on pg 32.
- Chromatographic system - monitors whether the individual parameters standard deviations mean values fall between specified limits - the *Overlay* mode must be enabled. For more details see the chapter **Validation of the chromatographic system** on pg 29.


When do I want to validate?

- During analysis (online) - results are automatically loaded to the **Chromatogram** window after the single analysis has ended or one sequence injection has been performed. For more details see the chapter **Online - Sequence (or Single Analysis)** on pg 26.
- Subsequently (offline) - chromatograms measured earlier are either loaded manually into the **Chromatogram** window or batch processed using the *Analysis - Batch...* command from the **Instrument** window. For more details see the chapter **Offline - Manual** on pg 27.

Note: In the **Clarity** station the *Overlay* mode allows more chromatograms to be simultaneously opened in the **Chromatogram** window. When the *Overlay* mode is switched off, only the currently opened chromatogram will be validated against specified limit values.


5.3.1 Online - Sequence (or Single Analysis)

During an online validation, the measured chromatograms will be gradually and automatically opened in the [Chromatogram](#) window. Validation results will automatically be updated with every new chromatogram.

- In the [Chromatogram](#) window switch to the [SST Results](#) tab and open the **SST** method using the *SST - Open...* command. If no **SST** method is prepared, create a new one (for more details see the chapter **Creating SST method** on pg 24).
- Close any chromatograms that are still open in the [Chromatogram](#) window using the *File - Close All* command.
- Check that the *Overlay* mode has been enabled. E.g. in the bottom-right corner of the [Chromatogram](#) window. If *Overlay* mode has been disabled (the word "Overlay" is shaded gray), enable it by double-clicking on it or using the *File - Overlay Mode* command or the  icon.
- Ensure that automatic loading of measured chromatograms into the [Chromatogram](#) window has been enabled; select checkbox *Open Chromatogram Window* in the [Single Analysis - Post Run Settings](#) tab.
- Switch to the [Sequence](#) window and run the prepared sequence. When running a single analyses, gradually run them in the [Single Analysis](#) dialog.
- All measured chromatograms will be automatically opened in the [Chromatogram](#) window and added to the validation.
- After measuring the final sample, switch to the [Chromatogram](#) window, then switch to the [SST Results](#) tab and check the validation results.

5.3.2 Offline - Manual

Manual validation can be performed on already measured chromatograms with the advantage that these chromatograms may be measured in various sequences. Manual validation is used for the ex-post verification of system suitability.

- In the [Chromatogram](#) window switch to the [SST Results](#) tab and open the **SST** method using the *SST - Open...* command. If no **SST** method is prepared, create a new one (for more details see the chapter **Creating SST method** on pg 24).
- Close any chromatograms that are still open in the [Chromatogram](#) window using the *File - Close All* command.
- Check that the *Overlay* mode has been enabled. E.g. in the bottom-right corner of the [Chromatogram](#) window. If *Overlay* mode has been disabled (the word "Overlay" is shaded gray), enable it by double-clicking on it or using the *File - Overlay Mode* command or the  icon.
- Open all chromatograms that are to be included in the validation using the *File - Open...* command.

Note: More files can be selected simultaneously by left-clicking on them while holding down on the **Ctrl** key (file-by-file selection) or **Shift** key (continuous selection). You can select all files in the directory using the **Ctrl** + **A** keyboard shortcut. For more details see the description of [Open Chromatogram](#) dialog in the **Reference Guide**.

- Check the [SST Results](#) tab for validation results.

5.3.3 Offline - Batch reprocess

Batch opening and processing of chromatograms can be used for “ex-post” validation that works similarly to simple opening of chromatograms in the [Chromatogram](#) window with the *Overlay* mode enabled.

Note: Use batch opening if you want to batch adjust opened chromatograms prior to a validation, otherwise opening chromatograms in the [Chromatogram](#) window is more suitable (for more details see the chapter **Offline - Manual** on pg 27).

- Open the **SST** method in the [Chromatogram](#) window using the *SST - Open* command.

Caution: The *Overlay* mode in the [Chromatogram](#) window must be enabled.

- Switch to the [Instrument](#) window and open the [Batch](#) dialog using the *Analysis - Batch...* command.
- In the [Batch](#) dialog, select the chromatograms intended for the validation. For the description of the [Batch](#) dialog please see the **Clarity Reference Guide**.

Caution: The *Open Chromatogram Window* checkbox must be selected!

- Using the *Proceed* button will run a batch processing of selected chromatograms.
- All batch-processed chromatograms will be automatically opened in the [Chromatogram](#) window.
- You can view the validation result on the [SST Results](#) tab.

5.3.4 Validation of the chromatographic system

When validating a chromatographic system, the mean values or standard deviations of monitored parameters are checked for the compliance with specified criteria.

X	SST	Chromatogram	Retention Time [min]	Area [mV.s]
		Lower Limit	3,660	60,000
		Upper Limit		
		%RSD Limit		
		Mean	3,655	59,892
		RSD [%]	0,19	8,26
		Parameter Result	⊖	⊖
⊕	✓	Sample_Vial_6-1	3,660	63,390
⊖	✓	Sample_Vial_8-1	3,650	56,395

Fig 12: Chromatographic system parameters validation

Note: When validating a chromatographic system, the results of individual chromatograms found in the list of validated chromatograms under the bold line in the **Parameters Table** are irrelevant.

⊕	✓	Sample_Vial_6-1	3,660	63,390
⊕	✓	Sample_Vial_8-1	3,650	56,395
⊖	✓	Sample_Vial_8-2	3,643	51,378
⊖	✓	Sample_Vial_9-1	3,647	140,237
⊕	✓	Sample_Vial_9-2	3,660	148,283

Fig 13: List of chromatograms - irrelevant validation results

A chromatographic system can be validated **online** (running) or **offline** (loading of earlier measured chromatograms into the [Chromatogram](#) window).

5.3.5 Summary table of validated chromatograms

In the summary table of validated chromatograms, one can easily and quickly determine if a chromatogram is deviating out of line.

The procedure for setting the **SST** method is the same as in the chromatographic system validation (for more details see the chapter **Validation of the chromatographic system** on pg 29).

Caution: The *Overlay* mode in the **Chromatogram** window must be enabled.

In the parameters table you can set limit values for the measured results to comply with.

Only the **List of chromatograms** in the **Parameters Table** and icons to the left of it are relevant. All other results (overall validation result, results in the **Component Table**, as well as results in the **Parameters Table** - above the bold line) refer to the chromatographic system validation.

X	SST	Chromatogram	Retention Time [min]	Area [mV.s]
		Lower Limit	3,650	56,000
		Upper Limit		
		%RSD Limit		
		Mean	3,653	79,861
		RSD [%]	0,22	57,45
		Parameter Result	0	0
✓	✓	Sample_Vial_6-1	3,660	63,390
✓	✓	Sample_Vial_8-1	3,650	56,395
✗	✓	Sample_Vial_8-2	3,643	51,378
✗	✓	Sample_Vial_9-1	-	-
✓	✓	Sample_Vial_9-2	3,660	148,283

Fig 14: List of validated chromatograms

5.3.6 Storing results in DBF

Results saved in a database file can be freely used, for example, for a system validation according to special validation methods, to monitor trends in results, etc.

Note: Setting of the *Overlay* mode has no effect on results saved in the database file.

- Open the **SST** method in the **Chromatogram** window using the *SST - Open...* command.
- Open the **SST Properties - General** dialog using the *SST - General...* command.
- Here, select the *Log SST Result into DBF file* and fill-in the database file name into the *File Name* field.
- Use the **Clarity** station either *online* (measuring) or *offline* (opening of saved chromatograms) to validate the desired chromatograms and save the results into the *.DBF file.

Note: Only validation results of chromatograms opened in the **Chromatogram** window will be saved in the database file.

- Open the created database file in the database program. The database file is automatically created in the current project directory.

Caution: If you open the database file in another program, the **SST Extension** cannot write into it. Work with the database file only after completing work with **Clarity**, thereby avoiding an unwanted locking of the file while saving results.

5.3.7 Chromatogram validation

During the chromatogram validation, it is determined whether or not the parameters of individual chromatograms meet the specified criteria. Individual chromatograms can be validated either online (running) or offline (loading of earlier measured chromatograms into the [Chromatogram](#) window). With the *Overlay* mode disabled, the [Chromatogram](#) window will always show only one chromatogram. Regardless of from where we open a chromatogram ([Single Analysis](#), [Sequence](#), [Open Chromatogram](#), [Batch](#)), every result will always be related to the current chromatogram.

Note: The overall validation result indicates whether or not the chromatogram has complied with all criteria for all components; the **Component Table** displays results for individual components.

- Open the **SST** method in the [Chromatogram](#) window using the *SST - Open...* command.

Caution: The *Overlay* mode in the [Chromatogram](#) window must be disabled.

- Using any arbitrary method (from an analysis or, eventually, from a file), open a chromatogram in the [Chromatogram](#) window.
- All data on the [SST Results](#) tab relate to the currently opened chromatogram.

6 Workarounds

6.1 Stopping a running sequence

To set a sequence follow the same procedure as described in the chapter "**Online - Sequence (or Single Analysis)**" on pg 26., inserting the following two steps after the setting of the *Overlay* mode:

- Select the *SST - Events...* command in the **Chromatogram** window.
- In the displayed **SST Properties - Events** dialog select the *Stop Sequence* in the *If SST result is FAILED* section.

Note: Stopping a sequence can be also done together with other pre-defined action, such as running a program, sending an e-mail etc.).

6.2 Sequence summary table

Outputs from **SST** can, to a certain extent, replace the summary tables function for measuring sequences. The means and standard deviations of monitored parameters are calculated directly in the **SST Extension** of the **Clarity** station.

A separate table containing summaries of individual parameters is prepared for each component.

If the **SST Extension** is only used for summary results, no limit values need to be set - you simply have to determine which components are to be monitored.

Overall SST Result : ?				Component Name: Chloroform, Calculate by: EP (N)				
Used	OK	Component Name	Retention Time	X	SST	Chromatogram	Retention Time [min]	Amount
1	<input checked="" type="checkbox"/>	? Chloroform	3,717			Mean	3,652	1,864
2	<input checked="" type="checkbox"/>	? Trichloroethane	4,053			RSD [%]	0,21	47,04
3	<input type="checkbox"/>	Tetrachloroethane	4,387			Parameter Result	?	?
4	<input type="checkbox"/>	Trichloroethylene	5,960	?	<input checked="" type="checkbox"/>	Sample_Vial_6-1	3,660	1,266
5	<input checked="" type="checkbox"/>	? Bromodichloroethane	6,870	?	<input checked="" type="checkbox"/>	Sample_Vial_8-1	3,650	1,251
6	<input type="checkbox"/>	ISTD	7,107	?	<input checked="" type="checkbox"/>	Sample_Vial_8-2	3,643	1,157
7	<input type="checkbox"/>	Tetrachloroethylene	9,567	?	<input checked="" type="checkbox"/>	Sample_Vial_9-1	3,647	2,842
8	<input checked="" type="checkbox"/>	? Dibromochloroethane	9,930	?	<input checked="" type="checkbox"/>	Sample_Vial_9-2	3,660	2,806

Fig 15: Summary Table substitution

- Open all measured chromatograms in the **Chromatogram** window.

Caution: The **Chromatogram** window must be switched to the *Overlay* mode.

- Switch to the **SST Results** tab.
- Create a new **SST** method using the *SST - New* command.
- Transfer the components from the calibration connected to the current chromatogram, into the **SST** method using the *SST - Update from Calib* command.

Caution: The chromatograms being validated must be calibrated.

- In the *Used* column of the **Component Table**, select which components should be summarized.
- Select which parameters will be monitored using the *SST - Parameters...* command.
- Switch to the **Subparameters** tab and uncheck the selection of all three items - *Lower Limit*, *Upper Limit* and *%RSD Limit*.
- Close the **SST Properties - Subparameters** dialog using the *OK* button.
- Using the *SST - Show All Columns* and *Show All Rows* commands you can hide inactive rows and columns in the **Parameters Table**.
- Now the **Parameters Table** will display a summary table of all loaded chromatograms for the currently selected component in the **Component Table**.
- Using the *File - Report Setup...* command, open the **Report Setup** dialog and switch to the **SST** tab.
- Select the *Print* command in the top-left corner.
- Select the **Parameters Table**.

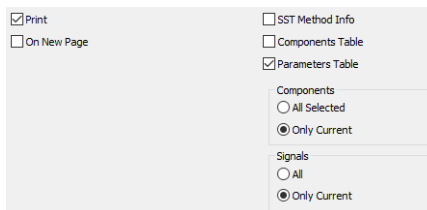


Fig 16: Setting of the Report Setup - SST tab

- Select the *Only Current* option in the *Components* section to print the **Summary Table** only for the currently selected component or select the *All Selected* option to print all summarized components (see above).
- Set the *Signals* section to select whether the **Summary Table** will be constructed using *Only Current* signal or *All* signals.

6.3 Using DBF in MS Excel

An experienced user can program selected reports in a database program (Access, dBase, FoxPro, SQL). However, not everyone is a database-programming expert. What if we open a database file containing validation results in a common application, for example **Microsoft Excel**? At first, a complex table will be displayed. Can it be processed in such a way to make it useful?

One option of working with an **SST *.DBF** file in **Excel** is to use what is known as **Contingency tables**:

- Select the whole table in **MS Excel**, e.g. by using the **Ctrl** + **A** key combination.
- Select the *Contingency tables and graphs* command in the *Data* menu.

- A “*Contingency table wizard*” will appear to help you prepare the table (and graph, if applicable).
- In the wizard, you gradually select the required data (database) and location where the contingency table and graph should be created (a new spreadsheet is a suitable option).
- In the last step (3/3) click the layout button and drag the field names onto the table picture to determine the look of the table layout.
- Using the *Finish* button, create the contingency table and graph.

Note: This procedure was created for use with **MS Excel 2000** and may vary for other versions.

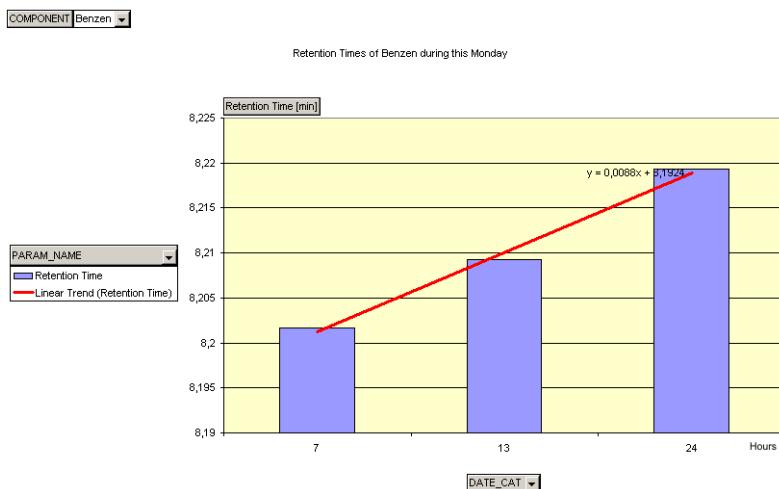


Fig 17: Example of a contingency graph in Excel

Using a contingency table you can, for example, display a detailed overview of the monitored component amount in chromatograms, sort data according to the validation date to compare results for various measurements (e.g. for periodically repeated measurements, or to monitor trends), and so on. Graphs can also be generated simply from the contingency tables using the wizard.

Note: Detailed information on working with contingency tables can be found in the **MS Excel** online help section.

7 Appendix - Mathematical Formulas

Tab 4: Mathematical formulas

	USP	EP	JP
Efficiency	$16 \left(\frac{RT_i}{W_i} \right)^2$	$5.54 \cdot \left(\frac{RT_i}{W_{h/2_i}} \right)^2$	$5.55 \cdot \left(\frac{RT_i}{W_{h/2_i}} \right)^2$
Assymetry	$\frac{b_i}{a_i}$		
Symmetry/ Tailing	$\frac{A_{05_i} + B_{05_i}}{2 \cdot A_{05_i}}$		
Capacity	$\frac{RT_i - T_M}{T_M}$		
Eff/I	$\frac{Efficiency_i}{ColumnLenght}$		
HETP	$\frac{ColumnLenght}{Efficiency_i}$		
Resolution	$2 \frac{RT_i - RT_{i-1}}{(W_{i-1} + W_i)}$	$\frac{1.18 \cdot (RT_i - RT_{i-1})}{(W_{h/2_{i-1}} + W_{h/2_i})}$	

Fig 18: Formula for parameter calculation

where:

Tab 5: Description of symbols

Symbol	Description	Symbol	Description
RT	- Component retention time	b	- Right half-width at 10% height
$W_{h/2}$	- width at half height	A_{05}	- Left half-width at 5% height
W	- Baseline width	A_{05}	- Right half-width at 5% height
a	- Left half-width at 10% height	T	- Retention time of the unretained solute.
		M	

RSD [%]

Percentage standard deviation (variation coefficient).

$$RSD [\%] = \frac{100}{\bar{X}} \cdot \sqrt{\left[\frac{1}{n-1} \sum_{i=1}^n (X_i - \bar{X})^2 \right]}$$

where:

Tab 6: Description of symbols

Symbol	Description
n	number of validated (loaded) chromatograms
X_i	the value of a monitored parameter of the i-th chromatogram
\bar{X}	arithmetic mean of a monitored parameter of all validated (loaded) chromatograms