PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
Title: Good Chromatography Practices	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

To lay down a procedure for Good chromatography to be followed by analyst during chromatography analysis and reviewer has to consider parameter during reviewing of the reports.

2.0 SCOPE:

This SOP is applicable for Good chromatography practices in Quality Control Laboratory.

3.0 RESPONSIBILITY:

Analyst - Responsible for following SOP during analysis.

Reviewer / Section Head - Responsible for following SOP during reviewing of Sequence, chromatograms and reports.

Head QC-Responsible for implementation and compliance of SOP.

4.0 PROCEDURE:

4.1 This procedure is applicable to chromatographic analysis by HPLC and GC, and includes tests for assay, related substances/compounds (impurities), content uniformity, dissolution, chromatographic purity, residual solvents and any other test as applicable.

4.2 Analyst shall ensure correct specification and standard test procedure is selected for the analysis of under test sample.

4.3 Analyst shall be verifying the availability of column, working standard, reference standard, reagents, chemicals and any specific requirement such as filter etc. as per requirement of standard test procedure.

4.4 Analyst shall connect the column in HPLC/GC for washing/conditioning with recommended solvents/gas.

4.5 Analyst shall prepare the mobile phase as per standard test procedure and before confirmation or adjustment of pH of mobile phase analyst shall verify the calibration status of pH meter. If the pH meter not calibrated then analyst shall calibrate the pH meter as per pH meter calibration standard operating procedure.

4.6 Analyst shall degas the mobile phase with help of vacuum filtration method or ultra-sonication

method. Analyst shall conform that there is no air bubbles evolves from the mobile phase.

4.7 Analyst shall select the correct method (program) in HPLC/GC analysis as per standard test procedure from the available method database in HPLC/GC. If the method is not available in method database then reviewer shall create the method (program) in HPLC/GC as per the standard test procedure, for the relevant test.

4.8 While selecting or creating the method (program) in HPLC/GC, reviewer shall conform the correctness of column, wavelength, flow rate and column temperature (if applicable) etc. as per standard test procedure.

4.9 Analyst shall load the mobile phase in HPLC and equilibrate the column with mobile phase till stable base line is achieved.

4.10 Analyst shall prepare the solutions as per the respective standard test procedure.

4.11 Analyst shall create the sequence in HPLC/GC up to system suitability criteria injections and run the sequence.

4.12 Analyst shall monitor the running sequence to meet the system suitability criteria for chromatographic condition/ parameter such as retention time, resolution, tailing factor, theoretical plates, relative retention time, peak shape, scaling, peak marking, peak height, run time, base line drift etc as applicable.

4.13 If the system suitability criteria meet as per respective standard test procedure, then analyst shall append the sequence and continue the analysis after authorization of sequence and method (program) file print out to the respective reviewer.

4.14 If the system suitability criteria does not meet as per standard test procedure analyst shall inform to respective reviewer/supervisor.

4.15 Reviewer/Supervisor shall verify standard test procedure, preparation of mobile phase, standard solution, and chromatographic conditions etc. and based on the chromatographic understanding Reviewer/Supervisor suggest corrective action to achieve the required criteria and Reviewer/Supervisor shall put the invalidate stamp with remark on sequence.

4.16 When taking above corrective action, while the system is ready, create the sequence with new version and follow the step 4.11 to 4.15.

4.17 If the system suitability criteria does not meet as per respective standard test procedure than

supervisor in consultation with QC Head and allow changing the chromatography conditions as per Annexure - I.

4.18 After restoration of chromatographic condition, append the injection in same sequence or if require create new sequence. If system suitability criteria meet as per respective standard test procedure, then analyst shall append the sequence and continue the analysis after authorization of sequence and method (program) file print out to the respective reviewer.

4.19 Put the "INVALIDATE" stamp on all the chromatograms and sequences which are not considering for calculation and keep with raw data.

4.20 On completion of analysis of HPLC/GC, analyst shall verify the chromatogram for conformance of the chromatographic condition/ parameter such as retention time, resolution, tailing factor, theoretical plate, relative retention time, peak shape, scaling, peak marking, peak height, run time, base line drift etc as applicable.

4.21 Analyst shall integrate all the chromatograms as per respective quantification method.

4.22 Incase if any of the peaks are not integrated as per respective quantification method, analyst shall inform to the Reviewer/Supervisor.

4.23 Reviewer/Supervisor shall be modifying the respective quantification method using the pertinent integration parameters given as per Annexure – II. During this Reviewer/Supervisor has to ensure that all the require peaks have been integrated.

4.24 While the disregard limit and LOD value is specified in the respective standard test procedure, then reviewer shall be considering the same value for integration, if desired.

4.25 **Manual Integration:**

4.25.1 Incase if any of the peaks are not integrated using the integration parameters as per annexure II, then manual integration can be done by the Reviewer/supervisor.

4.25.2 Reviewer/supervisor shall be, reprocessing the chromatogram to integrate the peaks using manipulate peaks integration parameter (manually peak integration) under "Data Reduction privileges" into its own user ID after authorization from QC head or his designee.

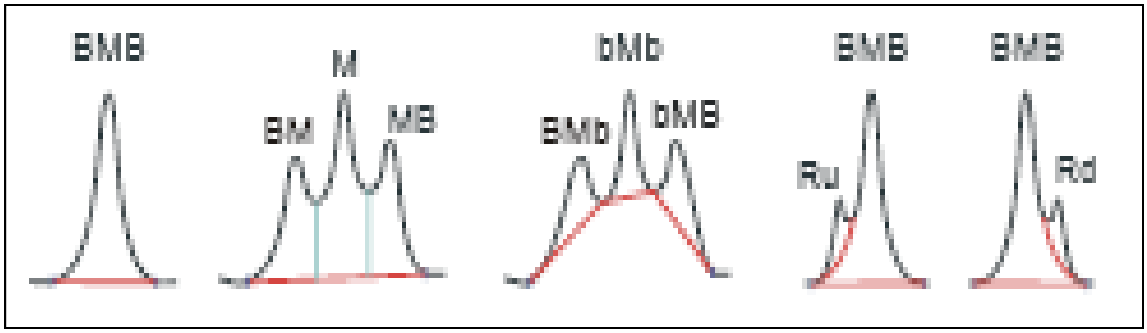
4.25.3 Manual integration is valid in the following cases but not limited,

1. Overlapping peak
2. Peaks are misidentified.

3. Peaks have been "missed" by chromatography software.
4. There are well-defined peaks on the shoulders of other peaks (riders)
5. Excessive peak fronting or tailing due to a rise or fall in the baseline.
6. Any other situation that is reasonably justifiable using basic chromatographic principles

4.25.4 When the reviewer/supervisor integrates any peak manually the same shall be displayed with "*"mark in the type under peak information table and modifier user displayed with date time on the chromatogram. Re-process chromatogram consider for calculation. Take zoom copy of this manually integrated part and the Initial integrated chromatogram put the INVALIDATE stamp and keep with raw data.

Peak type displayed as:



4.26 Analyst/Reviewer shall take the print out of all chromatograms and calculate the result as per standard test procedure.

4.27 After completion of analysis and calculation, analyst shall submit the raw data and report to supervisor for review.

4.28 On review of the raw data and chromatograms reviewer shall verify all the entries.

4.29 During the review if any abnormality found in integration of the chromatograms, reviewer shall re-process the chromatograms using pertinent integration parameters or privileges after authorization from QC head or his designee. Re-processed chromatogram shall be considered for calculation. Put the INVALIDATE stamp on Initial integrated chromatogram and keep with raw data.

4.30 **Adjustments/variations of operating conditions to meet system suitability:**

4.31.1 Adjustments of operating conditions to meet the system suitability requirements are necessary; Adjustments/variations can consider as per given in annexure - I, unless otherwise directed in the monograph/respective STP.

4.31.2 Adjustments are permitted only when suitable standards (including reference standards) are available for all compounds used in the suitability test and are used to show that the adjustment have improved the quality of the chromatography in meeting system suitability requirements.

4.31.3 Adjustments to chromatographic systems perform in order to comply with system suitability requirements should not be made to compensate for column failure or to circumvent replacing a deteriorated column.

4.32 General points to consider:

4.32.1 The sample and standard should be dissolved in the mobile phase. If that is not possible, then avoid using too high level of the organic solvent as compared to the level in the mobile phase.

4.32.2 The sample and standard concentration should be close. Weight of sample and standard should be taken within 10 % criteria of the mentioned weight.

4.32.3 During running of the chromatographic system sample solution (compartment) & column oven temperature shall be set at 25°C, otherwise specified in procedure.

4.32.4 The samples should be bracketed by standards during the analytical procedure.

4.32.5 If standard solution is not stable then inject new standard solution as per respective test procedure and check Similarity factor using following formula;

$$\text{Similarity factor} = \frac{\text{Mean Area of initial standard}}{\text{Area of bracketing standard}} \text{ X } \frac{\text{Weight of bracketing standard}}{\text{Weight of initial standard}}$$

Similarity factor shall not be more than % RSD criteria given for replicate standard as a system suitability in the specific test procedure.

Ex.

If % RSD limit is NMT 2.0 then Similarity factor should be Between 0.98 and 1.02

If % RSD limit is NMT 5.0 then Similarity factor should be Between 0.95 and 1.05

4.32.6 Filtration of the samples before injection is occasionally observed. Filtration will remove particulates (Centrifugation performs the same function) that may clog the column. Adhesion of the analyte to the filter can also happen.

4.32.7 The absolute difference between two results injected from same vial in % assay by HPLC test should not be more than ± 2.0 %

4.32.8 **Unknown peaks in residual solvents (GC) in raw material:**

If unknown peaks observed in Residual solvent analysis in Raw material (API and Excipient), QA/QC shall refer this for identification to R & D. Once identification is completed, following are the options to be considered by QA/QC to release or reject the batch of the raw material.

1) If identified solvents are class-1 (as per ICH Q3C) or genotoxic, solvents, batch should be taken up for further investigation.
2) If the unknown peaks falls under the category of class-2, class-3 or other than class-2, class-3, such solvents should be quantified and if the results are under 10% of ICH limit, batch will be released based on the impact which is very negligible (below 10% of ICH limits).
3) Quantification: These solvents shall be quantified by QC by using the same method (wherein these were reported as unknown) by using the respective standard at 10% of ICH limit in triplicate with the RSD of not more than 15% as criteria.

Note: If RSD criteria does not comply not more than 15% for standard at 10% of ICH limit, then prepare standard at 30% of ICH limit and inject in triplicate with RSD of not more than 15% criteria & quantify the solvents with this standard.

5.0 ANNEXURE (S):

Annexure – I : Adjustments/Variations parameters of Operating Conditions to meet system suitability

Annexure – II : Detection parameters

6.0 REFERENCE (S):

CQA Guideline : Guideline for Chromatographic Practices , BP, and USP

SOP: Preparation, approval, distribution, control, revision and destruction of Standard Operating Procedure (SOP).

7.0 ABBREVIATION (S)/DEFINITION (S):

SOP - Standard Operating Procedure.

HPLC - High Performance Liquid Chromatograph.

GC - Gas Chromatograph.

TLC – Thin Layer Chromatography

RSD - Relative Standard Deviation.

UV - Ultra Violet.

°C - Degree Centigrade.

ICH – International conference on Harmonization

R & D – Research and development

BP – British Pharmacopoeia

USP – United State Pharmacopoeia

mm - Millimeter

mL – Milliliter

nm – Nanometer

NMT – Not more than

STP – Standard test procedure

REVISION CARD

S. No.	REVISION No.	REVISION DATE	DETAILS OF REVISION	REASON (S) FOR REVISION	REFERENCE CHANGE CONTROL No.
1	00	---	---	New SOP	---

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ANNEXURE I

ADJUSTMENTS/VARIATIONS PARAMETERS OF OPERATING CONDITIONS TO MEET SYSTEM SUITABILITY

I. ISOCRATIC ELUTION LIQUID CHROMATOGRAPHY				
1	**Flow Rate**			
	i. Without change the column dimension.	Adjust up to ± 50 % of the stated flow rate.	Adjust up to ± 50 % of the stated flow rate.	Adjust as per USP and BP
	ii. Changing the column dimension.	Adjustment more than 50% acceptable if $F_2 = F1 \frac{l_2 d_2^2}{l_1 d_1^2}$ Where, F_1 = Flow rate stated in monograph in mL/min F_2 = Adjusted flow rate in mL/min l_1 = Length of the column stated in the monograph in mm l_2 = Length of the column used in mm d_1 = Internal diameter of the column stated in the monograph in mm d_2 = Internal diameter of the column used in mm	Adjustment as per, $F_2 = F1 \frac{l_2 d_2^2}{l_1 d_1^2}$ Where, F_1 = Flow rate stated in monograph in mL/min F_2 = Adjusted flow rate in mL/min l_1 = Length of the column stated in the monograph in mm l_2 = Length of the column used in mm d_1 = Internal diameter of the column stated in the monograph in mm d_2 = Internal diameter of the column used in mm	Adjust as per USP
2	**Column**			
	i. Stationary phase	Not defined	No change permitted of stationary phase. (No replacement of C_{18} by C8).	As per BP

	ii. Length	Adjust up to ± 70 % of the stated	Adjust up to ± 70 % of the stated	Adjust as per USP and BP
	iii. Inner (Internal) Diameter	Can be adjusted if the linear velocity is kept constant.	Adjust up to ± 25 % of the stated	Adjust as per BP
	iv. Particle size	Can be reducing up to 50 % of stated. Increasing of particle size not permitted.	Can be reducing up to 50 % of stated. Increasing of particle size not permitted.	Adjust as per USP and BP
3	**Detector (Wavelength)**	No adjustment permitted. The procedure specified by the detector manufacturer, or another validated procedure is used to verify that error in the detector wavelength is, at most, ± 3 nm.	No adjustment permitted.	Adjust as per USP.
4	**Column Temperature**	± 10°C of the stated. Column thermo stating is recommended to improve control and reproducibility of retention time.	± 10°C of the stated, unless otherwise prescribed.	Adjust as per USP and BP
5	**Injection Volume**	The injection volume can be reduced as far as is consistent with accepted precision and detection limits. Increasing of injection volume not permitted.	The injection volume can be decreased provided detection and repeatability of the peak(s) to be determine satisfactory. Increasing of injection volume not permitted.	Adjust as per USP and BP
6	**Mobile phase**			
	i. pH of the aqueous component of the mobile phase	± 0.2 of the stated value or range for the aqueous buffer used in the preparation of the mobile phase.	± 0.2 pH of the stated, unless otherwise prescribed, or When non-ionisable substances pH	Adjust as per BP

			± 1.0.	
	ii. Concentration of salts in the buffer component of a mobile phase	± 10 % if the permitted pH variation is to be meeting.	± 10 % of stated.	Adjust as per USP and BP
	iii. Ratio of components in the mobile phase	Minor component of the mobile phase specified at 50% or less. The amount of minor solvents may be adjusted ± 30% relative. No any component is change by more than ± 10% absolute. (In relation to the total mobile phase).	The amount of minor solvents may be adjusted by ± 30% relative or ±2 % absolute, whichever is larger. No other component is altered by more than 10% absolute.	Adjust as per BP
	iv. Binary mixture	Specified ratio of 50 : 50 30% of 50 is 15 % absolute, but this exceeds the maximum permitted change of ± 10 % absolute in either component. Therefore, mobile phase ratio may be adjusted only within the range of 40 : 60 to 60 : 40.	The amount of minor solvents may be adjusted ± 30% relative or ±2% absolute, whichever is larger. No other component is altered by more than 10% absolute.	Adjust as per USP
		Specified ratio of 2 : 98 30% of 2 is 0.6 % absolute. In this case an Absolute adjustment of – 2 % is not allowed because it would reduce the amount of the first component to zero. Therefore the maximum allowed adjustment is within the range of 1.4 : 98.6 to 2.6 : 97.4.	The amount of minor solvents may be adjusted ± 30% relative or ±2% absolute, whichever is larger. No other component is altered by more than 10% absolute.	Adjust as per USP

	v. Ternary mixture:	Specified ratio of 60: 35: 5 For second component, 30 % of 35 is 10.5 % absolute, but this exceeds the maximum permitted change of ± 10 % absolute in any component. Therefore, the second component may be adjusted only within the range of 25 % to 45 % absolute. For the third component, 30% of 5 is 1.5 % absolute. Therefore, mixture ranges the maximum allowed adjustment is of 50:45:5 to 70:25:5 or 58.5:35:6.5 to 61.5:35:5.5.	The amount of minor solvents may be adjusted ± 30% relative or ±2% absolute, whichever is larger. No other component is altered by more than 10% absolute.	Adjust as per USP
II. GRADIENT ELUTION LIQUID CHROMATOGRAPHY				
1	**Flow Rate**			
	i. Without change the column dimension.	Adjust up to ± 50 % of the stated flow rate.	---	As per USP
	ii. Changing the column dimension.	$F_2 = F1 \frac{l_2 d_2^2}{l_1 d_1^2}$ Where, F_1 = Flow rate stated in monograph in mL/min F_2 = Adjusted flow rate in mL/min l_1 = Length of the column stated in the monograph in mm l_2 = Length of the column used in	$F_2 = F1 \frac{l_2 d_2^2}{l_1 d_1^2}$ Where, F_1 = Flow rate stated in monograph in mL/min F_2 = Adjusted flow rate in mL/min l_1 = Length of the column stated in the monograph in mm l_2 = Length of the column used in	As per USP and BP

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		mm d_1= Internal diameter stated in the monograph in mm d_2 = Internal diameter of the column used in mm	mm d_1 = Internal diameter stated in the monograph in mm d_2 = Internal diameter of the column used in mm	
2	**Column**			
	i. Stationary phase	---	No change permitted of stationary phase. (No replacement of C_{18} by C8).	As per USP and BP
	ii. Length	Adjust up to ± 70 % of the stated	Adjust up to ± 70 % of the stated	Adjust as per USP and BP
	iii. Inner (Internal) Diameter	Can be adjusted if the linear velocity is kept constant.	Adjust up to ± 25 % of the stated	Adjust as per BP
	iv. Particle size	The particle size can be reducing by 50 % of stated, Increasing of particle size not permitted.	No adjustment permitted.	Adjust as per USP
3	**Detector**	Deviation of wavelengths specified in the procedure is not permitted. The procedure specified by the detector manufacturer, or another validated procedure is used to verify that error in the detector wavelength is, at most, ± 3 nm.	No adjustment permitted.	Adjust as per USP
4	**Column Temperature**	± 10°C of the stated. Column thermo stating is recommended to improve control and reproducibility of retention time.	± 5°C of the stated, unless otherwise prescribed.	Adjust as per USP

5	**Injection Volume**	The injection volume can be reduced as far as is consistent with accepted precision and detection limits. Increasing of Injection Volume not permitted.	The injection volume can be decreased provided detection and repeatability of the peak(s) to be determine satisfactory. Increasing of Injection Volume not permitted.	Adjust as per USP and BP
6	**Mobile phase**			
	i. pH of the aqueous component of the mobile phase	± 0.2 of the stated value or range for the aqueous buffer used in the preparation of the mobile phase	No adjustment permitted.	Adjust as per USP
	ii. Concentration of salts in the buffer compon-ent of a mobile phase	± 10 % of the permitted pH variation is to be meeting.	No adjustment permitted.	Adjust as per USP
	iii. Ratio of components in the mobile phase	No adjustment permitted. If adjustments are necessary, only column changes or dwell volume adjustments are recommended.	Minor adjustments of the composition of the mobile phase and the gradient are acceptable provided that: - The system suitability requirements are fulfilled. - The principal peak(s) elute(s) within ± 15 % of the indicated retention time(s). - The final composition of the mobile phase is not weaker in elution power than the prescribed composition.	Adjust as per BP

			Where compliance with system suitability requirements are not achieved, it is often preferable to consider the dwell volume or to change the column.	
	iv. Dwell volume	If the dwell volume used during elaboration of the monograph is given in the monograph, the time points (t min) stated in the gradient table may be replaced by adapted time points (t_c min). $t_c = t - \frac{(D - D_0)}{F}$ D = Dwell volume in mL D_0 = Dwell volume used for development of the method, in mL F = Flow rate, in mL/minute	If the dwell volume used during elaboration of the monograph is given in the monograph, the time points (t min) stated in the gradient table may be replaced by adapted time points (t_c min). $t_c = t - \frac{(D - D_0)}{F}$ D = Dwell volume in mL D_0 = Dwell volume used for development of the method, in mL F = Flow rate, in mL/minute	Adjust as per USP and BP
III. GAS CHROMATOGRAPHY				
1	**Flow Rate**	Adjust up to ± 50 % of the stated	Adjust up to ± 50 % of the stated	Adjust as per USP and BP
2	**Column**			
	i. Length	Adjust up to ± 70 % of the stated	Adjust up to ± 70 % of the stated	Adjust as per USP and BP
	ii. Internal Diameter	Adjust up to ± 50 % of the stated	Adjust up to ± 50 % of the stated	Adjust as per USP and BP
	iii. Particle size	If the chromatography meets the system suitability, changing from larger to smaller and smaller to larger particle size GC mesh support acceptable.	Maximum reduction of 50 % of stated, Increasing not permitted (packed column).	Adjust as per USP

	iv. Film thickness	Adjust - 50 % to + 100 % (capillary column)	Adjust - 50 % to + 100 % (capillary column)	Adjust as per USP and BP
3	**Oven Temperature**			
	i. Isothermal	Adjust up to ± 10°C of the stated.	Adjust up to ± 10 % of the stated.	Adjust as per USP and BP
	ii. Programming temperature	Adjust ± 10°C of the stated. The specified temperature must be maintained or when the temperature must be changed from one value to another, an adjustment of up to ± 20 % is permitted.	Adjust ± 10 % of the stated.	Adjust as per USP.
4	**Injection Volume and split volume**	May be adjusted if detection and repeatability are satisfactory.	May be adjusted, provided detection and repeatability are satisfactory.	Adjust as per USP and BP
IV. THIN LAYER CHROMATOGRAPHY				
1	**Application volume**	Not defined	10-20 % of the prescribed volume if using fine particle size plates (2-10 mm)	Adjust as per BP
2	**Mobile phase**			
iii	i. pH of the aqueous component of the mobile phase	Not defined	± 0.2 pH of the stated, unless otherwise prescribed, or when non-ionisable substances pH ± 1.0	Adjust as per BP
	ii. Concentration of salts in the buffer component of a mobile phase	Not defined	± 10 % of stated.	Adjust as per BP
	iii. Ratio of components in the mobile phase	Not define	The amount of minor solvents may be adjusted ± 30% relative or ±2%	Adjust as per BP

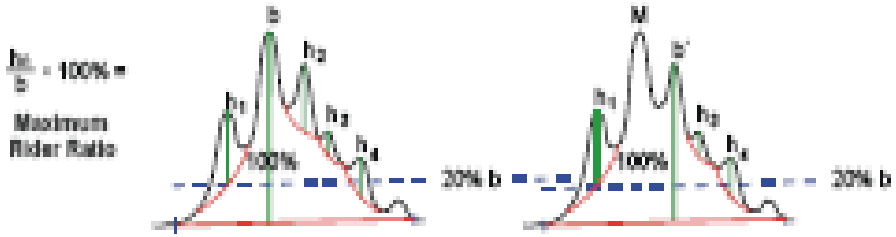
			absolute, whichever is larger. For a minor component at 10 % of the mobile phase, a 30 per cent relative adjustment allows a range of 7-13 per cent whereas a 2 per cent absolute adjustment allows a range of 8-12 per cent, the relative value being therefore the larger. For a minor component at 5 per cent of the mobile phase, a 30 percent relative adjustment allows a range of 3.5-6.5 per cent whereas a 2 per cent absolute adjustment allows a range of 3-7 per cent, the absolute value being in this case the larger. No other component is altered by more than 10 per cent absolute.	

ANNEXURE II
DETECTION PARAMETERS

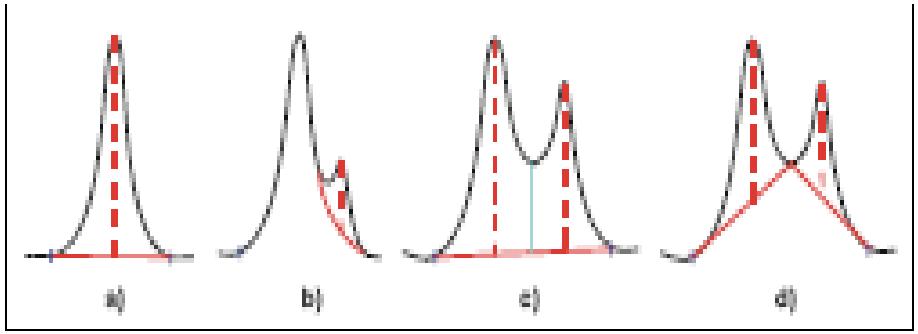
S.No.	Parameters	Description	Function
1.	Baseline Point	The Baseline Point detection parameter	This command has priority over Lock Baseline and Valley to Valley, but not

S.No.	Parameters	Description	Function
		defines a baseline point at the indicated time.	over Type (Peak Type) from the peak table.
2.	Detect Negative Peaks	The Detect Negative Peaks parameter enables and disables detection of negative peaks. When the parameter is enabled, negative as well as positive peaks are detected.	Enabling detection of negative peaks automatically enables the Lock Baseline parameter. Disabling negative peak detection locks the baseline to the default again. In the result report, the area of negative peaks is indicated as a positive value.
3.	Front Riders to Main Peaks	Select the Front Riders to Main. Peaks detection parameter to change rider peaks on the leading edge of a peak into main peaks.	Set the parameter value to On to change upward riders to main peaks even if riders are enforced; for example, Rider Threshold = 0.00%, Maximum Rider Ratio = 100.0%).
4.	Inhibit Integration	The Inhibit Integration detection parameter serves to fade out certain chromatogram areas. When set, peak detection is disabled.	If the value is set to On before the first peak to inhibit, peak detection will not take place until the parameter is disabled (Off); that is, no peaks are recognized in this area. The chromatogram is displayed on screen, but it is not integrated.
5.	Lock Baseline	If the Lock Baseline parameter is set to Off, the detection program calculates the baseline below non-resolved peaks according to a complex pattern recognition process. The other values are used to lock the baseline on different levels.	Off Under non-resolved peaks, the baseline is determined automatically. The Valley-to-Valley parameter is effective in this mode, only. On

S.No.	Parameters	Description	Function
			In case of non-resolved peaks, the baseline is not pulled up to the relative minima (valleys). The baseline connects the start of the first with the end of the last non-resolved peak. If one of the valleys in between is located below the baseline, the baseline is connected with this minimum to avoid cutting off a peak foot. The Valley-to-Valley parameter is not effective. At current level The baseline is fixed at the current signal level and is extrapolated horizontally to the right. The Valley-to-Valley parameter will not take effect.
6.	Maximum Area Reject	Select the Maximum Area Reject parameter to limit the number of integrated peaks. This parameter is very similar to Minimum Area.	The parameter defines the maximum peak area up to which a peak is rejected. No peaks with a peak area below the defined value are integrated.
7.	Maximum Height Reject	Select the Maximum Height Reject parameter to limit the number of integrated peaks. This parameter is very similar to Minimum Height.	The parameter defines the maximum peak height up to which a peak is rejected. Not all peaks with a peak height below the defined value are integrated. (Determination of the height is always relatively to the baseline.)
8.	Maximum Peak Height	Positive peak identification is performed using the Minimum Height parameter. All peaks above this height value are	All peaks above the specified peak height are classified as Unknown; that is, despite positive identification, they are not assigned a name from the peak

S.No.	Parameters	Description	Function
		identified.	table. (The height is always determined relative to the baseline.) This option is very useful when working with two detectors switched in series. Peaks that are clearly identified in the channel of the first detector
9.	Maximum Rider Ratio	If one or several peaks are above the Rider Threshold in a series of non-resolved peaks, determines via the Maximum Rider Ratio detection parameter, whether a peak is classified as main peak or Rider.	For this, the height of the peak to classify is put in relation to the height of the greatest peak in the series (reference peak).  If the ratio h/b multiplied by 100 percent produces a value larger than the one defined as maximum rider ratio, the peak is a main peak. If the ratio h/b multiplied by 100 percent produces a value smaller than the one defined as maximum rider ratio, the peak is a rider peak. Starting with the largest peak in the series (reference peak), all adjacent peaks are then classified. As soon as another main peak is recognized, this peak automatically becomes the new reference peak (b´). The maximum rider ratio

S.No.	Parameters	Description	Function
			is recalculated considering b´. The remaining peaks are classified again. The process is continued until all peaks of the series are classified. Riders can be recognized as such by the skimming tangent drawn on the chromatogram plot and by a Ru (Rider up) or Rd (Rider down) entry in the Type column of the report.
10.	Maximum Width	The Maximum Width detection parameter defines the maximum width above which peaks are ignored during peak detection. The parameter must be enabled before the peak end. The maximum width influences peak recognition and thus the baseline.	The peak width is measured on the baseline. For peaks that do not reach the baseline, the width is extrapolated.
11.	Minimum Area	The Minimum Area detection parameter is used as a minimum criterion determining the area threshold below which peaks are ignored during peak detection or integration. This parameter must be enabled at a retention time before the start of a peak. It influences peak recognition and, therefore,	Should only this inhibition criterion be applied, peak numbers in the integration report table may not be consecutive, because two detection phases are carried out for a chromatogram; that is, for peak detection and area calculation. The minimum area parameter might be effective in the second run only; that is, a peak may be sorted out only then, the result being a gap. The No. column of the result file thus indicates which criterion was responsible for inhibiting a peak.

S.No.	Parameters	Description	Function
		also the baseline.	
12.	Minimum Height	The Minimum Height detection parameter is used as a minimum criterion determining the height threshold below which peaks are ignored. Enable this parameter at a retention time before the start of the peak. The parameter influences peak recognition and, therefore, also the baseline.	The peak height of main peaks is measured relative to the baseline (a). For rider peaks, the height measurement is relative to the curve of the main peak (b). The baseline of unresolved peaks strongly influences the height (c, d). 
13.	Minimum Width	The Minimum width detection parameter is used as a minimum criterion defining the minimum width below which peaks are ignored during peak detection. Enable this parameter at a retention time before the start of the peak. The parameter influences peak recognition and, therefore, also the baseline.	The peak width is measured on the baseline. In the case of peaks not reaching the baseline, the width is extrapolated.

S.No.	Parameters	Description	Function
14.	Peak Group Start/End	Select the Peak Group Start and Peak Group End detection parameters to identify several successive peaks as one peak group. A peak group that has been defined in such a way is treated as one single peak. The peak maximum of the largest peak becomes the peak maximum of the entire group. For the group, only one name and number is entered in the chromatogram. Parameters such as area value, peak height, etc. are determined and displayed only once.	The Peak Group Start parameter (Peak Group End parameter, respectively) defines the start (end) of such a group. On the Detection tab page of the QNT Editor, set the start of the peak group automatically (Auto). If select the Fixed mode, the baseline will start and end exactly at the mentioned retention times. The setting made for the Peak Group Start parameter is also adopted for Peak Group End. The system toggles between Peak Group Start and Peak Group End when appending a line.
15.	Peak Purity Start/End Wavelength (PWl Start/PPWI End)	The Peak Purity Start Wavelength and Peak Purity End Wavelength detection parameters limit the wavelength range for peak purity calculations.	For calculating the Peak Purity Index (PPI) and the Peak Purity Match Factor, only the wavelength range within the values limited by start and end is used.
16.	Peak Purity Threshold (PP Threshold)	The Peak Purity Threshold detection parameter determines the threshold for the signal height above which spectra comparison is performed for the UV and Mass Spectra. The parameter is important for the Peak Purity Analysis (PPA) and the Peak Ratio.	Peak Purity Analysis (PPA) The value is stated in percent of the peak height that is at the value 20%, only the spectra are used to determine the match factor for which the signal height of the peak is at least 20%. The smaller the selected value, the wider the match factor curves. The calculation may include spectra that no longer have an optimum Signal-to-

S.No.	Parameters	Description	Function
			Noise Ratio. The match factor curve then has "fringes" at the margins (peak start and peak end). This can be avoided by a smaller threshold value.
17.	Peak Shoulder Threshold	The Peak Shoulder Threshold parameter defines a threshold value for peak shoulder recognition.	First, the average baseline curvature is determined for the entire chromatogram. The curvature threshold value is the product of the average baseline curvature and the threshold value. Detected peak shoulders with a maximum curvature above this threshold value are not considered. Select the Sensitivity detection parameter to influence the determination of the average baseline curvature. Higher sensitivity results in a smaller average baseline curvature and thus effects peak shoulder recognition.
18.	Peak Slice	The Peak Slice detection parameter determines the width (= time span) from which several successive data points are interpreted as peak or as noise. The presetting is 5/100 seconds.	General Rule If a specific peak should "just" be interpreted as a peak and if this was not reached via the Auto setting, a manual peak slice value can be specified by selecting the peak width. Note: This parameter only refers directly to peak detection and indirectly to integration. For area calculation, the original signal is used. (The parameter influences peak start and peak end. In this way, the peak area is influenced, also.)

S.No.	Parameters	Description	Function
			To change the variables, Sensitivity and Peak Slice, is only necessary in case of chromatograms comprising very wide (many minutes) or very narrow (< 0.1 s) peaks. Under normal conditions, the default values should not be changed.
19.	Rider Skimming	This parameter indicates how Rider Peaks are skimmed.	Tangential at lower peak end (default and the common skimming method): For ascending rider peaks, the peak start, and for descending rider peaks, the peak end is defined in such a way that rider skimming is tangential to the chromatogram. Tangential at both peak ends: Peak start and peak end are determined so that rider skimming is tangential at both chromatogram ends. Exponential: The chromatogram is approximated by an exponential function, so that the slope of the chromatogram and the exponential function correspond at the peak start and the peak end of the rider peak. This option clearly distinguishes from the two others. In most cases, Exponential maps the actual course of the curve very accurate. With this option, the rider peak will usually receive a more realistic larger area. You can use this option only if a sufficient number of data points is available.

STANDARD OPERATING PROCEDURE

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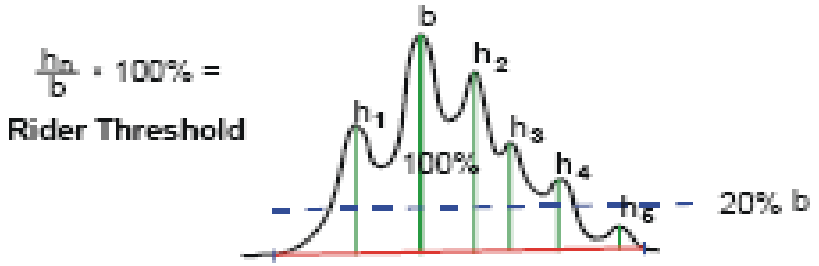
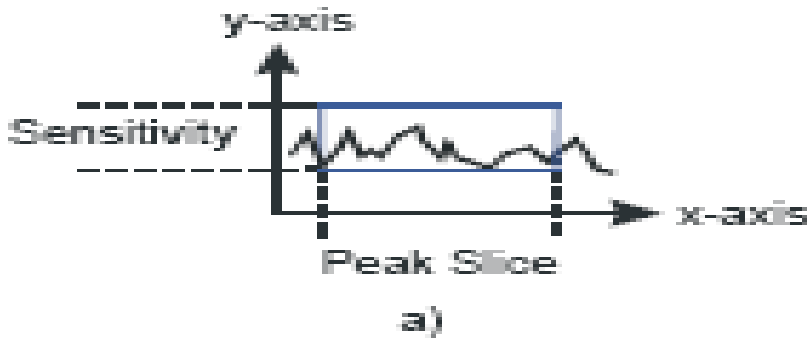
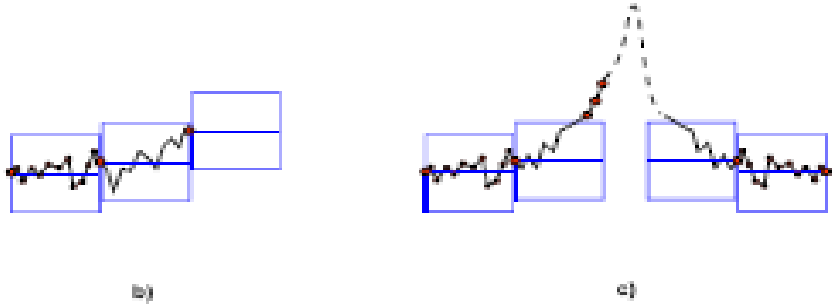
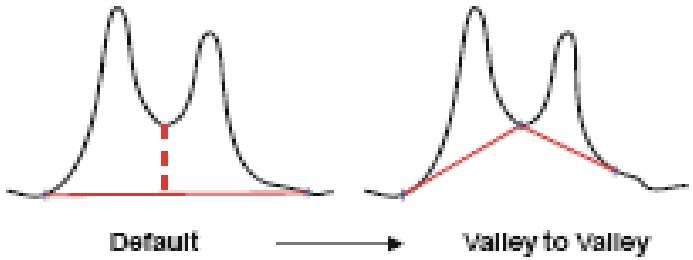
S.No.	Parameters	Description	Function
20.	Rider Threshold	The Rider Threshold and Maximum Rider Ratio variables determine whether a single peak in a series of non-resolved peaks is classified as a Rider Peak (skimming peak) or as main peak.	The ratio between the Peak Height of the single peaks (here: h1 to h5) and the height of the largest peak (here: b) determines whether a peak is classified as rider peak or main peak.  If the height ratio h/b multiplied by 100 percent produces a value below the defined rider threshold, the corresponding peak is a main peak by definition. In this example, this is only the case for the h5 peak. If the height ratio h/b multiplied by 100 percent produces a value above the defined rider threshold, the Rider Threshold criterion is not sufficient for a clear classification. In this case, the Maximum Rider Ratio is established. The resulting value allows you to classify the remaining peaks as rider peaks or main peaks. Riders can be recognized as such by the skimming tangent drawn on the

S.No.	Parameters	Description	Function
			chromatogram plot and by a Ru (Rider up) or Rd (Rider down) entry in the Type column of the report (Integration tab page).
21.	Sensitivity	Together with the Peak Slice parameter, the Sensitivity parameter determines the granularity of the peak detection algorithm; i.e., when using small values, even the smallest signals are interpreted as peaks; for example, pump pulsation, whereas such signals are ignored when using higher values.	Peak recognition is performed using a rectangle formed by the granularity of the y-axis (Sensitivity) and the granularity of the x-axis (Peak Slice)  a) As shown in the picture, this rectangle is placed with its lower left corner on the first data point, and is duplicated by mirroring in the direction of negative y-values. As long as none of the subsequent data points is above or below the mirrored rectangle, the last data point will be used for positioning further rectangles (b). The measured signal is interpreted as noise.

S.No.	Parameters	Description	Function
			 **Manually Setting the Parameters Sensitivity and Peak Slice:** Manually modify the granularity of the peak recognition; that is, can be determine the size of the rectangle or the bandwidth. The larger the area, the lower the sensitivity of the system. Smaller peaks are likely to be interpreted as noise. General Rule If a specific peak shall "just" be interpreted as a peak and if this was not achieved via the Auto setting, specify a manual Sensitivity value of a third of the signal height of the peak.
22.	Tailing/Fronting Sensitivity Factor	This detection parameter is an implicit threshold for setting the peak end. In the	The following paragraphs describe the Tailing sensitivity factor options. The Fronting sensitivity factor behaves in the same way.

S.No.	Parameters	Description	Function
		case of the Fronting sensitivity factor, the options refer to the peak start.	Off (=0): The peak end is moved on the time axis until the slopes of the chromatogram and of the baseline correspond in the peak end. Factor 1 - 100: First, the time for the peak end as described above is determined for Off (=0), then the right baseline width is determined, using the Baseline Level parameter of the Right Width peak variable, or peak.right_width(0)). The value, which is the upper limit for the distance between the peak retention time and the peak end, is multiplied by the Tailing sensitivity factor. If this limit is exceeded, the peak end is corrected and reset accordingly.
23.	Valley to Valley	Usually, an algorithm determines the baseline automatically. The Valley to Valley parameter enables baseline treatment from valley-to-valley, that is, from peak minimum to peak minimum, in a series of non-resolved peaks.	When the parameter is enabled, the baseline below non-resolved peaks leads from peak end to peak end. 

S.No.	Parameters	Description	Function
24.	Void Volume Treatment	Use this parameter to hide a negative peak at the chromatogram start. This is especially useful for the negative water peak in ion chromatography. If Void Volume Treatment is set to On, peak detection is disabled for the range of the negative water peak.	The system searches for the negative water peak in the retention time range between Void Volume Treatment = On and Void Volume Treatment = Off. The negative water peak is the peak with the lowest signal at the retention time. Peak suppression is enabled for the time between Void Volume Treatment = On and the end of the negative water peak. The chromatogram is recorded, but it is not integrated. (This corresponds to Inhibit Integration = On.) Peak detection is enabled again only after the peak end of the negative water peak has been detected. (This corresponds to Inhibit Integration = Off.)