

Pre-Programmed Monitoring of System Performance with Bruker Gas Chromatographs and Mass Spectrometers Connected to Bruker Workstation

by:

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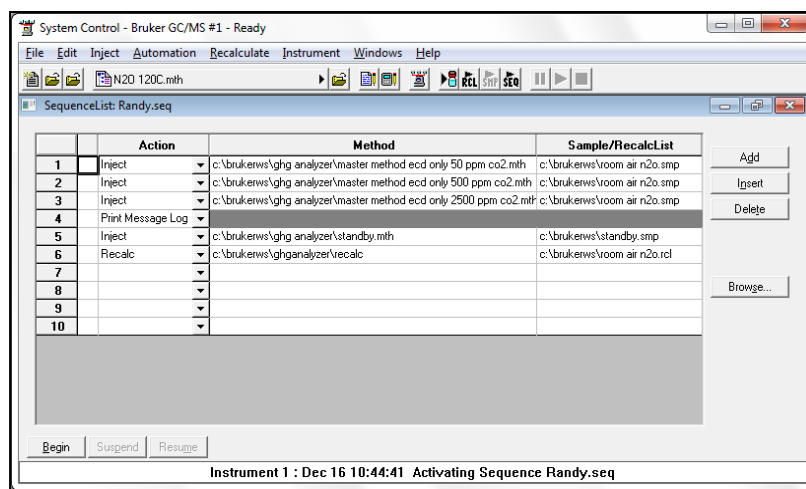
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Many gas chromatographs and mass spectrometers are actively used for monitoring and quality control applications. They frequently have automated samplers to allow unattended operations, for high sample throughput with minimal operator interventions. Gas chromatographic results are very dependent on operating conditions of the instrument, including reproducible injection processes, consistent column flow rates and temperatures, stable detector performance, and proper computations of the detector signals. Changes in any of these can directly impact the significance of results. Typically, an operator must tediously review stacks of data reports to be assured that results are reported accurately. The Bruker MS Workstation has built-in judgments that can be activated to monitor operations for every run, often duplicating wearisome manual examinations by the data reviewer. Many of these decisions have been in the long history of forerunners to the Bruker Workstation, going back to the Varian CDS111 in 1974.

Numerous method protocols list extensive quality control measures to ensure that correct results are reported. A common procedure is the proper use of internal standards to assist in applying mathematical corrections for some of these variables. Other approaches include regular insertion of control samples, and frequent recalibration of responses with fresh standards. All of these must be monitored by the operator to avoid collection of faulty sample results. Often vital is an assurance that the system is operating properly when processing time-critical samples or analysis of limited sample quantities; if the system is **not** performing as expected, these sample runs are wasted and sometimes cannot be reanalyzed. The Bruker Workstation for both GC detector and mass spectral data has incorporated safeguards to automatically perform user-defined checks on the quality of results, and can trigger a shutdown of operations with discovered harmful faults, without user intervention. As a result, samples are not allowed to be processed through an instrument that falls short of expected performance.

A. Validation of SampleLists, RecalcLists and Sequences for Proper Names and Pathways Used for Locating Methods and Data Files

Bruker Workstation has convenient provisions for building up a series of samples to be executed by construction of SampleLists and Sequences. SampleLists are created to indicate the order in which samples are to be run, and entries can be made here to activate methods at selected points in the list. Sequences allow for expanded operations where methods are tied to specific SampleLists and RecalcLists¹, and additional actions involving repetition of the same samples with different methods, especially applicable in method development, and printed documentation of all operations (MessageLog - see Section P below).

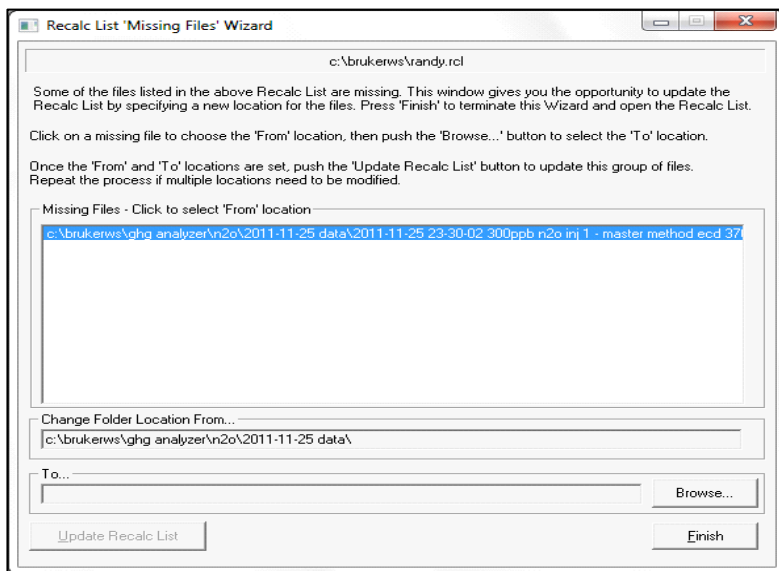


Critically important with setting up these lists is the accuracy of file names and their path ways to locate them. If an error is made with entry of their name and location, operations could be interrupted in the middle of an expected process without warning. To prevent this catastrophe, programmed checks at the onset of execution of SampleLists, RecalcLists and Sequences are made to ensure that every specified file is available.

¹ RecalcLists are usually automatic registers of sample data files created from execution of a SampleList. This allows easy postrun recomputations of a series of runs after modifications to a method. File naming for a RecalcList is typically the SampleList with an extension of .rcl, colocated in the same folder.

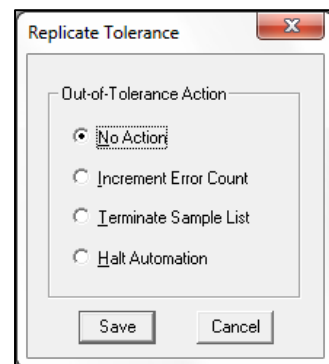
If all is well, activation of a SampleList, RecalcList or Sequence proceeds without a hitch. Hidden from view is the automatic confirmation of all specified methods, SampleLists and RecalcLists and their pathways. If a file cannot be located, several error messages can be generated before full implementation commences, including “Method does not exist!”, “SampleList does not exist!” and “RecalcList does not exist!” The operator can then relocate the suspect file and reinitiate the execution. Often the issue with not finding the required files is an inaccurate path for locating the file, especially entry by hand. Most screens asking for methods, SampleLists and RecalcLists have Browse and Recent Files buttons to simply click on the desired file.

For processing a series of files with RecalcList operations, .RUN files can be located in Windows Explorer and then “click-n-drag” these into the RecalcList pane to assure that file names are legitimate. If files cannot be located when a RecalcList is executed, a special ‘Missing Files’ Wizard is activated and the operator is allowed to rectify the file names or delete unfounded ones.



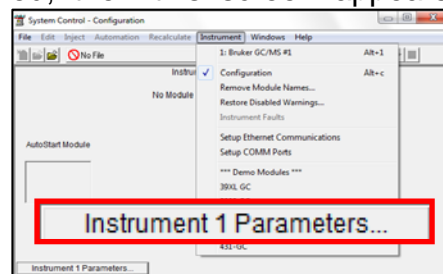
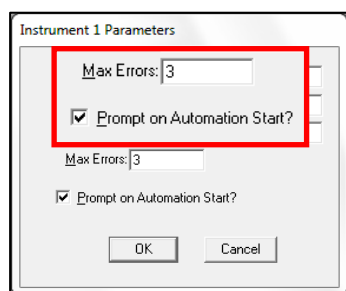
B. “Out of Tolerance” Error Actions

The Bruker Workstation has two classes of operational errors. A “fatal” error stops operations either before the run starts or upon completion of that run, and all subsequent samples are not examined. “Non-fatal” errors usually apply to one run and should not trigger a stoppage; only after consecutive runs with the same error, operations can be halted if specified. The operator sets in the number of consecutive runs with the same “non-fatal” error to convert to a “fatal” error and stoppage of operations.



Sometimes failure to achieve results within the specified tolerance window needs to be listed only as a warning, and no further action is needed; here the user specifies “No action” and the run sequence continues unabated.

If an unexpected perturbation occurs with a single run in a series, this temporal fault should not necessarily stop operations with only this one malfunction. However, if three consecutive runs, for example, fail to meet the same specified judgment, then a serious system flaw is likely to have occurred, and the run series should be stopped so the problem can be rectified. Here “Increment Error Counter” is selected, and the number of consecutive runs to trigger termination of operations - “Max Errors” - is listed in “Instrument Parameters” either found in System Control → Configuration, or if “Prompt on Automation Start?” is checked, then this screen appears whenever automation is started. If, however, the problem is considered serious with only one episode, the system can be directed to either terminate the active SampleList and proceed on the next one listed in a sequence, or halt all operations.



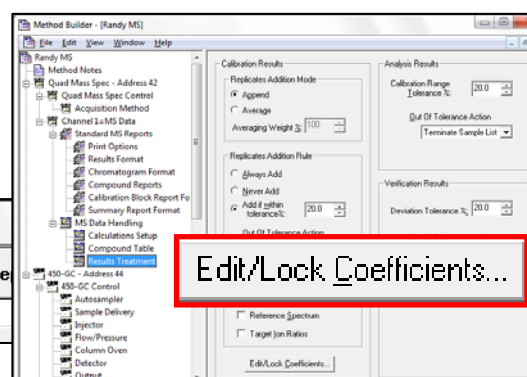
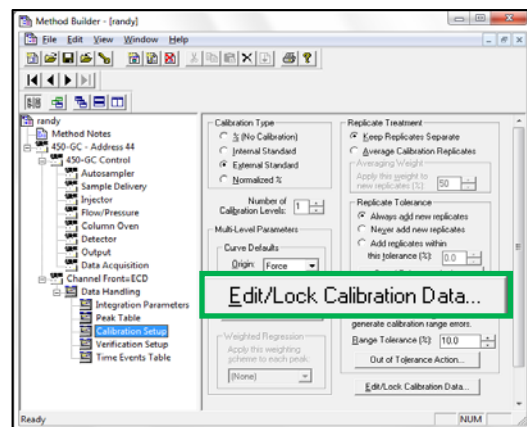
C. Missing Calibration Run - At Least One Peak Has Wrong Calibration Calc Type

The Bruker Workstation mandates that a full calibration process must be executed for all listed peaks before samples can be reported using External Standard, Internal Standard, or Norm% computations. If not, then an error is triggered, the message “At Least One Peak Has Wrong Calibration Calc Type” is displayed, and operations are halted.

Even if the calibration had been performed and then a new peak is added into the Peak Table, a calibration must be performed for that new peak; simply entering new response factors into the coefficients table does not release this requirement. One route around this error is to lock all coefficients and then execute a “dummy” calibration on any chromatogram to set the internal flag that the method has had a calibration performed for all peaks. Be sure to unlock coefficients when finished to allow later updates to response factors.

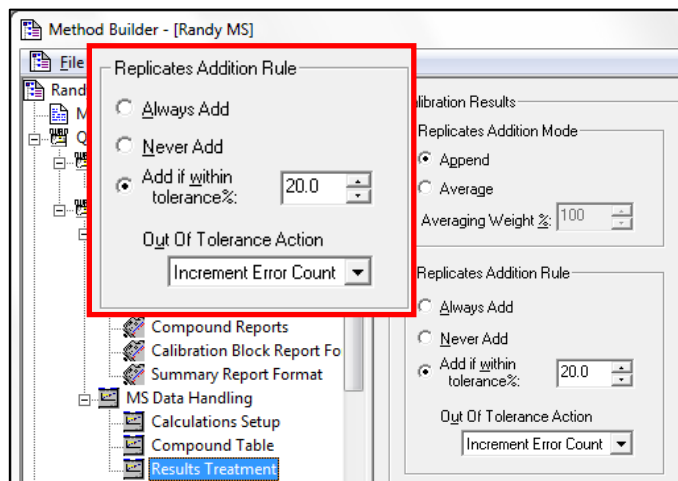
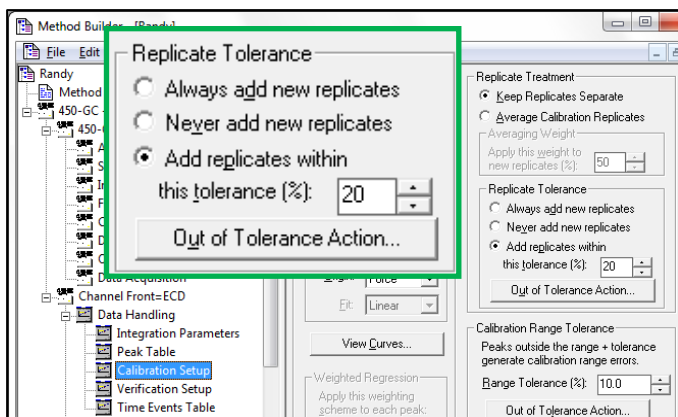
Coefficients					
	Retention Time	Peak Name	Lock Coeffs.	X	Intercept
1	14.205	Nitrous Oxide	<input checked="" type="checkbox"/>	4892.5	0

Lock Coeffs.



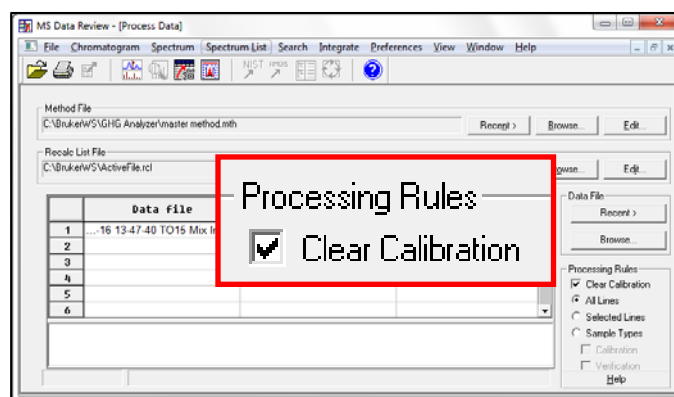
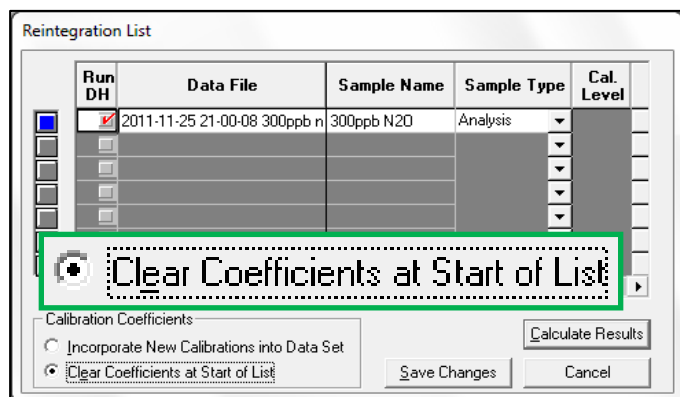
D. Replicate Tolerance (GC Only) and Replicate Addition Rule (MS Only)

To prevent bad data from encroaching into a calibration data set, the operator can define acceptable repeatability for peak response factors for the new run to be added into the running average. If the new point for the current run meets the specified tolerance, based on the previous running average, the new point is processed and accepted into the average. If the test fails, then the point is not included, and the error “Calib. Out of Tolerance” is posted with the specified action undertaken. The first calibration run with a new method is always presumed to become the target for subsequent standard runs. After the first run completes, the operator must check that the first run was success.



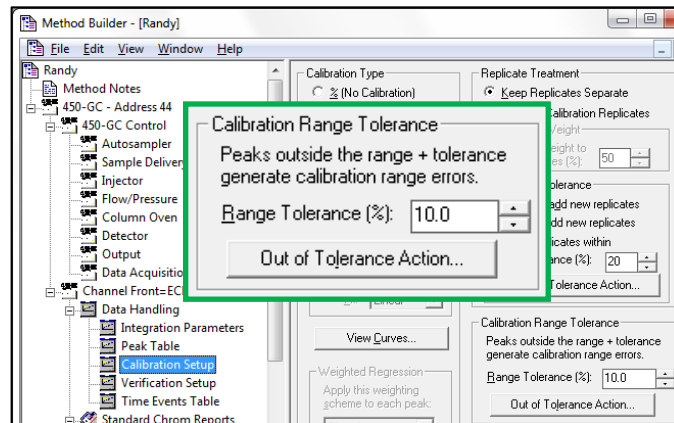
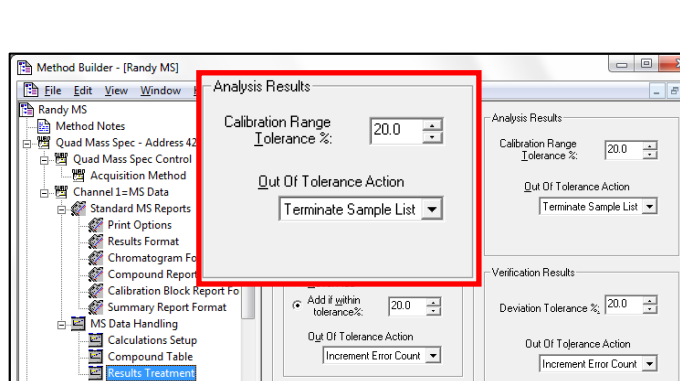
E. New Calibration Block - SampleList or RecalcList

Once a calibration process is performed, the computed response factors remain part of the method and new data points are added into the mix, unless the operator chooses to start a “New Calib Block” in the Sample List or clear coefficients in MS Data Review or Interactive Graphics for GC. Previous calibration data is also discarded when the calibration type is changed, such as a switch from external standard mode to internal standard.



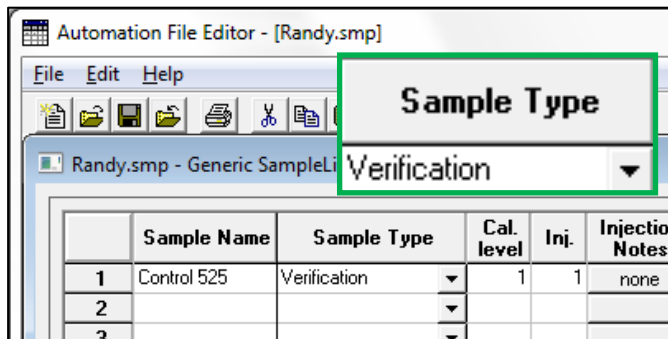
F. Over/Under Calibration Range Tolerance for Unknown Samples

The calibration range is usually valid only for the concentration interval used in calibration operations. Results for unknowns that are outside this range should be considered suspect. A flag can be set to trigger an error when a sample result exceeds either the upper or lower limits of the calibrated range, as a percentage of those limits. An Out of Tolerance Action can be initiated if this test fails.



G. Verification Tolerance for Control Samples

Once a calibration operation is completed, the curve fit should be validated with a check sample to confirm performance of the standards. The expected answers are entered into the Compound Table or Peak Table, either as one of the standards or as a separate level amount. When a Sample Type is labeled as "Verification", the generated results are compared with the expected ones. If the reported values exceed the tolerance specified, an error "Verification Failure" is generated. As another check, a verification run can be performed at the end of a sample series, to validate that the system performance has been maintained through the series. An Out of Tolerance Action can be initiated if this test fails.



Print Date: 14 Feb 2009 19:41:25 Target Compound Report for #11 from ...45 pm toxics 6 minutes sms - Page 11

Sample ID:	0.1 ppbv std	Operator:	RSB
Instrument ID:	Varian Ion Trap	Last Calibration:	2009-02-14 19:31
Measurement Type:	Area	Calibration Type:	Internal Standard
Acquisition Date:	2008-05-07 20:02	Data File:	...toxics 6 minutes sms
Calculation Date:	2009-02-14 19:31	Method:	...urve_cal_10_2_08.mth
Sample Type:	Verification		
Inj. Sample Notes:	None		

Compound:	Sample Type:	Verification
Peak Name:	11	Compound Number: 9
Result Index:	CAS Number: None	Identified

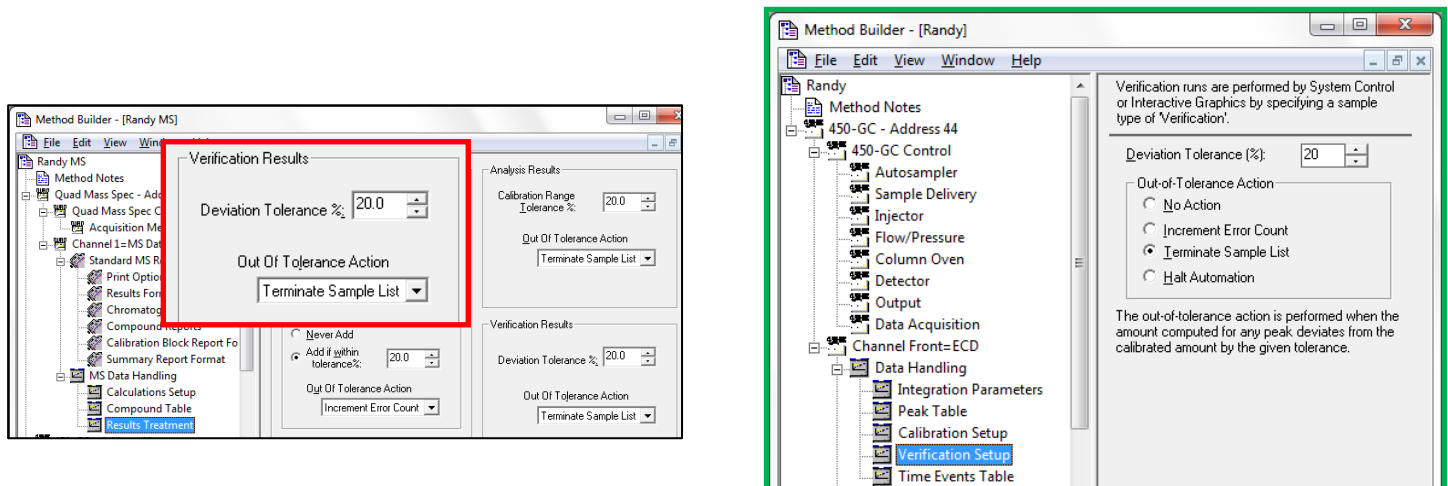
Identification	Parameter	Specification	Actual	Status
	Search Type	Spectrum		
	Retention Time	20.044 +/- 0.100	20.051 min.	Pass
	Match Result	N-R >= 800	874	Pass

Integration and Quantitation	Parameter	Specification	Actual	Status
	Quan Ions	101.0		
	IS Peak Name	Bromochloromethane		
	Calibration Equation	Linear, Force, 1/n ²	y = +5.0748x	
	Area	>=100	172238	Pass
	Height		32375	
	Amount	>= 0.005 ppbv	0.089 ppbv	Pass
	Verification Deviation	+/- 30.00%	8.51%	Pass

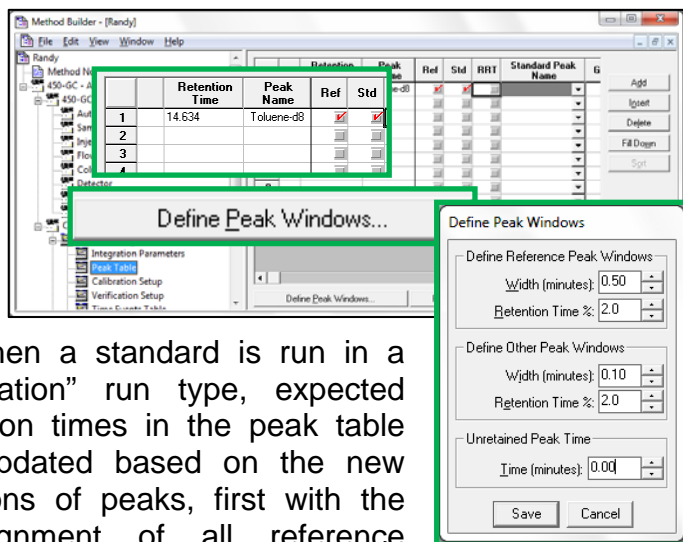
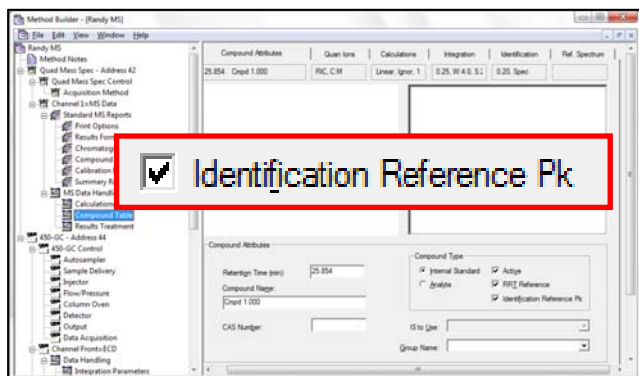
Verification Deviation	+/- 30.00%	8.51%	Pass
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H. Retention Time Adjustments with Reference Peaks

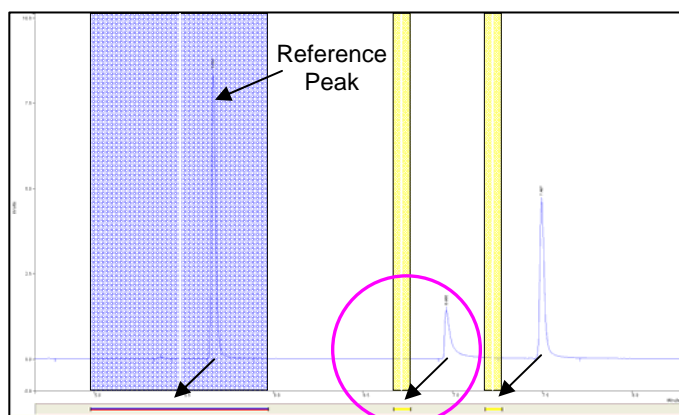
Measurements in chromatography cannot be considered exacting, as random and systematic errors can introduce some variability in retention times (along with area measurements). To help correct for systematic deviations in retention times, certain compounds can be assigned as Reference Peaks. Any movement in time of these peaks is detected and a correction based on this change is applied proportionally to all non-reference or "Other" peaks, based on their relative position to reference peaks. If multiple Reference Peaks are assigned, then the new expected retention times are proportioned based on their proximity to these reference peaks.



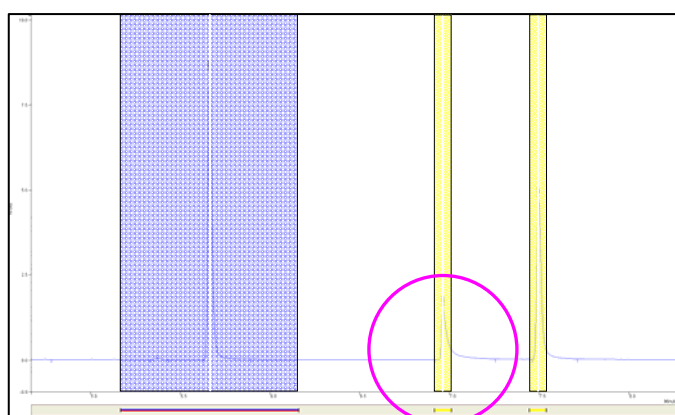
Reference Peaks **always** must be present in **every** sample and standard in a series, and must be the **largest** peak within the Window; typically internal standards are perfect candidates. Non-reference peaks are picked out as the **closest** peak to the non-reference target Retention Time, after time adjustment from a Reference Peak correction.



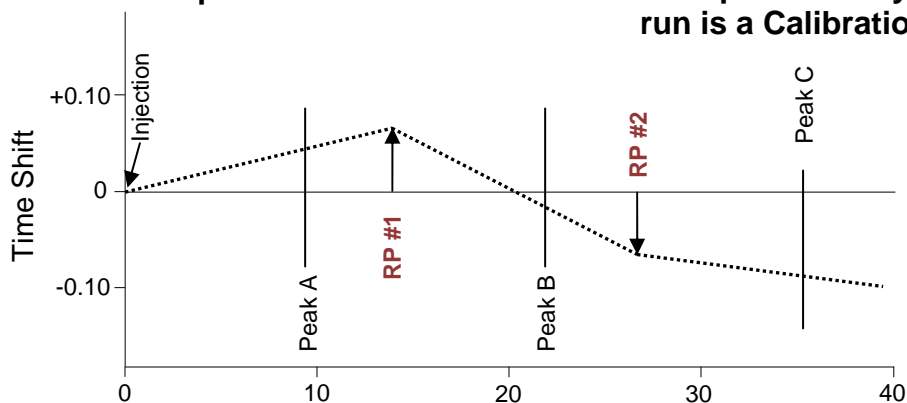
When a standard is run in a “calibration” run type, expected retention times in the peak table are updated based on the new locations of peaks, first with the reassignment of all reference peaks, followed by locating the non-reference peak locations after adjustments from the shift in reference peaks.



Normal variations in chromatographic conditions can shift retention times away from the expected locations. And adjacent peaks can be mislabeled and wrong results are reported.



Proper use of Reference Peaks can sense a shift in retention times and allow corrections to move the expected retention times over to the correct peaks. Peak Table is permanently updated only if run is a Calibration type.



Multiple Reference Peaks (for example RP #1 and RP #2) can provide time corrections over the whole chromatogram. New expected Retention Times for unknown peaks are adjusted proportionally to their positions relative to adjacent reference peaks. This synthetic example illustrates the corrections involved with two reference peaks.

Compound	Expected Retention Time	Measured Retention Time	Measured Shift	Correction	New Expected Retention Time
Peak A	9.374	--		+0.045	9.419
RP #1	13.841	13.907	+0.066	--	--
Peak B	21.844	--		-0.015	21.829
RP #2	26.752	26.816	-0.064	--	--
Peak C	35.395	--		-0.064	35.331

I. Missing Reference Peaks

If all Reference Peaks are not identified in the chromatogram during a calibration sequence, the quantitation process is terminated with a fatal error message “Reference Peak Not Identified”, and all results are reported as Area%. The operator then is required to remedy the cause of the error by fixing the chromatographic conditions or by readjusting expected retention times for reference peaks in the Peak or Compound Tables, and then reprocessing the data runs.

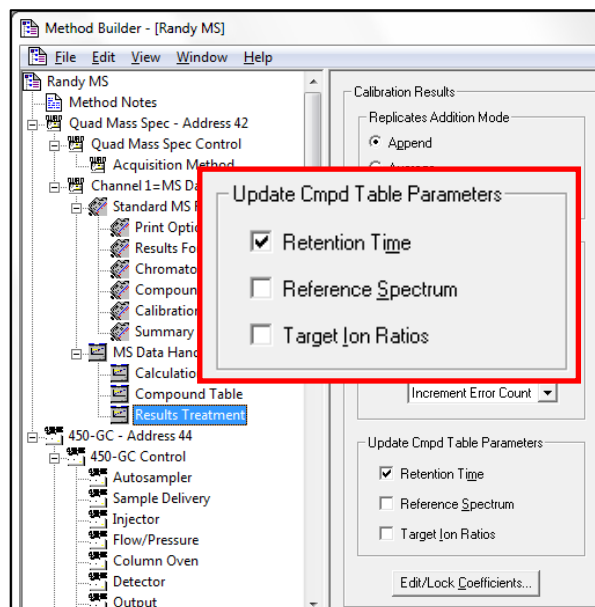
With “analysis” run types, a missing reference peak generates an error “Reference peak not identified correctly” and all results divert back to “Area%”, and the error counter is incremented for each episode. An Out of Tolerance Action can be initiated if this test fails.

J. Update Compound Table Parameters (MSOnly)

The operator can choose what parameters can alter the peak table settings for retention times, reference spectra or target ion ratios, as a result of calibration runs. For example, if peak retention times shift slightly, then these times in the peak table can be updated from the last standard. This operation is similar to those with reference peaks (discussed above), but does not need to have reference peaks identified.

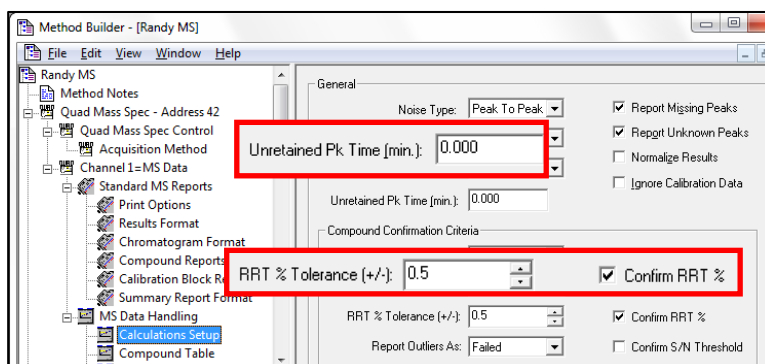
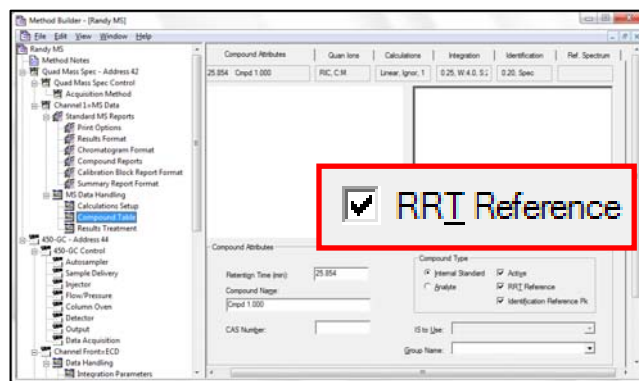
Occasionally, these updates can pick a closely eluting isomer as the target compound and adjust the identifying criteria based on this wrong peak. The user should be careful here and use a wider integration window to properly integrate the peak, but select a narrow identification window to only pick out the proper peak. Only the apex of the specified peak needs to be within the identification window to be chosen; any peak outside will not be considered.

To ensure that a “normal” data set is used in this updating process and not an extreme one (either low or high), a calibration sequence should be performed with a mid-level standard as the last one. For example, the levels, in increasing concentrations, should be 1,2,4,5, then 3. Thus, the sequence would end with information that should apply to nearly all peak concentrations.



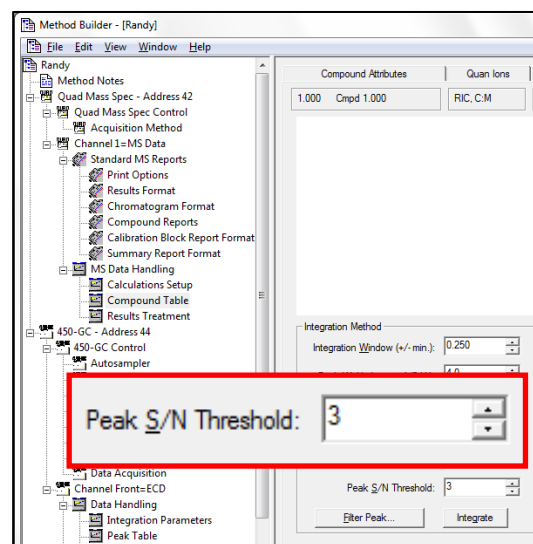
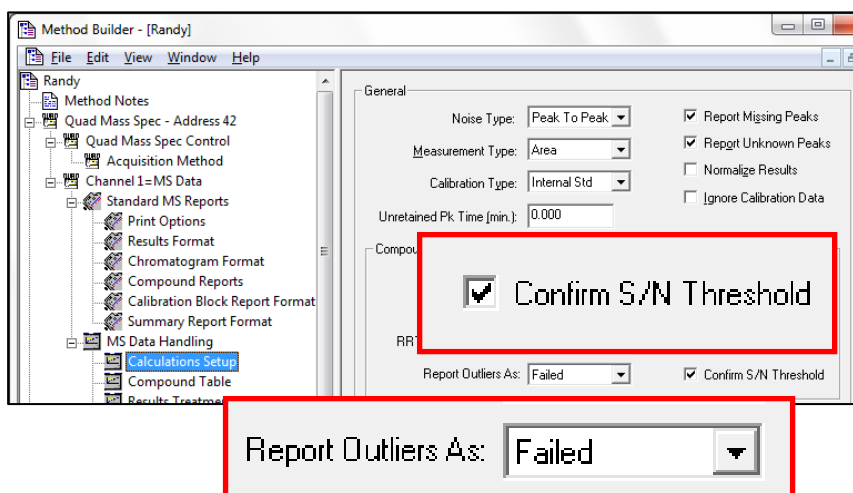
K. Relative Retention Time Tolerance (MS Only)

Peaks can move around a bit due to subtle changes in chromatography, but their shifts can be correlated by monitoring their positions relative to an identified Relative Retention Peak, often an internal standard, corrected with Unretained Peak Time value. By selecting a compound to be the RRT Reference peak, relative retention times for all of the other peaks detected are computed and compared with the corresponding relative times in the Compound Table. Then any shift that affects all of the peaks, like a small change in carrier flow rate, peaks are properly located and quantitation is performed appropriately. If computed relative times do not match within the specified RRT % Tolerance window, the peak is not reported in the final report, as it has shifted outside the indicated window and is not to be considered as that analyte. Retention times set in the Compound Table are not altered by this process.



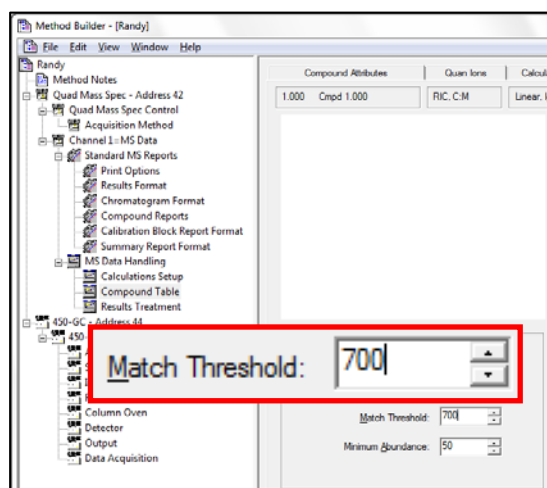
L. Confirm S/N Threshold (MS Only)

Minor peaks can often be detected at levels well below assigned reporting limits. To keep these insignificant peaks out of final reports, a judgment can be made on their exclusion based on the detected signal-to-noise - S/N - value found. Each compound can be assigned a S/N Threshold value that is activated by selecting Confirm S/N Threshold in the method Calculations Setup. If the detected signal-to-noise is less than the specified tolerance, the peak is reported as Failed or Missing, depending on the selection of "Report Outliers As",

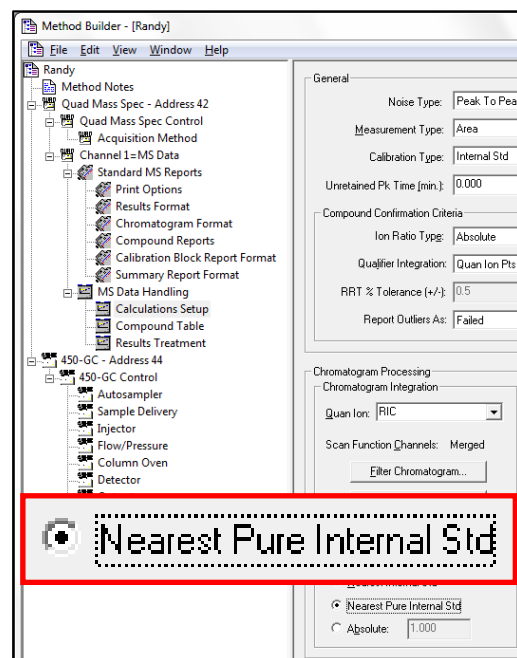


M. Nearest Pure Internal Standard (MS Only)

A danger with using Internal Standard calculation mode occurs when the internal standard coelutes with another compound that possesses common ions. As a result, the detected ions from the intruder can skew the peak identification and assigned area. One mechanism to prevent this error is to select a judgment on the purity of the Internal Standard peak and assigning a tolerance threshold. By checking that the internal standard peak must match the reference spectrum within a minimum value, using "Nearest Pure Internal Std", the detected peak within the assigned Internal Standard time window is compared from an intensity-weighted averaged spectrum generated from scans across the target peak from peak start to peak end. Then this spectrum is

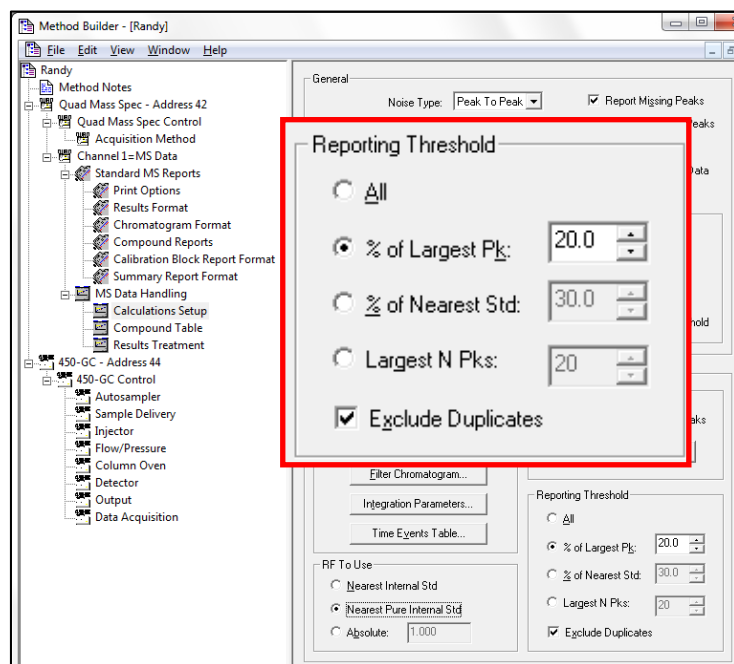


compared to the reference spectrum of the corresponding internal standard in the Compound Table. The peak is accepted as pure if it meets the Target Compound Match Threshold specification specified. If the threshold is missed, this internal standard peak will not be used, and the nearest internal standard peak passing the test will be applied instead.



N. Reporting Threshold (MS Only)

These parameters specify whether to exclude some integrated unknown peaks from associated reports. They are applied to all detected peaks that exceed the Pass Size Reject specification. Reporting Threshold specifications are designed to eliminate unwanted peaks before any library searching is performed. This checking can significantly shorten processing time for library searches of unknown peaks.



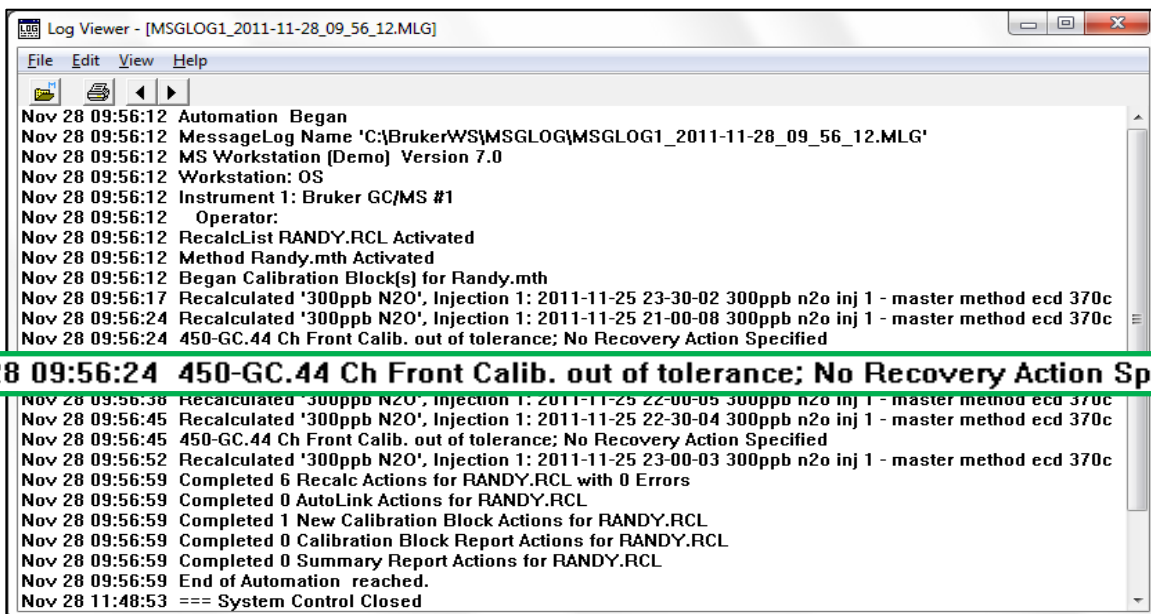
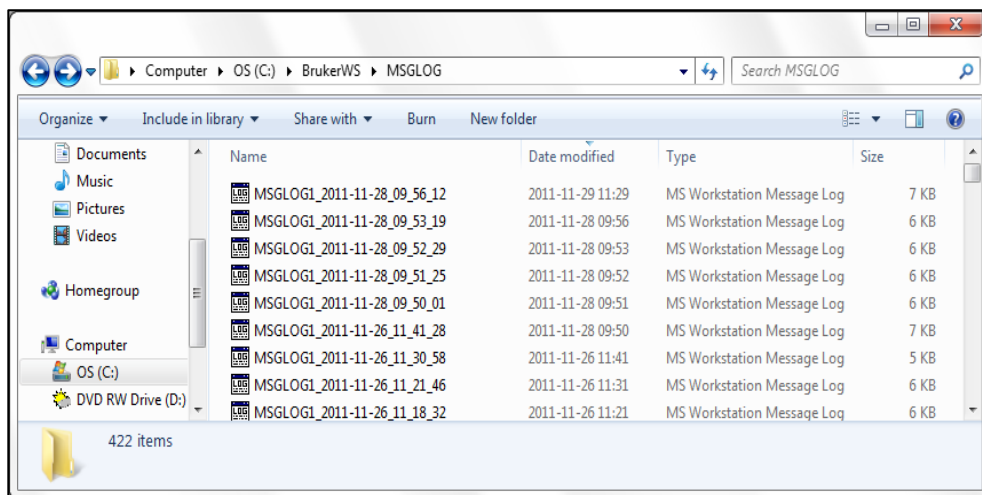
O. Error Listings

Errors are documented in locations related to the type of fault. For example, all errors related to operations in System Control can be found in the related Message Log. Others related to the quality of the results, such as Verification failures, are reported in the report of results. Others are not indicated with a message. For instance, problems with Reference Peak adjustments can result in analyte peaks not being identified and reported, with no error code, except maybe for “Missing Peak”, if its reporting parameter had been selected.

P. Message Log

Every operation through System Control is fully documented, with a date/time stamp, in an automatically created Message Log archived in C:/BrukerWS/MSGLOG. A fresh file is created whenever a new sample sequence is executed. This report can be employed as a convenient sample log of all details associated with the execution of sequence or recalculation actions.

Many errors triggered during operations are listed here. Examples included missing peaks, calibration errors and other actions triggered from an Out of Tolerance action.



Q. List of Possible Notifications and Errors (listed alphabetically)

1. **At Least One Peak Has Wrong Calibration Calc Type** - See Section C.
2. **Calib. out of range** - Section F.
3. **Calib. out of tolerance; Halting Automation** - See Section B.
4. **Calib. out of tolerance; Incrementing Error Counter** - See Section B.
5. **Calib. out of tolerance; No Recovery Action Specified** - See Section B.
6. **Calib. out of tolerance; Terminating SampleList** - See Section B.
7. **Data Handling: All Coefficients for All Peaks are Zero** - See Section E.
8. **Data Handling: At Least One Curve has Improper Solution!** - See Section E.
9. **Data Handling: Default to A%** - See Section C.
10. **Data Handling: Cannot Calibrate Over 10 Replicates/Level** - See Section E.
11. **Data Handling: Non-reference peak not identified** - See Section H.
12. **Data Handling: Reference peak not identified correctly** - See Section H and I.
13. **Data Handling: Standard peak not found** - See Section H.
14. **End of Automation reached** - See Section B.
15. **Error Counter at 2 Errors; Ended SampleList** - See Section B.
16. **Method does not exist!** - Section A
17. **RecalcList does not exist!** - Section A.
18. **Report Codes: b = RRT out of tolerance** - See Section K.
19. **Report Codes: C = Out of calibration range** - See Section F.
20. **Report Codes: h = No Reference Peaks. Reporting Peak Size** - See Section H and I.
21. **Report Codes: M = Missing peak** - See Section H.
22. **Report Codes: N = No internal stds found to quantitate Unknowns** - See Section H.
23. **Report Codes: V = Out of verification tolerance** - See Section G.
24. **Same Fatal Error has Occurred Twice in Automation** - Section B.
25. **SampleList does not Exist!** - Section A
26. **Verification Failure** - See Section G.

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