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LABORATORY POLICY REGARDING MANUAL CHROMATOGRAPHIC PEAK INTEGRATION

FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION

LABORATORY PROGRAM

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LABORATORY POLICY REGARDING MANUAL CHROMATOGRAPHIC PEAK INTEGRATION

1. Scope and Application

This document establishes the policy of the laboratory regarding manual chromatographic peak integration.

1.1. Deliberate tampering of integration files to allow "failed" QC to "pass" is illegal and constitutes fraud. The manual manipulation of raw data to achieve some desired outcome, a practice known as "peak shaving", is one of the primary concerns being addressed in this policy statement. This policy allows manual integration only under specific circumstances.

1.2. Documentation requirements and specific chromatographic situations where manual peak integration is often required are described. The appropriate and consistent use of manual integration is essential for cases where automated integration does not adequately reflect the true peak area or is not feasible.

1.3. References are provided from EPA and instrument manufactures describing peak integration algorithms and methodology. Analyst reporting data must be familiar with the principles of operation for proper peak integration as set forth in these references.

2. Summary

It is the policy of the laboratory to report automated peak integration results, except in situations where the chromatography software has made an obvious error in the determination of the peak area or the analytical method has specific integration requirements. Manual peak integration must never be used solely to meet QC criteria. Corrective action regarding instrumentation or computer software must be taken if manual integrations become common for an analysis or instrument that normally uses automated peak integration. The policy for reporting manually integrated results is given in general terms since there may be analytical methods or situations with requirements exceeding the purview of this SOP.

It is expected that situations will arise where automated quantitation procedures in the chromatographic software do not integrate peaks properly. Software integration problems usually occur when there are compound coelutions, baseline noise, baseline irregularities or matrix interferences. General technical approaches to these specific situations are described.

Documentation of manual integrations must be maintained with the original quantitation or analysis data files. A flag will suffice for simple manual integrations. More complex manual integrations require additional documentation including a

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written, signed and dated explanation. Exceptions to the documentation requirement include the reporting of Tentatively Identified Compounds (TICs) and the reporting of data in the FL-PRO method. Reporting of TICs is already a rough estimate and does not fall under the directives of this policy. The FL-PRO method has a method specific requirement for quantitation that involves integration over a range of compounds. Integration over a range of compounds may routinely require manual intervention, depending on the capabilities of the chromatographic software. Similarly, there are several other analytical methods that use multiple peak integration by a whole area approach that are exempt from the documentation requirement. Always refer to the specific analytical SOP for special instructions.

3. Quantitative Chromatography: Analysis Based on Peak Area

Quantitative analysis requires that the relationship between the magnitude of the detector signal and concentrations of calibration standards be established. The calibration concentration range is normally set to conditions where the detector response is linear, which means that the area of a peak is proportional to the amount of each corresponding component. The size of the detector signal is measured by its peak area, as determined by an automatic or manual integration routine. Automated peak area determination is considered the default method for performing quantitative analysis in most chromatography measurements. Peak area measurements are easily automated and are independent of peak broadening and asymmetry effects that are frequently associated with chromatographic analysis. Automation of the peak area measurement also increases efficiency and removes potential biasing of the data. However, as with any automated analytical technique, there are limitations and potential complications that may arise as chromatographic conditions vary. Manual integration of the peak area will become necessary in situations where the automated software provides inadequate quantitation.

In the process of integration, each analyte peak area must be measured by integrating from the start-to-end points where the peak intersects the baseline. The integrated area must not be extended to include baseline noise beyond the intersection points. Likewise, the peak integration must not exclude area under the peak prior to the baseline intersection. Due to analytical noise and other factors, there is always some uncertainty in determining which data points represent the start and end points. In some borderline cases, the data may need to be qualified as an estimate. The data must be qualified as an estimate if the uncertainty in the start and end points of the integration causes the quantitation to fall outside the method acceptance limits.

4. Chromatographic Conditions where Manual Integrations may be Warranted

4.1. Peak Co-Elutions - Baseline resolution of each target analyte provides the best quantitative results, but is not always achievable due to limited chromatographic separation ability or sample matrix effects. In most cases, the computer software can be setup to automatically establish the baseline of partially resolved peaks. The laboratory must demonstrate that each target analyte is

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resolved adequately during calibration and can meet all method quality control criteria. Co-eluting peaks with a valley less than half the peak height of the smaller peak can normally be manually integrated by separating the two peaks with a line drawn from the valley bottom to the projected baseline as shown by the manual integration in Figure 1. Otherwise, the co-eluting analytical results must be reported as a sum of the peaks. Methods that utilize mass spectrometry are less affected by resolution problems because overlapping peaks may often be mass-resolved. However, in the case of positional isomers that are not mass resolvable, the data must also be reported as a sum of the peaks. The use of a secondary confirmation column with a different stationary phase is another tool that can be used to help resolve quantitation problems related to peak co-elution.

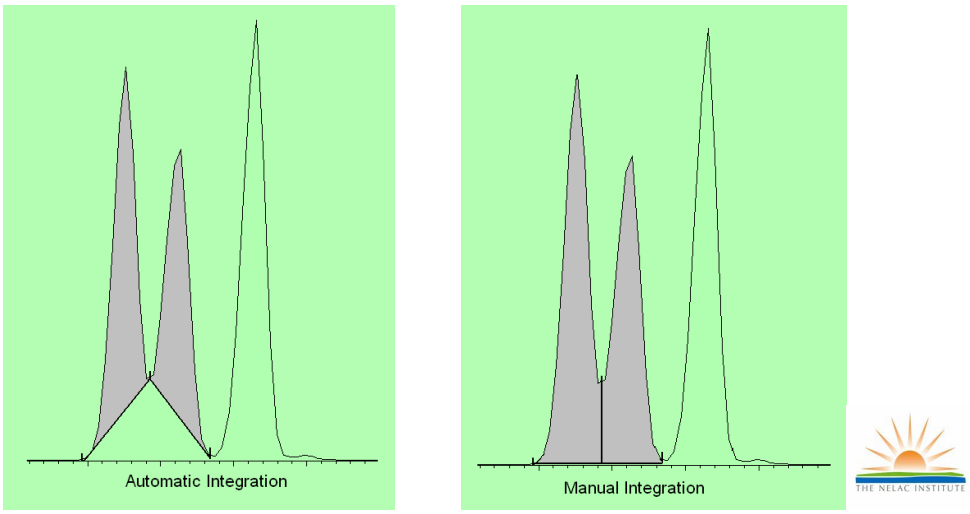


Figure 1: Co-eluting Chromatographic Peaks

4.2. Leading or Tailing Peaks - Severely leading or tailing peaks make it difficult to determine the beginning or ending of a peak from the baseline. Peak symmetry can be measured at 10% peak height as the ratio of the forward part of the peak to rear part of the peak, relative to a tangent drawn at the peak apex. A symmetry ratio of 0.90 to 1.2 is considered acceptable for most chromatography. A leading or tailing peak may frequently be corrected by adjusting the temperature program, adjusting column flow rate or reducing the column load. Manual integrations exceeding the symmetry ratio of 0.90 to 1.2 are considered irregular and must be accompanied by documentation, unless noted otherwise in the method SOP. There may be some compounds that always have tailing integration problems as seen in Figure 2 below. Problematic compounds should be specifically addressed in the method SOP and have detailed quantitation instructions.

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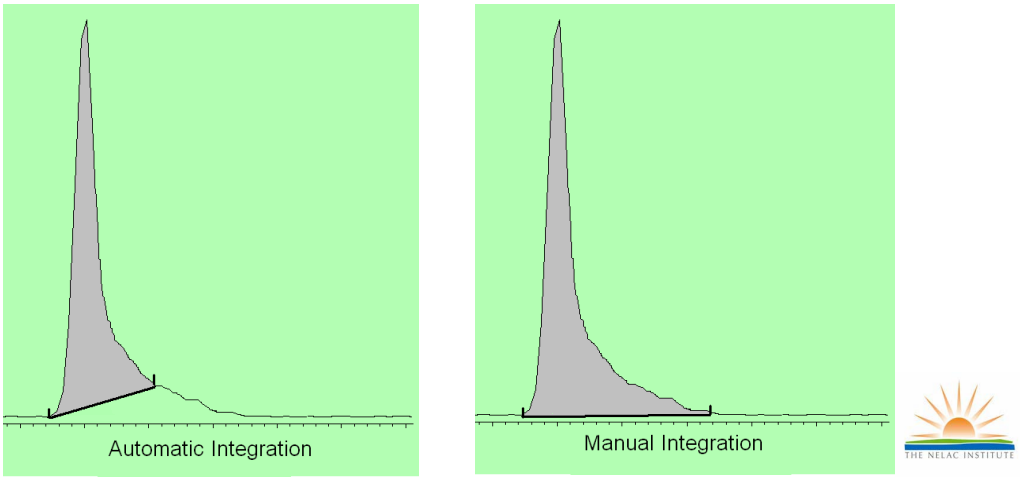


Figure 2: Excessive Chromatographic Peak Tailing

4.3. Baseline Interferences - The way the baseline is drawn will substantially affect the quantitation results of an analysis. Peak areas must reflect accurately the concentrations of the analytes they represent. If the baseline is too far above or below the contours of each peak, the area attributed to the peaks will not represent the concentration of the corresponding analytes. Baselines may be elevated, drifting, sloped, humped or excessively noisy. Poor baseline quality is sometimes a sign of inadequate sample clean-up or poor instrument performance. Corrective action for these conditions must be taken to minimize or eliminate baseline anomalies prior to sample analysis. In the case of a slope, the baseline reference must be projected horizontally under each peak to the next baseline reference point during quantitation. Figure 3 and 4 demonstrate manual integrations in situations with excessive noise and sloped backgrounds, respectively.

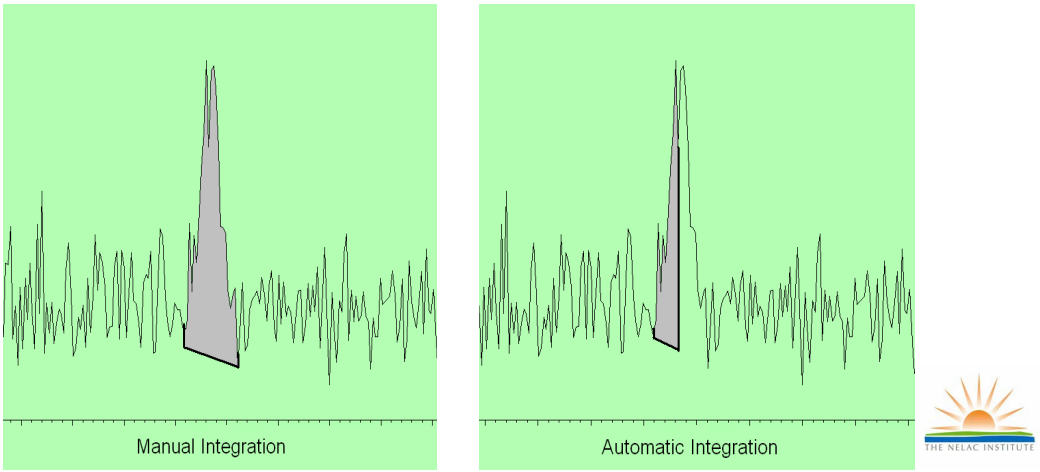


Figure 3: Excessive Chromatographic Baseline Noise

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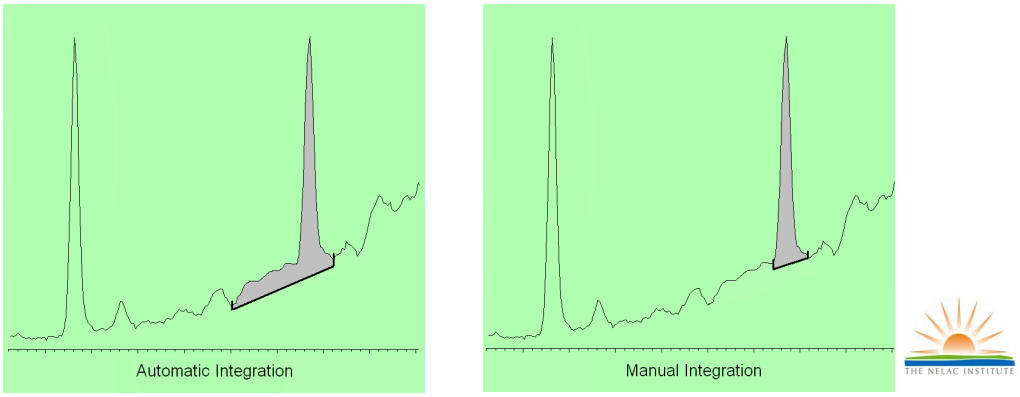


Figure 4: Chromatographic Baseline Drift

4.4. Matrix Interference – In chromatography the presence of matrix interference frequently appears as extra peaks or as an elevated or sloping background. Additional sample cleanup, sample dilution, analysis on a second column or analysis by another technique may be required if the peaks are not resolvable according to Section 4.1 or 4.3. Figure 5 shows a peak integration on an extremely large shoulder interference.

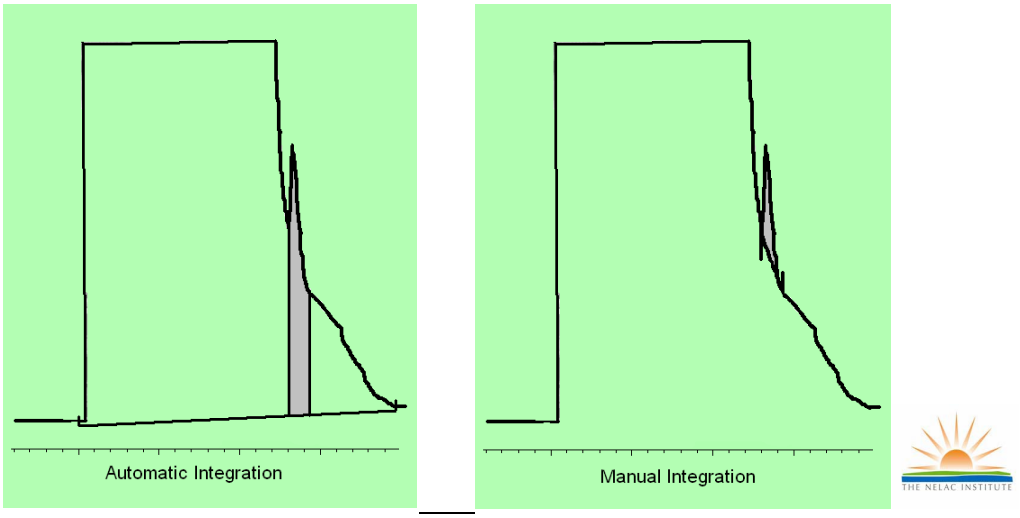


Figure 5: Chromatographic Target Compound on Shoulder

4.5. Negative Peaks - Negative peaks (dips) before or after target peaks are common with some chromatographic instruments and analysis conditions, and may complicate the integration of nearby target peak areas. These negative dips are frequently associated with air and moisture introduced during the initial injection, system leaks, or detector contamination. Negative peaks may cause a significant drop in the chromatographic baseline, leading to serious quantitative errors. The data must be qualified as an estimate if a negative peak co-elutes with a target analyte since the area under the peak that was lost to the dip is unknown and the baseline reference point on the affected side of the peak may

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not be well established. The baseline beginning and ending reference points may be established by:

- For Peaks Followed by a Dip - extend the baseline from the beginning of the peak to the end point of the dip.

- For Peaks Preceded by a Dip - extend the baseline from the beginning of the dip to the end point of the peak.

The manual integration is then drawn using that baseline reference point and the results are always reported as estimates. Refer to Figure 6 for an example.

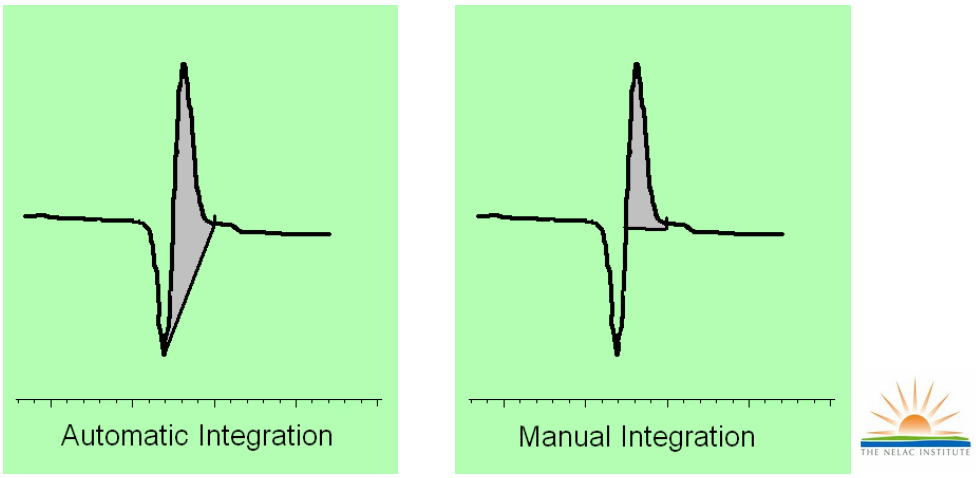


Figure 6: Chromatographic Negative Peaks

4.6. Inappropriate Peak Integrations - Manual integrations must never be performed in an attempt to make the laboratory results meet method acceptance criteria. Figure 7 provides examples in inappropriate integrations known as "peak shaving" and "peak enhancement".

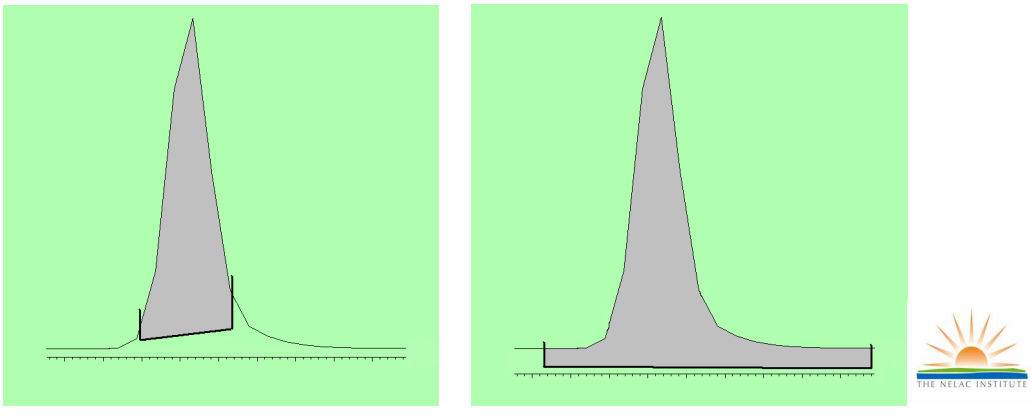


Figure 7: Chromatographic "Peak Shaving" and "Peak Enhancement"

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5. Documentation Requirements for Manually Integrated Peaks

5.1. Manual integrations must be clearly identified, dated, and the analysts name must appear on the quantitation or analysis report. It is enough for the date and name to be entered electronically on the data and appear in the final hardcopies.

5.2. A flag will suffice for simple manual integrations. A manual integration may be considered “simple” when the peak is clearly defined, has good symmetry, and does not have significant co-elution or baseline issues.

5.3. More complex manual integrations require additional documentation including a written, signed, and dated explanation. The explanation must be complete and compelling to justify the use of manual integration.

5.4. Borderline cases must be approved and documented by the supervisor. Supporting data (duplicates, dilutions, second column confirmation, or second method confirmation) may be required to settle a borderline situation. If the supporting evidence is inconclusive, then the affected data must be reported as an estimate.

6. References

6.1. “Statement of Work for Organic Analysis”, OLM04.2, U.S. Environmental Protection Agency, Contract Laboratory Program, Sections 11.2.1.9 and 11.2.1.10, May 1999.

6.2. US EPA Method 8000D, "Determinative Chromatographic Separations", Test Methods for Evaluating Solid Wastes, Fifth Edition, SW-846 Update VI, March 2018.

6.3. "Agilent OpenLAB Data Analysis" - Reference to Data Analysis, Agilent Technologies, M8370-90020, October 2012.

6.4. "Manual Integration", The NELAC Institute Presentation, Assessment Forum, January 2008.

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