DEPARTMENT OF AGRICULTURE

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Approval Signatures

Name/Signature	Title	Date	Meaning/Reason
Treeske Ehresmann (TEHRESMA)		05 Jun 2019, 03:24:01 PM	Approved

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Work Instructions for Manual Integration for Chromatography – Chem/Tox Unit

Purpose:

To provide a procedure for how and when to perform manual integration of chromatography. In the Chemistry Toxicology unit, this work instruction applies to chromatography generated using HPLC, GC, IC and FIA.

Frequency:

All chromatography should be carefully reviewed by the analyst and data reviewer to determine if manual integration is needed.

Responsibilities:

1. Laboratory analysts are responsible for following the integration instructions in the applicable method, in this WI and in accordance with the instrument data collection software. Training is required of all analysts performing chromatographic manual integration.

2. The unit supervisor is responsible for ensuring that analysts performing chromatography are trained on this work instruction.

3. Lead workers are responsible for training analysts and providing technical expertise and troubleshooting guidance to analysts.

4. Peer data reviewers are responsible for reviewing data integrations (when applicable) and communicating concerns with manual integrations to the analyst and unit supervisor.

References:

1. The NELAC Institute: Manual Integration Assessment Form Presentation, January 2008.

2. Standard Operating Procedure for Manual Integration, STL Burlington, 2000.

3. Manual Integration Policy and Technical Assistance Document (NC WW/GW LC Policy 04/09/2010).

4. ALACC Guidelines: Appendix C: Chemistry, Revised August 2018.

Supplies:

1. Computer with chromatographic software for integration of chromatograms

Definitions:

1. **Baseline:** The chromatographic signal plotted as a function of time in the absence of signal construction from components of interest. Signal consists of electronic “noise”, substances in the eluent, carrier gas, septum bleed or chromatographic column bleed. These materials produce a constant signal noise.

2. **Chromatography:** A separation technique involving differential retention of components between stationary and mobile phases.

3. **Chromatographic Peak:** An increase in signal response from baseline to a maximum and then back to baseline.

4. **Coelution:** The concurrent elution of two or more compounds to the detector at the same time.

5. **Integration:** The process used by chromatographic software to determine peak area or height used for quantitation.

6. **Interference Peaks or Fused Peaks:** Peaks that partially or totally coelute with the peak of interest.

7. **Manual Integration:** The process employed by the data user to provide accurate quantitation of peak area where the original integration or identification provided by the chromatographic software is in error. User integrates peaks manually to assure consistency between standards and samples.

8. **Negative Spikes:** Negative spikes in chromatograms are sudden upsets in the chromatographic signal below the normal baseline. Can result from matrix mismatch between eluent and injected sample, or air in system.

9. **Normal (Gaussian) Peak Shape:** Symmetric chromatographic peaks with the highest concentration of detected material at the midpoint of the peak in the selected retention time range.

10. **Peak Enhancement:** Adjusting the baselines for integration to increase the area of a peak. The enhancement of sample areas over the standards can increase the calculated amount in the sample. If reduction is required, the enhancement of the standards only is performed. This in an inappropriate manual integration technique.

11. **Peak Splitting:** When a Gaussian peak gets a shoulder or a twin. The splitting can affect all peaks or just one, and different effects can be attributed to different causes.

12. **Peak Tailing:** The delayed return of a peak to chromatographic baseline and could be related to delay of compound elution from the chromatographic system by adsorption or dead volume effects

13. **Peak Trimming, Shaving or Clipping:** Manually placing the baselines to reduce the peak area on integration to enhance the analyte amount in a sample (only the standards are shaved) or reduce the amount of analyte reported (by shaving the sample and not the standards). This in an inappropriate manual integration technique.

14. **Retention Time:** The time for any compound to move through the chromatographic column and be detected. The retention time is usually established at the apex of a chromatographic peak. Ideally the peak retention range begins at the time of positive baseline inflection and ends when the slope of the negative inflection returns to baseline.

Procedure:

Manual integration is required for chromatography that has not been integrated appropriately by the instrument software's automatic integration procedures. It is an acceptable and appropriate procedure when it can be technically justified.

Manual integration is not a substitute for poor instrument optimization, lack of maintenance, poor technique / sample prep / sample clean-up. Excessive amounts of manual integrations, even when done properly and for the right reasons, should be evaluated for possible corrective action.

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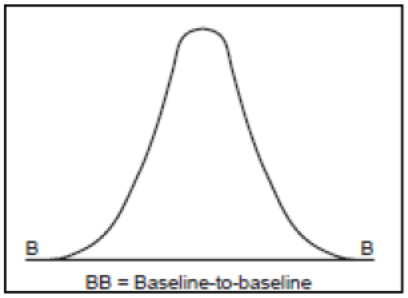
1. Carefully review chromatograms integrated by the instrument's software. Review chromatograms at an appropriate scale (e.g., enlarged to discern baseline noise, etc.).
   - 1.1 Manual integration is appropriate for situations including, but not limited to:
     - 1.1.1 Peak splitting.
     - 1.1.2 Area added due to a co-eluting interferant.
     - 1.1.3 Failure to detect a peak or the wrong peak is identified by the chromatographic software.
     - 1.1.4 Baseline noise (the signal-to-noise ratio is less than 3:1).
     - 1.1.5 Matrix interference.
     - 1.1.6 Well-defined peaks on the shoulders of other peaks.
     - 1.1.7 Negative spikes in the baseline.
     - 1.1.8 Excessive peak tailing due to failure of the instrument response to return to baseline or a rise in the baseline.
     - 1.1.8 Failure to separate closely eluting peaks.
   - 1.2 Manual integration is NOT appropriate for the following situations:
     - 1.2.1 For the sole purpose of meeting quality acceptance criteria.
     - 1.2.2 Peak trimming, shaving or clipping.
     - 1.2.3 Peak enhancement.
     - 1.2.4 Baseline elevated above the signal.
     - 1.2.5 Baseline dropped below the signal.
     - 1.2.6 Selectively adjusting integration.
2. Perform manual integration using a consistent approach. Standards, samples and quality control **MUST** be integrated the same way.
3. Use the same techniques for manual integration that are automatically used by the chromatographic software.
   - 3.1 **Baseline to Baseline integration:** The peak integration baseline is a continuation of the existing baseline. Begin integration when the signal first begins to rise above the baseline and end when the signal returns to the original baseline.



   - 3.2 **Baseline to Valley Integration (Baseline Projection):** This integration is used for two overlapping chromatographic peaks. Draw a vertical line from the valley of the two peaks to the original baseline. Integrate the first peak from when the baseline starts to rise and end at the vertical line. Integrate the second peak from the vertical line to when the signal returns to the original baseline.

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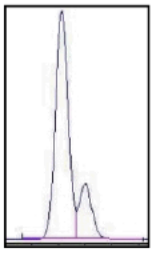
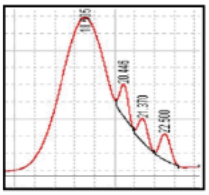
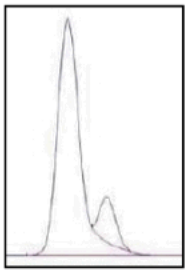


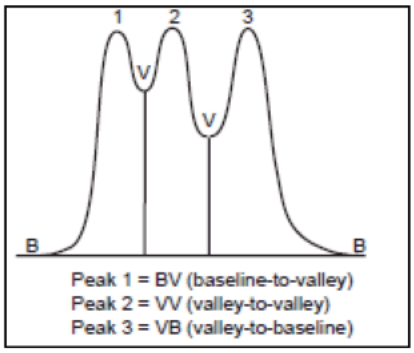
3.3 **Tangent Skim:** This integration is used for two overlapping chromatographic peaks. Draw parent to the baseline. Separate the small peak(s) from the parent peak with separate baseline. Small peak is labeled as skim, shoulder or rider peak.



3.4 **Exponential Skim:** This integration is used for two overlapping chromatographic peaks. Used to create curvature in the skim line to approximate the underlying baseline of the parent peak.



3.5 **Drop Perpendicular:** This integration is used for multiple overlapping chromatographic peaks. Draw a baseline connecting the real baseline before and after the peak group. A tangent is dropped from the valley of each intersecting peak to the original baseline.



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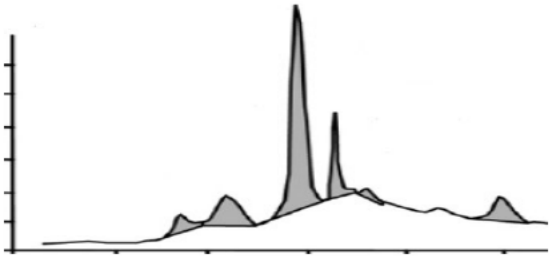
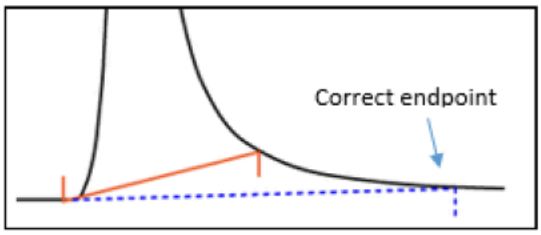
3.6 **Valley to Valley Integration:** This integration is used for multiple overlapping chromatographic peaks. The first and last peaks are integrated baseline to valley. All other peaks are integrated Valley to Valley



Note: When deciding whether to use baseline projection, tangent skim or exponential skim, drop perpendicular, or valley to valley integration, analyst judgment and experience are critical. Which method to use depends on the relative size of the peaks and their resolution. Check to see how the calibration standards were integrated and use the same method. If unsure, check with lead worker.

3.7 **Peak Tailing**: Check that the correct endpoint is selected.

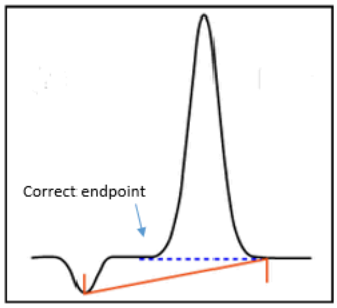


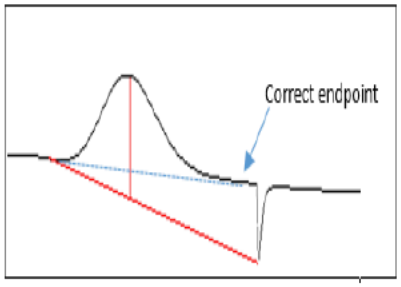
3.8 **Negative Peaks near Peak of Interest**: Draw baseline from the stable portion of the baseline. May have to draw baseline backwards.

3.8.1 In Flow Injection chemistry negative peaks tend to be due to chemistry (e.g., pH) mismatch between carrier solution and the sample solution.

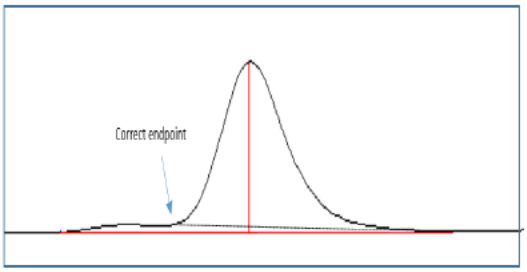
3.8.2 In Ion chromatography negative peaks can be due to:

- Dead volume or air trapped in system.
- Contaminated eluent solution.

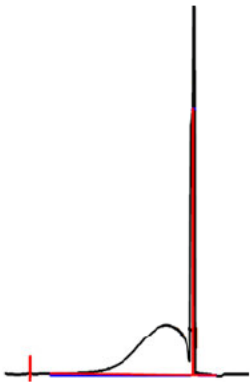




3.9 **Area Added Due to a Co-eluting Interferant:** compare baseline and peak shape of the standards to figure out if there is co-eluting interferants in sample peak.



3.10 **Air Spike in Peak:** When air spike appears in the peak there is air being injected along with the sample. Check the timing of the injection, verify that the sample loop is being filled correctly and/or that solutions are properly degassed. Do not report peaks with air spike.



4. When manual integration is performed, document the following.
   - 4.1 Date and initials of person performing the manual integration.
   - 4.2 Reason the manual integration was performed.
   - 4.3 Identity of the manually integrated peaks / compounds. Using instrument software to identify manually integrated peaks is preferred but notes are also acceptable
5. Retain both the original and manually integrated chromatograms.

Collaborators:

Name	Role
Kristy Broten **Juan Garcia** **Michele Swarbrick**	Lab Analysts
Treeske Ehresmann	Unit Supervisor
Brian Miller	Quality Assurance Officer