CHROMATOGRAPHIC PEAK INTEGRATION PROCEDURES

CONTENTS

1.0 SCOPE AND APPLICATION

The purpose of this standard operating procedure (SOP) is to describe scientifically sound, legitimate and acceptable procedures to be utilized, as guidelines, for analytical data reduction and manipulation activities pertaining to chromatographic peak integration. This SOP is applicable to all chromatographic data generated at the United States Environmental Protection Agency/ Environmental Response Team (USEPA/ERT) Scientific, Engineering Response and Analytical Services (SERAS) Contract laboratory.

2.0 METHOD SUMMARY

In general, procedures in the laboratory use peak area and not peak height for quantitation. Most of the discussion and all the examples provided in this SOP are for determining appropriate peak area. The principles and the specific procedures for completing, documenting and reviewing manual integration generally apply to both peak area and peak height.

Chromatographic peaks generated will generally be integrated using automated methods using software provided by the instrument manufacturer. The analyst will review the automated integration and make adjustments and manually integrate the peaks, if necessary. The analyst is expected to use proper judgment in selecting methodology for peak integration based on this SOP. All data manipulations will be documented and peer reviewed to ensure compliance with this SOP. Under no circumstances will data manipulation, such as peak shaving etc. are used for meeting QC criteria.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

This section does not apply to this SOP.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

Incorrect results may be obtained due various errors that could result when using the automated peak integration program such as peak splitting, adding area due to a coeluting interferant, failure to detect a peak, excessive peak tailing due to failure of the instrument response to return to baseline or a rise in the baseline, and failure to separate peaks. Failure to follow the procedures outlined in this SOP for manual integration may result in incorrect results.

5.0 EQUIPMENT/APPARATUS

This section does not apply to this SOP.

6.0 REAGENTS

This section does not apply to this SOP.

7.0 PROCEDURE

The USEPA/ERT SERAS laboratory utilizes software developed by instrument manufacturers or third party suppliers to reduce data from an analog signal to a concentration present in the sample. Most of the software used for chromatography is capable of quantitating using either peak area or peak height and employs mathematical algorithms related to the slope of the response to detect the beginning and end of peaks. Depending upon the

sophistication of the software package the analyst has flexibility within the parameters of the software to designate internal standards for either quantitation, qualification or both; establish peak thresholds (at an appropriate level above instrument noise); and similar data processing guidelines.

The analytical SOPs include the optimal instrument parameters that allow for automatic integration by the data system in most cases. However, regardless of the sophistication of the software, instances occur when the automated software does not integrate a peak correctly. The failure of the software to appropriately integrate a peak is usually obvious from visual inspection of the chromatogram (at an appropriate scale). Various errors can occur which include, but are not limited to, peak splitting, adding area due to a coeluting interferant, failure to detect a peak, excessive peak tailing due to failure of the instrument response to return to baseline or a rise in the baseline, and failure to separate peaks. The software packages invariably provide a procedure where by the analyst can review the individual data file and provide peak specific instructions on integration to correct these problems. This procedure is referred to as "manual integration" and relies solely upon the experience and judgment of the analyst to determine proper integration for each peak.

All data shall be integrated consistently in standards, samples and QC samples. Integration parameters - both automated and manual - shall adhere to valid scientific chromatographic principles. Manual integration will be employed to correct an improper integration performed by the data system and must always include documentation clearly stating the reason the manual integration was performed, the name of the analyst, and the initials of the supervisor approving the manual integration. Under no circumstances should manual integration be performed solely for the purpose of meeting quality control criteria. In other words, peak shaving, peak enhancing, or manipulations of the baseline to achieve these ends must never occur as this results in an improper integration rather than correcting a data system error.

Procedures for determining an "out of control" state for an analytical system are provided in the associated analytical SOP. With respect to integration procedures, the analyst must pay particular attention to integration problems encountered for the analysis of standards or blanks, which, in theory, should be free of contamination and interfering peaks. Unusual baseline characteristics, unidentified peaks, splitting peaks, excessive tailing or other problems may indicate a need for instrument maintenance or other corrective action.

For compounds such as benzoic acid, which produce non-Gaussian peaks, the analyst may use automatic integration parameters in the method, which are different from the other compounds in order to obtain proper peak areas. All chromatographic data shall be integrated consistently for all standards, samples and QC samples. Integration parameters, both automated and manual, must adhere to valid scientific chromatographic principles. Manual integration shall be performed to accurately measure the area under the peak and shall not be performed solely for the purpose of meeting quality control criteria. Eliminating part of the subject peak area or including peaks not belonging to the subject peak is inappropriate manipulation of the analytical data and will not be tolerated.

An example of improper manual integration is peak shaving or peak enhancement to make failed calibrations, surrogates, or internal standards appear to meet QC criteria. Conducting peak shaving to eliminate part of the subject area or including area counts not belonging to the subject peak is inappropriate manipulation of analytical data.

7.1 Proper Integration of Chromatographic Peaks

Appropriate integration includes all the subject peak area and no extraneous area due to noisy baseline, coeluting peaks or changes in baseline configuration. Common integration techniques include baseline-to-baseline, valley-to-valley or a combination of these procedures. Complicated chromatograms with coeluting peaks may require peak

skimming or other appropriate techniques to calculate the area. When determining integration procedures the analyst should consider the underlying peak shape and provide an integration, which includes all the peak area of the subject peak while excluding any area due to interfering peaks or a noisy baseline.

The following examples provided in Appendix A refer to properly integrated peaks.

- Figure 1 is a properly integrated single peak. The peak is symmetrically shaped and exhibits no indication of coelution. The baseline is stable and returns to the same level (i.e., the baseline is flat). This is an example of baseline-to-baseline integration. Peaks of this nature are usually appropriately integrated automatically by the software. On occasion, the analyst must integrate a peak of this nature manually due to a retention time shift, which causes the data system to incorrectly determine that the peak is not a target analyte. The analyst must justify peak identification for such out-of-window peaks, usually based on changes in the retention time of standards.

- Figure 2 is a proper integration of several peaks which are not completely resolved (i.e., the response does not return to the baseline between peaks). In this instance the lowest point between the two peaks, the valley, is selected as the appropriate end point for the peaks.

- Figure 3 provides several examples of peaks with slight interferences either just prior to or immediately after the target peak. These interfering peaks are not resolved and may be included in the automatic integration. Figure 3 demonstrates examples of proper integration of these peaks.

- Figure 4 is an example of a peak, which requires the use of sophisticated software to remove the area due to a co eluting peak. Depending on the sophistication of the data system, it may be possible to remove the additional area. It is necessary that the resulting integration area preserve the Gaussian peak shape.

7.2 Improper Integration of Chromatographic Peaks

Inappropriate integration is any integration, either automated or manual, which excludes area, which should be associated with the target peak or includes area not reasonably attributable to the target peak such as area due to a second peak or excessive peak tailing due to a noisy baseline. Intentionally excluding peak area is commonly referred to as peak shaving.

The following examples provided in Appendix B illustrate improper integration. Occurrences such as these are unacceptable and prohibited.

- Figure 5 provides an example of an improperly integrated peak. The “tailing” side of the peak has been removed eliminating significant area, which should be included in the peak. This is not an example of removing an excessive area due to peak tailing because the Gaussian shape of the peak has clearly not been preserved in the integration.

- Figure 6 is an example of an improperly elevated baseline. This clearly excludes a large area of the peak, which a baseline-to-baseline integration would correct.

- Figure 7 is an improperly integrated peak, which includes both elevating the baseline and eliminating the leading and tailing edge of the peak.

- Figure 8 is an example of a peak tail, which had not been included in the integration due to a noisy baseline. The tail of the peak should be integrated assuming a Gaussian peak shape as

clearly indicated by the shape of the secondary ion trace.

- Figure 9 is an example of adding baseline area to a peak.

7.3 Manual correction of erroneous automated data system integrations

Appendix C provides examples of data system integrations that are inappropriate and the manual integration performed to correct the errors.

- Figure 10 is an example of a noisy baseline resulting in poor integration by the data system that is attempting to integrate using a valley-to-valley integration procedure. The appropriate integration of the peak eliminates area associated with baseline changes and integrates only the target peak.

- Figure 11 is an example of coelution of the tailing edge of a peak resulting in additional area being included in the automated integration. The manual integration is performed to preserve the peak shape and eliminate the additional area.

- Figure 12 is an example of automated integration that can occur when detector response is noisy. The baseline is integrated at an excessively high level and the peak is split due to the noise observed at the top. (The peak is shown superimposed over a normal peak for comparison purposes.) The manual integration of this peak includes all the area reasonably attributable to the peak while excluding the noisy baseline.

- Figure 13 is an example of automated integration where the baseline has shifted and the data system used the original system baseline to set the integration baseline. Correct integration uses the new baseline.

8.0 CALCULATIONS

As described in Section 7.0.

9.0 QUALITY ASSURANCE/QUALITY CONTROL

The analyst must submit a legible copy of all manual integrations that show the entire peak and baseline. The analyst must initial and date the printout of the manual integration, indicating acceptance not only of the procedures used by the analyst to reintegrate the peak but also the reasoning used to determine that reintegration was necessary and adequate.

In addition, the following documentation must be submitted:

- Data packages must contain the quantitation report and chromatogram (or total-ion chromatogram for GC/MS data) of each standard, blank, sample and QC sample.

- GC/MS data: For each target analyte, tentatively identified compound, internal standard and surrogate identified by GC/MS, the analyst must submit mass chromatograms of at least two characteristic ions and a background-subtracted mass spectrum. For standards, matrix spike samples, laboratory control samples and dilutions, detailed reports showing each target compound are not necessary; however, the analyst must submit a summary report (i.e., a quantitation report and a total-ion chromatogram).

- To ensure appropriate quality control review, all manually integrated analytical data output shall be designated with an "M". If the data system does not automatically flag the values as such, the analyst must clearly mark the report as such.

10.0 DATA VALIDATION

Manual integration will be employed only to correct an improper integration performed by the data system and will always include documentation clearly stating the reason the manual integration was performed and the name of the analyst. The entire data package including the manual integration will be peer reviewed in the laboratory, and dated and initialed by the Group Leader, before release to the data validation group. The analytical data package generated will be reviewed and validated in accordance with the applicable SERAS data validation SOP's, before release.

11.0 HEALTH AND SAFETY

This Section does not apply to this SOP.

12.0 REFERENCES

USEPA Region 9 SOP #835, "Chromatographic Integration Procedure," Revision 0, July 1, 1998.

California Military Environmental Coordination Committee, "Best Practices for the Detection and Deterrence of Laboratory Fraud," March 1997, Version 1.0.

United States Environmental Protection Agency, "Test Methods for Evaluating Solid Waste, Method 8000B Determinative Chromatographic Separations", SW-846 Third Edition, December 1996.

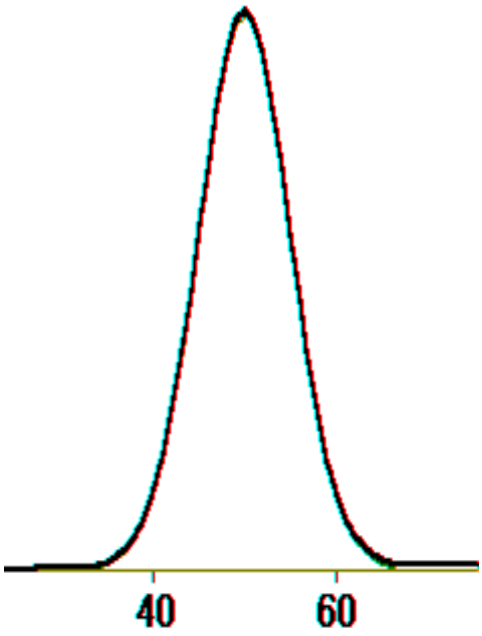
13.0 APPENDICES

Appendices
SOP# 1001
January 2000

Appendix A
Figure 1 Properly integrated single peak
SOP#1001
January 2000

Figure 1 Properly integrated single peak

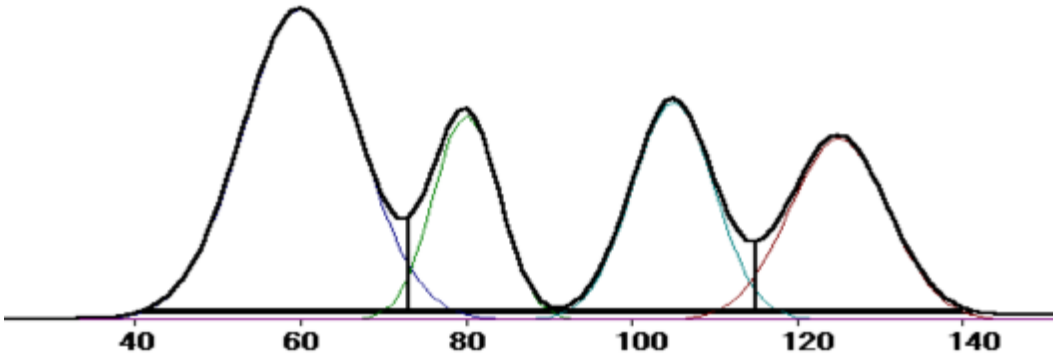
The peak is symmetrically shaped and exhibits no indication of coelution. The baseline is stable and returns to the same level (i.e., the baseline is flat). This is an example of baseline-to-baseline integration. Peaks of this nature are usually appropriately integrated automatically by the software. On occasion, the analyst must integrate a peak of this nature manually due to a retention time shift that causes the data system to incorrectly determine that the peak is not a target analyte.



Appendix A
Figure 2 Properly integrated unresolved peaks
SOP#1001
January 2000

Figure 2 Properly integrated unresolved peaks

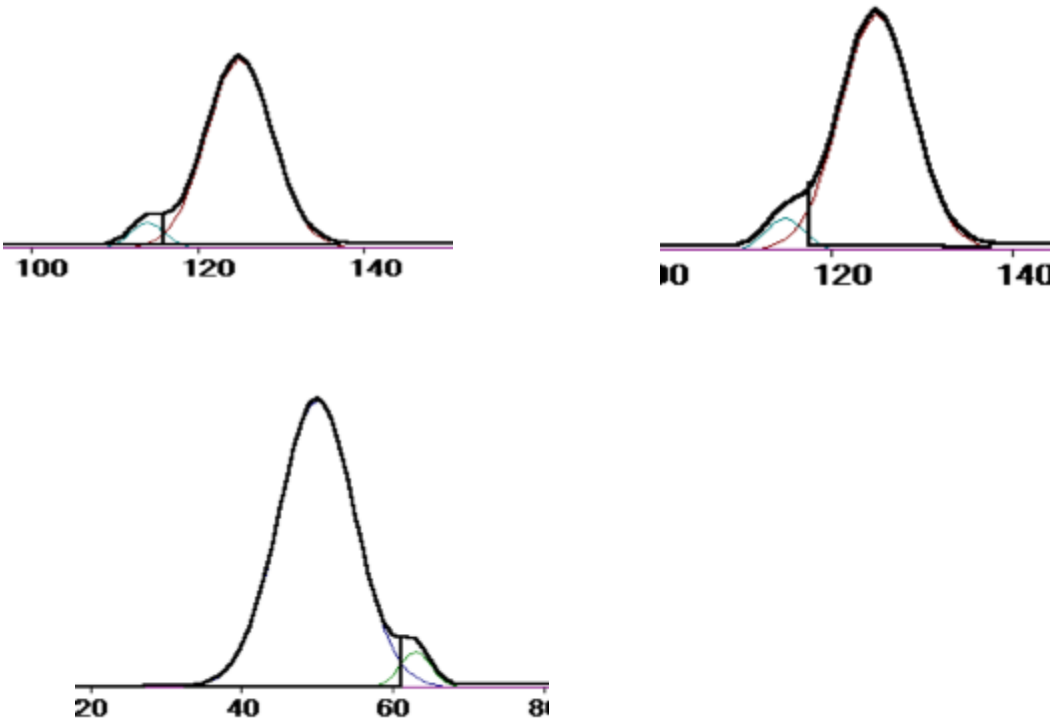
Proper integration of several peaks which are not completely resolved (i.e., the response does not return to the baseline between peaks). In this instance the lowest point between the two peaks, the valley, is selected as the appropriate end point for the peaks.



Appendix A
Figure 3 Proper integration to remove interfering peaks
SOP#1001
January 2000

Figure 3 Proper integration to remove interfering peaks

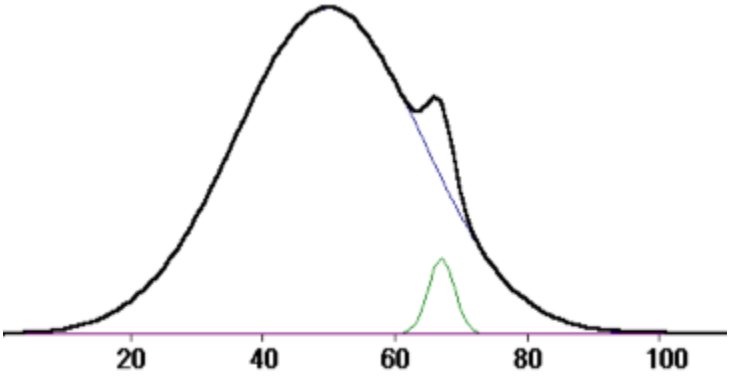
Several examples of peaks with slight interferences either just prior to or immediately after the target peak. These interfering peaks are not resolved and may be included in the automatic integration. Figure A-3 demonstrates examples of proper integration of these peaks.



Appendix
Figure 4 Peak shape requiring use of peak skimming
SOP#1001
January 2000

Figure 4 Peak shape requiring use of peak skimming

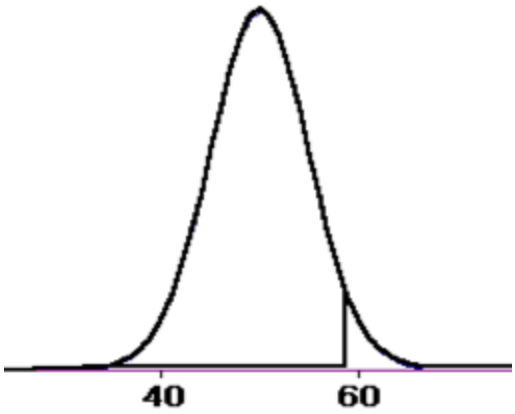
This is an example of a peak that may require the use of more sophisticated software to remove the area due to a co eluting peak. Depending on the sophistication of the data system, it may be possible to remove the additional area. It is necessary that the resulting integration area preserve the Gaussian peak shape**.**



Appendix B
Figure 5 Peak shaving by removing tail
SOP#1001
January 2000

Figure 5 Peak shaving by removing tail.

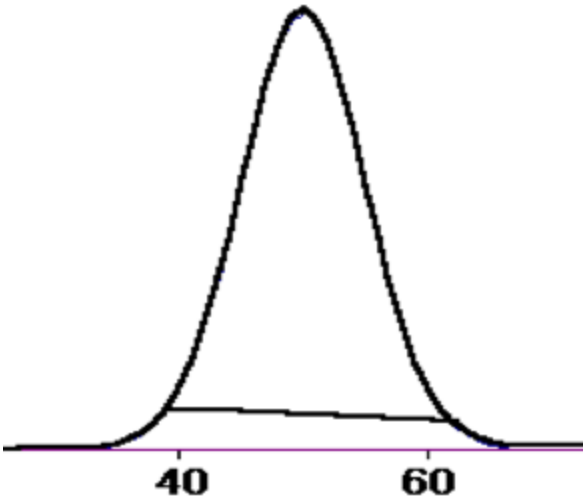
This is an example of an improperly integrated peak. The "tailing" side of the peak has been removed eliminating significant area that should be included in the peak. This is not an example of removing an excessive area due to peak tailing because the Gaussian shape of the peak has clearly not been preserved in the integration.



Appendix B
Figure 6 Peak shaving through elevating the baseline
SOP#1001
January 2000

Figure 6 Peak shaving through elevating the baseline

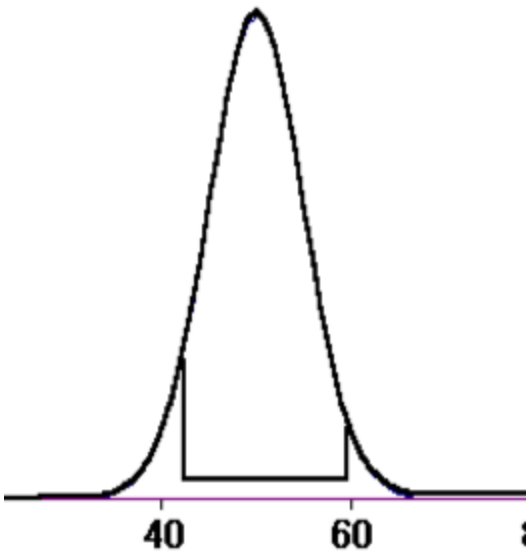
This is an example of an improperly elevated baseline. This clearly excludes a large area of the peak that a baseline-to-baseline integration would correct.



Appendix B
Figure 7 Gross peak shaving
SOP#1001
January 2000

Figure 7 Gross peak shaving.

This is an improperly integrated peak that includes both elevating the baseline and eliminating the leading and tailing edge of the peak.

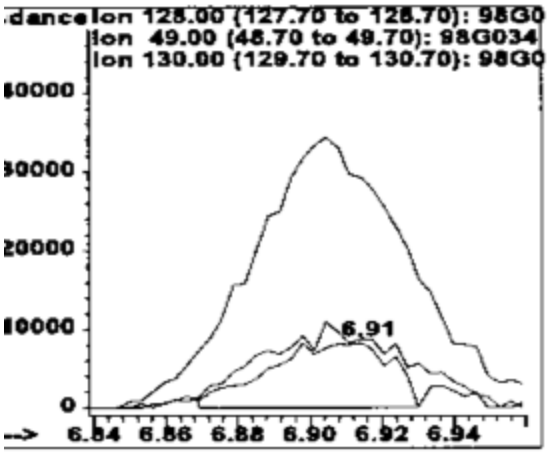


Appendix B

Figure 8 Including area due to a noisy baseline
SOP #1001
January 2000

Figure 8 Including area due to a noisy baseline

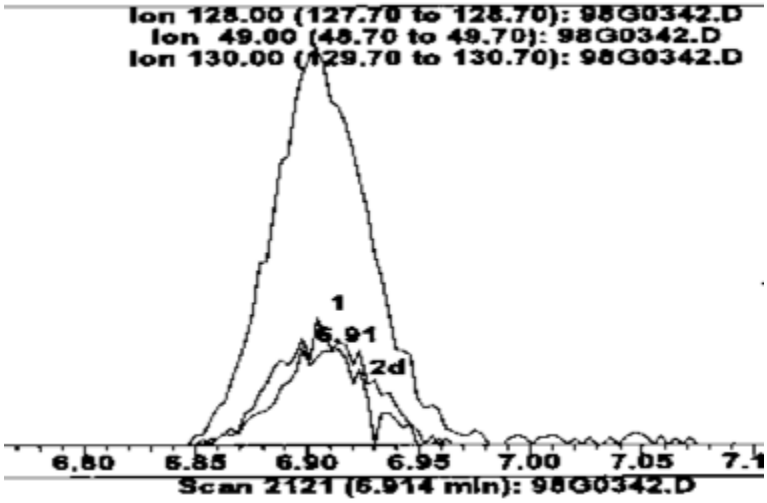
This is an example of a peak tail that had not been included in the integration due to a noisy baseline. The tail of the peak should be integrated assuming a Gaussian peak shape as clearly indicated by the shape of the secondary ion trace.



Appendix B

Figure 9 An example of adding baseline area to a peak
SOP #1001
January 2000

Figure 9 An example of adding baseline area to a peak

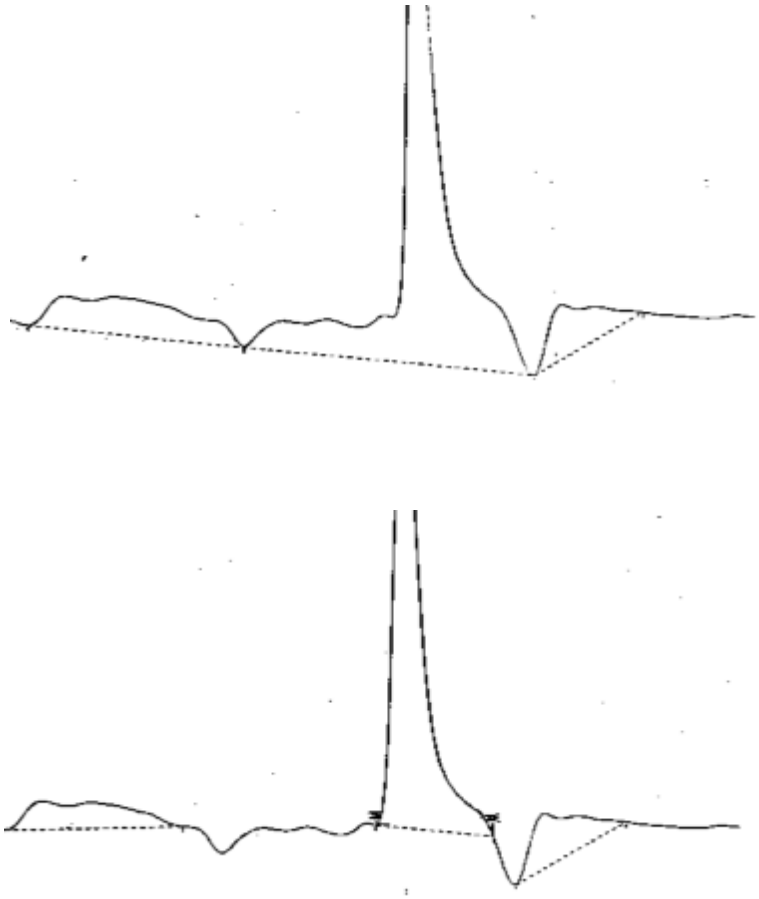


Appendix C

Figure 10 Noisy baseline.
SOP #1001
January 2000

Figure 10 Noisy baseline

This is an example of a noisy baseline resulting in poor integration by the data system that is attempting to integrate using a valley-to-valley integration procedures. The appropriate integration (shown in the second figure) of the peak eliminates area associated with baseline changes and integrates only the target peak.



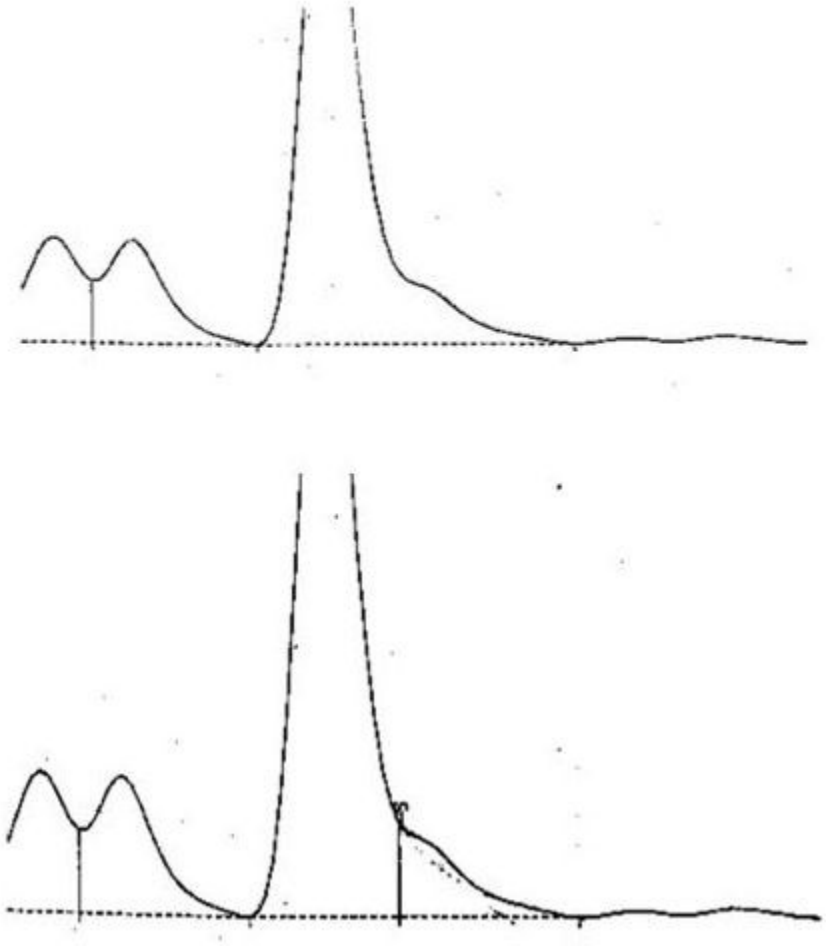
App

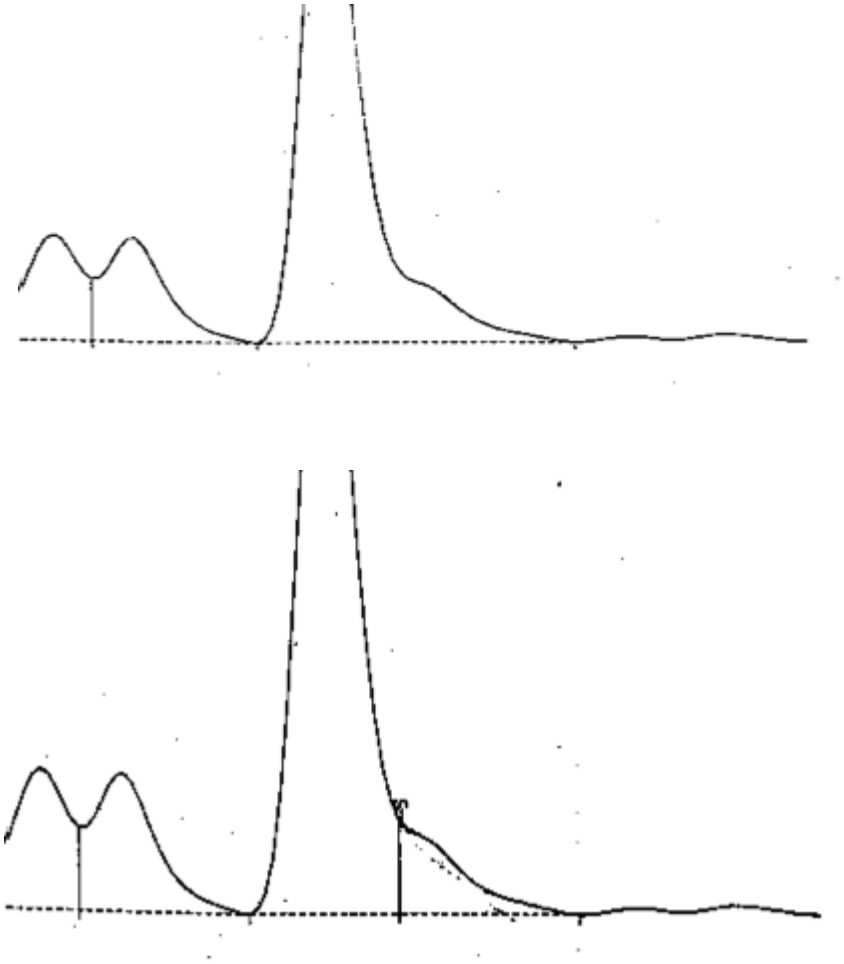
endix C

Figure 11 Addition of co-eluting peak

Figure 11 Addition of coeluting peak.

This is an example of coelution of the tailing edge of a peak resulting in additional area being included in the automated integration. The manual integration is performed to preserve the peak shape and eliminate the additional area.

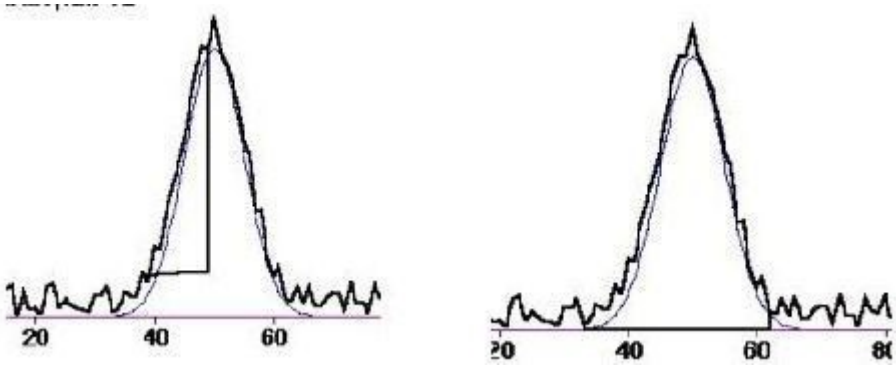




Appendix C
Figure 12 Peak splitting.
SOP#1001
January 2000

Figure 12 Peak splitting.

This is an example of automated integration that can occur when detector response is noisy. The baseline is integrated at an excessively high level and the peak is split due to the noise observed at the top. (The peak is shown superimposed over a normal peak for comparison purposes.) The manual integration of this peak includes all the area reasonably attributable to the peak while excluding the noisy baseline.



Appendix C
Figure 13 Baseline Shift
SOP#1001
January 2000

Figure 13 Baseline Shift

Ion chromatogram where data system used original baseline to integrate peaks after water dip. Correct integration compensates for baseline shift.