Lab Director Approval: Mark Tolbert / 08/19/2021

QA Manager Approval: Jeffrey Moore / 08/19/2021

Standard Operating Procedure for Manual Integration

Access to this SOP shall be available within the laboratory for reference purposes; the official copy of this SOP resides on the official Georgia EPD website at https://epd.georgia.gov/about-us/epd-laboratory-operations. Printed copies of this SOP will contain a watermark indicating the copy is an uncontrolled copy.

1 Scope and Application

1.1 This SOP is a guide for manual integration of peaks in various types of chromatography. It provides several examples of common problems that occur when analyzing various matrix for one or more analytes.

1.2 This SOP is not intended to be all encompassing. There are an endless number of variations that can occur when a peak is integrated. However, the most common problems will be discussed here. The main rule to follow is consistency. If a standard has co-eluting peaks, all integration should be the same. It is far worse to constantly change an integration style for the same peak than to pick a less desirable integration.

1.3 Even with today's sophisticated software, bad integration occurs quite often. The software is not intuitive enough to compensate for unusual circumstances caused by matrix interferences or baseline noise. The analyst must determine the most representative chromatogram to provide reasonable results for unusual circumstances.

2 Definitions

2.1 Manual Integration – The use of data system tools to manually determine the beginning and ending of integration for a specific peak or any other manipulation of peak area performed at the discretion of the analyst. This is also referred to as re-integration.

2.2 Peak Shaving – Using manual integration to remove a legitimate portion of a peak. This is not necessarily a bad practice. There are occasions where it is legitimate. Overlapping peaks require a small amount of shaving to represent each individual peak correctly. However, chromatographic peaks are never manually integrated, by the subtraction of peak area, to allow an instrumentation response to pass quality control criteria. Analysts are advised that purposely modifying peaks in a biased manner is considered non-ethical and may constitute laboratory fraud.

2.3 Baseline Noise – Any interference that causes irregularities in the baseline of a chromatogram. This can be from a solvent peak, non-target compounds, etc.

2.4 Gausian Peak Shape – A "perfect" peak shape. It is bell shaped and is symmetrical if a line is dropped from the peak tip to the baseline.

3 Quality Control

3.1 Any questionable manual integration should be documented. If a problem peak is manually integrated a "before" and "after" picture should be made for review, if necessary, by a supervisor or manager.

3.2 A random audit of data packages will be conducted by the individual Lab Managers during internal audits of analytical methods. The inspection of manual integration, among other things, will be checked and documented on the internal audit forms. Audit forms will be submitted to and kept on file in the QA Office.

4 Procedure

4.1 Method specific peak acquisition and interpretation procedures are covered in individual SOPs for each analytical method.

4.2 Analytical methods may have different integration parameters for compounds such as benzoic acid, which produces non-Gausian peaks. Other peaks will be integrated by standard method integration procedures. This will ensure more accurate peak areas. However, each compound will be integrated consistently for all standards, spikes, and samples. Integration parameters, both manual and automated, 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 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.

4.3 Examples of improper manual integration are peak shaving and peak enhancement to make failed calibrations for 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.

4.4 Appropriate integration must include all subject peak area and no extraneous area due to noisy baseline or coeluting peaks. Common software settings for integration are baseline-to-baseline, valley-to-valley, or a combination of these procedures. Complicated chromatograms with coeluting peaks may require peak skimming or more difficult techniques to appropriately calculate the area. This will also depend on the limitations set by the software integration program. The analyst should consider the underlying peak shape when determining the appropriate integration procedure. The integration should include all the peak area

while excluding any area due to interfering peaks or a noisy baseline.

4.5 Appendices A-C give visual examples of Good (A), Bad (B), Common Software Errors (C).

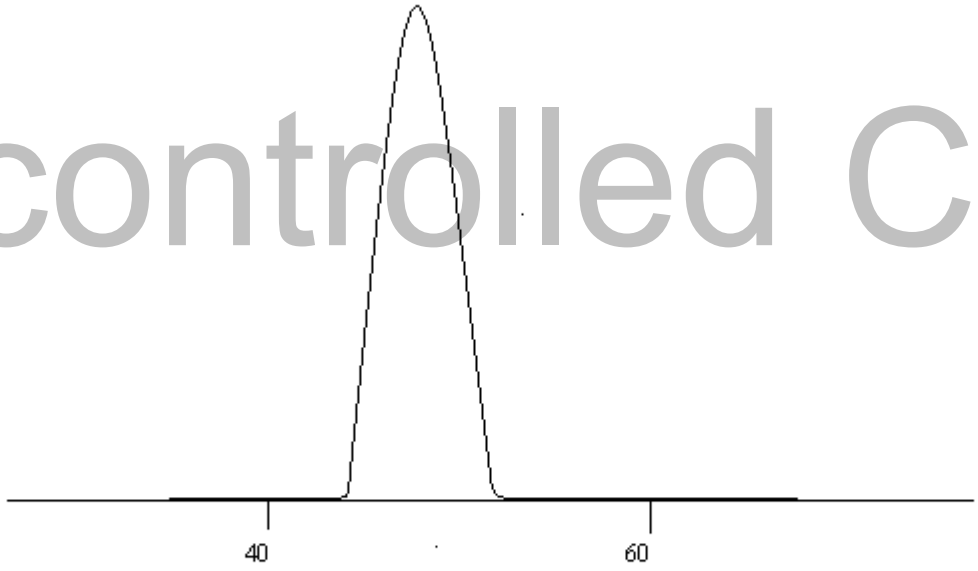
5 References

5.1 Chromatographic Peak Integration Procedures, USEPA Region 5 CRL Standard Operating Procedure, 11/09/99, rev. 0

Appendix A

Figure A-1 Properly integrated single peak

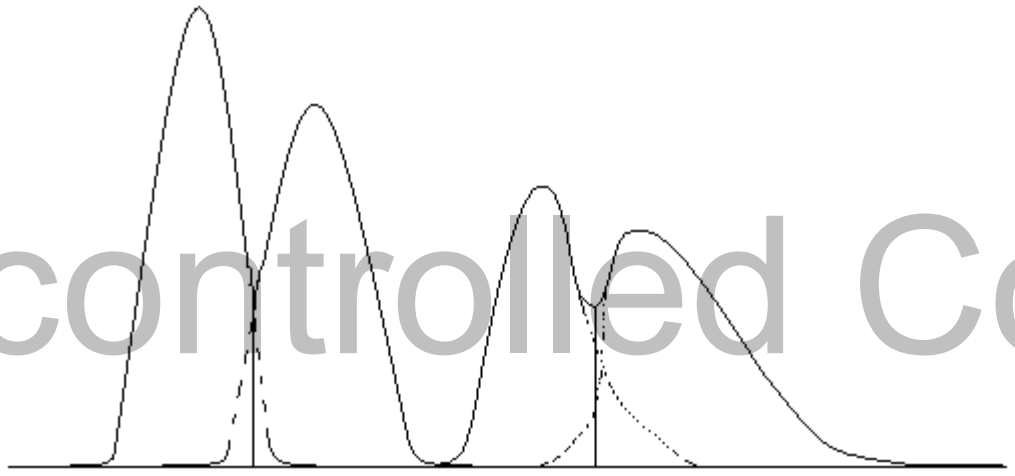
The peak is symmetrically shaped and exhibits no indication of collusion. The baseline is stable and returns to the same level (i.e., the baseline is flat). This is an example of baseline-to-baseline integration. Peaks of this nature are usually appropriately integrated automatically by the software. 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.



Appendix A

Figure A-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

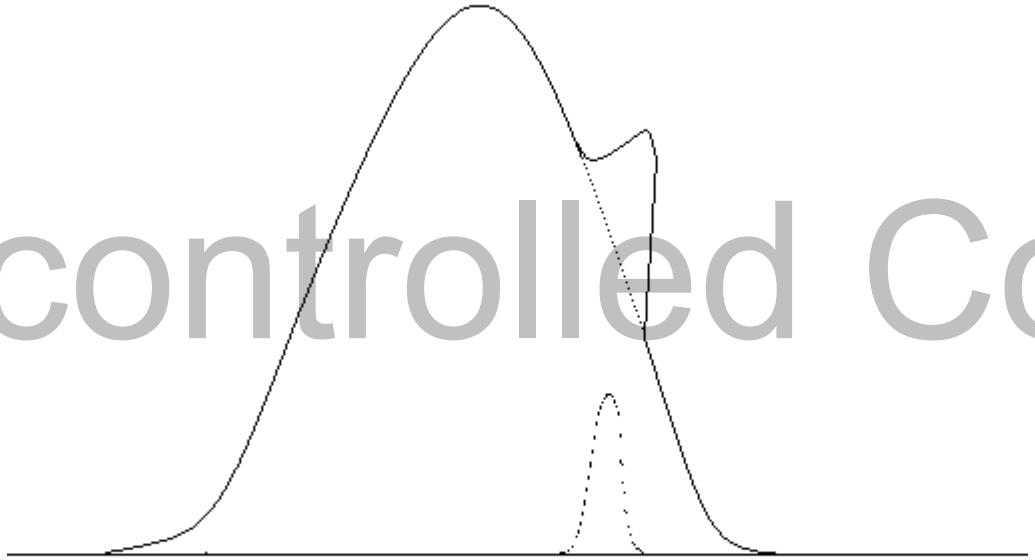
Figure A-3: Proper integration to remove interfering peaks

Two 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 the proper integration of these peaks.

Appendix A

Figure A-4: Peak shape requiring use of peak skimming

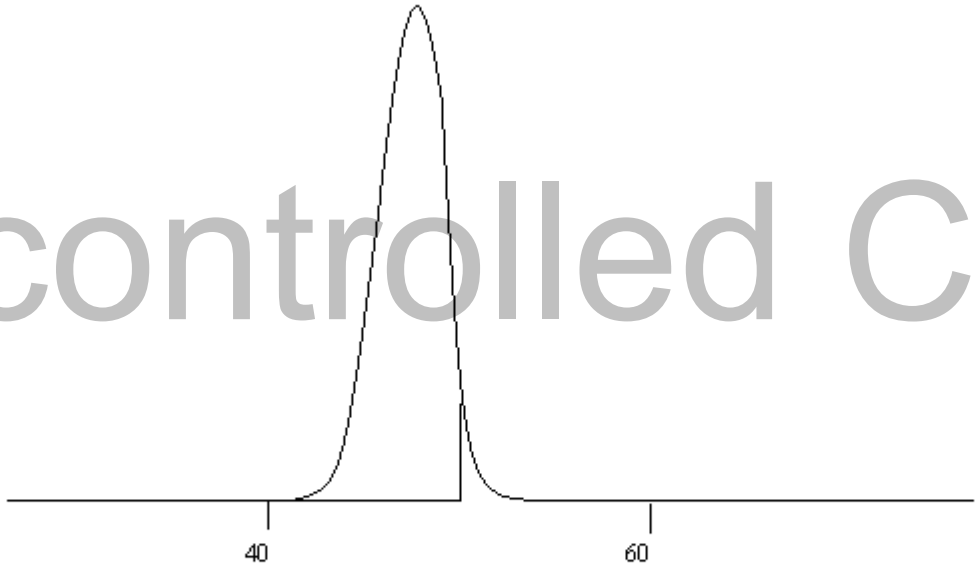
This is an example of a peak which may require the use of more sophisticated software to remove the area due to a co-eluting peak. Depending on the sophistication of the data system it may be possible to remove the additional area. It is necessary that the resulting integration area preserve the Gausian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



Appendix B

Figure B-1: Peak shaving by removing tail

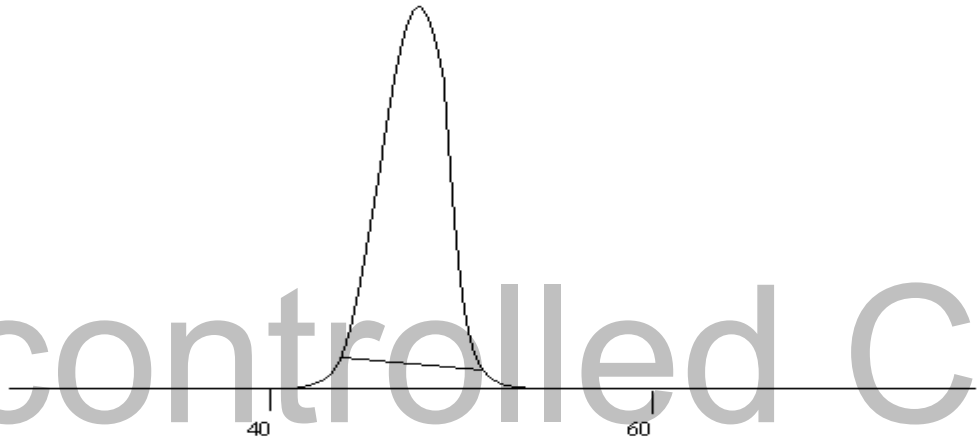
This is an example of an improperly integrated peak. The "tailing" side of the peak has been removed eliminating significant area which should be included in the peak. This is not an example of removing an excessive area due to peaking tailing because the Gausian shape of the peak has clearly not been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



Appendix B

Figure B-2: Peak shaving through elevating the baseline

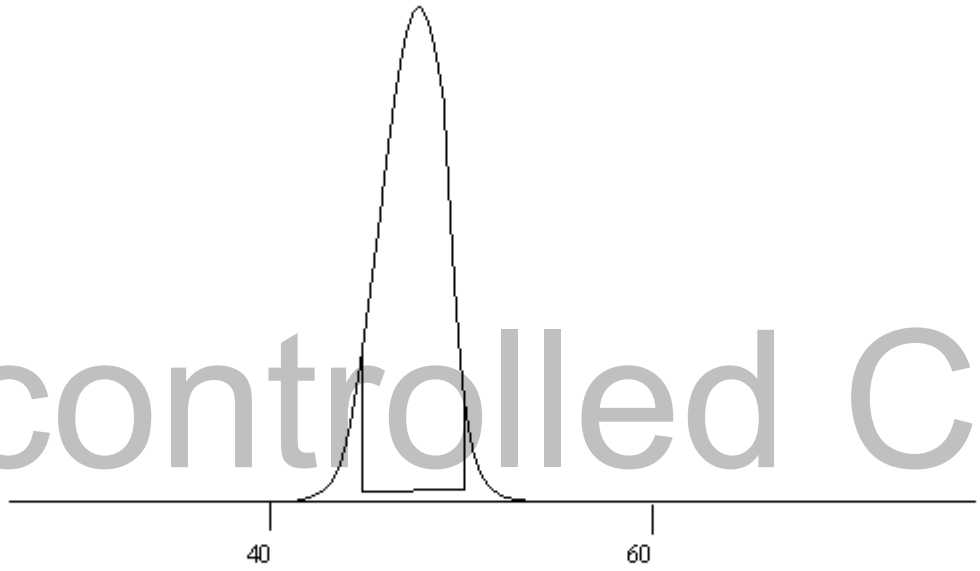
This is an example of an improperly elevated baseline. This clearly excludes a large area of the peak which a baseline-to-baseline integration would correct.



Appendix B

Figure B-3 Gross peak shaving

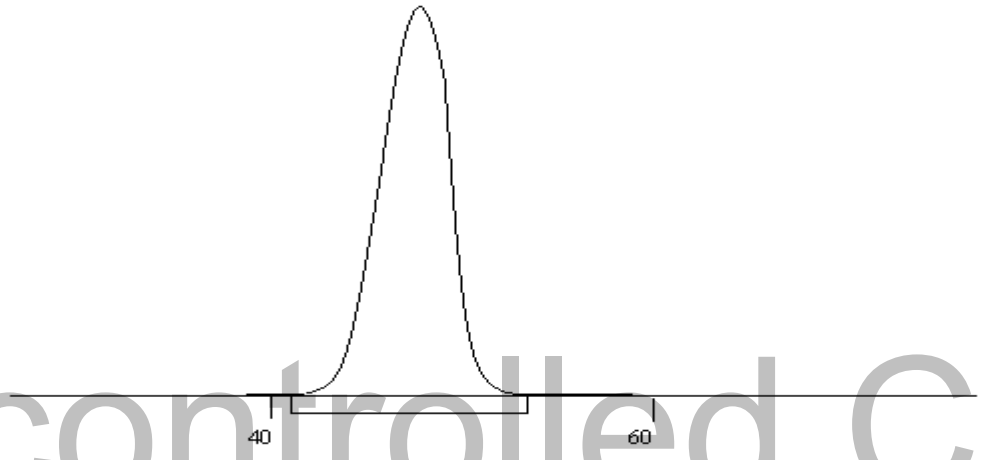
This is an improperly integrated peak which includes both elevating the baseline and eliminating the leading and tailing edge of the peak.



Appendix B

Figure B-4: Adding baseline area to a chromatographic peak

Figure B-5 is an example of adding baseline area to a peak.



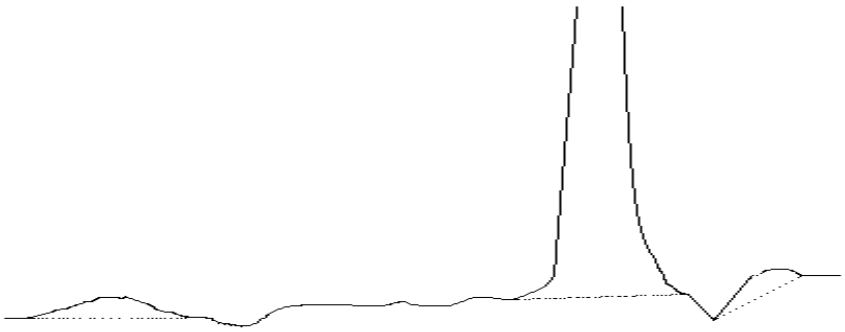
Appendix C

Figure C-1: Noisy baseline

Figure C-1 A is an example of a noisy baseline resulting in poor integration by the data system which 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 C-1B).

Figure C-1 A:

Figure C-1 B:



Appendix C

Figure C-2 Addition of co-eluting peak

Figure C-2 A is an example of co-elution 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 C-2 B).

Figure C-2 A:

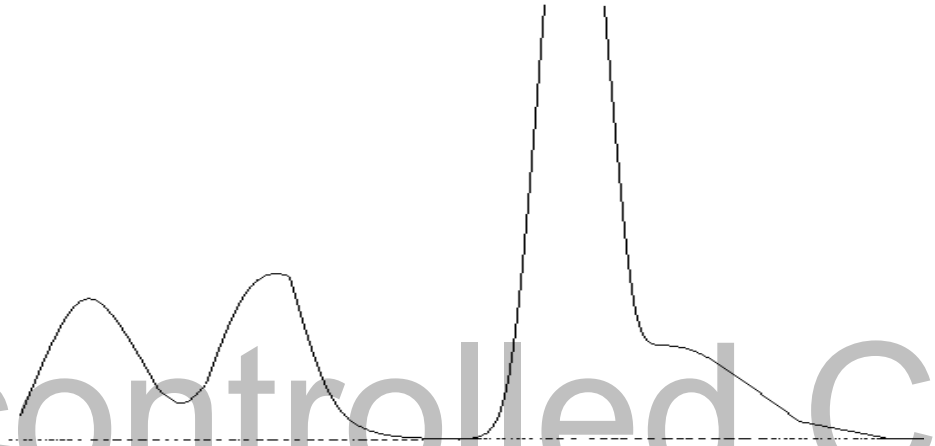
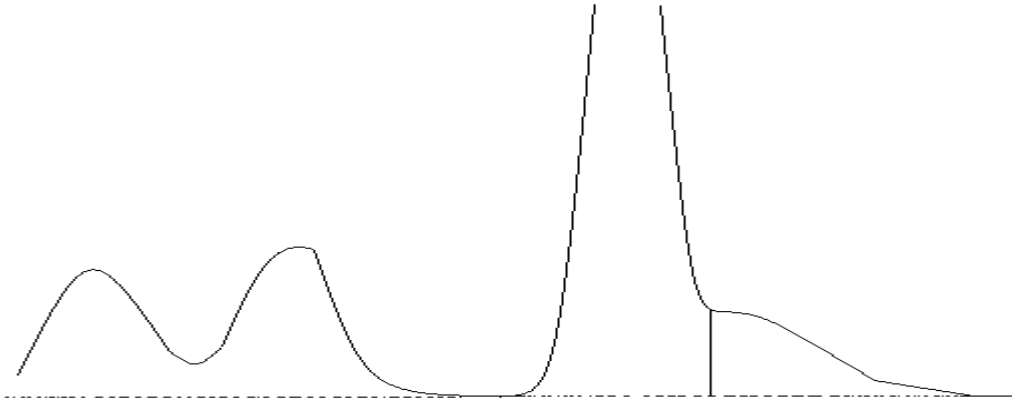


Figure C-2 B:



Appendix C

Figure C-3: Peak splitting

Figure C-3 A is an example of automated integration which can occur when detector response is noisy, two compounds or ions have similar detector responses, or from poor resolution. The baseline is integrated at an excessively high level and the peak is split due to the noise observed at the top. The manual integration of this peak includes all the are reasonably attributable to the peak while excluding the noisy baseline (Figure C-3 B).

Figure C-3 A

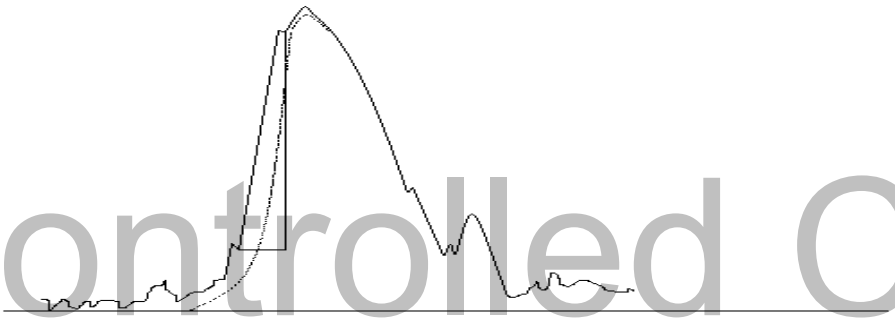
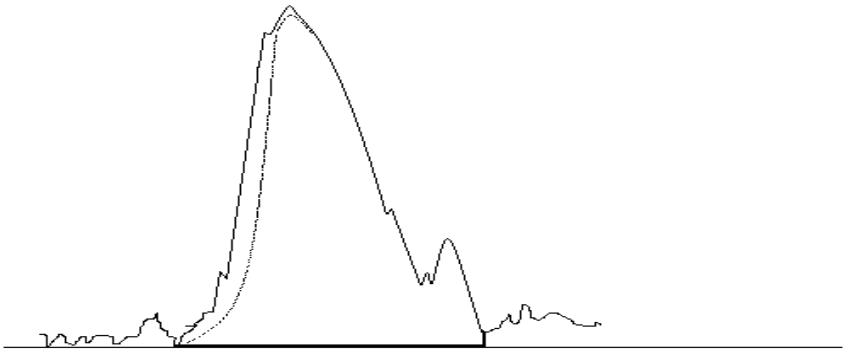


Figure C-3 B



Appendix C

Figure C-4: Baseline Shift

Figure C-4 A is an Ion chromatogram where the data system used the original baseline to integrate peaks after the water dip. Correct manual integration compensates for baseline shift (Figure C-4 B).

Figure C-4 A:

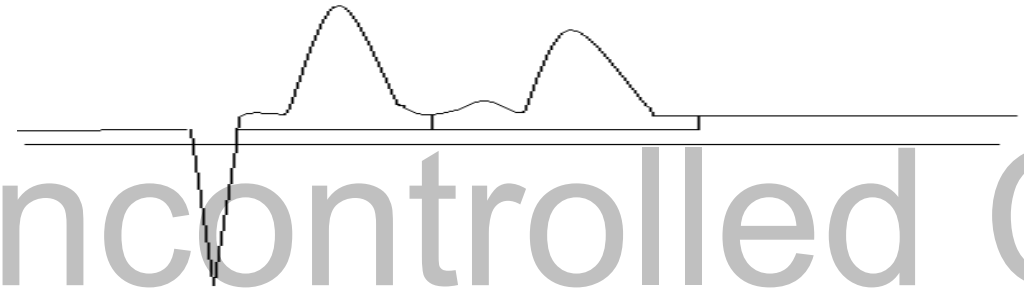


Figure C-4 B: