

Homework – Internal Standard Calibration

Problem:

GC-MS analysis of several compounds was performed. Several calibration solutions (mixtures A-F) were prepared using a serial dilution of calibration standards and addition of internal standard (*o*-terphenyl) as shown in the Table 1.

Table 1. Concentrations ($\mu\text{g}/\text{mL}$) of target compounds and internal standard *o*-terphenyl in calibration solutions

	Formula	Mix F NA2-74-F	Mix E NA2-74- E	Mix D NA2-74-D	Mix C NA2-74-C	Mix B NA2-74- B	Mix A NA2-74-A
Guaiacol	$\text{C}_7\text{H}_8\text{O}_2$	0.064	0.320	1.60	7.99	40.0	200
Vanillic acid	$\text{C}_8\text{H}_8\text{O}_4$	0.064	0.319	1.60	7.99	39.9	200
1,2-Dimethoxy-4-[(2-methoxyphenoxy)methyl]benzene	$\text{C}_{16}\text{H}_{18}\text{O}_4$	0.064	0.320	1.60	8.00	40.0	200
Bicreosol	$\text{C}_{16}\text{H}_{18}\text{O}_4$	0.064	0.320	1.60	8.00	40.0	200
<i>o</i>-Terphenyl (IS)	$\text{C}_{18}\text{H}_{14}$	62.0	62.0	62.0	62.0	62.0	62.0

Each standard mixture was analyzed two times. Your task is to process the resulting data and to obtain main calibration parameters using the following steps:

- Identify compounds based on retention times and mass spectra and determine the major ion to be used for quantification.
- Obtain calibration data (peak areas) using HP ChemStation software for two target compounds of your choice and internal standard.
- Generate main calibration parameters, slope, intercepts, s_y , R^2 , charts with calibration curves using the internal standard method.
- Determine LOQs.
- Report your results.

1. Data processing steps in detail

To obtain peak areas for specific ions, the data need be processed in the computer lab on Windows based computers. Details how to work with HP Chemstation are listed as commands in Section 2. **Watching the videos listed in Section 3 is strongly recommended.** The following steps are essential for the homework completion:

Open GC-MS files. The GC-MS files are located in the computer lab on the hard-drives C:\MSDCHEM\1\Data\Chem443 homework\ or you may copy analyses files from the http://arts-sciences.und.edu/chemistry/kubatova-research-group/chrom_ms02.cfm. To open files in HP ChemStation software, click on the desktop the icon “MS data analysis 1.” To view the data file, go to “File\load data file”, find directory to which you have saved the data, and open the file. The commands for work with the data are listed in Section 2.

Obtain peak areas for two compounds and IS for selected ions. Higher accuracy and sensitivity are achieved when processing specific m/z ions instead of the total area.

- ✓ View mass spectra for target compounds, and select the m/z of the ions you intend to quantify for both analytes (Section 3, Viewing Data in MSD ChemStation)

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- ✓ Extract the selected ions for both analytes and internal standard (Chromatogram/Extract Ions)
- ✓ Ensure that you use the ChemStation Integrator (*Chromatogram/Select Integrator*)
- ✓ Integrate your peaks manually or automatically (*Section 3, Viewing Data in MSD ChemStation, Basic Integration*)

For automatic integration you may need to adjust integration parameters – mainly the threshold in *Chromatogram/MS Signal Integration Parameters*. For manual integration, you need to switch to the manual integration mode in *Tools/Options*.

- ✓ Obtain integration results (peak areas) in Chromatogram/Integration report. Copy the retention data and peak areas into the MS Excel spreadsheet for further data processing.

Process the data in MS Excel.

- ✓ First organize the data (compound names, retention times, concentrations, peak areas) in columns similarly as shown in videos in *Section 3 (MS Excel processing, Internal standard calibration in Excel Part 1)*.
- ✓ Obtain slope and intercept, standard deviation of the linear curve (s_y), correlation coefficient and calibration curve charts. This should be done using the LINEST function, see pertinent videos on LINEST, in Section 3 (part 1). Note for the calibration you may use only points within the linear range with response above background noise.
- ✓ Obtain charts as shown in *Section 3 (MS Excel processing, Internal standard calibration in Excel Part 2)*.

Obtain limit of quantification for two analytes. The calibration points used for the determination of LODs and LOQ should be within one order of magnitude. This is typically three lowest concentrations, however occasionally only two lowest concentrations may be used (*Section 3 (MS excel processing, LOD and LOQs with LINEST in MS Excel)*).

Report your results in the form of MS Excel spreadsheet labeled with your name. The grading will be based on the results provided for items listed below, each for 5 pt, with total of 40 pt. You may be allowed one resubmission.

- ✓ Ion selection (explain why you selected specific ion) and integration
- ✓ Ensure labeling of all the variables (including specification which ion and compounds are you targeting)
- ✓ Use of significant figures
- ✓ Calculation of calibration parameters
- ✓ Calibration charts
- ✓ Calculation of limits of quantification
- ✓ Overall organization and graphic presentation
- ✓ Statements (full sentences) reporting LOQs, LLOQ values, and slope and intercept suitable for quantification (*ensure your data are reported with a correct number of significant figures*).

2. Commands for work with the GC-MS files in ChemStation

Copy chromatogram of Mass spectrum to Word = click Tools/Copy Window and select the appropriate number (2 for chromatogram and (1) for Mass spectrum).

Chemstation Integrator = “Chromatogram/Select Integrator”

Extract ions from single sample = “Chromatogram/extract ion chromatogram” type in m/z of interest

Extract ion from multiple samples (data files) = “Tools/Options/Overlay Chromatograms” “and overlay either TIC or selected ion chromatograms

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Integrate = “Chromatogram/Integrate or autointegrate”

Integrate manually = “options/manual integration”, then you can integrate by using the right key on the mouse (click and drag)

Integration parameters = “Chromatogram/Integrate Parameters”

Integrate smaller peaks = “Chromatogram/Integration Parameters”, change the threshold to lower number
ENTER “Chromatogram/Integrate”

Integration report = “Chromatogram/Integration results”

Magnify = Left click, drag and view

Mass spectra of the peak = Right double click (if it does not work, go to Tools/options and make sure that the manual integration is off)

Select library = “Spectrum/select library” type in “?” select NIST98.L (or 2005)

Search spectra in the library = Right double click on the spectra

Subtract spectra = right double click on the peak, right double click on the background “Spectrum/subtract”

View extracted ions overlaid (on/off) = “Chromatogram/Display ion chromatograms in merged format”

Zoom = left drag over the area to be zoomed

Zoom out = left double click

3. Instructional Videos - Tutorials

Viewing Data in MSD ChemStation

- Transferring GC-MS data to another computer <https://youtu.be/Odmnqid1hpl>
Fine name: Data_Storing_Transfer.mp4
- Chromatograms and mass spectra: <http://youtu.be/yZ6mkfQUgZg>
File name: Chemstation_View Data.mp4
- Basic Integration: <http://youtu.be/PoR7YhT3v4Q>
File name: ChemStation_Simple Integration in MSD Chemstation.mp4

ChemStation for Advanced Users

- Creating Quant method <https://youtu.be/ish26zecnZE>
File name: ChemStation_QMethod_Setup_Quantification method.mp4
- Creating database for peak areas in ChemStation (Custom reports) <https://youtu.be/IXGXGRJ6bMw>

MS Excel data processing

- Internal standard calibration in Excel Part 1 <http://youtu.be/bDgXHxO906g>
File Name: MS Excel_Internal_standard_calibration_in_Excel_2015-03-08.mp4
- Internal standard calibration in Excel Part 2: Charts <http://youtu.be/6W5IH25E-KY>
File Name: MS Excel_Internal_standard_calibration_in_Excel_Part2_charts_2015-03-08
- LOD and LOQs with LINEST in MS Excel <http://youtu.be/ap-Ewhseyl>
File name: MS Excel_LOD_and_LOQs_with_LINEST_2015-03-09.mp4