Homework – Internal Standard Calibration   Processing in MassHunter

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 (µg/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	$C_7H_8O_2$	0.064	0.320	1.60	7.99	40.0	200
Vanillic acid	$C_8H_8O_4$	0.064	0.319	1.60	7.99	39.9	200
1,2-Dimethoxy-4-[(2-methoxyphenoxy) methyl]benzene	$C_{16}H_{18}O_4$	0.064	0.320	1.60	8.00	40.0	200
Bicreosol	$C_{16}H_{18}O_4$	0.064	0.320	1.60	8.00	40.0	200
o-Terphenyl (IS)	$C_{18}H_{14}$	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 MassHunter software for two target compounds of your choice and internal standard.

- Generate main calibration parameters, slope, intercepts, s_y, R^2, and 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 needs be processed in the computer lab on Windows-based computers. Details of how to work with MassHunter 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 may copied as analysis files from the http://arts-sciences.und.edu/chemistry/kubatova-research-group/chrom_ms02.cfm.   To open files in the MassHunter software, Open the MassHunter "Qualitative software"   located on the desktop in the computer lab.

  To view the data file, go to "File\open data file", find the directory to which you have saved the data, and open the file. The commands for working with the data are listed in Section 2 or follow videos in section 3.

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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 MassHunter*)

- ✓ Extract the selected ions for both analytes and internal standard (Chromatogram/Extract Ions)

- ✓ Ensure that you use the MassHunter Integrator (*Chromatogram/Select Integrator*)

- ✓ Integrate your peaks manually or automatically (*Section 3, Viewing Data in MassHunter, Basic Integration)*

- ✓ 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 the 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. These are typically the three lowest concentrations, however, occasionally only the 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 an MS Excel spreadsheet labelled with your name. The grading will be based on the results provided for items listed below, each for 5 pt, with a total of 40 pt. You may be allowed one resubmission.

- ✓ Ion selection (explain why you selected a specific ion) and integration.

- ✓ Ensure labelling of all the variables (including specification of 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*).

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

- **Copy chromatogram of Mass spectrum to Word** = Right-click on the chart of interest and select **Copy to clipboard.** Or by clicking on the Edit tab, and then selecting **Copy to clipboard.**
- **Integrator** = Right-click on the Extracted ion Chromatogram peaks and select "**integrate chromatograms**"
- **Extract ions from a sample** = Right-clicking on the chromatogram and then selecting **Extract Chromatograms→Type→EIC,** then type in the ions based on the mass spectra.
- **Integrate** = Right-click on the Extracted ion Chromatogram peaks and select "**integrate chromatograms**"
- **Integration list**= Click on this icon
- **Magnify** = Right click, drag.
- **Mass spectra of the peak** = Left double-click
- **Select library** = **Method Editor** menu in the **workflow tab,** select **options and check identify by library.**
- **Search spectra in the library** = Right-click on the spectra and then select search library
- **Subtract spectra** = **Data Navigator → right-click on mass spectrum → subtract any chromatogram**
- **View extracted ions overlaid (on/off)** =
- **Zoom =** Right- drag over the area to be zoomed
- **Zoom out** = Click on

3.   Instructional Videos - Tutorials

Viewing Data in Mass Hunter

https://youtu.be/nAeURNPKzmo Navigating MassHunter Qual 10

https://youtu.be/QlnOZztk6No Viewing data in MassHunter (scaling and zoom)

https://youtu.be/_20GFh-p_l4 Identification in GC-MS analysis

https://youtu.be/Xg_h1gIp6FY General peak integration (TIC)

https://youtu.be/otz-L9e5rco Quantification using extracted ion current (EIC) chromatograms.

MassHunter for Advanced Users

- **There is a separate instruction for advanced user guide for MassHunter "Quantitative analysis"**

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/6W5lHz5E-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