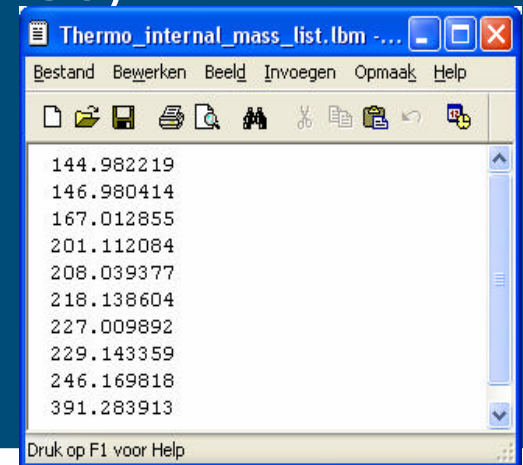


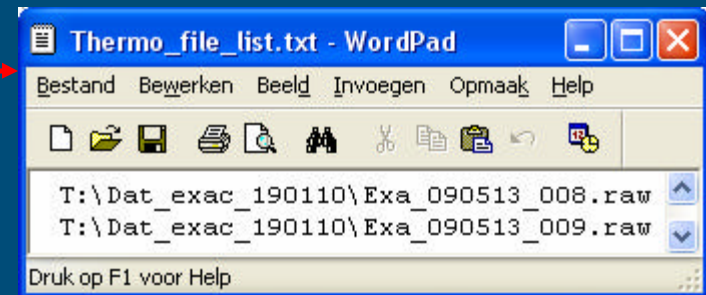
Subppmconverter method: see also Search_LCMS.ppt and Subppmconverter.ppt!

- Start by looking for background masses in eluents which are plausible for internal lock mass. Average each mass over multiple scans in the same file. Check that the intensity is mostly above 20000 (ca. 5x noise).
- Make a list in wordpad and call the file “Thermo_internal_mass_list.lbm”



Example experiment used:

- Sample A: containing ca. 220 known reference compounds. This sample is used for external mass correction/calibration.
- Sample B: preprocessed and concentrated urine sample.
- Injection order:
 - 1 injection of sample A
 - 1 injection of sample B



Go to Subppm_converter.exe

The screenshot shows the 'Thermo_subppm_converter' application window. It has several sections for data processing:

- List of Thermo Files:** Includes a 'Show List of Files to Convert' button and a 'Select' button. A red arrow points to the 'Select' button.
- INTENSITY RELATED MASS CORRECTION:** Contains the formula $PPM\ ERROR = A \times (10\log\ Intensity) + C$. It has input fields for 'Slope A:' (set to 0) and 'Constant C:' (set to 0). Red arrows point to both of these fields.
- INTERNAL MASS CORRECTION:** Includes an 'Extract scans with scan filter as scan:' field (set to 1) and a 'Minimum Amplitude of Background Masses to Use:' field (set to 20000). A red arrow points to the '1' in the scan filter field. Another red arrow points to the '20000' in the amplitude field.
- List of Background Masses:** Shows a text box with 'T:\Thermo_internal_mass_list.lbm', a 'Select' button, and an 'Edit' button.
- Convert:** A large button in the center of the window. A red arrow points to it.
- EXTERNAL MASS CORRECTION:** Includes a '.csv_polyfit file:' text box, a 'Select' button, and another 'Convert' button.
- Exit:** A button at the bottom left.

■ Select raw data sets (data in previous slide)

■ Intensity dependant mass correction: set to zero!

■ Put in the scan filter to use; check original data for this.

■ Set a minimum threshold value for internal lock masses; minimum threshold = ca. 6x noise.

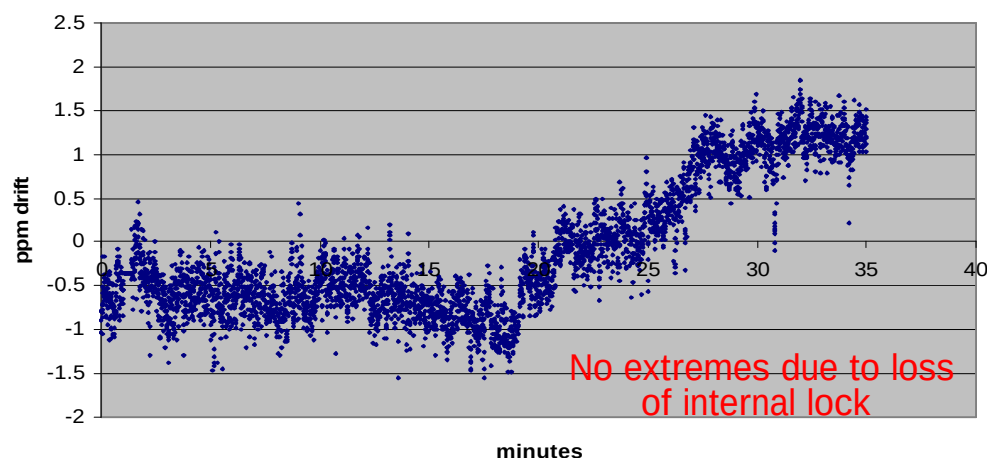
■ Note : profile raw data become centroid netCDF data; correction for internal masses

■ Note : profile raw data and centroid netCDF data are in the same folder.

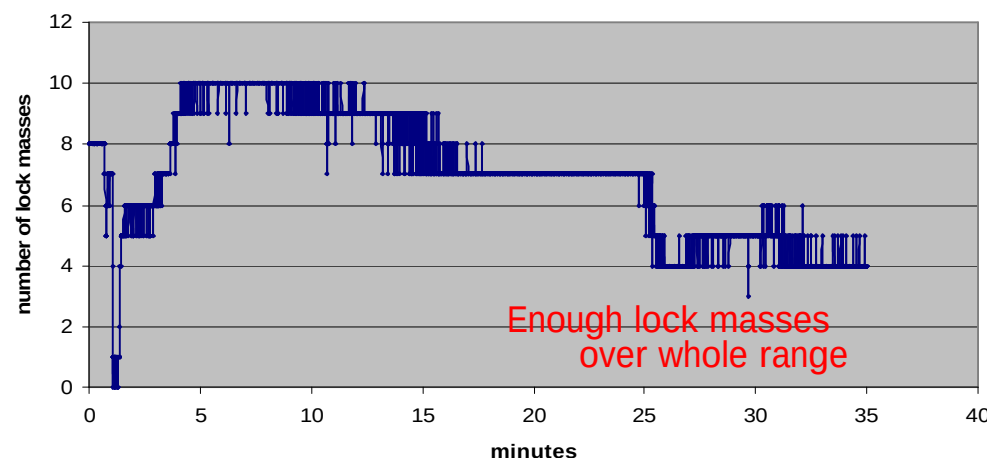
Perform conversion and use the output netCDF in metAlign.

Evaluate multiple internal lock correction: Besides netCDF output there is a csv output: create evaluation graphs in Excel

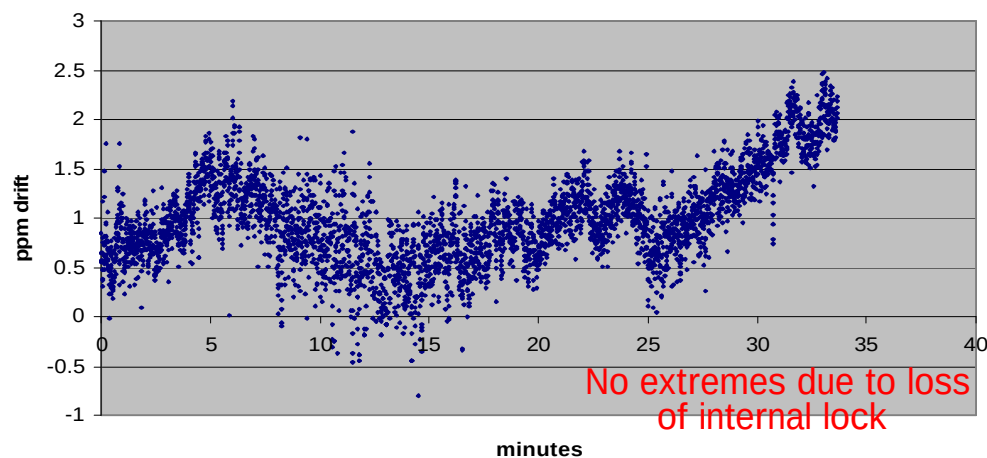
ppm drift vs. time for sample A



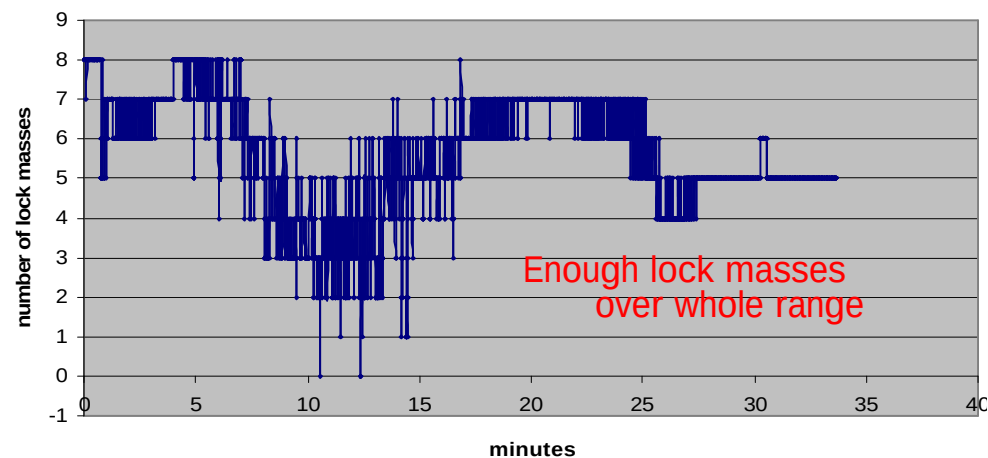
number of lock masses vs. retention for sample A



ppm drift vs. time for sample B



number of lock masses vs. retention for sample B



Use metAlign to produce redms_acc files from the sample A data.

- Only part A is needed
- Only sample A data (1 files)
- Run Button 11

Metalign = RIKILT program for full scan MS comparison; Free Permanent License Version 230110

PART A: PROGRAM CONFIGURATION, DATA SET SELECTION AND BASELINE CORRECTION

1A. Program configuration

1B. Mass Resolution/Bin

Accurate Mass Mode

Mass Res = 50000

SELECT INPUT DATA SETS

2A. Group 1: List of Data Sets

2B. Select

Clear

and / or

3A. Group 2: List of Data Sets

3B. Select

Clear

BASELINE AND NOISE ELIMINATION PARAMETERS

4. Retention Begin (Scan nr) 1

5. Retention End (Scan nr) 10000

6. Maximum Amplitude 1000000000

7. Peak Slope Factor (x Noise) 1

8A. Peak Threshold Factor (x Noise) 2

8B. Peak Threshold (Abs. Value) 3000

9. Average Peak Width at Half Height (Scans) 5

10. Keep Peak Shape (no alignment) ☐

11. Run Baseline Correction

Button 1A

Configuration

Start from a Previous MetAlign Session

Import Parameters, Configuration and/or Data from a Previous Session

Browse

Definition of Folders

Final Results Folder T:\Ex_190110\metalign_220909_fp20000_all_lock\Default_ Browse

Original Data Folder T:\Ex_190110\metalign_220909_fp20000_all_lock\Default_ Browse

Temporary Folder T:\Ex_190110\metalign_220909_fp20000_all_lock\Default_ Browse

Data Format and Function Selection

INPUT FORMAT

MassLynx Dbridge

NetCDF

HP/Agilent Chemstation

Xcalibur Xconvert

INPUT FUNCTION SELECTION

Total number of functions: 1

Function number to be used: 1

SCAN FILTER OF 1ST DATASET

Use filter of scan:

OUTPUT FORMAT

MassLynx

NetCDF

HP/Agilent Chemstation

Xcalibur

Cancel Save

Button 1B

Accurate or Nominal?

SELECT DATA TYPE

Accurate mass data

Mass Resolution is Defined as Mass Divided by the Mass Width at Half Height

Mass Resolution: 50000

WARNING: Wrong mass resolution leads to peak loss!

Amplitude range for accurate mass determination:

From 20000 to 999999999

TOR without DRE (saturation effects on mass) ☐

Suppression

Interval around mass peak: 0.01

Percentage of amplitude of mass peak: 5

Pre-suppression

Interval around mass peak: 0.5

Percentage of amplitude of mass peak: 3

Interval offset from mass peak: 0

Set to 99900000 if the mass should be taken from the apex.

Button 3A

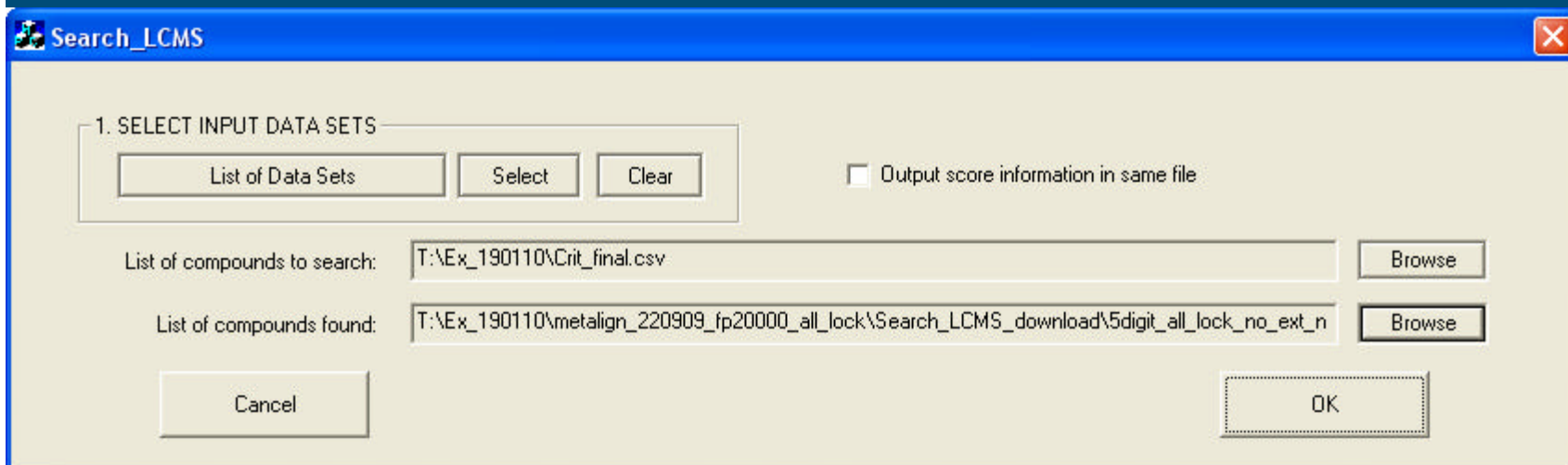
list_group1.txt - WordPad

Bestand Bewerken Beeld Invoegen Opmaak Help

T:\Dat_exac_190110\Intern_all_lock_20000\Exa_090513_008.cdf

Druk op F1 voor Help

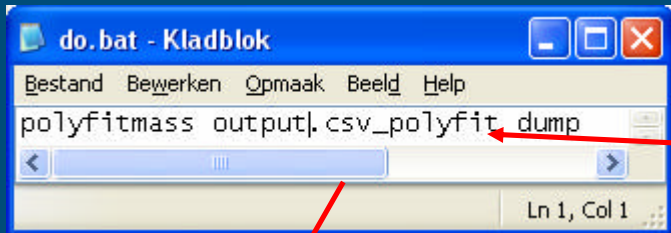
Use Search_LCMS to collect data on reference compounds in sample A and to make a file for external calibration.



- See Search_LCMS.ppt for instructions;
- Redms_acc file input are from the baseline folder from metalign output

Note : Crit_final.csv (search criteria) is easily made using large retention windows and copying back from first search output.

In Search_LCMS folder: Use polyfitmass and the Search_LCMS file ,“output.csv_polyfit”, to obtain polyfit_curve.csv.



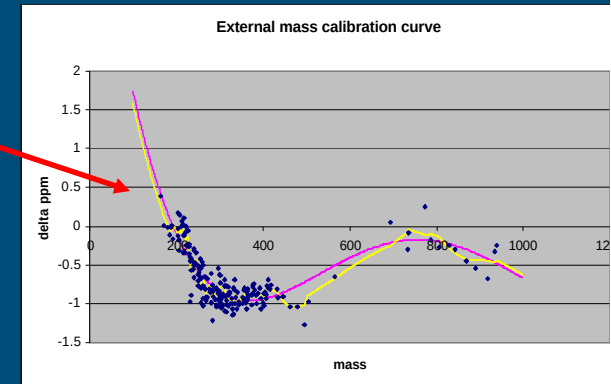
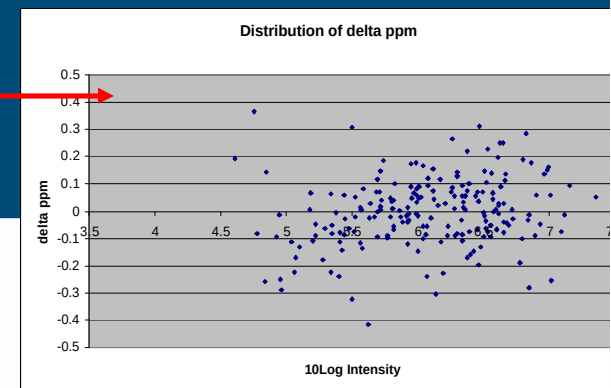
■ Make a small batch file as shown and execute this by double click.

■ Polyfit_curve.csv is created

■ Use excel to create a calibration graph

■ Top: Column A and C: blue
■ Bottom: Col A and B : pink
■ Bottom: Col A and C : yellow

■ Top: Log (Col B) and Col E: blue



Note : Bottom Col C is used for external calibration of raw data

Go to Subppm_converter.exe

Thermo_subppm_converter

List of Thermo Files :

INTENSITY RELATED MASS CORRECTION: $PPM\ ERROR = A \times (10\log Intensity) + C$

Slope A: Constant C:

INTERNAL MASS CORRECTION

Extract scans with scan filter as scan: Minimum Amplitude of Background Masses to Use:

List of Background Masses:

EXTERNAL MASS CORRECTION

.csv_polyfit file:

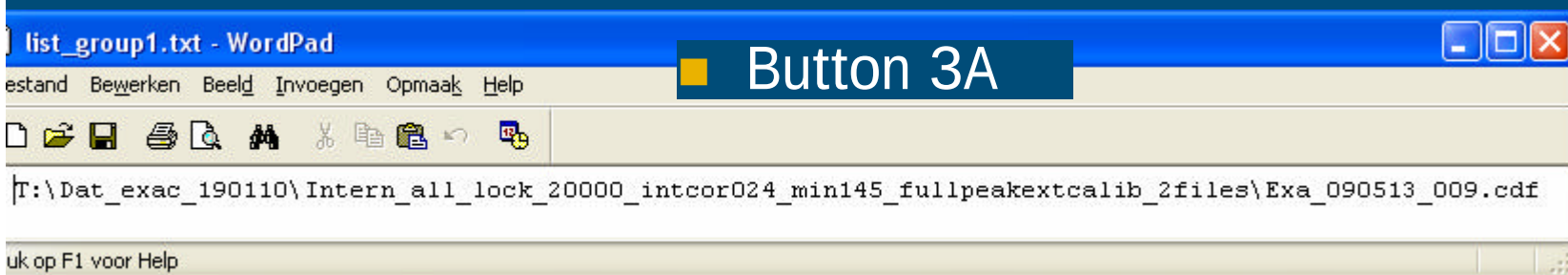
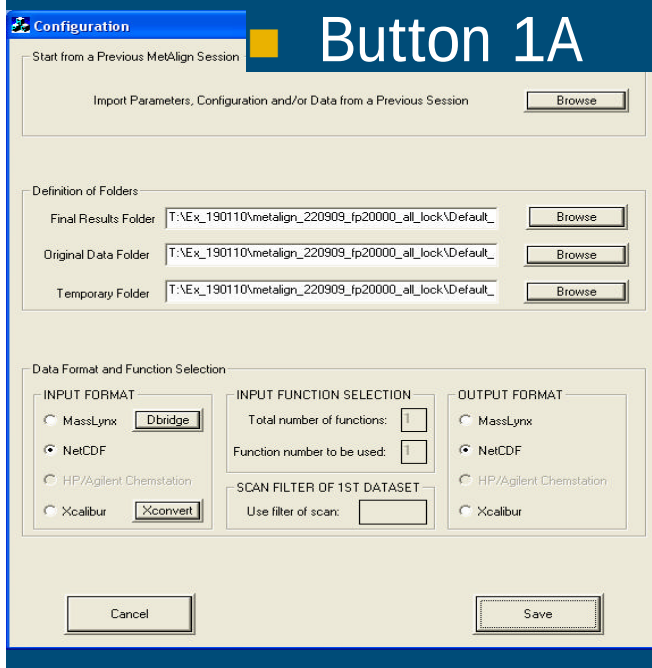
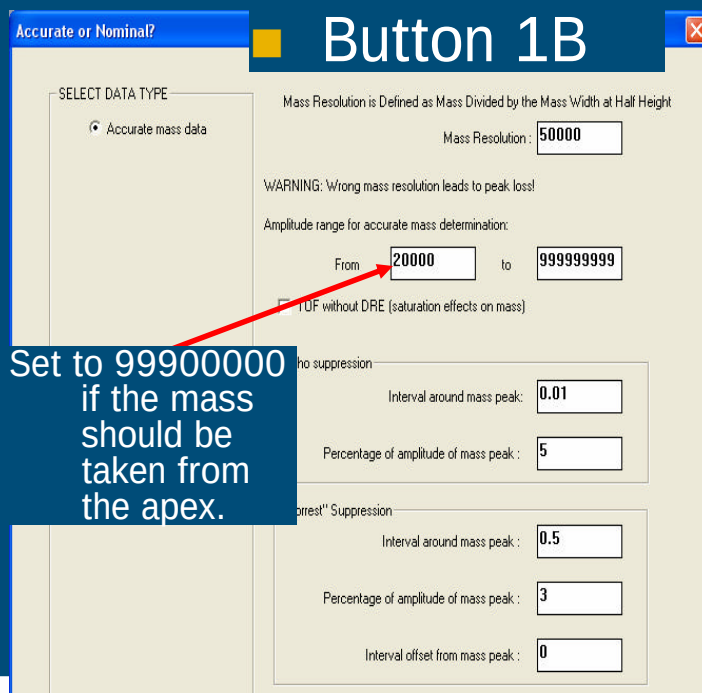
- See slide 2 and 3 (use all files)
- Include a “.csv_polyfit” file for external calibration (from the Search_LCMS folder)

- Note : profile raw data become centroid netCDF data; correction for internal and external masses.

Perform conversion and use the output netCDF in metAlign.



Use metAlign to produce the redms_acc file for the sample B data



Only sample B data (1 file)

Use metAlign to produce the redms_acc file for the sample B data

Metalign = RIKILT program for full scan MS comparison; Free Permanent License Version 230110

PART A: PROGRAM CONFIGURATION, DATA SET SELECTION AND BASELINE CORRECTION

1A. Program configuration

1B. Mass Resolution/Bin

Accurate Mass Mode

Mass Res = 50000

SELECT INPUT DATA SETS

2A. Group 1: List of Data Sets

2B. Select

Clear

and / or

3A. Group 2: List of Data Sets

3B. Select

Clear

BASELINE AND NOISE ELIMINATION PARAMETERS

4. Retention Begin (Scan nr) 1

5. Retention End (Scan nr) 10000

6. Maximum Amplitude 1000000000

7. Peak Slope Factor (x Noise) 1

8A. Peak Threshold Factor (x Noise) 2

8B. Peak Threshold (Abs. Value) 3000

9. Average Peak Width at Half Height (Scans) 5

☐ 10. Keep Peak Shape (no alignment)

11. Run Baseline Correction

PART B: SCALING AND ALIGNING DATA SETS

12. SCALING OPTIONS

☒ No Scaling

☐ Autoscaling on Total Signal

☐ Scale on Marker Peak

From 1st Data Set, Group 1:

Mass 100

Scan Nr. 200

13. INITIAL PEAK SEARCH CRITERIA

Scan Nr. Max. Shift

Begin of 1st Region 499 60

End of 1st Region 30000 60

Begin of 2nd Region 0 0

End of 2nd Region 0 0

14. TUNING ALIGNMENT OPTIONS AND CRITERIA

☐ No Pre-align Processing (Rough)

☒ Pre-align Processing (Iterative)

Calculation Criteria for Chromatography Shift Profiles

15. Maximum Shift per 100 Scans 35

Mass Peak Selection 1st Iteration Last Iteration

16. Min. Factor (x Noise) 3 2

17. Min. Nr. of Masses 4 3

SELECT MIN. NR. PER PEAK SET

18. Group 1: 0 max.=1

or

19. Group 2: 0 max.=0

20. Run Scaling and Alignment

21. Detailed Ascii Output
Excel Compatible Output
Differential Retention Display

PART C: PEAK SELECTION AND EXPORT TO MS SOFTWARE FORMAT FOR VISUALISATION

PEAK SELECTION CRITERIA

22. Significance Percentage 95.00000

23. Minimum Ratio between Means 2

24. Minimum S/N Ratio 2

☐ 25. Either in Gr. 1 or Gr. 2: >= 1 Masses/Compound

26. FILTER ON CONDITION

☐ Group 2 > Group 1

☒ Group 1 > Group 2

27. Run Peak Selection

28. Detailed Ascii Output
Excel Compatible Output
Masslynx Include List Output

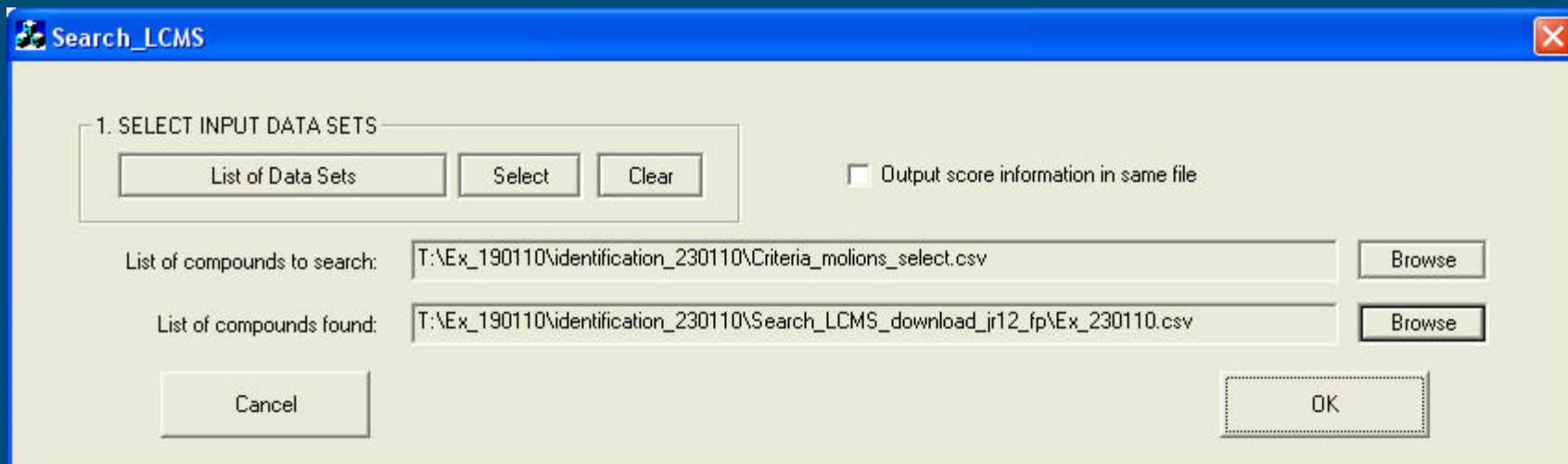
TOTAL PROCESSING

29. Total Processing

30. Save and Exit

■ Run button 11 to do PART A

The “sample_B.redms_acc” can now be searched by the Search_LCMS module for compounds of interest.



- See Search_LCMS.ppt for instructions;
- Redms_acc file input is from the baseline folder of the metalign output

Intensity-related mass correction? Is this necessary?

- See slides 14,15 and 16 in
Subppmconverter_multiple_file_averaging_method.ppt

redo this procedure from the start using an intensity correction of the mass

The screenshot shows the 'Thermo_subppm_converter' window. It has several sections: 'List of Thermo Files' with a 'Show List of Files to Convert' button and a 'Select' button; 'INTENSITY RELATED MASS CORRECTION' with a formula $PPM\ ERROR = A \times (10\log Intensity) + C$, 'Slope A' set to 0.24, and 'Constant C' set to -1.45; 'INTERNAL MASS CORRECTION' with 'Extract scans with scan filter as scan' set to 1, 'Minimum Amplitude of Background Masses to Use' set to 20000, a file path 'T:\Thermo_internal_mass_list.lbm', and 'Select' and 'Edit' buttons; a 'Convert' button; and 'EXTERNAL MASS CORRECTION' with a '.csv_polyfit file' field, a 'Select' button, and another 'Convert' button. An 'Exit' button is at the bottom left. Red arrows point from the text on the right to the 'Select' button, 'Slope A' field, 'Constant C' field, 'Minimum Amplitude' field, and the 'Convert' button in the 'INTERNAL MASS CORRECTION' section.

- Select raw data sets (data in slide 2)
- Intensity dependant mass correction: set to 0.24 and -1.45 (see previous slide)!
- Put in the scan filter to use; check original data for this.
- Set a minimum threshold value for internal lock masses; minimum threshold = ca. 6x noise.

- Note : profile raw data become centroid netCDF data; correction for internal masses

Perform conversion and use the output netCDF in metAlign.

Continue with slide 4 again!



Continue with slide 4.

