MS-ESQUIRE-A: Bruker Esquire 3000+ Ion Trap MS

This instrument is based on an Agilent 1200 HPLC connected via an ESI-interface to an ion-trap type of mass spectrometer. The ion-trap collects ions and keeps them for a short period in a circular path. By varying the radiofrequency field of the trap, ions can be further fragmented and the weight of the fragments can be determined by a scanning process.

The instrument is suitable both for peptides and small molecules, and can be made to do MS^n-analysis (i.e., select fragments for further fragmentation), to give more information about the structure. Typically, it will give an accurate mass with one decimal.

This document can be downloaded from
http://drug.ku.dk/research/facilities/analytical-core-facility/ms-esquire-a/.

Contents

Prepare the sample(s)

Dissolve the sample in a suitable solvent

The compounds should be dissolved completely. Neat acetonitrile or methanol is ok as long as small volumes are injected. Best is to use a 50:50-mixture of acetonitrile (or methanol) and water. Any solid particles must be filtered away (use 22 µm nylon filters).

Suitable amounts and concentration

Keep the concentration below or at 1 mg/mL. The instrument is sensitive, so it is better to start with small amounts than overloading the electrospray interface and detector. Salty samples should be avoided, and the total concentration (including salts and buffers) must be less than 20 mM.

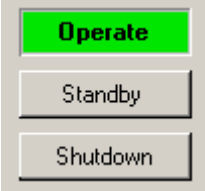
If you want to analyze a reaction mixture it is enough with ~10 µL (use an open ended capillary tube) and adding this to ~1 mL of solvent in an HPLC-vial.

Vials

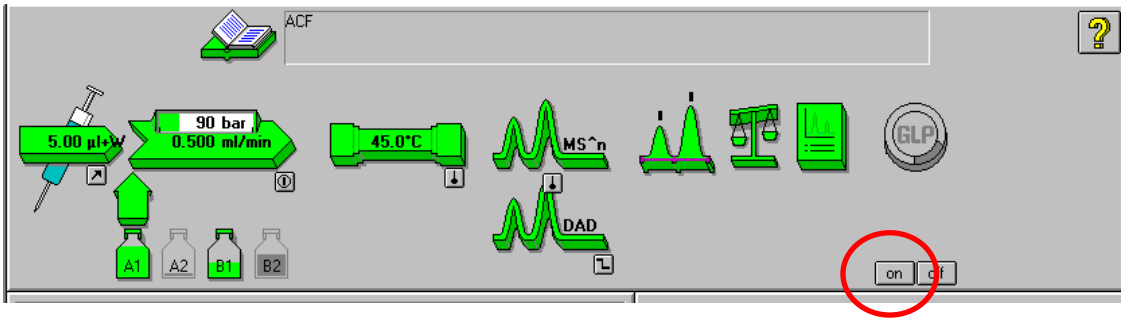
Use vials of suitable size and volume. The auto sampler can handle vials without caps or caps with septum.

Check system

- Esquire MS-control screen on the right screen should be running. Click on “Operate” (should turn green).



- The `Chemstation` program should be running on the left screen and all units should be green[1]. The instrument might be switched off (gray) and in that case press the small on-button below the schematic figure on the screen.



[1] Except the MS-unit. It might be gray if a shutdown method is loaded.

Check solvents

Check solvent bottles on top of the instrument. Analyses will automatically stop if the volume in the solvent bottles falls below 100 mL. Solvents are filled up by a SuperUser (see list of users by the instrument).

- Solvent A: 95% MilliQ-water + 5% MeCN. 0.1% formic acid.
- Solvent B: 5% MilliQ-water + 95% MeCN. 0.1% formic acid.

Check the waste bottles on the floor under the instrument. Make sure the waste lines are secured in the waste bottles.

Place your sample in a free position.

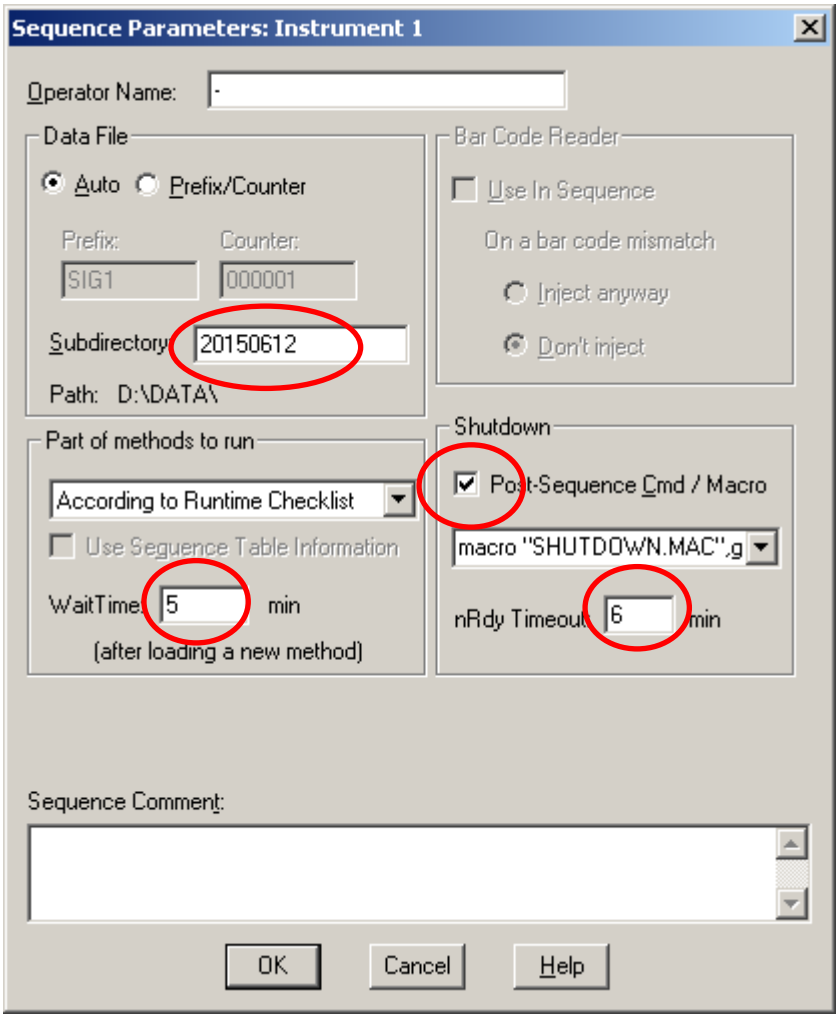
The **front plate** (P1) is for samples you don't want to keep. Sample vials are removed (put into waste bin by the instrument) when tray is full or at least once a week.

The **back plate** (P2) is for standard samples and for samples you want to keep. Mark sample with initials (according to the list by the instrument). Unmarked samples will be removed.

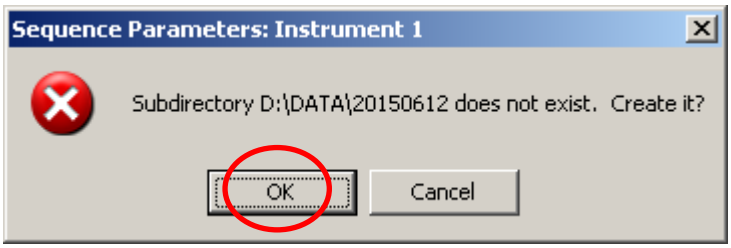
Positions are designated by a plate number, a row (letters A-F) and a column number as in the following examples: `P1-A-01`, `P2-F-12`. Use shorthand notation when entering positions in the sequence list – '`p1a1`' will be automatically expanded to '`P1-A-01`'.

Enter sequence parameters

- Click in the left hand panel (or use the menu ‘Sequence’) to bring up the sequence parameters. Enter the current date as ‘Subdirectory’ (YYYYMMDD, e.g., 20150612). Make sure that the shutdown-macro is activated.

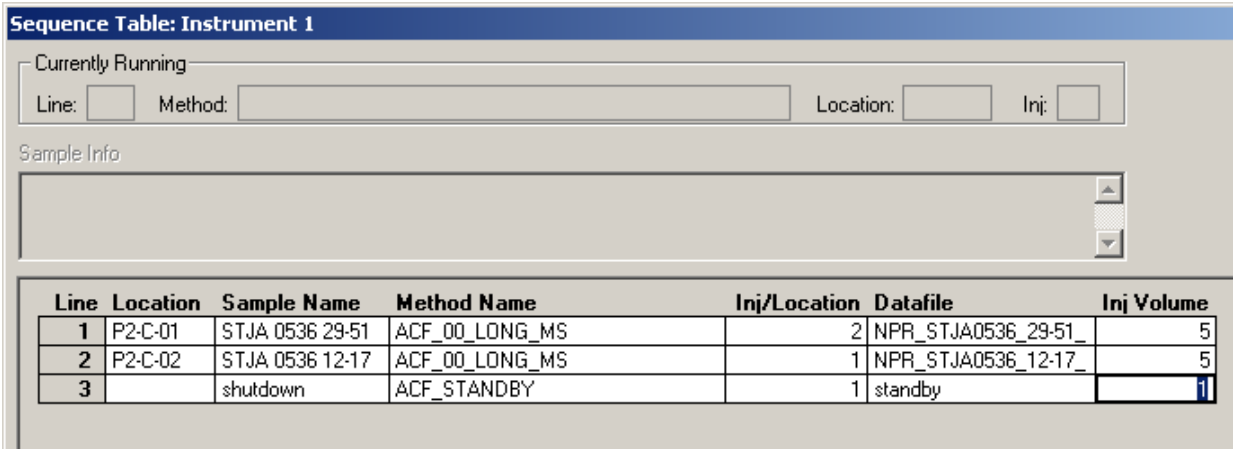


- Acknowledge the creation of the new directory.



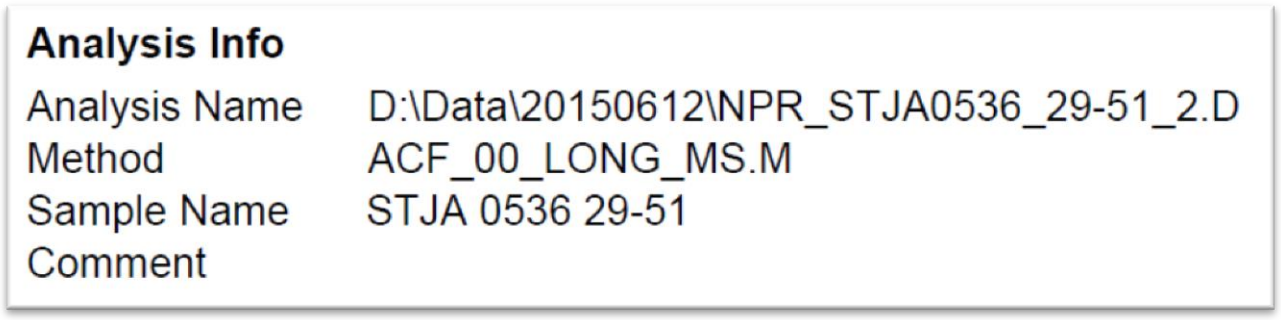
Enter sequence table

- Click in the left-hand panel and select ‘Sequence table…’.
- Enter location, sample name (identifier in printed reports), method name, number of injections and data file name.



Line	Location	Sample Name	Method Name	Inj/Location	Datafile	Inj Volume
1	P2-C-01	STJA 0536 29-51	ACF_00_LONG_MS	2	NPR_STJA0536_29-51_	5
2	P2-C-02	STJA 0536 12-17	ACF_00_LONG_MS	1	NPR_STJA0536_12-17_	5
3		shutdown	ACF_STANDBY	1	standby	1

The entered information will be printed in the report header as the example shown below.



Location: Enter plate (`P1` or `P2`), row (`A-H`) and column (`1-9`). Enter as '`p2c1`' or '`P1-C-01`'. Leave empty for no injection (blank run).

Sample name: This is a text field that will be printed on the reports.

Method name: Use a predefined method according to the table below. Start with '`ACF_40_SHORT_MS`' when in doubt.

Type of sample	Method	Description	MS
Polar compounds, many different compounds	ACF_00_LONG_MS	Starts with 0% B and increases to 100% B over 30 minutes.	Positive mode, up to 1500 m/z, optimized for 600 m/z.
Polar compounds, pure or a few different compounds.	ACF_00_SHORT_MS	Starts with 0% B and increases to 100% B over 10 minutes.	Positive mode, up to 1500 m/z, optimized for 600 m/z.
Fatty substances, complex mixtures	ACF_40_LONG_MS	Starts with 40% B and increases to 100% B over 30 minutes.	Positive mode, up to 1500 m/z, optimized for 600 m/z.
Fatty substances, relatively pure	ACF_40_SHORT_MS	Starts with 40% B and increases to 100% B over 10 minutes.	Positive mode, up to 1500 m/z, optimized for 600 m/z.
Wash	ACF_WASH_SYSTEM	15 minutes, 100% B	Flow from column to waste.
Shutdown	ACF_STANDBY	Blank injection, reduces flow to 0.05 mL/min.	No analysis, reduces gas flow to standby values.

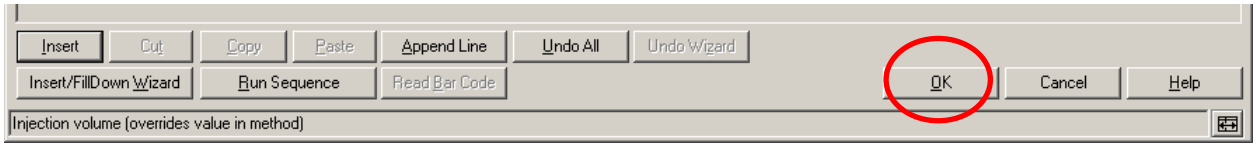
Type of sample	Method	Description	MS
Polar compounds, many different compounds	ACF_00_LONG_HIGHMS	Starts with 0% B and increases to 100% B over 30 minutes.	Positive mode, up to 2800 m/z, optimized for 1500 m/z.
Polar compounds, pure or a few different compounds.	ACF_00_SHORT_HIGHMS	Starts with 0% B and increases to 100% B over 10 minutes.	Positive mode, up to 2800 m/z, optimized for 1500 m/z
Fatty substances, complex mixtures	ACF_40_LONG_HIGHMS	Starts with 40% B and increases to 100% B over 30 minutes.	Positive mode, up to 2800 m/z, optimized for 1500 m/z.
Fatty substances, relatively pure	ACF_40_SHORT_HIGHMS	Starts with 40% B and increases to 100% B over 10 minutes.	Positive mode, up to 2800 m/z, optimized for 1500 m/z

Number of injections: Enter 1 (one) or, if it is the first analysis for the day, 2 (two).

Datafile: Enter file name. Use Group name, initials and sample identifier. Use underscores (not space) and normal characters (A-Z, a-z, 0-9, _). Note that it is possible to overwrite previous data if the datafile entries are identical to data already present in the save directory.

Injection volume: Use a small number (1 µL) if you are unsure about the concentration. Use up to 10 µL if you have very dilute samples.

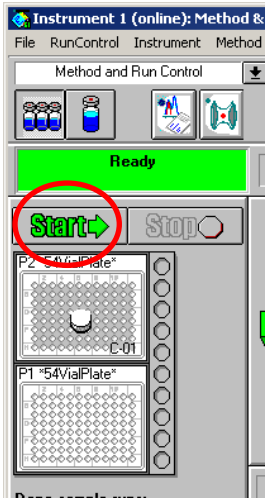
- Enter a last line according to the example to put the instrument in standby mode. You do not have to save the sequence. Just press 'Ok'.



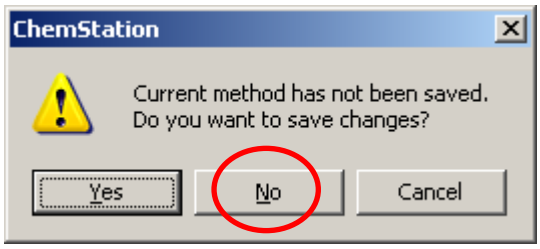
The sequence does not need to be saved, but if you want to save the sequence, use the generic file name "MS-ESQUIRE-A.S".

Start the analysis

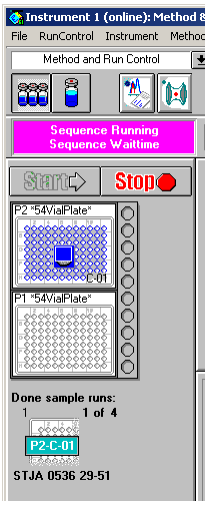
- Press the Start button on the main screen.



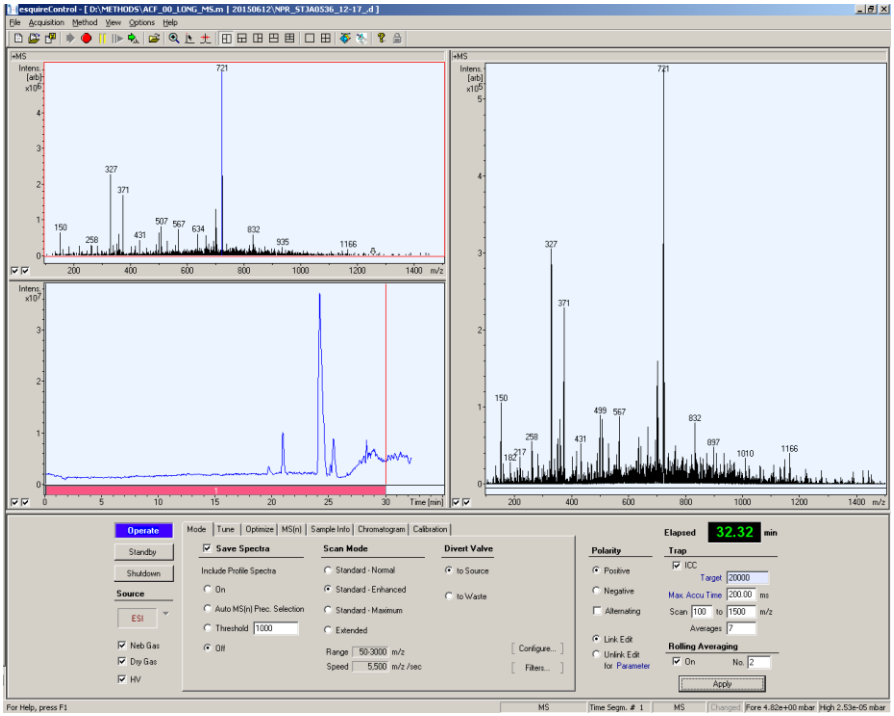
- Any question about saving the method should be answered with ‘No’.



The instrument will start with an equilibration time countdown and then start with the analysis.

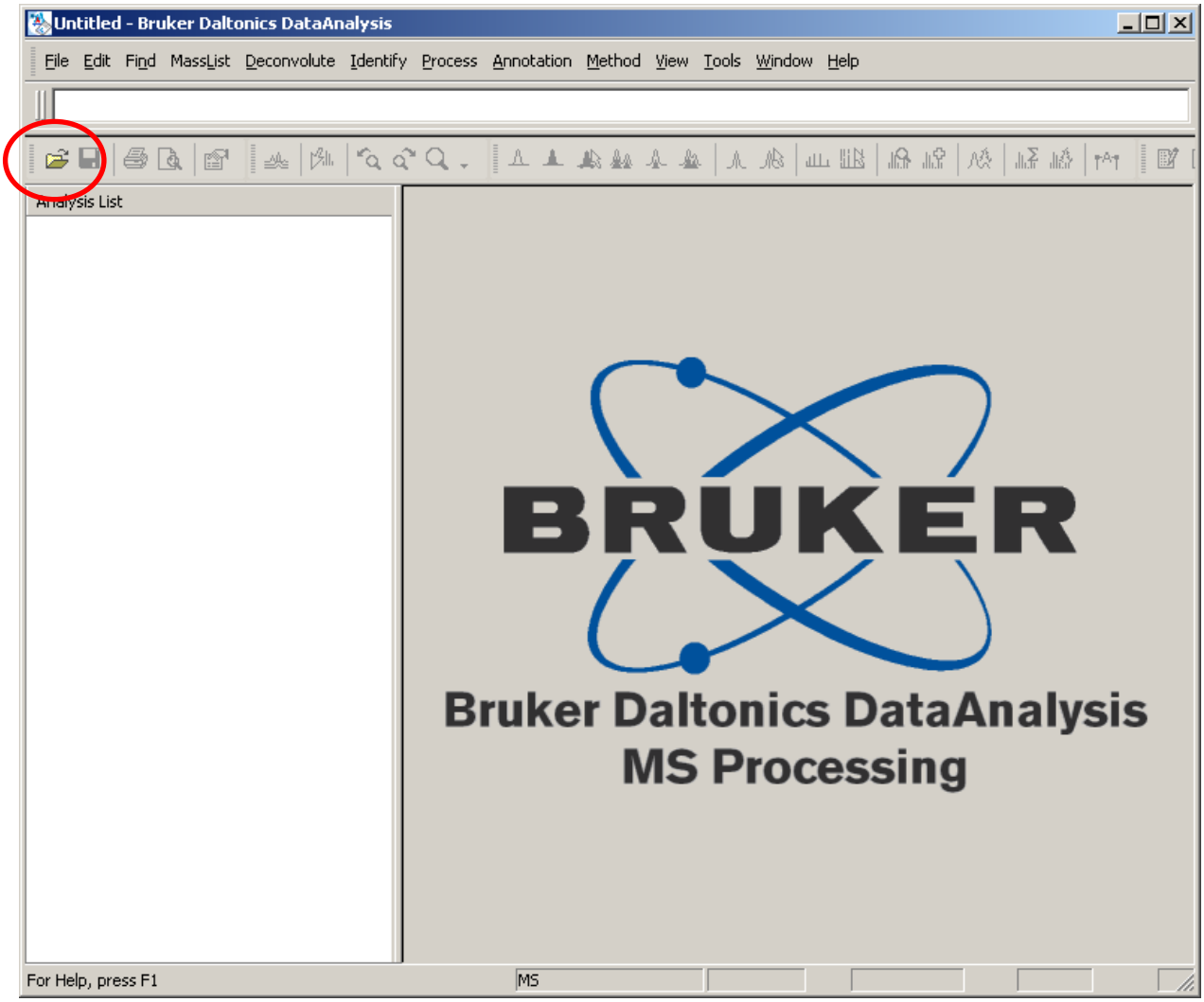


The MS-instrument (right screen) will look like below under analysis.

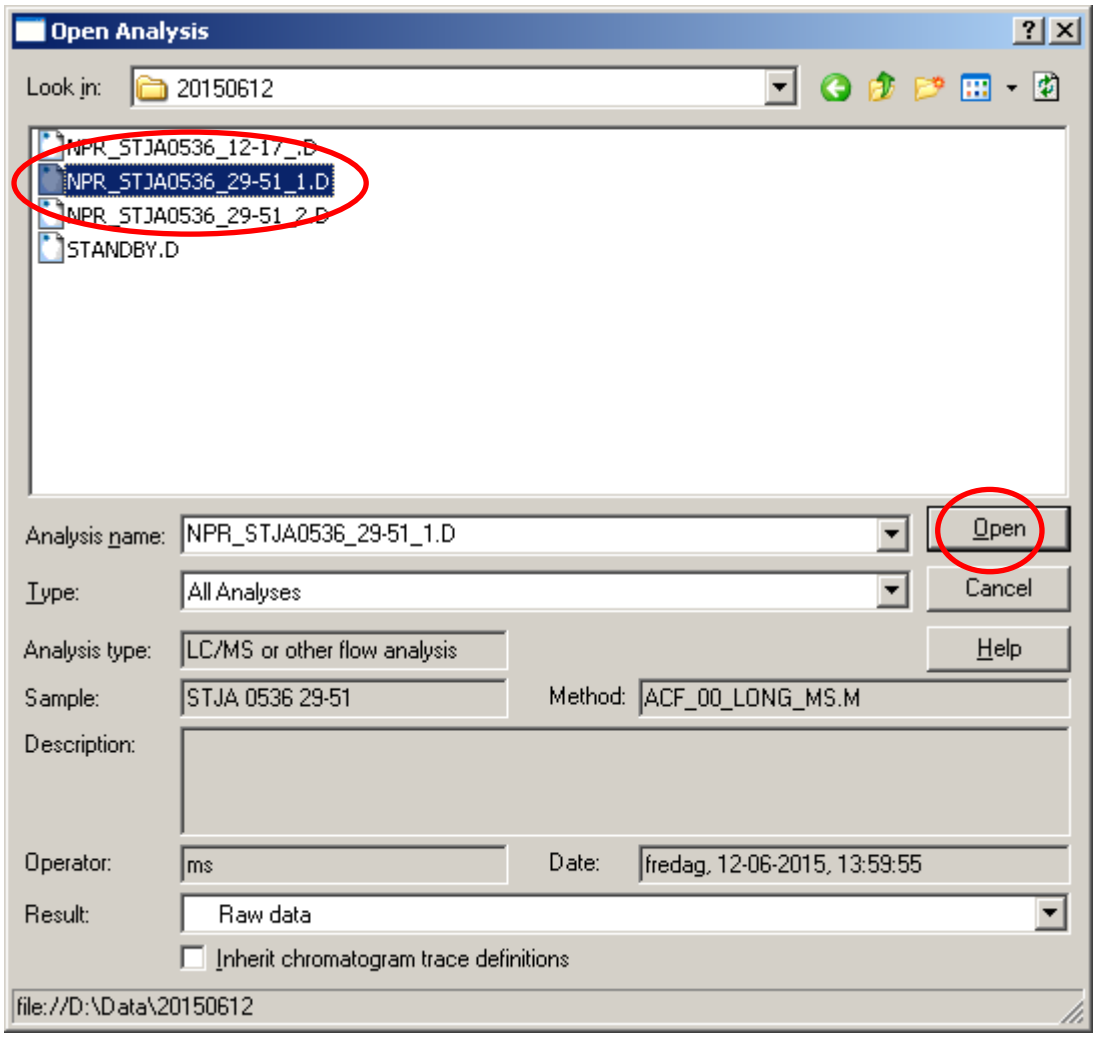


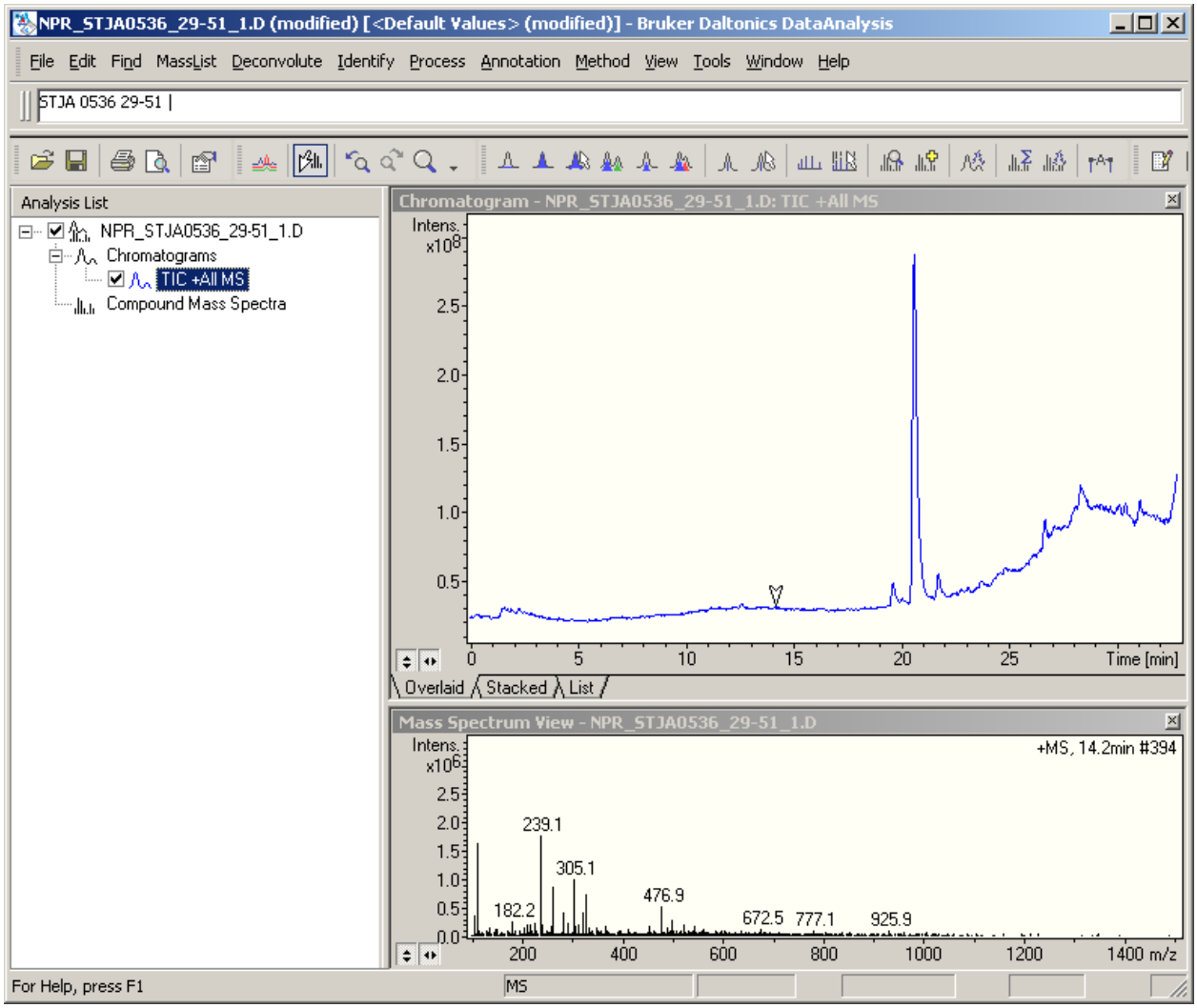
Data processing

Process the data using the software 'DataAnalysis' on the workstation. The most basic processing is shown below.



Open the data

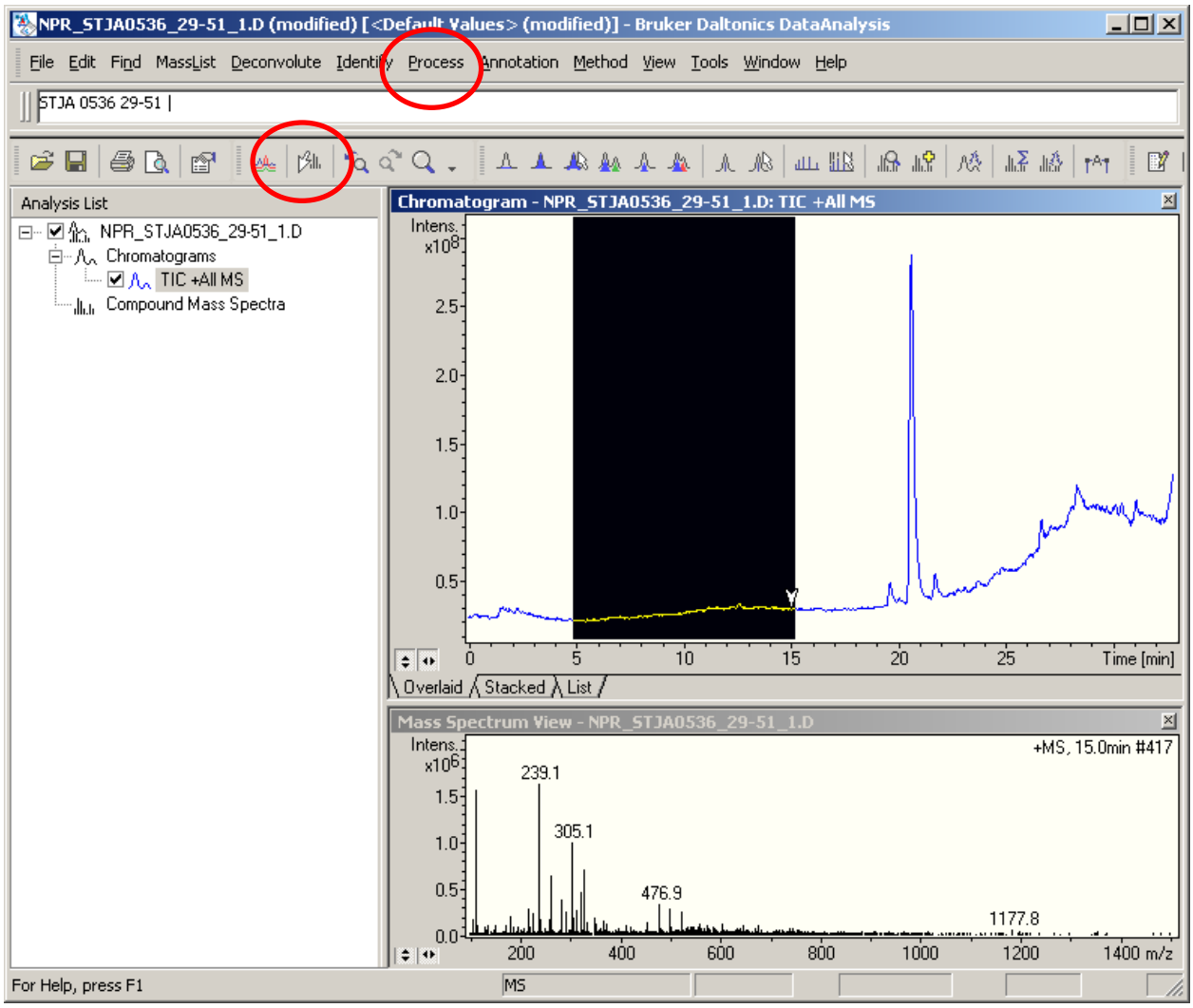



NPR_STJA0536_29-51_1.D (modified) [<Default Values> (modified)] - Bruker Daltonics DataAnalysis
File Edit Find MassList Deconvolute Identify Process Annotation Method View Tools Window Help
STJA 0536 29-51 |
Analysis List
NPR_STJA0536_29-51_1.D
Chromatograms
TIC +All MS
Compound Mass Spectra
Chromatogram - NPR_STJA0536_29-51_1.D: TIC +All MS
Intens.
x10^8
2.5
2.0
1.5
1.0
0.5
0
5
10
15
20
25
Time [min]
Overlaid Stacked List
Mass Spectrum View - NPR_STJA0536_29-51_1.D
Intens.
x10^6
+MS, 14.2min #394
239.1
305.1
476.9
182.2
672.5
777.1
925.9
200
400
600
800
1000
1200
1400 m/z
For Help, press F1
MS

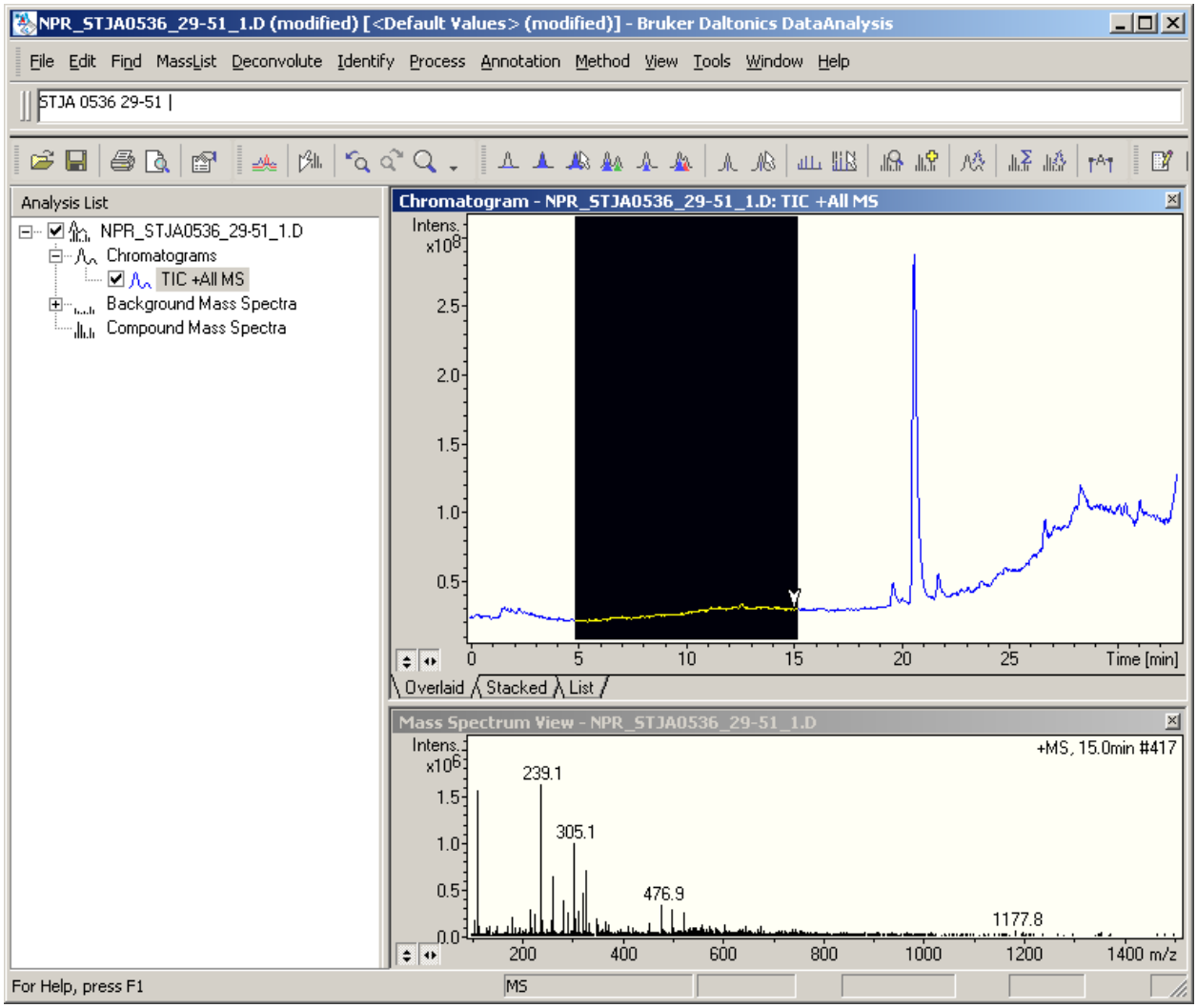
Define background spectra

This is sometimes necessary due to impurities introduced when several different researchers are using the instrument, while only a few actually cleans the interface.

Find an empty range in the chromatogram and mark this (there is a button that should be toggled to the inactive state before it is possible to mark ranges).



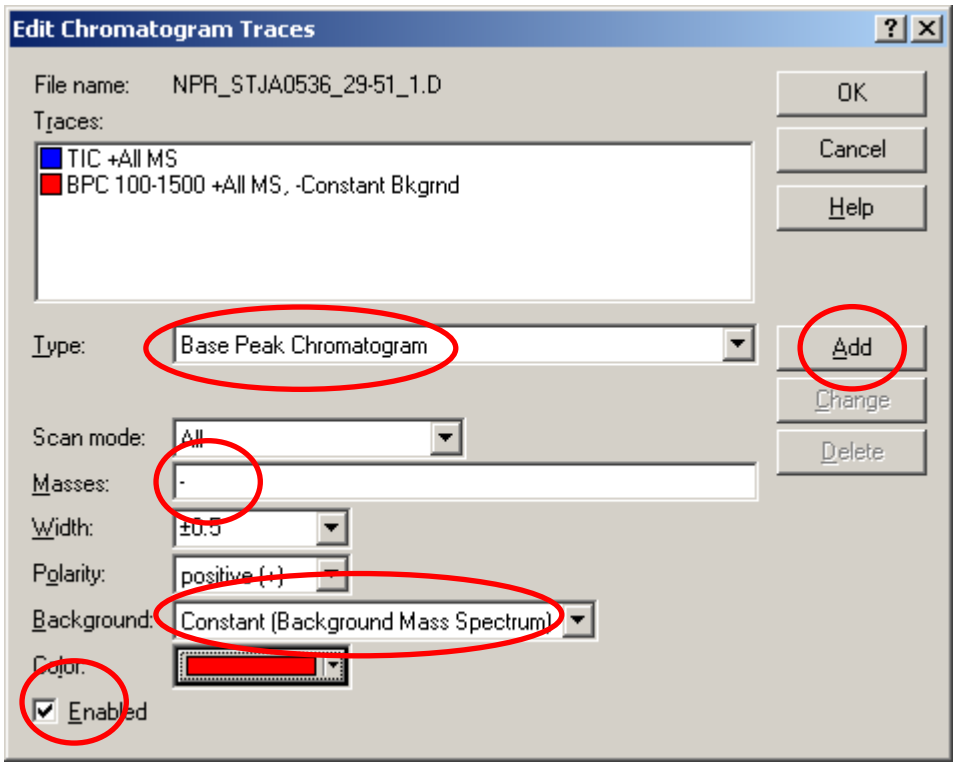
Select `Process` ⇨ `Define Background Mass Spectra.`

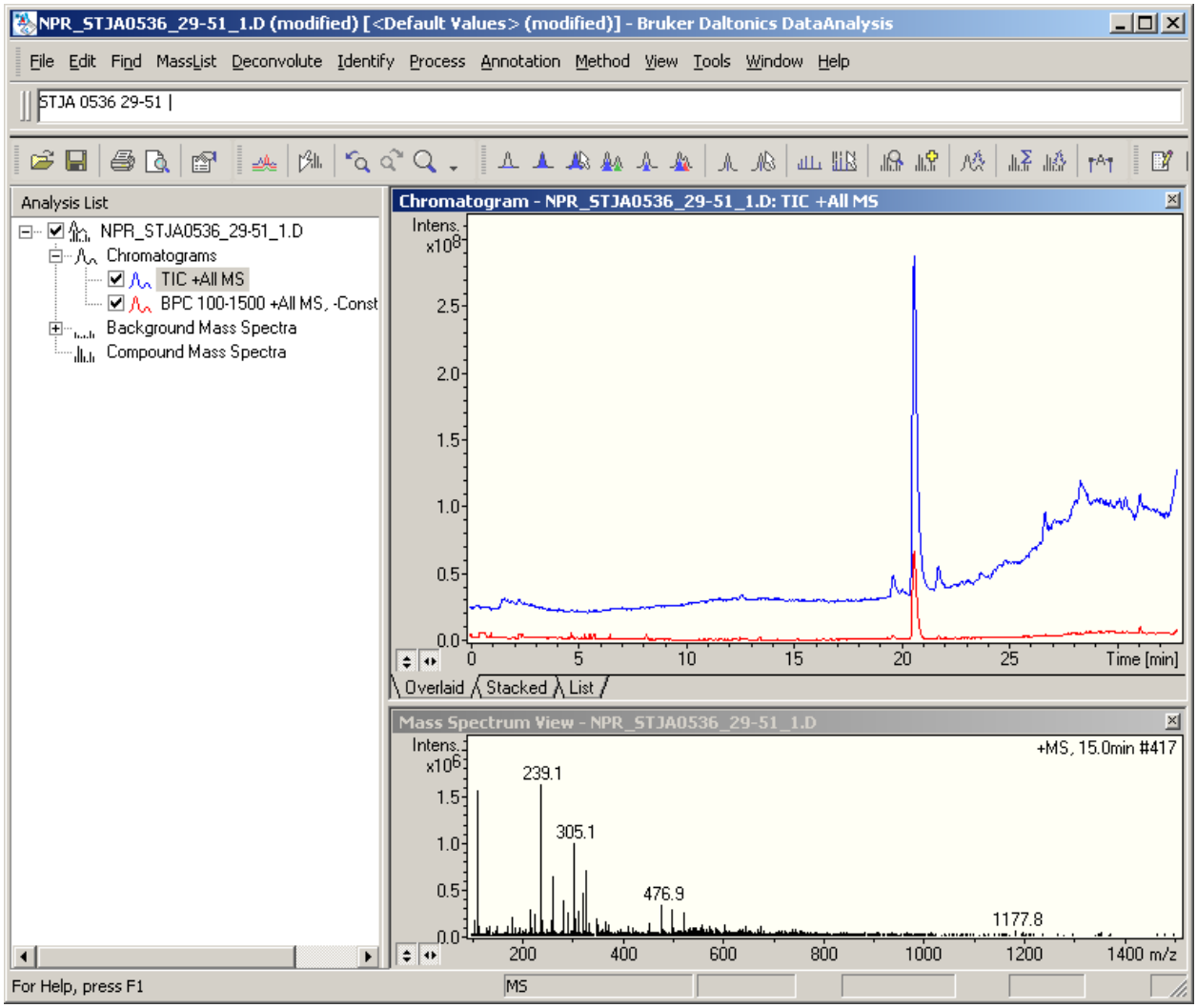

NPR_STJA0536_29-51_1.D (modified) [<Default Values> (modified)] - Bruker Daltonics DataAnalysis
File Edit Find MassList Deconvolute Identify Process Annotation Method View Tools Window Help
STJA 0536 29-51 |
Analysis List
NPR_STJA0536_29-51_1.D
Chromatograms
TIC +All MS
Background Mass Spectra
Compound Mass Spectra
Chromatogram - NPR_STJA0536_29-51_1.D: TIC +All MS
Intens.
x10^8
2.5
2.0
1.5
1.0
0.5
0
5
10
15
20
25
Time [min]
Overlaid Stacked List
Mass Spectrum View - NPR_STJA0536_29-51_1.D
Intens.
x10^6
+MS, 15.0min #417
239.1
305.1
476.9
1177.8
1.5
1.0
0.5
0.0
200
400
600
800
1000
1200
1400 m/z
For Help, press F1
MS

Define new chromatography traces

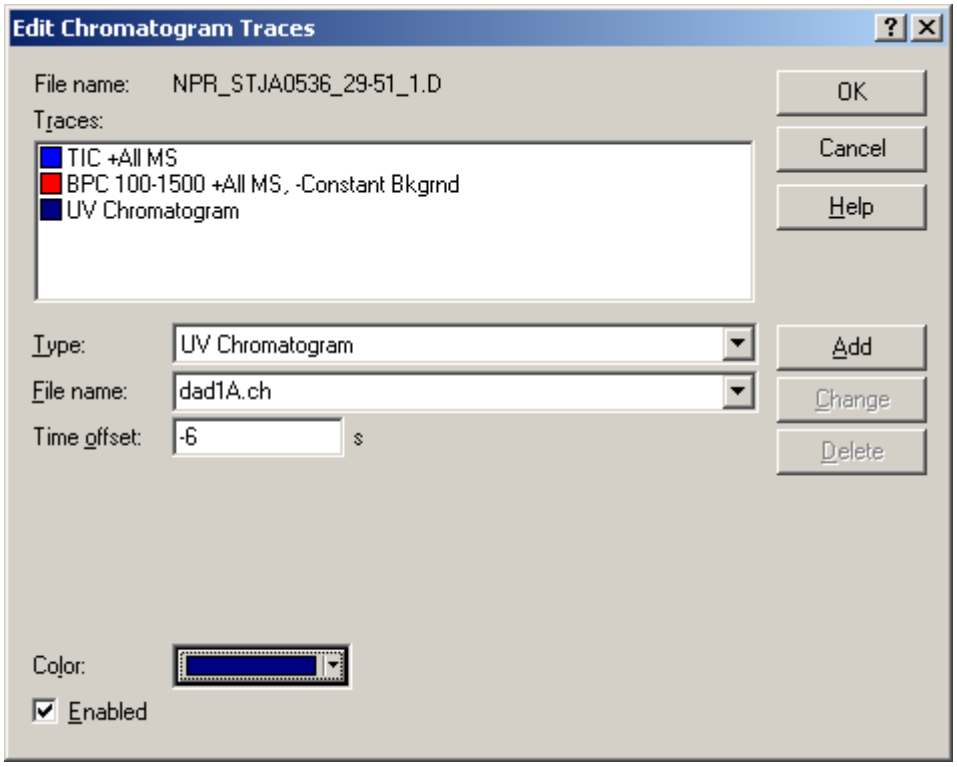
Press F7 and add a base peak chromatogram (BPC, intensity of the largest MS-signal for each time point is plotted against retention time).

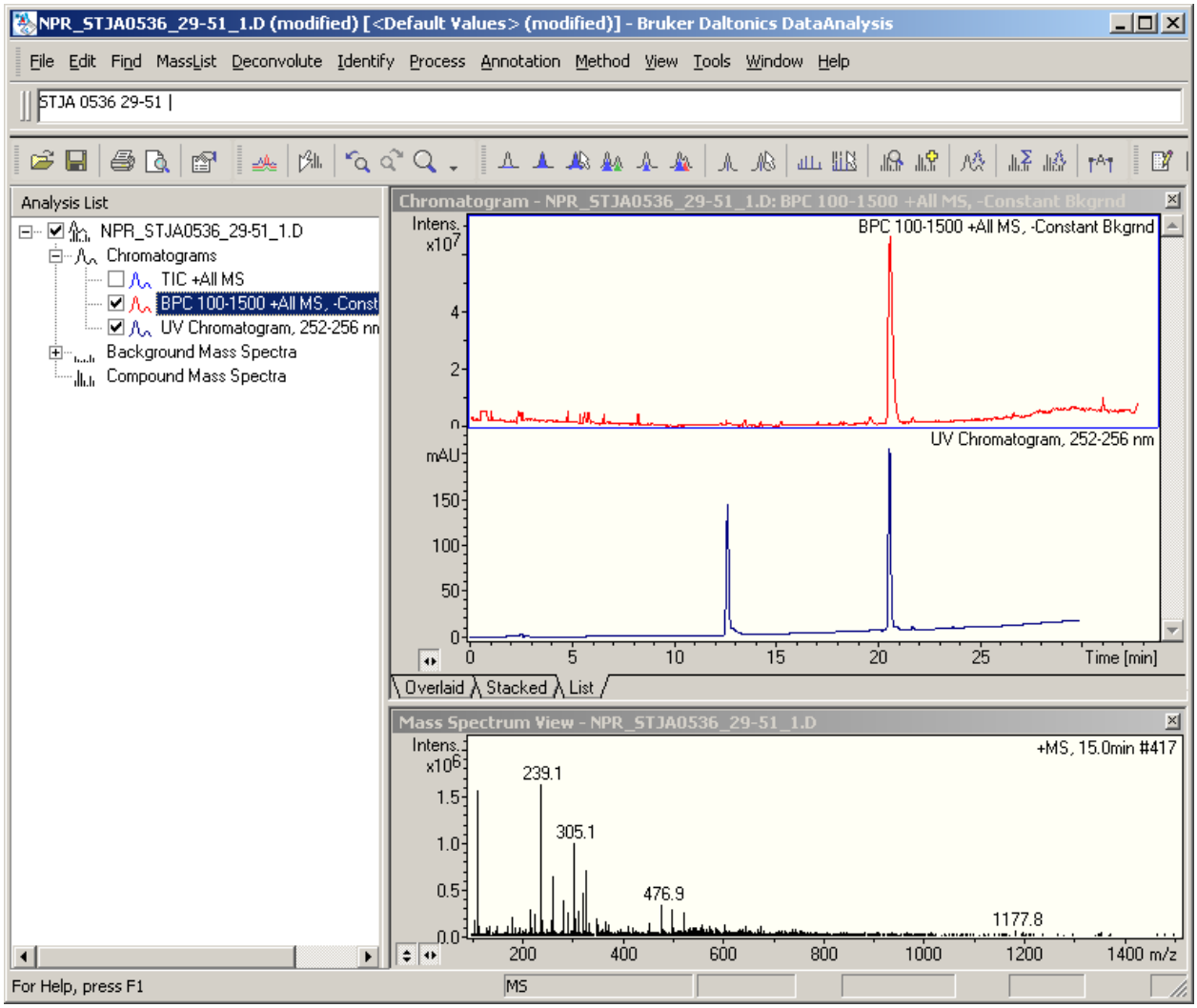
A BPC often gives a good looking chromatogram, but sometimes a peak might disappear completely. Specify a range of masses or use a TIC (Total ion chromatogram) instead.




NPR_STJA0536_29-51_1.D (modified) [<Default Values> (modified)] - Bruker Daltonics DataAnalysis
File Edit Find MassList Deconvolute Identify Process Annotation Method View Tools Window Help
STJA 0536 29-51 |
Analysis List
NPR_STJA0536_29-51_1.D
Chromatograms
TIC +All MS
BPC 100-1500 +All MS, -Const
Background Mass Spectra
Compound Mass Spectra
Chromatogram - NPR_STJA0536_29-51_1.D: TIC +All MS
Intens.
x10^8
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
Time [min]
Overlaid Stacked List
Mass Spectrum View - NPR_STJA0536_29-51_1.D
Intens.
x10^6
+MS, 15.0min #417
239.1
305.1
476.9
1177.8
1.5
1.0
0.5
0.0
200
400
600
800
1000
1200
1400 m/z
For Help, press F1
MS

Press F7 again to set up UV-traces.

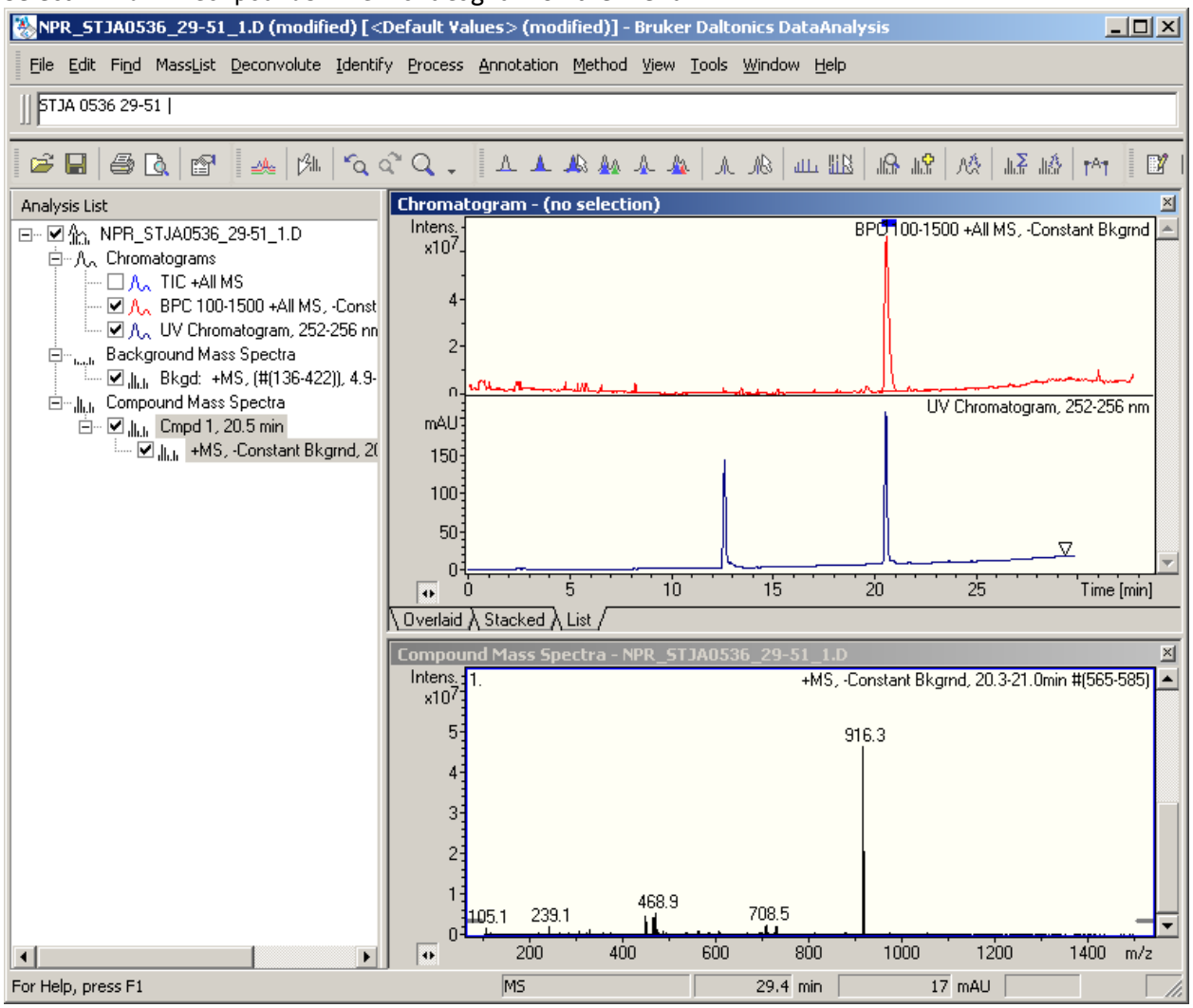



NPR_STJA0536_29-51_1.D (modified) [<Default Values> (modified)] - Bruker Daltonics DataAnalysis
File Edit Find MassList Deconvolute Identify Process Annotation Method View Tools Window Help
STJA 0536 29-51 |
Analysis List
NPR_STJA0536_29-51_1.D
Chromatograms
TIC +All MS
BPC 100-1500 +All MS, -Const
UV Chromatogram, 252-256 nn
Background Mass Spectra
Compound Mass Spectra
Chromatogram - NPR_STJA0536_29-51_1.D: BPC 100-1500 +All MS, -Constant Bkgrnd
Intens.
x10^7
BPC 100-1500 +All MS, -Constant Bkgrnd
UV Chromatogram, 252-256 nm
mAU
Time [min]
Overlaid Stacked List
Mass Spectrum View - NPR_STJA0536_29-51_1.D
Intens.
x10^6
+MS, 15.0min #417
239.1
305.1
476.9
1177.8
m/z
For Help, press F1
MS

Pick chromatographic peaks

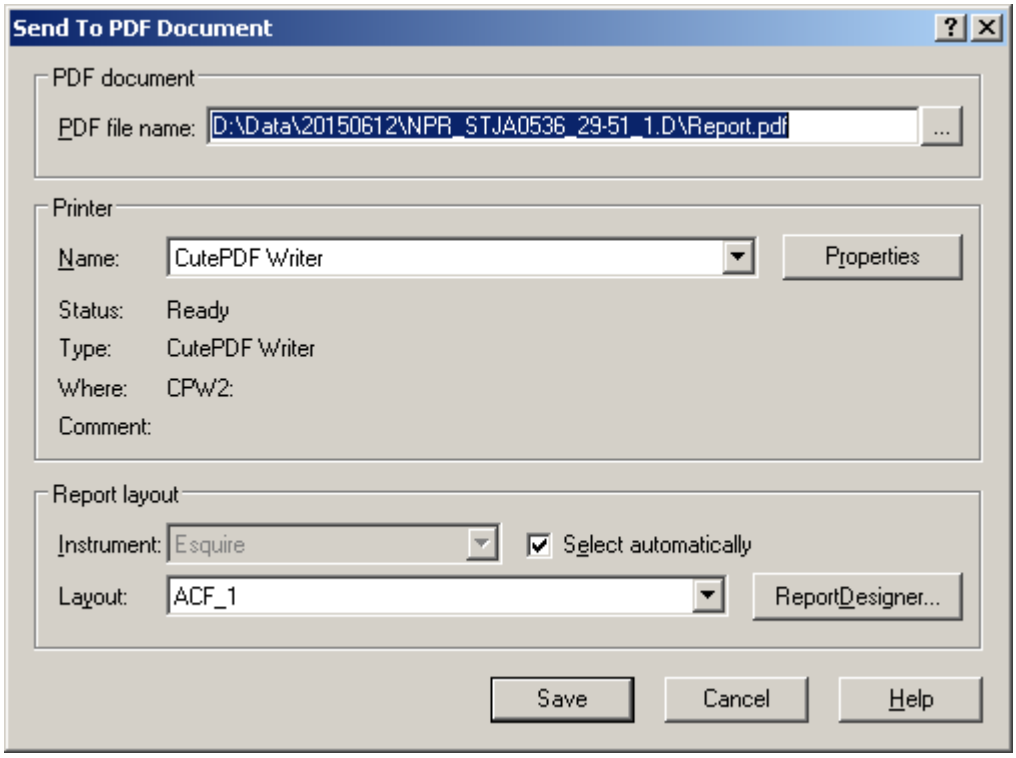
Mark the base peak chromatogram in the left pane.

Select `Find` ⇨ `Compounds – Chromatogram` on the menu.



Save results as a pdf.

Select `File` ⇨ `Send to` ⇨ `PDF document…` on the menu. Select the layout `ACF_1` and save the pdf together with the data (keep the suggested name).

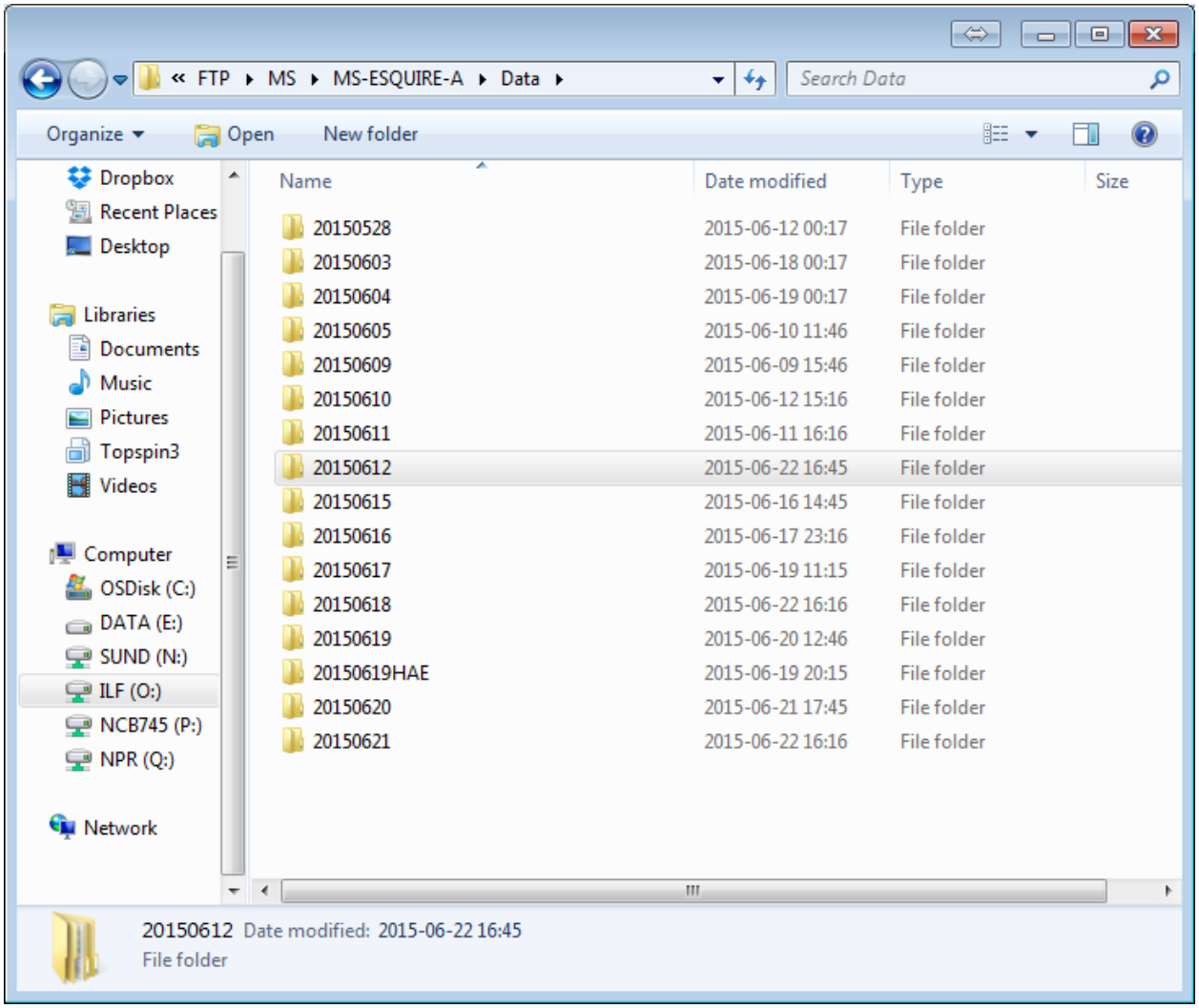


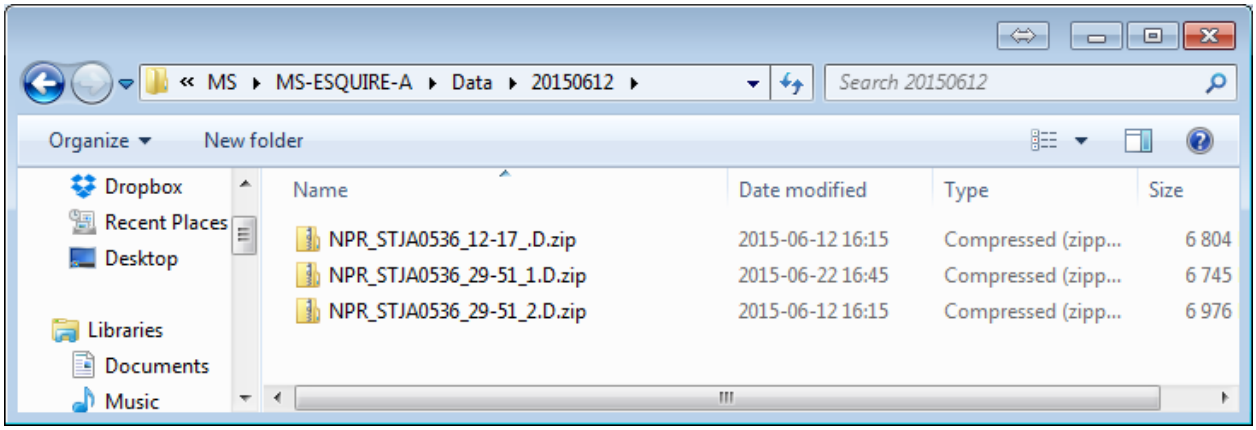
Data retrieval

The MS-data is automatically zipped and copied to the O-drive of the SUND-network whenever new data is acquired, processed or a pdf is saved in the data folder. Hence, there is no need to manually copy the data using an USB-device. The script responsible for the data transfer is currently running twice an hour.

Access to the ILF O-drive is administered by the IT-department on SUND. Use cable or log in via https://vpn.sund.ku.dk (employed or SUND-computers) or http://remote.sund.ku.dk (students or non-SUND computers).

Browse to `O:\FTP\MS\MS-ESQUIRE-A\Data` and find the appropriate data folder (date). It will contain one zip-file per analyzed sample. Copy and unpack the zip-files to another location, possibly on your own hard drive or your P-drive. Find the relevant pdf-files and print them from your own computer. The workstation by the instrument is currently not connected to a printer.





Data handling and archiving

Data is not deleted from the workstation. Old data (a few months) is moved to a subfolder named `ILF2014`, `ILF2015`, `ILF2016`,.. in the folder `OldData`-folder on the D-drive. Occasionally, the data is backed up, but it is the users own responsibility to make sure that own data is copied to a more secure location where it is backed up on a regular basis (like the P-drive).

Data copied to the O-drive is automatically deleted after 14 days. This storage is only meant for easy transfer of the data.

Restart guide

Sometimes a part of the program crashes, leaving other components hanging without the option to close them. Follow the guidelines here to restore the program to working order. Please, send a mail to Nils Nyberg (nn@sund.ku.dk) when you have done this.

1. `Start` ⇨`Programs` ⇨`Bruker Daltonics` ⇨ `Utilities` ⇨`ProcessCleaner`
2. Make sure there are no USB-drives attached
3. `Start` ⇨`Shutdown` ⇨ `Restart`
4. When the messages '`512 -- Chassis fan not detected`' and '`F1: Boot`' appears: **press F1**.
5. Log in (`Ctrl – Alt – Delete`). Username and password can be found in small print on the left screen (low left).
6. `Start` ⇨`Programs` ⇨`Agilent ChemStation` ⇨`Instrument 1 online` (icon also on desktop).
7. Move Esquire control window to the right screen.
8. `Instrument 1 (online)` ⇨`View` ⇨ Set a mark by "`System Diagram`".
9. `Instrument 1 (online)` ⇨`Sequence` ⇨ `Load Sequence` ⇨ `MS_ESQUIRE_A.S`.