The workflow of the screening procedure

MS1 analysis

Instrumental analysis

- Perform sample (e.g., wastewater extract) analysis by direct injection HRMS approach in both polarities.
- This will yield two HRMS spectra.
- Export spectra as two separate CSV files.
- CSV files have to contain two columns: 1st column: m/z value, 2nd column: the intensity of corresponding m/z signal.

MS1 data processing

R environment (vai Rstudio IDE)

- Open **Pharm_MS_MSMS_screener.R**. Run the code in Rstudio IDE sequentially by pressing Ctrl+Enter.
- Make sure that the database file (Database_pharmaceuticals_environment.xlsx) is placed in ~/Database/.
- Line 25: Define the name of the sample (variable: ***Sample.name***).
- Line 146: Place the measured Full MS CSV files in ~/Samples/***Sample.name***/MS1_spectra.
- Filename for negative mode: ***Sample.name***-NEG.csv, filename for positive mode: *Sample.name*-POS.csv.
- Line 243: Define the number of MS2 scans you will be performing per compound by changing the value of ***No.MS2.spectra*** variable (in the example it is 3).
- Line 292: Assign the collision energy (CE) levels that will be measured by changing the value of ***CE.levels*** variable (examples are shown in Line 271 – 290).
- Line 315: Stop here. At this point you will have two precursor lists at ~/Samples/***Sample.name***/Precursor lists/ that contain information about compounds that have been detected in samples and need to be investigated by means of MS2. The precursor lists contains information how each separate MS2 containing CSV file must be named so that the R code can read the files.

MS2 analysis

Instrumental analysis

- Perform sample (e.g., wastewater extract) analysis by direct injection HRMS – MS2 approach in accordance to the generated precursor lists: Example_sample-Precursor_mass_list_target.xls and Example_sample-Precursor_mass_list_suspect.xls.
- Place target MS2 spectra in ~/Samples/***Sample.name***/MS2_spectra_target/
- Place suspect MS2 spectra in ~/Samples/***Sample.name***/MS2_spectra_suspect/
- CSV files have to contain two columns: 1st column: m/z value, 2nd column: the intensity of corresponding m/z signal.

MS2 data processing

R environment (vai Rstudio IDE)

- Make sure you have placed the MS2 files in correct folders.
- Continue running the code from line 351.
- Line 315-415: Will automatically create results files in ~/Samples/***Sample.name***/Results/ folders for both target and suspect screening.
- Line 418-456: Will visualize the main parameters for detected compounds (e.g., Q1 relative mass error (ppm), Q2 relative mass error (ppm), Q2/Q1 relative ratio error, number of detected MS2 ions that were successfully matched against the given library, Q1 m/z signal intensity.
- The database can be edited/supplemented manually. However, column names and sheet order must not be changed.