IsoX: Isotopolog data eXtraction from Orbitrap RAW files

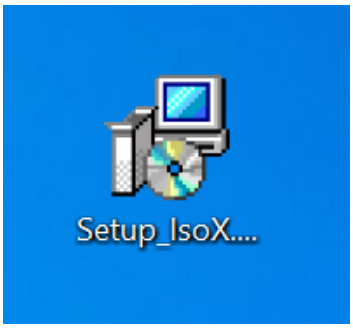
(Version 2022; 03-MAY-2022)

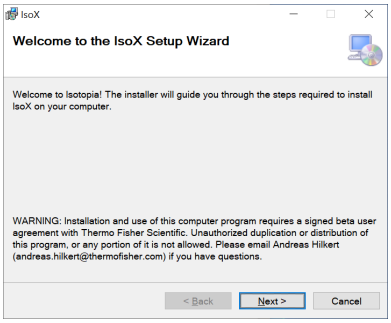
For questions, bug reports, feature requests, please contact: Caj Neubauer (123caj@gmail.com)

For beta testing licenses, please contact: Andreas Hilkert (andreas.hilkert@thermofisher.com)

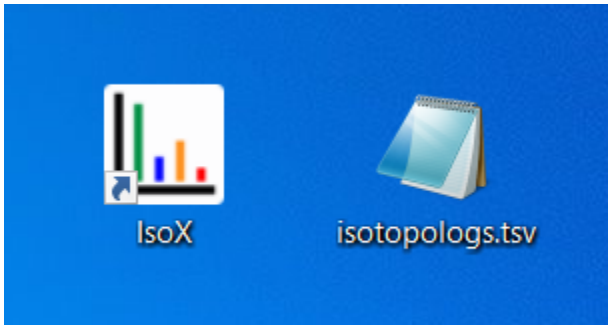
This software runs on ***Microsoft Windows*** (utilizing the ***.Net Framework***).

- To install ***IsoX***, double click the ***IsoX*** installer (`Setup_IsoX.msi`) and follow the installation prompts.

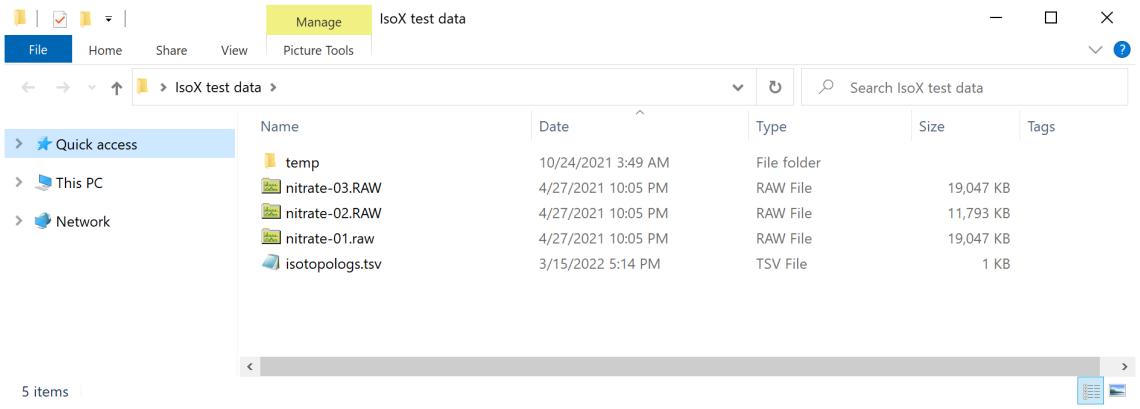




- After the installation two new files should appear on the desktop.



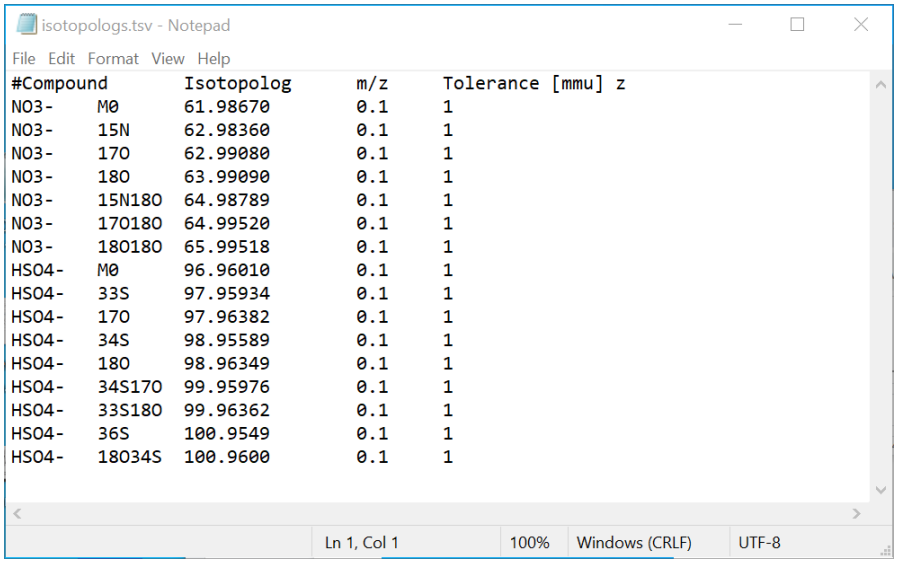
- Copy `isotopologs.tsv` into the directory that contains `RAW` files you want to process.



- Open and edit `isotopologs.tsv` in ***Notepad*** (preferred). If you edit the file in ***Excel***, make sure to save it again in the tab-delimited file format with the file ending `.tsv` (eg, not as `isotopologs.tsv.txt`)

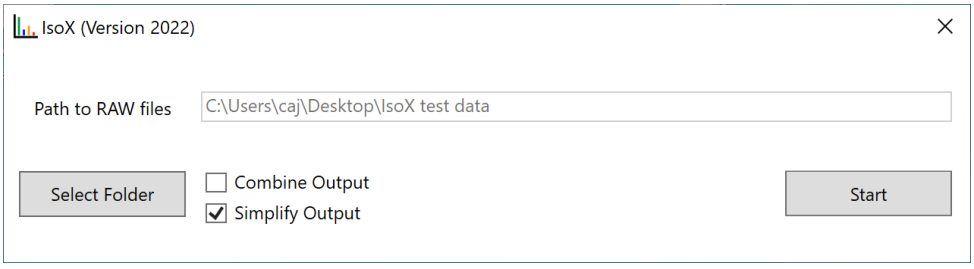
The input file has five columns:

1) **Compound**: name of the analyte
2) **Isotopolog**: name of the isotopolog
3) **m/z**: mass-to-charge ratio (*m/z*) of the isotopolog
4) **Tolerance [mmu]**: +/- tolerance for the mass-to-charge ratio (in mmu)
5) **z**: the charge state of the ion (typically 1). This value is used when calculating the number of ions.



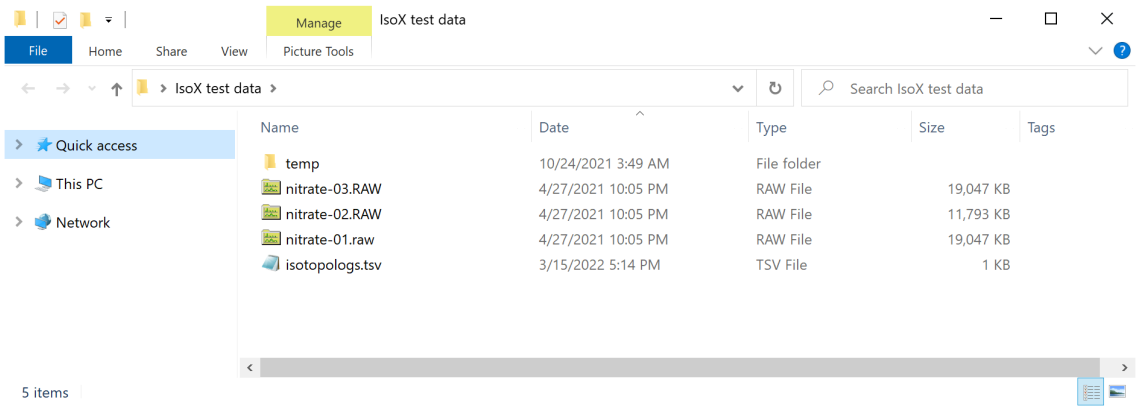
IsoX will look for all isotopologs defined in the input file (in the example above, NO_3^- and HSO_4^- ions). However, in practice for many data acquisitions the mass range is restricted by the isolation window of the quadrupole mass filter. It is therefore feasible to use '`isotopologs.tsv`' as a simple database for analytes of interest in different experiments. (Ideally data is collected by using a lockmass for the target analyte, so that observed *m/z* are not varying between data collected on different days. Then the input file `isotopologs.tsv` does not have to be manually edited.)

- Open ***IsoX*** by double clicking the icon on the desktop (the program, as well as an original version of `isotopologs.tsv` can also be found in the program folder: `C:\Program Files\IsoX`).



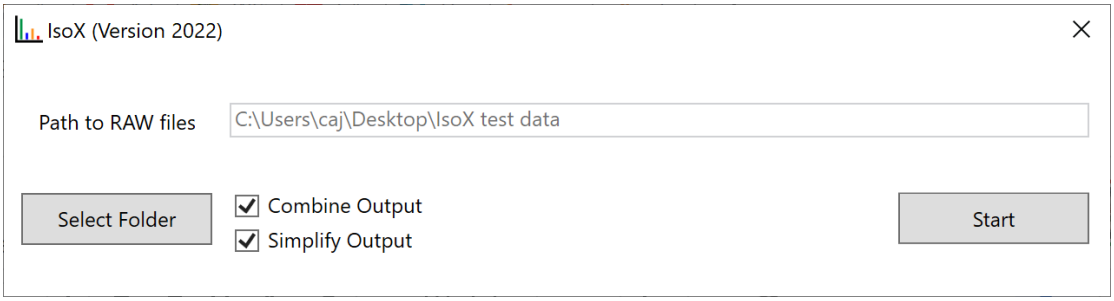
- Click 'Select Folder' and select a `RAW` file in the folder that contains your data and `isotopologs.tsv`.

In this example, the folder `IsoX test data` contains 3 `RAW` files with MS data for nitrate (NO_3^-) and `isotpologs.tsv` for quantifying its isotopologs.

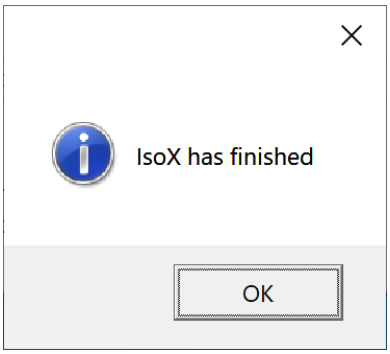


IsoX offers two options:

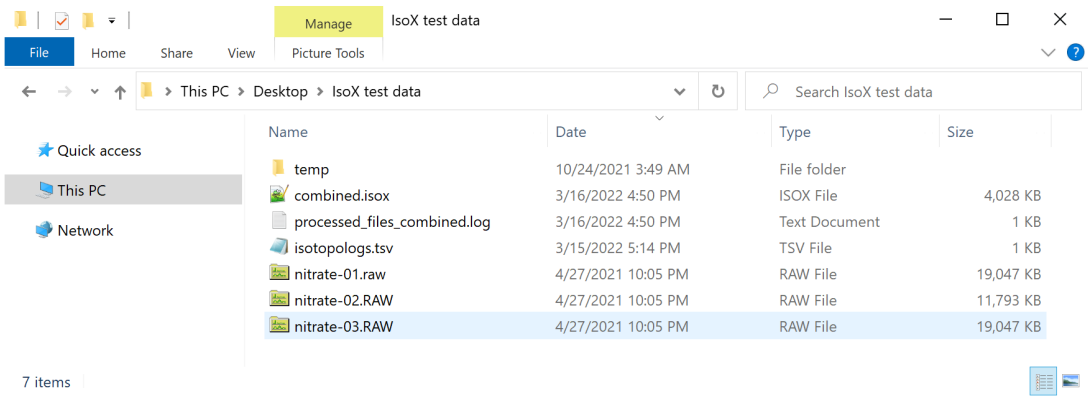
1) **Combine Output**: Combines data from all `RAW` files into one output `.isox` file (`combined.isox`). If unchecked, ***IsoX*** creates one `.isox` output file per `RAW` file (`nitrate-01.isox`, `nitrate-02.isox`, etc.)
2) **Simplify Output**: If checked, ***IsoX*** reports only basic data that is needed for isotope ratio analysis in the `.isox` file. If unchecked, ***IsoX*** reports additional columns that are not strictly necessary for isotope ratio analysis, but may be useful for some advanced users (eg, for data quality control or to remove outliers).



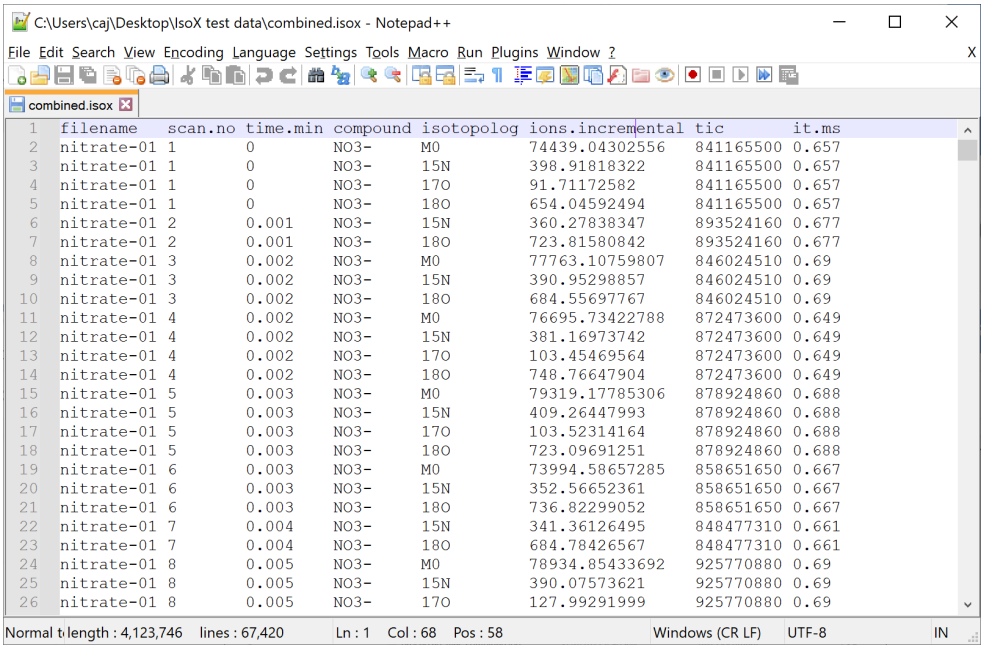
- When clicking 'Start' ***IsoX*** will extract information relevant to isotopic analysis from the `RAW` files. When the program is finished (usually after a few seconds) it shows the following prompt.



The data folder now contains two new files, `combined.isox` contains the extracted information and `processed_files_combined.log` provides a record of which `RAW` files have been processed so far (this is useful in case more `RAW` files are added to the folder at a later time). The `.log` files also keeps track which version of IsoX was used.



The file `combined.isox` also is a simple tab-delimited file and looks like this when opening in ***Notepad++*** (it can be opened also in ***Excel, Notepad***, etc):



The output is provided in a '[tidy data](#)' format, ideally suited for import into data science software such as ***R*** or ***Python***.

The basic output contains 8 column:

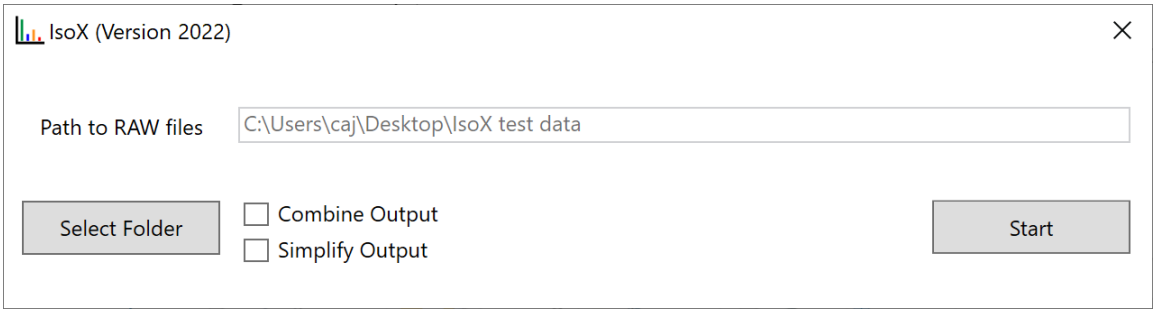
1) **filename**: name of the processed `RAW` file
2) **scan.no**: number of the processed scan
3) **time.min**: time in minutes

4) **compound**: name of the compound as defined in `isotopologs.tsv`
5) **isotopolog**: name of the isotopolog as defined in `isotopologs.tsv`
6) **ions.incremental**: a calculated number of ions for the signal. This value is calculated using the following approximation:

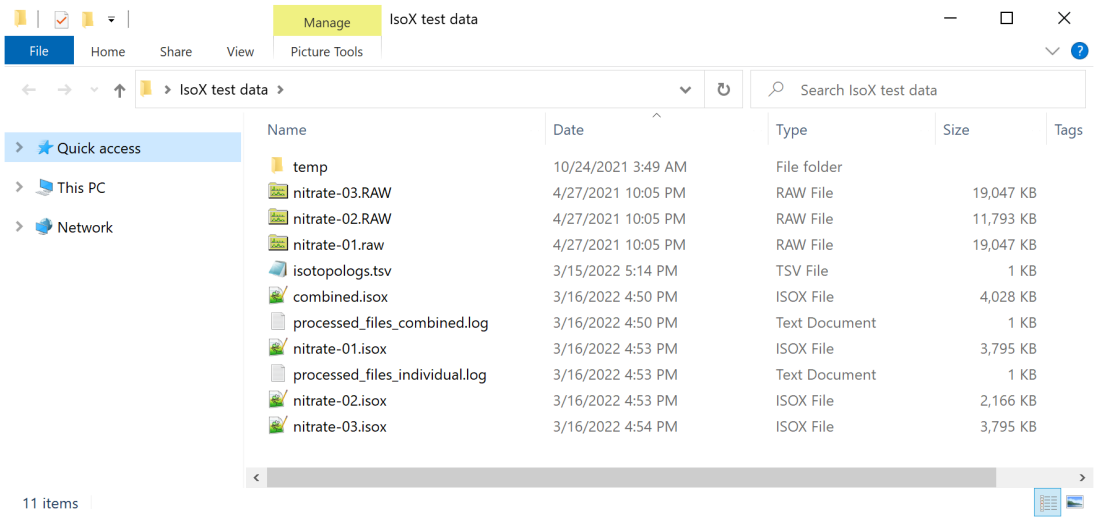
   *ions. incremental = (Intensity/Peak.Noise) * 3 * sqrt(240000/FT.Resolution) * sqrt(Microscans)*

7) **tic**: total ion current
8) **it.ms**: injection time in milliseconds (tic*it.ms is an estimate of the ions per scan. This is often a useful filter to remove scans that are outliers due to an unusually small or large number of ions entering the Orbitrap.)

For illustration, we here rerun the program in the same data folder with the following settings:



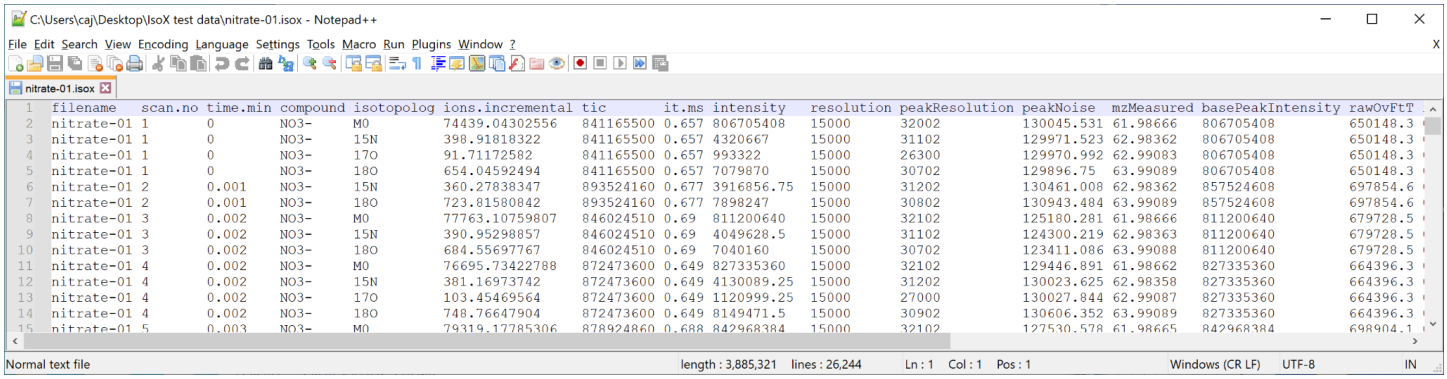
Four new files have been created. One new log file (`processed_files_individual.log`), and three new `.isox` files (one for each `RAW` file):



Since the option 'Simplify Output" has been deselected, new .isox files contains additional data columns. These data columns can be explored by advanced users to better understand aspects of the experiment that may affect isotope ratios.

- **intensity:** the measured intensity of the isotopolog signal
- **resolution:** the mass resolution setting of the Orbitrap

- **peakResolution:** the mass resolution of the isotopolog signal
- **peakNoise:** the noise of the isotopolog signal
- **mzMeasured:** the noise of the isotopolog signal
- **basePeakIntensity:** the intensity of the most intense signal in the scan
- **rawOvFtT:** TIC estimation done with the Orbitrap
- **intensCompFactor:** intensity compensation factor
- **agc:** AGC setting during acquisition
- **agcTarget:** AGC target used
- **microscans:** number of micro scans
- **numberLockmassesFound:** number of lock masses found in scan
- **analyzerTemperature:** temperature of the Orbitrap
- **baseline:** Baselines



The `.isox` files are best viewed and analyzed in a data science software such as ***R*** or ***Python***.

A note of caution:

Please be careful when rerunning ***IsoX*** in the same data folder. `RAW` files that have previously been processed (recorded in the .log file) will not be re-processed. This enables efficient processing of long sample queues with many `RAW` files.

This default can cause inconsistencies when the input file `isotopologs.tsv` has been modified in the meantime. Other inconsistencies can be caused when ***IsoX*** is re-run with additional `RAW` files but different settings for the 'Simplify Output' option are used. In such a scenario, some data will have basic output columns only while others contain extra columns.

To avoid inconsistencies it is good practice to delete old output files created by ***IsoX*** and rerun the program once again with all data and the preferred settings in ***IsoX*** and `isotopologs.tsv`.

Why are sometimes several rows reported per scan & isotopolog?

IsoX extracts data from `RAW` files for all signals that occur within the *m/z* range specified in `isotopologs.tsv` (*m/z* range = m/z ± Tolerance [mmu]). If there are multiple signals in the *m/z* range ***IsoX*** will simply report multiple rows. All data are reported, since there could be an artifact, contaminant or issue with the settings used in `isotopologs.tsv`. Looking at the respective scan in ***XCalibur QualBrowser*** or ***Freestyle*** will be helpful to identify what is going on.

Often the signal of interest is the most abundant isotopolog. Appropriate filters can be readily applied in data science software. For example in ***R*** a command like the following can be used:

```
df.filtered <- df %>% group_by(filename, compound, isotopolog) %>%
filter(ions.incremental = max(ions.incremental))
```

Notepad++

This text viewer is particularly useful to view tab delimited files on Windows PCs. It can be downloaded for free (https://notepad-plus-plus.org/downloads/). To visualize all characters use `View-> Show Symbols -> Show All Characters`. To align tab-separated files neatly with columns, consider adding the plugin `Elastic Tabstops` via the menu option `Plugins -> Plugins Admin`. ***Notepad++*** can then be set as the default program to open files with the `.isox` extension.