



QuiC 5 User Manual

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1 General Information

1.1 Scope of QuiC

QuiC is Biognosys' quality control (QC) application designed to monitor near real-time MS instrument parameters to obtain immediate and accurate instrument performance diagnosis. It provides an intuitive user interface, it is easy to set up, and it supports automated monitoring and data processing. QuiC is especially useful for simultaneous performance tracking of multiple MS instruments. It supports acquisition metrics for DDA, DIA, MRM, and PRM experiments from a variety of vendors. A list of all quality control parameters monitored by QuiC is available in [Appendix 5.1 Quality control parameters monitored by QuiC](#). The core features of QuiC include:

- Modifiable QC analysis workflows
- Automated folder monitoring and data processing
- Support for iRT peptides and additional background peptides
- Metadata annotation and event tag handling
- Export of the QC plots to a PDF report

1.2 QuiC 5 Release Features

QuiC 5 comes with several improvements that make your quality control assessment faster and more efficient than ever.

- ❖ Enhanced timsTOF folder monitoring support, ensuring seamless integration
- ❖ Faster overall processing, saving you valuable time
- ❖ Rapid filtering of runs based on acquisition date for precise data management
- ❖ Quick and hassle-free addition and removal of pending tasks for enhanced workflow flexibility

1.3 Computer System Requirements

The recommended and minimum system requirements for QuiC are listed in Table 1:

Table 1. System specifications

Specifications	Minimum	Recommended
Operating System	Windows 10, x64	Windows 10 or higher, x64
CPU	Intel® Core™ CPU, 2.7 GHz (quad core) or similar	Intel® Core™ CPU, 3.4 GHz (octa core) or similar
Hard drive	200 GB free space	500 GB free space, solid state drive (SSD)
RAM	16 GB	32 GB or more
Software	.NET Framework 4.7	.NET Framework 4.7 or higher

QuiC is only available for Windows operating systems.

NOTE: Starting from January 2020 Microsoft will no longer support Windows 7. We recommend that you upgrade to Windows 10 for the best performance.

1.4 Supported Mass Spectrometers and Data Acquisition Methods

QuiC supports MS instruments from the following vendors:

- Thermo Scientific™ Orbitrap Exploris™ 480 (FAIMS 15 – 75 CV)
- Thermo Scientific™ Orbitrap Eclipse™ Tribrid™
- Thermo Scientific™ Q Exactive™ Series
- Thermo Scientific™ Orbitrap Fusion™ Series
- Thermo Scientific™ Orbitrap Elite™
- Thermo Scientific™ TSQ Series™
- Thermo Scientific™ Orbitrap Astral
- SCIEX TripleTOF® Series
- SCIEX Triple Quad Series
- SCIEX QTRAP Series
- SCIEX API Series
- Bruker Impact II
- Bruker timsTOF, timsTOF Pro, timsTOF Pro II, timsTOF SCP and timsTOF Ultra
- Waters Xevo® G2-XS
- Waters Xevo® TQ-S

QuiC analyzes a large variety of different QC data formats from DDA, DIA, MRM, and PRM. The minimal requirement is that all runs are spiked with the [iRT Kit](#) from Biognosys. In case you experience technical problems with the software, or if you have feature suggestions, please contact support@biognosys.com.

1.5 iRT Kit and Input Resources

QuiC requires Biognosys' [iRT Kit](#) to enable fully automated, fast, and accurate QC monitoring of MS parameters. The kit needs to be spiked into each sample before MS measurement. It contains a mix of 11 non-naturally occurring synthetic peptides.

For both DDA and DIA data, there are two analysis options: either search MS data against a protein database (FASTA file) or by using a spectral library file.

The FASTA search is performed by Biognosys' proprietary search engine Pulsar. More information on how to import and manage FASTA files can be found in section [3.3.2 Protein Databases](#).

QuiC uses libraries in *.kit file format. The built-in default spectral library consists of Human (HeLa) peptides. QuiC also provides an easy interface for importing custom spectral libraries, as well as access to the Biognosys Spectral Library Online Repository to download standard spectral libraries. More information on how to import and manage spectral library files can be found in section [3.3.1 Spectral Libraries](#).

NOTE: For both DDA and DIA data, the FASTA file search on average takes longer than the spectral library search, and therefore we recommend using a spectral library if possible.

Table 2. Input resources required for the search of different types of raw data

Resource	DDA	DIA	MRM/PRM
iRT Kit file	yes	yes	yes
FASTA database file or Spectral Library file	yes (one or the other)	yes (one or the other)	no

2 Getting Started

2.1 Installing and activating QuiC

The QuiC software licenses are free of charge and can be requested through our [webpage](#). After requesting a license, you will get an email containing both the installer and the activation key for the software.

When you install and start QuiC for the first time, you will be asked to activate your software by inserting the activation key into the respective dialogue. If your computer can connect to our server, the activation is automatic. If your QuiC computer does not have access to our servers, you can also activate your version of QuiC offline. Instructions will appear after a few seconds if the online activation was not successful. Please save the generated file for offline activation on your computer and send it to support@biognosys.com. We will promptly send you a license file. To activate QuiC using an activation file, click on the "[Browse License File...](#)" button in the QuiC Activation dialogue.

NOTE: *The activation keys are bound to a single individual computer. If you need to install QuiC on more than one computer, you will need to request additional activation keys.*

2.2 If you are new to QuiC

Thank you for choosing QuiC 5! Since you are new to QuiC, this section will introduce you to the main features of the software so that you can fully leverage your experience as a user.

QuiC 5 will start with default settings, opening up in the QC perspective with a prepackaged demo data set, which allows you to familiarize yourself with the plotting options. The Runs and Plots tabs are shown by default, whereas the Events, Instruments, Thresholds, and Default Workspaces tabs can be expanded by clicking on the menu option next to the Runs tab. In the Events tab, you can create, edit, and merge tags that can be parsed from each run file name. For instance, if you change the UPLC column in the middle of your QC analysis, and this information can be parsed from the file name (e.g., file name: *_new-column.*), upon creation of the tag "new-column," this event will be noted above your plots. The Instruments tab allows the naming of your instruments as you conveniently identify them in your facility and the association of

instrument-related event tags. In the Thresholds tab, thresholds for normal operating values can be set per instrument and metric. Let's say that you would expect 15,000 +/- 1,000 peptides and 9,000 +/- 100 protein groups per QC run for instrument A. You can configure thresholds of +/- 1,000 peptides and +/- 100 protein groups for instrument A. As soon as a QC run violates the thresholds, this will be flagged as an outlier in the Identifications plot, thus making it easily spotted upon inspection. Finally, the Default Workspaces tab is an easy view and selection of your runs based on the instrument and acquisition method.

Before you move to the Monitor perspective to add the files to analyze or folders that you want to monitor, you can configure your QC Run Storage Path in the Global Settings to an empty folder (see section 3.2.1). We also recommend importing or downloading from the Databases Perspective the resources (e.g., spectral libraries, protein databases) necessary for the search of DDA/DIA runs (see section 3.3).

You are now ready to set up your first QC analysis, and we hope you will enjoy using QuiC! Please let us know at support@biognosys.com whether we have done a good job. Constructive feedback, whether positive or negative, is always appreciated!

2.3 If you are upgrading from QuiC 3

Thank you for choosing QuiC 5! Since you are an experienced user of QuiC 3, the main differences will be in an improved user experience.

For your convenience, here is a list of the new or changed features with the corresponding chapters in this manual:

1. Plots are reordered in a logical grouping instead of alphabetically. See 3.5.2
2. New plots, such as File Size, Productive Gradient Length, and Ion Mobility plots. See 3.5.2
3. Plot Presets for defining new defaults for performance metrics plots. See 3.2.3

When you first start QuiC 5, after an upgrade from QuiC 3, all your old QC runs, monitors, and configurations should be automatically migrated. It would even be easy to mistake QuiC 4 for the older version, but as soon as you click on the QC Perspective → Plots tab, you will see that there are new and improved icons for the plot groups. Additionally, the plots within a plot group are now reordered in a more logical grouping, e.g. the iRT,

Delta iRT, and Delta iRT per iRT peptide are now placed before their RT counterparts. This ordering is also the same for the PDF export and Plot Presets.

You can find a number of new plots in the Plots tab. File Size over time is added under General, a line depicting Productive Gradient Length is included in the Gradient Length plot (which can be disabled through Plot Presets), and a new plot group Ion Mobility is now available. To keep the number of plots manageable, we have also decided to remove a number of DDA Advanced plots that we personally found confusing. Please let us know at support@biognosys.com if you disagree with our assessment and removed your favorite plot!

And finally, there is now more configurability options for the plot available. Some were already available through the right-click menu, for instance Sort by Acquisition Time/Index, but in QuiC 3 it was not possible to save your preference. Now, in QuiC 5, you can set your new defaults in the Settings Perspective → Plot Presets tab. You can override the sorting order and what quantities you want to have in the plot. These choices can be saved in individual schemas and used for automatic PDF exports. The default schema will also be used for the PDF export.

Summarizing: if you are upgrading from QuiC 3

- You can reuse the old QC runs, monitors, and configurations.
- Plots are reordered and logically grouped instead of alphabetically
- There are now plots for Ion Mobility specific performance metrics
- You can now configure the default settings for the plots

We hope you enjoy using the new version of QuiC! Please let us know at support@biognosys.com whether we have done a good job. Constructive feedback, whether positive or negative, is always appreciated!

2.4 If you are upgrading from QuiC 2

Thank you for choosing QuiC 5! Since you are an experienced user of QuiC 2, a lot of functionalities will be familiar to you. This section will introduce you to the main differences in user experience between QuiC 2 and 5, so you can fully leverage your experience from QuiC 2 without feeling overwhelmed.

For your convenience, here is a list of the new features with the corresponding chapters in this manual:

1. Runs View; the replacement for the History view that was in QuiC 2. See [3.5.1](#)
2. Default Workspaces for ease of selecting a per-instrument view. See [3.5.6](#)
3. Thresholds for ease of identifying outliers in your QC history. See [3.5.5](#)
4. Event tags management, where you can merge (erroneous) tags, see [3.5.3](#)
5. Instrument management, where you name your instruments, assign event tags and map multiple (erroneous) instrument IDs to one instrument name. See [3.5.4](#)
6. Custom spectral library searching / FASTA searching for DDA and DIA. See [3.2.2](#)

The first thing you will notice when starting QuiC 5 is that you need to activate it with a new, different license. It will by default also install in a different folder, so it is possible to run (the latest versions of) QuiC 2 and 5 in parallel. Please manually update the shortcuts in the Windows taskbar if necessary.

QuiC 5 will then start with default settings, opening up with a prepackaged demo data set. You can then configure your QC Run Storage Path (see section [3.2.1](#)) and point it to your QuiC 2 folder. We recommend backing up your old QC runs folder by making a copy of that folder in a separate location, before restarting QuiC. The new QuiC version will then proceed to migrate the database to a newer version. Your old files will remain untouched. However, new runs analyzed with QuiC 5 will not show up in QuiC 2 nor vice versa. After this one-time migration, you will notice the decrease in loading times during startup, run selections, and plotting immediately. If you prefer to start anew, point your QC Run Storage to a new, empty folder.

QuiC 4 opens up in the QC perspective, where the Runs and Plots tabs appear. The Events, Instruments, Thresholds, and Default Workspaces tabs are collapsed and can be expanded by clicking on the menu option next to the Runs tab.

It is recommended to clean up some configurations migrated from QuiC 2. It is now possible to merge event tags that were erroneously created due to, for instance, typographical errors like capitalization (e.g., newColumn, NewColumn, newcolumn are logically the same event and can now all be mapped to NewColumn). It would also be a good time to rename your instruments with more easily identified names. If your instrument has changed IDs before, they can now be mapped to the same instrument name. Here, event tags can also be mapped to instruments.

The replacement of the History View is now a combination of the Default Workspaces tab and the Runs tab in the QC perspective. The selection of QC runs per instrument and method can simply be done by selecting the entry; the Runs View will automatically update with the selection. New instruments are automatically added to the Default Workspaces. The Runs table allows the configuration of user-managed filters. For instance, it is possible to select certain time frames by filtering on Analysis Date or Acquisition Date (however, if this setting is shared among instruments, we recommend setting this in the QC Plot History Length in Global Settings). The user-defined "workspaces" is the filter configuration that can then be saved on disk and loaded when needed.

Finally, thresholds for normal operating values can be set per instrument and metric on the Thresholds tab. Let's say that you would expect 15,000 +/- 1,000 peptides and 9,000 +/- 100 protein groups per QC run for instrument A. You can then configure thresholds of +/- 1,000 peptides and +/- 100 protein groups for instrument A. As soon as a QC run violates the thresholds, they will be flagged as outliers in the identification plot which can be easily spotted upon inspection.

Summarizing: if you are upgrading from QuiC 2

- A new license is necessary.
- You can reuse the old QC runs and databases.
- Some settings regarding events and instruments will be automatically transferred, but it is recommended to clean up redundant events and instruments.
- If you want you can run QuiC 2 and QuiC 5 in parallel, that would be possible. The analyses will not interfere between the two versions.
- QuiC tries to replicate the QuiC 2 user experience of the QC History view through Default Workspaces.
- QuiC allows for more advanced run selections, See QC/Runs chapter for the possibilities and how to easily save and load your workspaces.

We hope you enjoy using the new version of QuiC! Please let us know at support@biognosys.com whether we have done a good job. Constructive feedback, whether positive or negative, is always appreciated!

2.5 Post-installation recommendations

It is recommended to pre-configure QuiC after the first start to enable optimal performance (Figure 2.1).

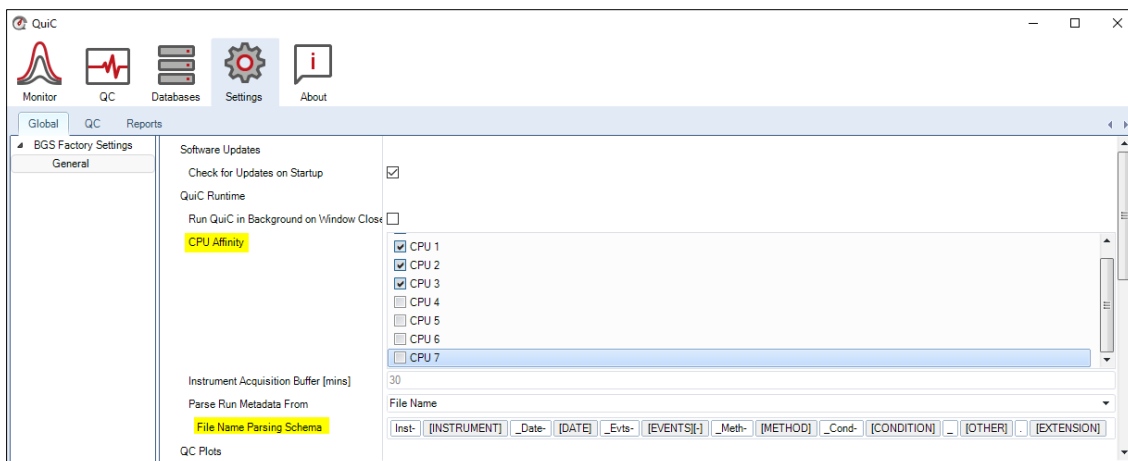


Figure 2.1. Setting up the number of CPU cores to be used by QuiC. By default, QuiC will use all the available CPUs in the system. Here one can define the File Name Parsing Schema to extract metadata information from raw file names.

1. CPU usage: to optimize the CPU usage, please select the desired number of CPU cores in the Settings Perspective → Global tab → General → CPU Affinity. In general, you can leave one CPU free to support other processes happening in the computer.
2. File naming: A very useful feature of QuiC is the possibility to extract metadata from the raw file names. It enables the use of "event tags" from file names, which will inform about the MS system maintenance that occurred. For example, instrument calibration, change of capillary, cleaning of a source or quadrupole, etc. You can specify the event tags parsing rule in the Settings Perspective → Global → General → File Name Parsing Schema. It is useful to set up a convenient schema before starting to use QuiC.
3. Directories (Figure 2.2): QuiC will set all directories in the C:\ drive by default. However, it is possible that the C:\ drive has a limited storage capacity. Thus, we strongly recommend changing the Temporary Directory to a local destination with enough available storage capacity. To do that, go to the Settings Perspective → Global → General → Temporary Directory.

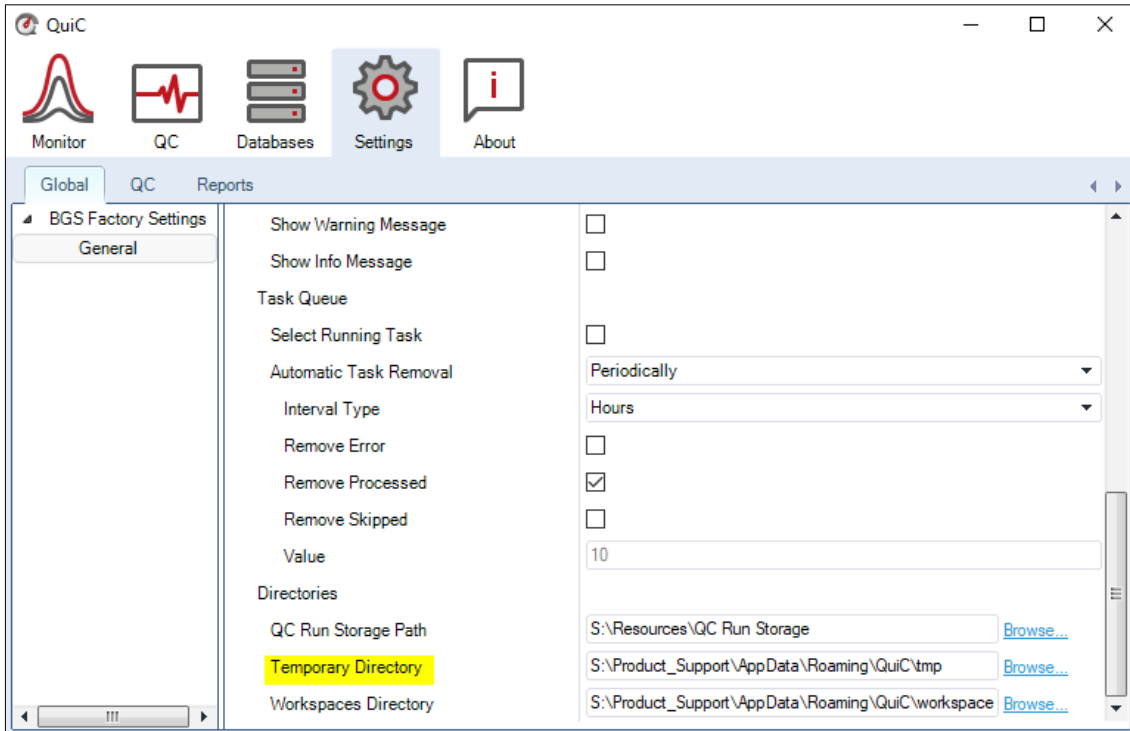


Figure 2.2. Setting up the Temporary Directory to a local destination with available storage space. Do not save permanent files in the Temporary Directory, as this gets cleared at the end of each analysis.

3 QuiC Usage

3.1 Structure of QuiC

QuiC is structured on several levels (Figure 3.1). The highest level is the perspective. Perspectives are mainly structured in tabs, which in turn are composed of panels. The left panel contains a menu with nodes, whereas the right panel contains information related to the selected node. For instance, the QC perspective visibly contains the two tabs “Runs” and “Plots” as well as a menu (top left corner) under which the “Events”, “Instruments”, “Thresholds”, and “Default Workspaces” tabs are collapsed. The “Plots” tab contains a plotting menu (left panel) and the respective visualization (right panel). Sometimes panels also contain actions that are highlighted in light blue. Actions typically allow to import/export files or edit/save changes.

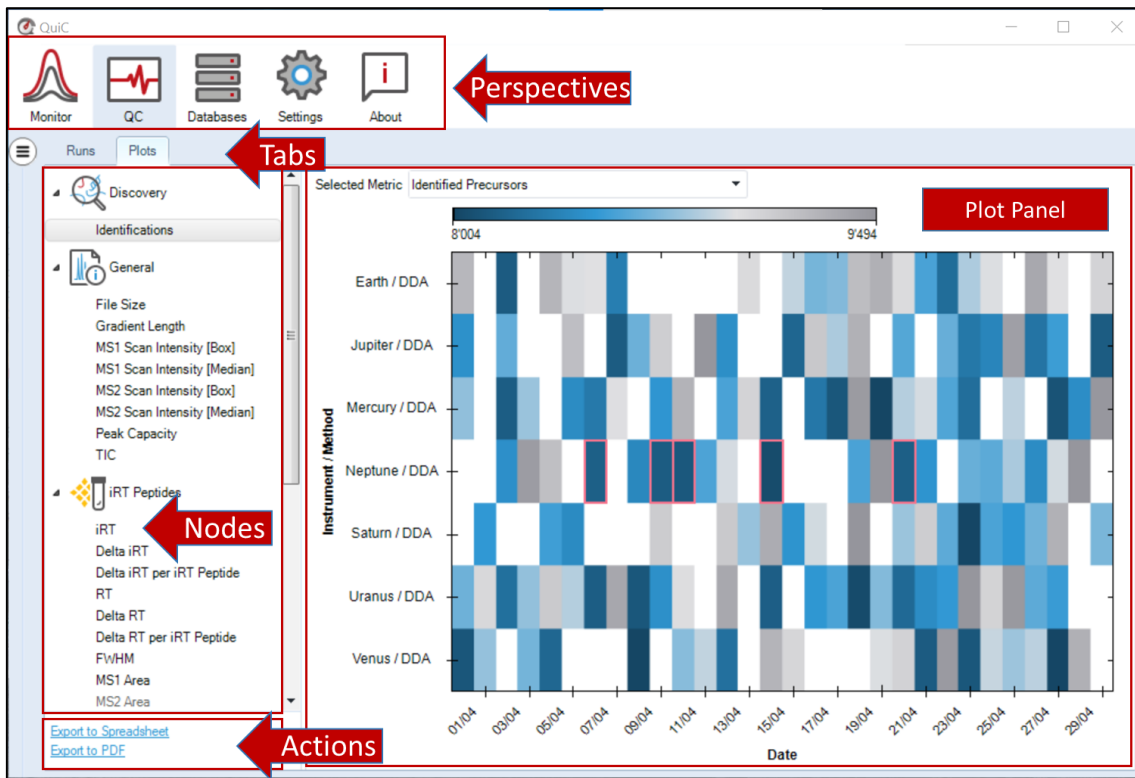


Figure 3.1. QuiC is structured in the following levels (ordered from highest to lowest): perspectives, tabs, panels (left and right), menu with nodes (left panel), information related to the selected node (right panel), and actions.

For usability, this manual will follow the logic of the workflow setup instead of the perspective-based structure of QuiC. The software always opens in the QC perspective, assuming that the user had already configured Settings, Databases, and Files/Folder to monitor. Here, we assume this is the first time you set up a QC analysis, and therefore, the manual guides you through the required pre-analysis configuration.

3.2 Settings Perspective

The Settings perspective is used to configure custom settings for QuiC processes included in the tabs Global, QC, and Reports. The Global tab groups various settings inherent to software functioning and computer configuration. In QC, one can custom-make the desired settings schemas for QC analysis by modifying predefined schemas as well as set new default ones. Saving settings schemas allow reusing the desired analysis parameters (no need to reset them every time). Finally, in Reports, one can define and save a selection of QC plots to export.

3.2.1 Global

The Global Settings tab is where the user changes the settings inherent to software functioning and computer configuration. Since there is only one instance of Global settings, this is the only part of the settings that don't allow the user to save separate schemas.

The settings are separated into different sections:

- Software Updates
- QuiC Runtime
- QC Plots
- Messaging
- Task Queue
- Directories

The Software Updates section only has one setting, which is the "Check for Updates at Startup". QuiC is regularly updated; with this setting turned on QuiC will check if it's currently running the latest version and will prompt you to update if it isn't. A working internet connection is needed for both the check and the download of the new version. It's recommended to turn on this setting.

The QuiC Runtime section has settings that determine how QuiC runs. Those are:

- Run QuiC in Background on Windows Close: whether you want QuiC to run analyses in the background when you close the window. One possible use case would be when you have a QuiC instance dedicated for analyses, while you look at the outputs from a remote QuiC instance.

- CPU Affinity: assigns the number of CPUs that QuiC can use. The more CPUs are assigned, the faster the analyses will finish. In general, you can leave one CPU free to support other processes happening in the computer.
- Instrument Acquisition Buffer [mins]: minutes to wait before QuiC can start the analysis of a freshly acquired run. This is to ensure that the instrument software closes the run file before QuiC starts analyzing it. The default setting of 30 minutes is usually sufficient.
- Parse Run Metadata From: where QuiC gets metadata information, such as instrument name, event tags, and experimental condition. The available options are "File Name" (default) and "Header Comments", where the latter is only available for Thermo instruments. The next field "File Name Parsing Schema" allows you to build up a definition on how to parse the information from the file name.

The QC Plots section contains the settings to limit the history you want to show in the QC plots and contains:

- History Length Units: possible units are "Days" and "Runs".
- History Length: a positive number in this field will limit the number of points shown in the QC plots. Depending on the previous field, it will be either days (till today) or the total number of runs respectively. A value of -1 indicates that no limit is set and the whole history will be shown.

The Messaging section determines what kind of messages QuiC will show. Messages are divided into the categories ERROR, WARNING, and INFO in order of decreasing priority. It is highly recommended to keep at least the ERROR messages on; it will help during the communication of issues with the Biognosys support desk (support@biognosys.com).

The Task Queue section has settings for the task queue that is displayed in the Monitor perspective. It allows you to set parameters for task removal to keep the queue clean. These are mostly cosmetic.

The Directories section allows you to specify the relevant directories where QuiC saves its data. By default, QuiC sets all directories in the C:\ drive. However, it is likely that the C:\ drive has limited storage capacity. Below are listed the directories and the recommended changes of their location:

- **QC Run Storage Path:** this path contains the QC history, that is, all the metrics of the analyzed. Run files stored in this directory are listed in the Runs tab of the QC perspective and can be visualized at any moment under Plots.
- **Temporary Directory:** this is where temporary data created during a QC analysis are stored. It is highly recommended to change this location to a local directory with plenty of storage space, i.e., at least 200 GB of free space. Please do not store permanent files in this location, as the temporary folder is cleared at analysis completion.
- **Workspaces Directory:** this is the storage location of Default Workspaces (see section [3.5.6](#)).

3.2.2 QC

The QC Settings Tab is where one can edit and save settings inherent to QC processes. We provide a Factory Settings schema as default for both Analysis and Batch Processing (Figure 3.2). A new settings schema can be generated by editing an existing one and by saving it with a new name ("[Save as...](#)" in the bottom left corner to give a name to your schema and click "[OK](#)"). The new schema will appear in the left menu, and can now be manually selected or set as the default (by right-clicking on the schema node) to use in any new analysis.

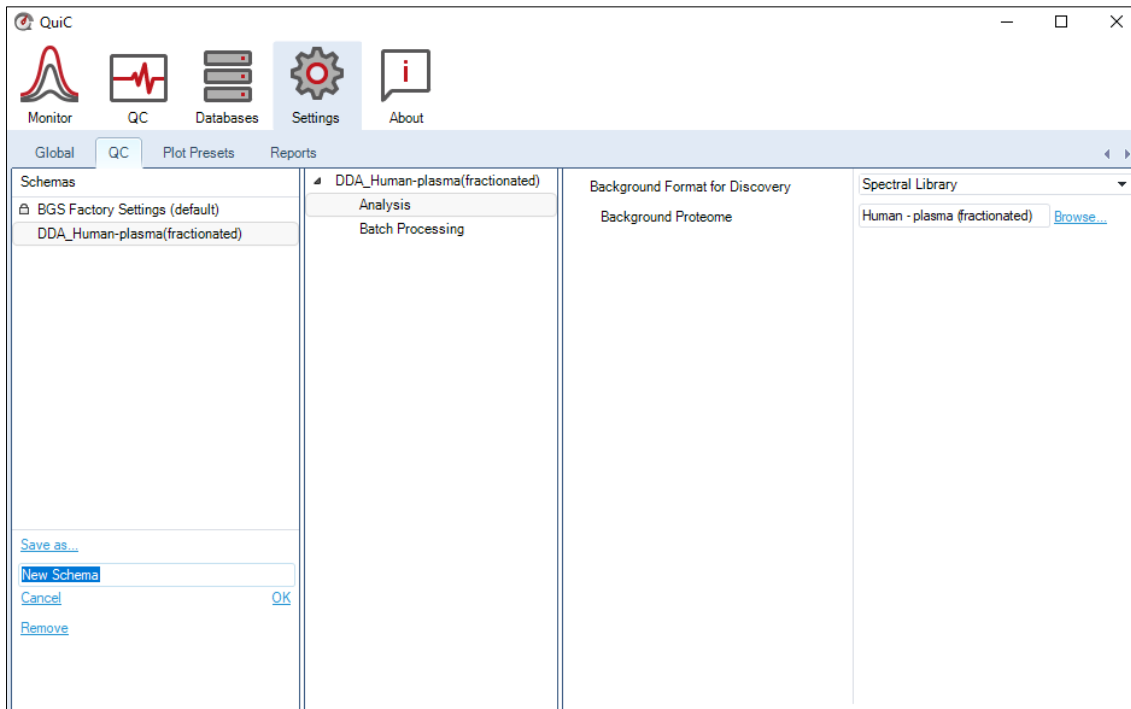


Figure 3.2. Under QC settings, the user can visualize the pre-configured Biognosys Factory Settings as well as custom-make new settings schemas to use for Analysis and/or Batch Processing. Settings schemas can be created or removed using the actions located at the bottom left corner of the tab. Any schema can replace the BGS Factory Settings and become the new default by right-clicking on the respective node.

Settings Schemas can configure both Analysis and Batch Processing. The Analysis node defines the spectral library or the protein database used for peptide search in Discovery pipelines (i.e., DDA or DIA). Even if configured, this setting will be ignored when dealing with targeted proteomics pipelines (i.e., MRM/PRM).

- Background Format for Discovery: specify here the resource to be used as background proteome. Spectral Library or protein FASTA.
- Background Proteome: When the option Spectral Library is selected, one can choose a spectral library that was previously imported or downloaded in the Database Perspective (section 3.3). The option of specifying a FASTA file as the background is new in QuiC 4. When selecting a FASTA file, the search space has to be defined as follows:

Table 3. Search space parameters

Digest Type	<p>Specific: both N- and C-terminus follow the specified digest rules</p> <p>Semi-specific: only one of the termini follows the specified digest rules</p> <p>Unspecific: no digest rules</p>
Enzyme/Cleavage Rules	Proteases used to <i>in-silico</i> digest the proteins from the protein database(s). Defined in Databases → Cleavage Rules
Maximum Peptide Length	Maximum number of amino acids allowed for a peptide
Maximum Variable Modifications	Maximum number of variable modifications allowed to happen in one peptide at the same time. The higher this number, the more possible combinations, and the bigger the search space and the longer the calculation time
Minimum Peptide Length	Minimum number of amino acids allowed for a peptide
Missed Cleavages	How many consecutive cleavage sites the protease could miss
Select Modifications	Maximum number of variable modifications allowed to happen in one peptide at the same time. The higher this number, the more possible combinations, and the bigger the search space and the longer the calculation time
Toggle N-terminal M	Pre-processing of the protein database by removing the N-terminal M (when there is one)

The Batch Processing node contains useful settings when monitoring several files in a folder. These settings can also be defined in the Monitor perspective (see section 3.4) every time that files or a folder are set to be monitored. However, setting schemas can only be saved, and therefore reused, if created here. These settings are the following:

- Age Filter (Days): the number of days counted back from today the monitor considers a file as valid. The default value of -1 considers all the runs valid. For example, a value of 30 will configure the file monitor only to consider run files more recent than 30 days.
- Include Subfolder: whether a file monitor only considers the configured folder or to include all subfolders as well.
- Monitor Folder: whether to keep monitoring a given folder and automatically add the new run files to the task queue.
- Skip Duplicate Runs: whether to consider files that were already analyzed. When this option is selected, files that already appear in the QC Perspective/Runs tab (see section 3.5.1) will be disregarded by the file monitor. To be reconsidered, these files should be manually deleted from the QC Perspective/Runs tab.
- Regex File Filter: only consider files that match this filter. For instance, `.*[qQ][cC].*` will consider all files that have any combination of QC in the file name.
- Thermo Header Filtering: only consider files that match this filter. This filter uses the Thermo header instead of the file name (only applicable for Thermo instrument files).
- Vendor Filter: whether to consider files from these vendors.

3.2.3 Plot Presets

The Plot Presets Tab is where one can edit and save settings relating the plots. Currently, we support toggling whether to Sort By Acquisition Time or Sort By Acquisition Index, and a Select Quantity... to plot. We provide a Factory Settings schema as default for all supported plots (Figure 3.3). A new settings schema can be generated by editing an existing one and by saving it with a new name ("Save as..." in the bottom left corner to give a name to your schema and click "OK"). The new schema will appear in the left menu, and can now be manually selected or set as the default (by right-clicking on the schema node) to use in any new analysis.

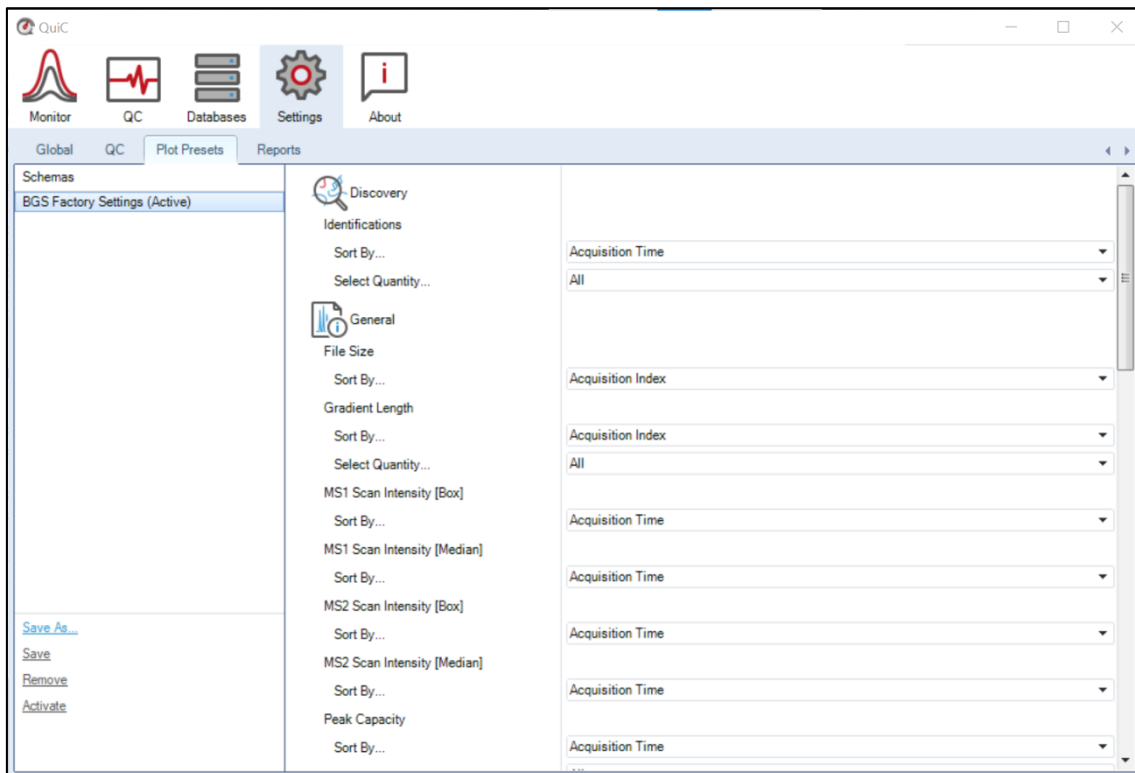


Figure 3.3. Under Plot Presets settings, the user can visualize the pre-configured Biognosys Factory Settings as well as custom-make new settings schemas to use for the plots. Settings schemas can be created or removed using the actions located at the bottom left corner of the tab. Any schema can replace the BGS Factory Settings and become the new default by right-clicking on the respective node.

3.2.4 Reports

It is possible to export all or a selection of plots generated in the Plots tab of the QC perspective to a PDF file to keep an external record of the instruments' performance. The settings for PDF generation can be edited every time a report is exported. However, if you want your reports always to contain the same selection of plots, here you can save a reporting schema.

For every schema, which can be saved, imported, and exported, one can select the plots to be reported. The plots selection can be modified in the central panel, and the new report schema is saved under a new name. Please note that not all plots have valid data for every instrument. Therefore, we recommend creating report schemas for your different instruments.

In the right panel, specify the Title, Username, and Path, where the report will be saved. You can also add a shared Description of the report. By selecting the Comments field, an additional Comment window will pop-up whenever you export a report in the QC

perspective (click on [“Export to PDF...”](#) chose the reporting schema and press [“OK”](#)). The option Open PDF will trigger a PDF viewer to open the file after export. The One PDF / Instrument option creates a separate report for each instrument instead of combining all the results in the same plots (default). The One PDF / Instrument option is only valid if multiple instruments or multiple method types are selected. Finally, note that Report schemas can be irreversibly deleted by pressing the [DELETE] keyboard command.

3.3 Databases Perspective

The Databases Perspective in QuiC allows the storage and management of the resources needed when setting up an analysis. Such resources include spectral libraries, protein databases, cleavage rules, and peptide modifications. Every resource added or edited (and saved) in the Databases Perspective becomes available when setting up an analysis in the Monitor Perspective.

3.3.1 Spectral Libraries

The two actions you can perform in the Spectral Libraries tab are:

1. Export a Spectral Library
2. Import a Spectral Library

To export a spectral library, click on [“Export Spectral Library...”](#) in the bottom left corner of the Spectral Libraries tab. The spectral library can be exported in two formats: tab-separated value (*.xls), and Biognosys kit (*.kit).

To import an external library into QuiC, click on [“Import Spectral Library...”](#) in the bottom left corner of the Spectral Libraries tab.

There are two ways of importing a library:

1. Importing a *.kit library (Biognosys' library format). In this case, no further curation is required. The library will be added to the Library tree.
2. Importing a compatible spreadsheet as a plain text, separated value format (*.txt, *.csv, *.tsv, *.xls). Headers defining your columns are mandatory in these files.

The [“Import Spectral Library...”](#) dialogue (Figure 3.4) will try to auto-detect column names. If there are new column names, QuiC will ask you whether or not you want to

store them as a recognized synonym for this column. This allows QuiC to automatically select these columns the next time you load a spectral library with a similar format.

The import function also allows you to refine your library. In the Library Settings panel, you can choose several options to be applied to your library (for details, see Appendix 5.2 Library Generation Settings). For example, you can perform protein inference again. To do this, go to the FASTA File panel and choose your protein database. You can also add Gene Ontology annotation information using the Gene Annotations panel.

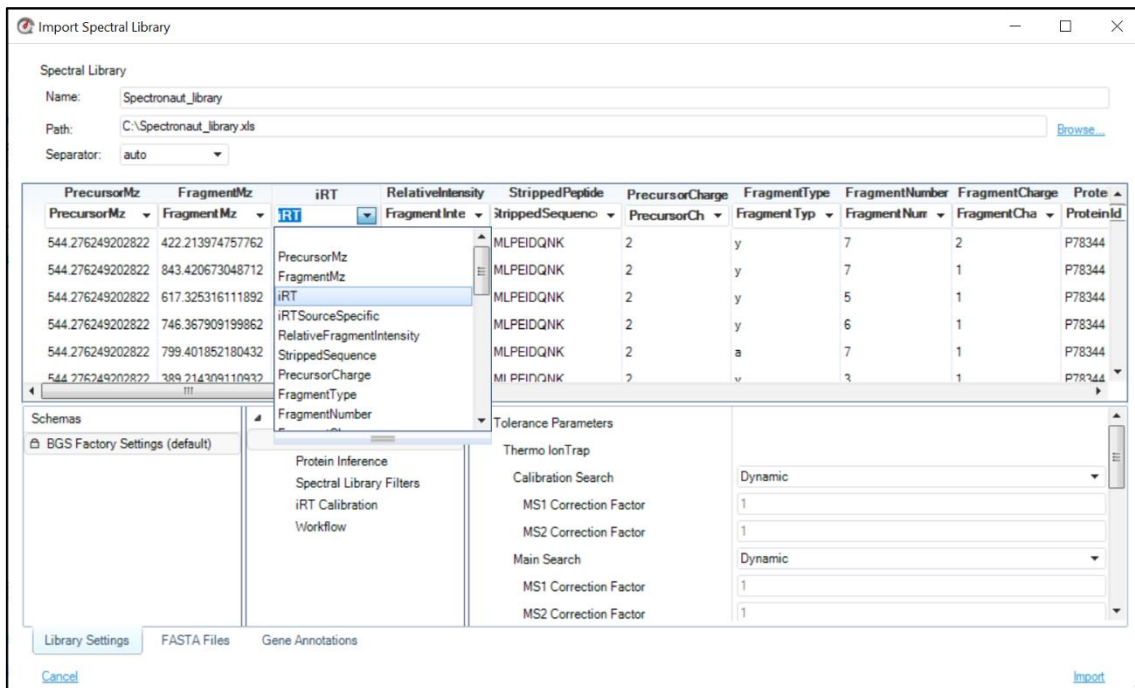


Figure 3.4. In the import spectral library dialogue, the user reassigns column headers to files in a separated value format to obtain a QuiC compatible spectral library. This is not necessary when importing *.kit libraries generated with other Biognosys software tools. In the spectral library dialogue, the library generation settings have to be specified, and both FASTA and Gene Annotations files can be appended.

3.3.2 Protein Databases

This tab allows you to import and manage your protein databases. QuiC uses protein databases (FASTA files) for peptide search and protein inference. The imported protein databases contain all the sequences, as well as meta-information extracted from the FASTA protein headers, using the specified parsing rule. QuiC already contains the UniProt parsing rule, but you can add new rules by clicking "New Rule..." in the Protein Databases overview or during the import (Figure 3.5).

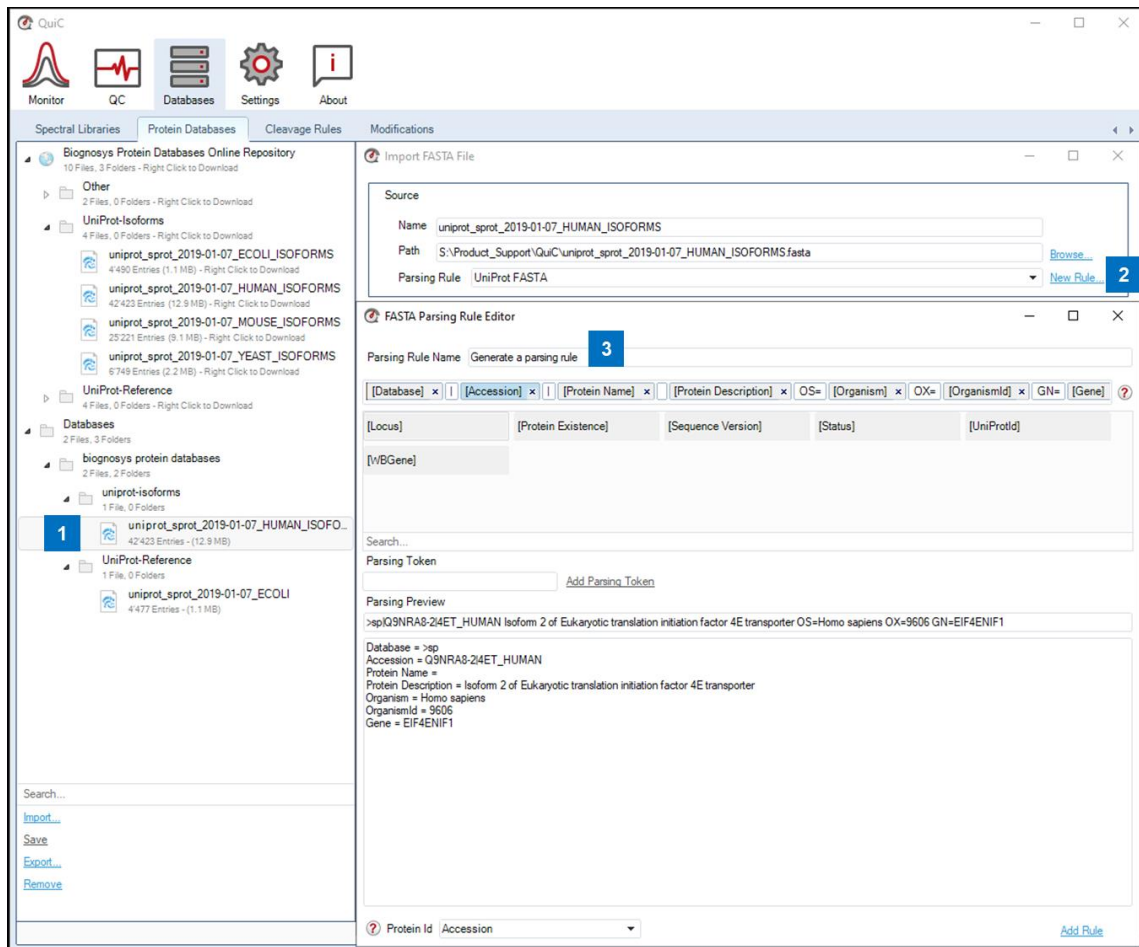


Figure 3.5. After import in QuiC, the new FASTA file appears in the database tree (left panel) and is available to be used. To generate a new parsing rule, the FASTA file has to be selected in the tree, and by clicking "New Rule..." the FASTA Parsing Rule Editor will open. A short tutorial will appear by hovering on the red question mark in the top right corner of the editor.

3.3.3 Cleavage Rules

The tool contained in this page allows you to define the rule to *in-silico* digest the proteins contained in your protein database(s) and include them in the search space. Cleavage rules are applied whenever you search in QuiC. The most frequent cleavage rules are already present in QuiC. Examples are Trypsin, Trypsin/P, and LysC.

To design your own rule, you have to click on an existing one, modify it, and click on "Save as..." in the bottom left corner (Figure 3.6). The rules are defined by which sites are cleaved by the enzyme. In the Digest Rule page, you will see a 20 x 20 matrix containing all possible combinations of amino acids. Select the combination where your enzyme cleaves and include a description in the dedicated field. Under the matrix, you will see a preview of how a sequence will look like after being cleaved following your digest rule; you can also paste a sequence of interest.

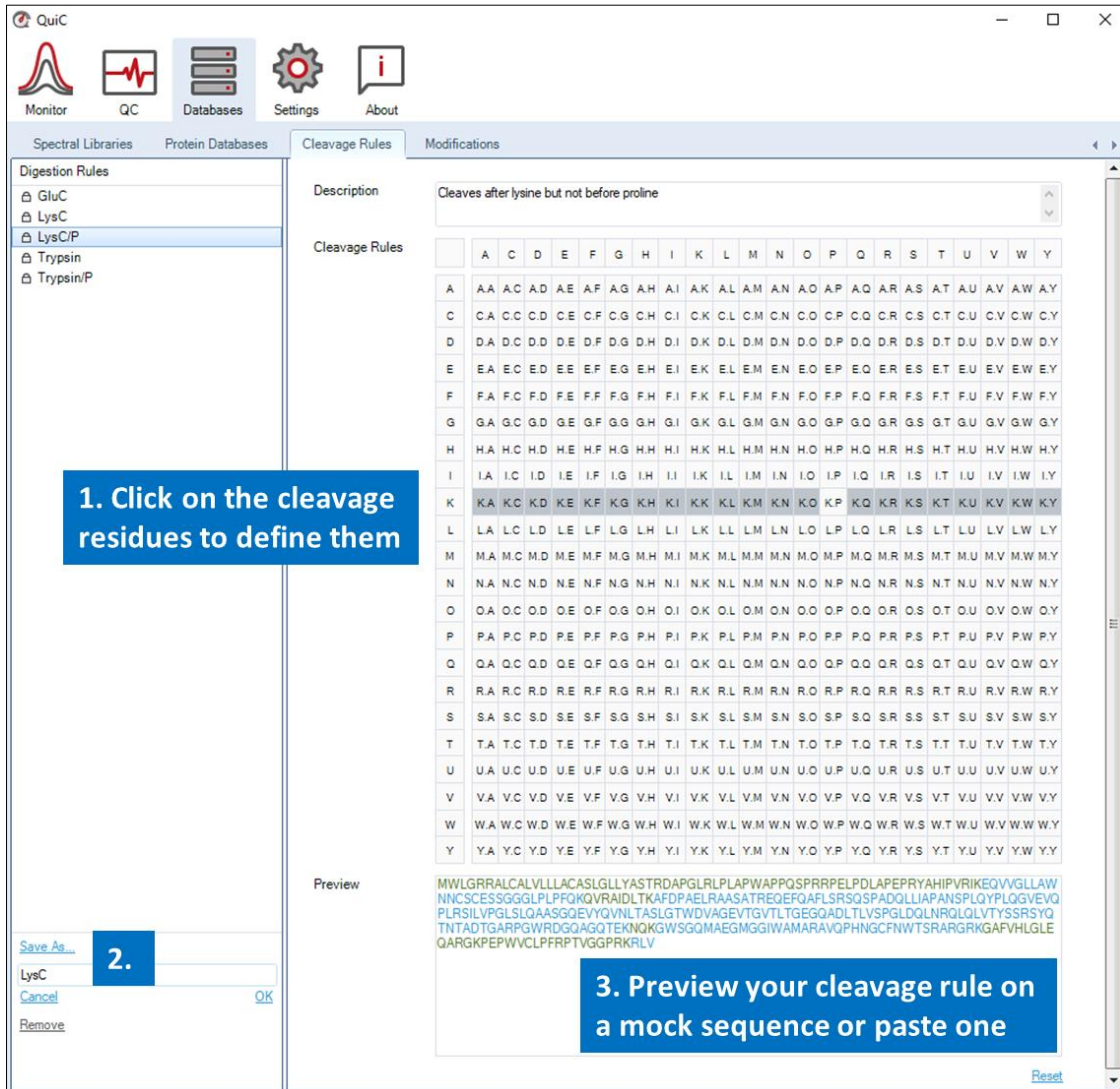


Figure 3.6. Define a new Cleavage Rule. As an example, the cleavage rule LysC was edited from LysC/P. The LysC/P rule specifies sequence cleavage after every lysine (K). The rule LysC specifies sequence cleavage after every lysine (K) unless followed by prolines (P). Therefore, to create the LysC from LysC/P, the rule "cleaving after K whenever it is followed by P" (K.P) was unselected.

3.3.4 Modifications

The QuiC Modifications panel provides a central location to manage modifications. Modifications contained here can be added to the Pulsar Search Settings when setting up an analysis. QuiC comes with a broad database of default modifications, but if you work with special ones, you can add them to the Modifications database. You can also edit an existing modification.

To create a new modification, click "New" in the bottom-left corner, give a name to your modification, and press "OK" (Figure 3.7). Create your new modification and press the "Save" action in the bottom-left corner. Another option is to edit an existing modification and press "Save As...".

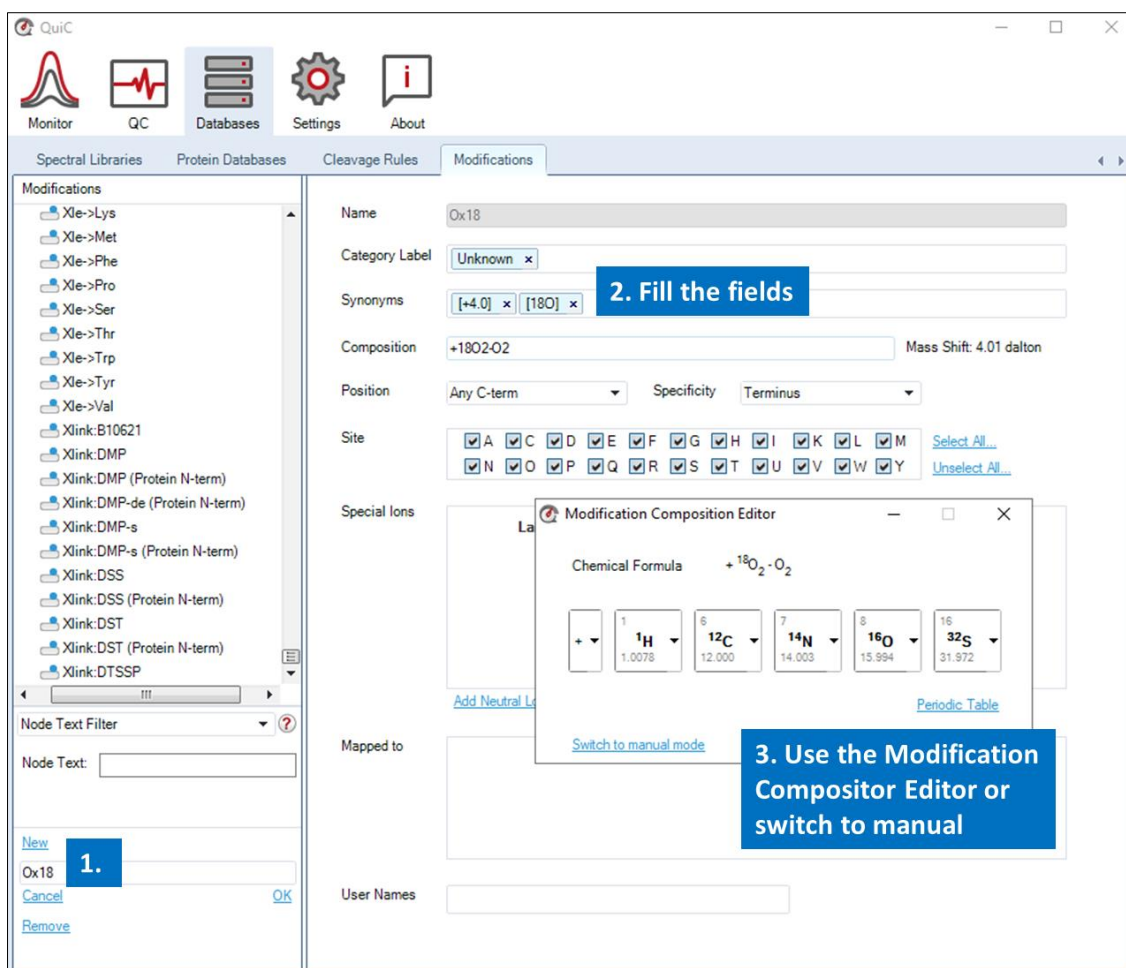


Figure 3.7. Add a new Modification to the database. QuiC has a comprehensive Modifications Database that can be searched in the field "Node Text". Nevertheless, if the database does not contain a modification of interest, this can be newly created in the Modifications tab or by editing an existing modification. Modifications can also be imported in *.xml format from other search engines.

3.4 Monitor Perspective

In the Monitor Perspective, the user can select individual runs, e.g. Thermo raw-files or Brukers .d folders, for a Single Analysis, or set up a Monitor to look for the appearance of new runs in a folder (Figure 3.8). When selecting a Single Analysis (by clicking "[Run Single Analysis...](#)"), a setup window containing the Analysis Settings appears. Here the user can select the BGS Factory Settings, modify the BGS Factory Settings, or choose a previously custom-made Settings Schema (see section 3.2.2). When monitoring a folder (by clicking on the dropdown arrow next to "[Run Single Analysis...](#)" and then selecting "[Add Monitor...](#)"), the Analysis Settings have to be specified as for files, but additionally, the option of Batch Processing applies. The Batch Processing option specifies the files to analyze in a folder. For instance, an MS instrument output folder can be monitored, and all (or a selection of) the run files can be automatically processed as soon as their acquisition completes (Figure 3.9). By confirming the Analysis and Batch Processing Settings (press "OK"), the QC analysis starts, and the execution is displayed in the left panel of the perspective (Figure 3.10). The completed files will appear in the Runs tab of the QC perspective.

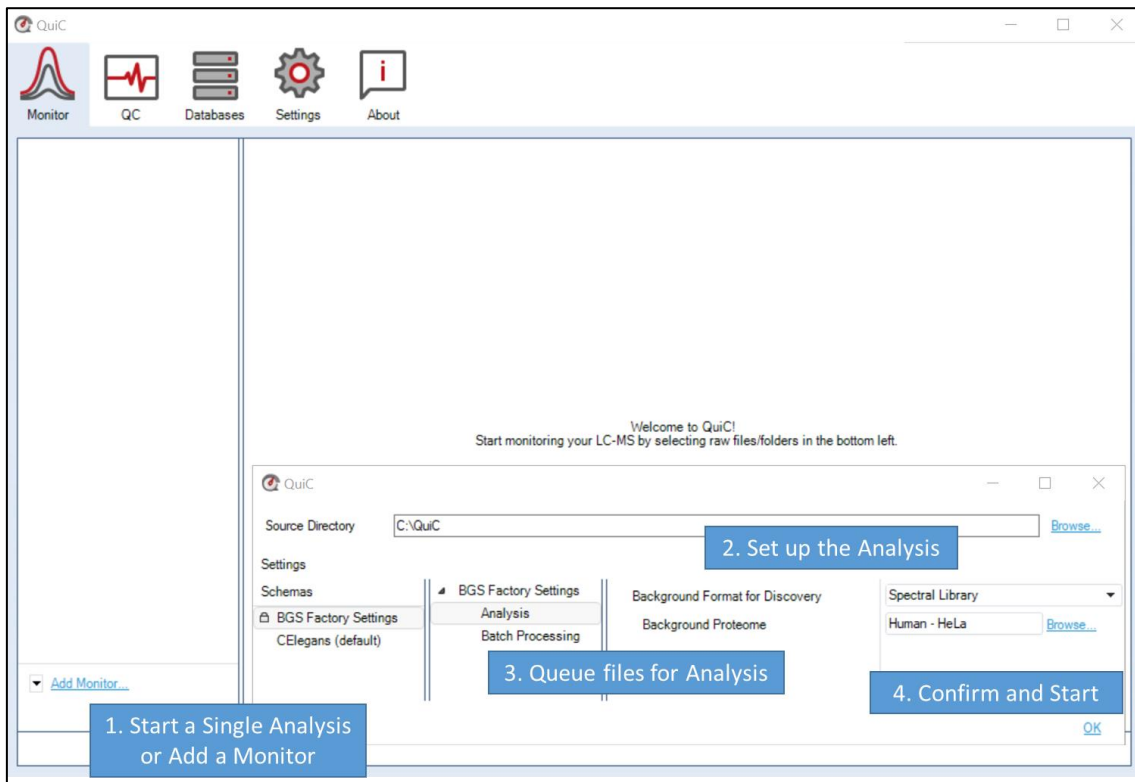


Figure 3.8. The Monitor Perspective allows the monitoring of individual files as well as of a folder. First, start a single analysis or setup a new monitor through the action “[Run Single Analysis.../Add Monitor...](#)”. Set up the Analysis Background in the Settings wizard and queue files that will be automatically analyzed as soon as added to the monitored folder with the Batch Processing option.

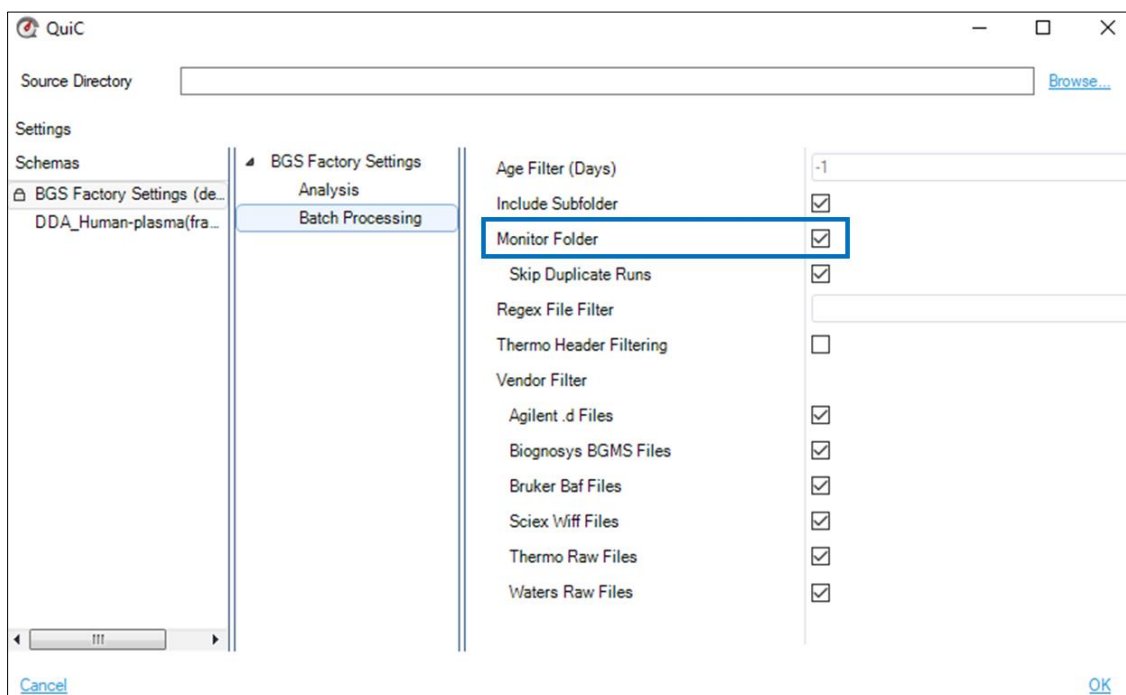


Figure 3.9. By selecting “Monitor Folder”, the Folder will be monitored even after all currently available files have been processed. This way that all newly created files (e.g., newly acquired runs) will be automatically processed.

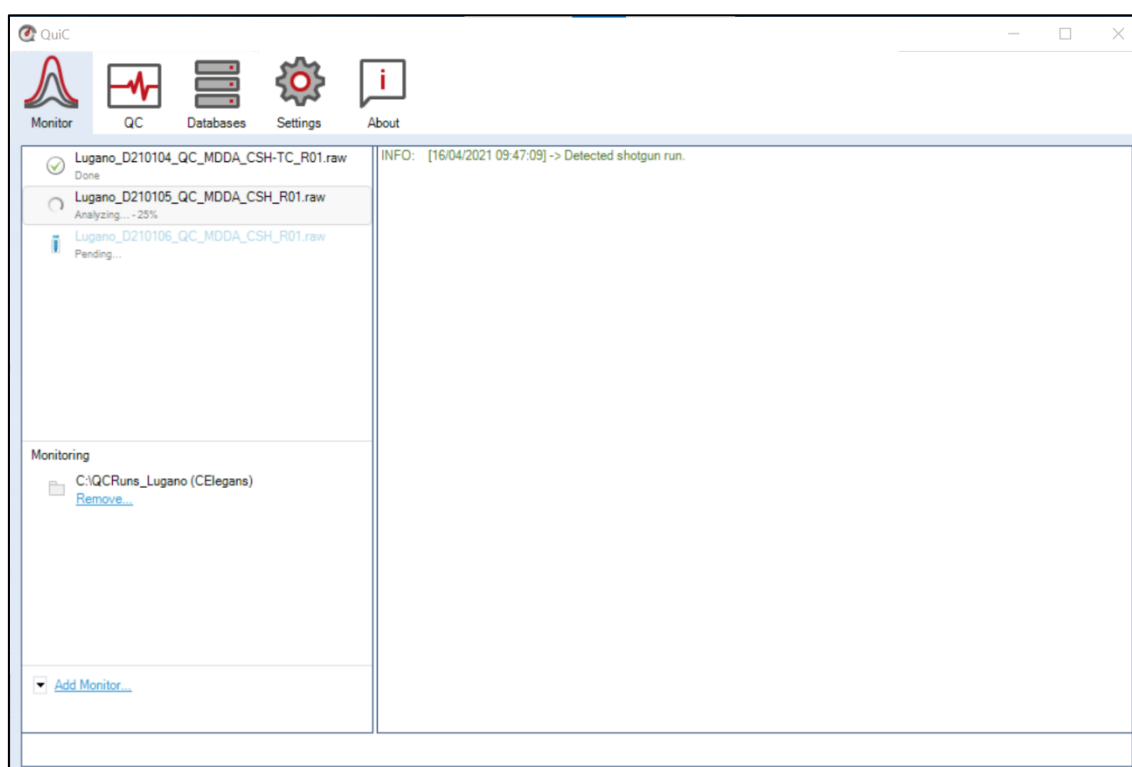


Figure 3.10. The Analysis execution is displayed in the left panel of the Monitor perspective. Completed files are marked with a green checkmark and will be automatically listed under Runs in the QC perspective.

3.5 QC Perspective

The QC Perspective is, as the name implies, the main perspective of QuiC. Through the Runs tab, the user can access the history of analyzed runs and visualize the results in the Plots tab. Through the top left menu, the Events, Instruments, Thresholds, and Default Workspaces tabs expand (Figure 3.11).

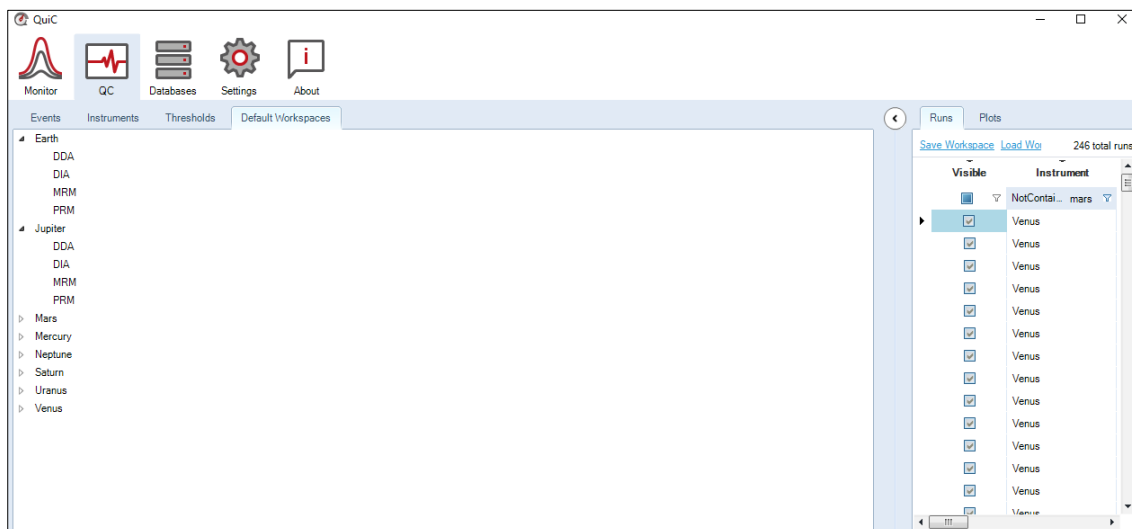


Figure 3.11. The QC Perspective is the main perspective of QuiC where Runs and Plots are saved. This perspective also contains the tabs Events, Instruments, Thresholds, and Default Workspaces, which by default are collapsed under the top left menu.

3.5.1 Runs

The Runs tab lists all the processed runs, which can be sorted and selected for plotting by means of the column filters (Figure 3.12). The selection of runs is manual, only one single run at a time can be selected. Selection is confirmed by a checkmark in the Visible column. The ticked runs are now displayed in the Plots tab. The non-ticked runs remain in the table but are greyed out and are not included in the plots. The table will keep the visible runs on top of the list and the non-visible ones at the bottom, even when you change the sorting order. That is, the visible and non-visible runs are sorted

independently. A selection of runs can also be saved as a workspace and loaded when needed.

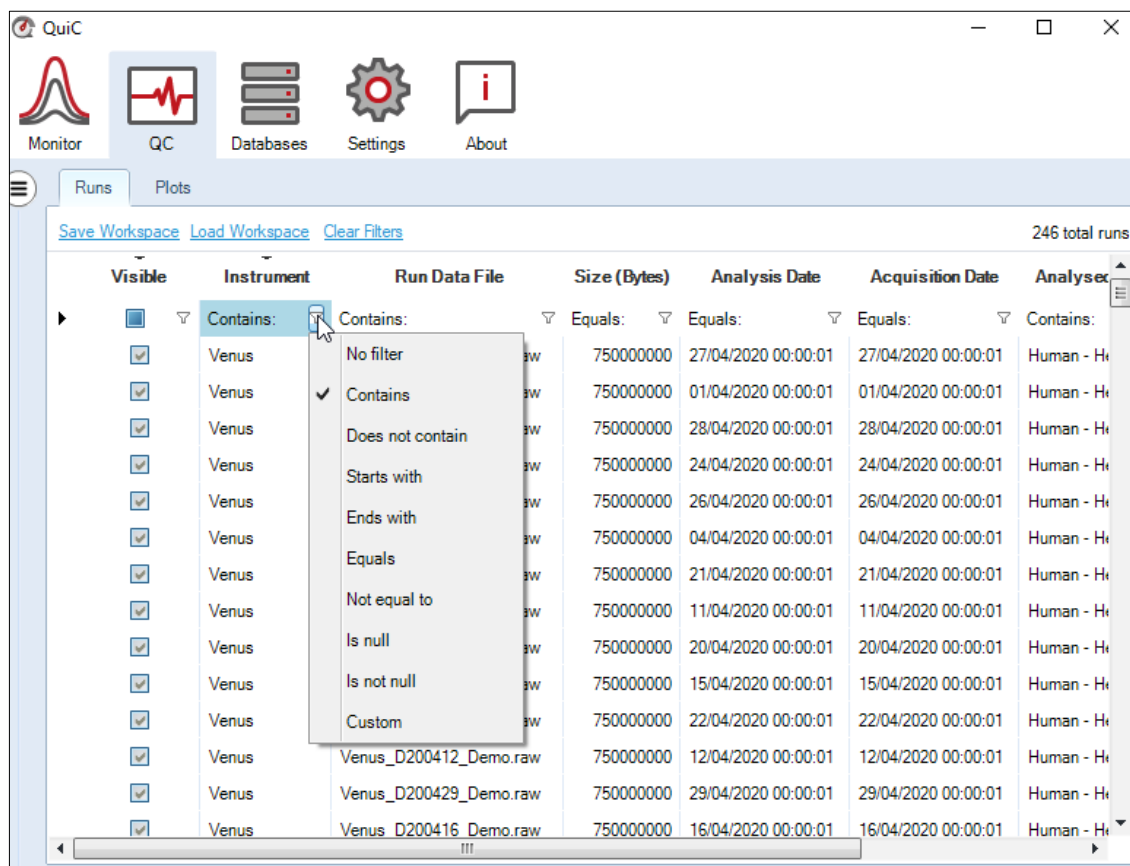


Figure 3.12. Every column of the Runs Tab can be sorted using filters. Every filter set in the Runs Tab also applies to the Plots Tab. In this way, only selected runs are visualized on plots. A filtering schema can also be saved as a “Workspace” and reloaded in future sessions.

When starting QuiC for the first time, none of the runs of the demo dataset will be selected. The user can choose a selection of runs either manually or with filters. The Visible column will then automatically update with the runs that meet this criterion, which will be highlighted. Clicking on the funnel symbol allows the user to change the filter type (e.g., Contains, Does not contain, Equals, Not equal to, etc.). The Custom filter combines multiple filters and allows specifying whether ALL or ANY of the criteria should be met. An example of its application is the selection of multiple instruments (e.g., Instrument A and B) in the analysis by setting a Custom filter: ALL, Contains Instrument A, Contains Instrument B.

QuiC will remember your last selection of runs when restarting. Nevertheless, we recommend saving the desired filter configuration (“Save Workspace”) for later use (“Load Workspace”). By default, workspaces are saved in the “Workspaces Directory”

specified in the Global Settings; this can be pointed to a new location (see section 3.2.1). Workspaces are transferable among different QuiC instances. However, be aware that filtering on the Instrument column, for instance, might not work if instruments are named differently in different instances.

Any filters that you have configured are highlighted under the column name in a light-blue shade. Clicking the "Clear Filters" button on top of the table will clear all applied filters. It is also possible to add comments to a run by typing in the Comments field. Finally, a run can be deleted by right-clicking on it. Be aware that by deleting a run, this will no longer be considered as a "duplicate run" in the Monitor Perspective, and it will be reanalyzed if present in the monitored folder (see section 3.2.2).

3.5.2 Plots

The Plots tab is the main QuiC output. Once the Events, Thresholds, and the Default Workspaces filters are defined, the Plots tab will display performance metrics according to your preferences.

All plots will be shown in either of two different formats, depending on whether you are looking at one instrument and acquisition method (e.g., Earth/DDA) or the combination of different instruments and/or acquisition methods (e.g., Earth/DDA and Jupiter/DDA):

1. One single instrument and acquisition method. Such plots are displayed when selecting an instrument and an acquisition method in the Default Workspaces or by manual runs selection in the Runs perspective. Possible representations are line charts, bar- and box plots.
2. Combination of instruments and/or acquisition methods. Such plots are displayed by manual selection of runs acquired on different instruments and/or with different acquisition methods in the Runs perspective. Here the metrics are displayed on heatmaps.

By right-clicking on the plots, the selection menu appears (Figure 3.13). The menu allows, among others, to save plots (as images or in vectorial format), display legends, rescale axes, or sort the metrics by the actual acquisition time (expressed in calendar dates), acquisition index (expressed in runs), or condition (if experimental conditions were set).

Reports containing all the plots (or a selection of them) can be exported by clicking on the actions (“[Export to Spreadsheet](#)” and “[Export to PDF](#)”) available at the bottom left corner of the Plots tab.

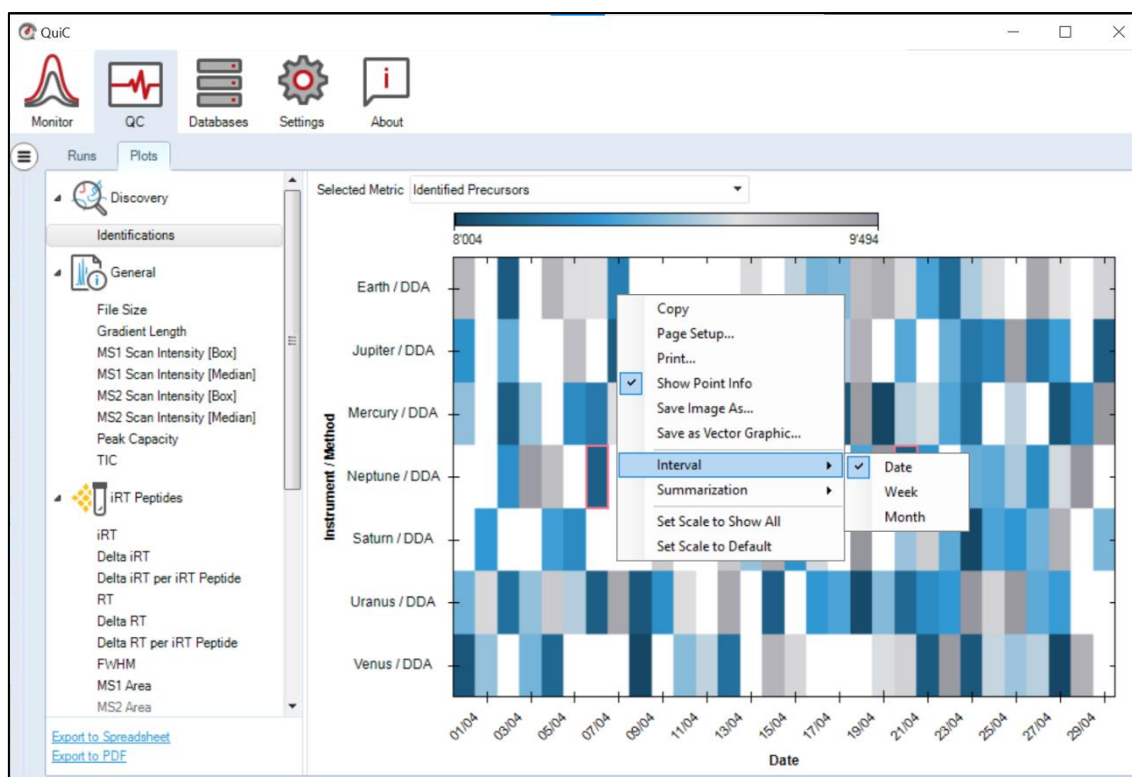


Figure 3.13. By right-clicking on the plots, the selection menu appears. The selection menu allows some plot modifications as well as saving them. The actions at the bottom left corner of the tab allow exporting all or a selection of plots.

3.5.2.1 Discovery parameters

The number of precursors and protein groups identified across runs for one (Figure 3.14) or more instruments and acquisition methods (Figure 3.15). For discovery methods (DDA and DIA), the number of identifications depends on the background proteome or FASTA file used. In the case of targeted methods (PRM and MRM), the number of identifications is based on the Biognosys iRT standard peptides spiked in the sample.

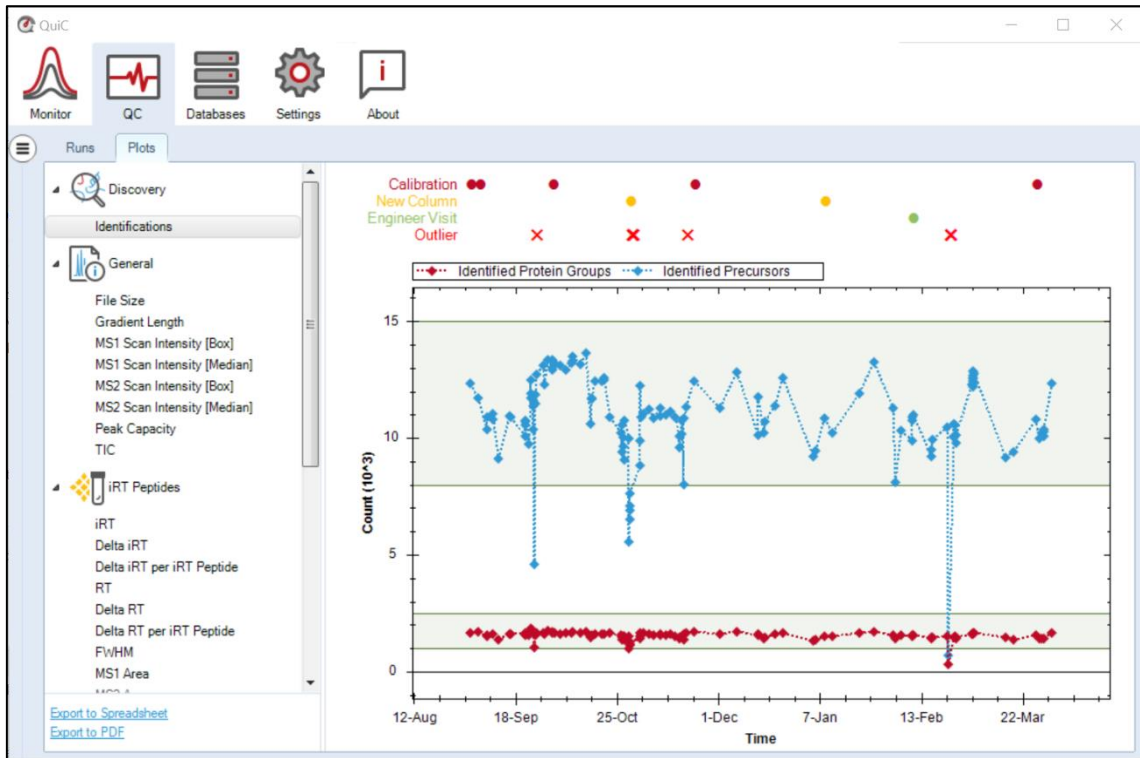


Figure 3.14. The Identifications plot shows the number of precursors (blue) and protein groups (red) identified across measurements (same instrument and acquisition method) over time. The green areas represent the thresholds set for identification. When QC runs violate the thresholds, they are flagged as outliers (red crosses above the plot). The Event Tags (i.e., Calibration, New Column, Engineer Visit) are also noted with different colors above the plot.



Figure 3.15. The Heatmap shows the identified precursors (or protein groups) across several instruments and/or acquisition methods. This overview allows for performance comparison of different instruments and acquisition methods over time. We recommend narrowing the time frame to a maximum of a few weeks of measurements to extrapolate meaningful performance information. The blue to gray gradient represents the number of identifications per run, and outlier runs (according to the set threshold) are framed in red.

NOTE: Because our analysis pipelines evolve, we allow the user to normalize identifications across QuiC version updates. This will mitigate visible increases or decreases in identifications in the plot due to a change in our algorithms. If the user thinks his IDs changed due to a QuiC software update (such changes are indicated in the event tag indicator area above the plot), they can right-click anywhere in the Identifications plot and select “Normalize Across QuiC Versions”. The formula for the normalized ID is given as follows:

$$ID_{\text{normalized}} = ID_{\text{median}} \cdot \frac{ID_{\text{unnormalized}}}{ID_{\text{version_median}}}$$

Where $ID_{\text{unnormalized}}$ is the original identifications value, $ID_{\text{version_median}}$ is the median ID value among all other ID values computed from the same version of QuiC as $ID_{\text{unnormalized}}$, and ID_{median} is the median ID overall. Note these values are only computed on the selected runs and not the entire set of runs. We suggest using this option only when you have a sufficient number of IDs per version.

3.5.2.2 General parameters

The general parameters' plots display information on the File Size, LC gradient length as well as common acquisition metrics, such as MS1 and MS2 scan intensities ([Box] and [Median]), peak capacity, and total ion current (TIC). Find a detailed list with all the QC parameters monitored by QuiC in [Appendix 5.1](#) and the supplementary plots in [Appendix 5.3](#).

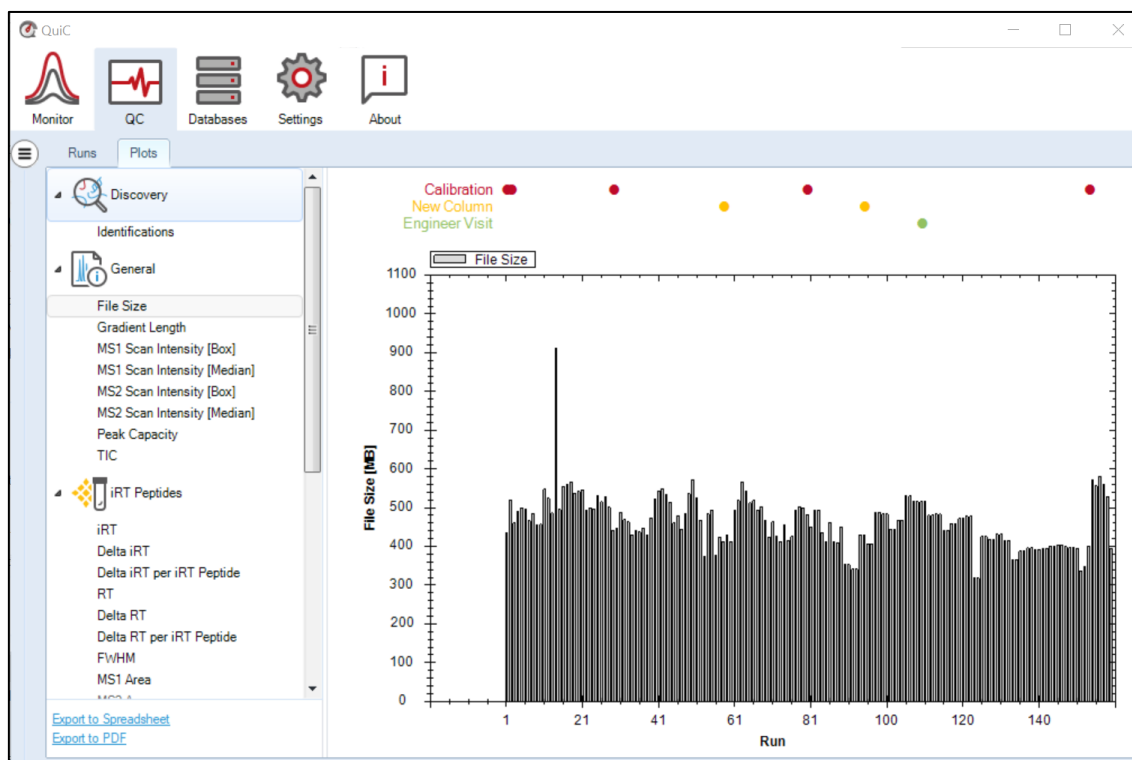


Figure 3.16. The File Size plot shows the file size on disk for selected run files. This plot informs if the acquisition saved a reasonable amount of data. It is recommended to sort the x-axis by the "Acquisition Index".

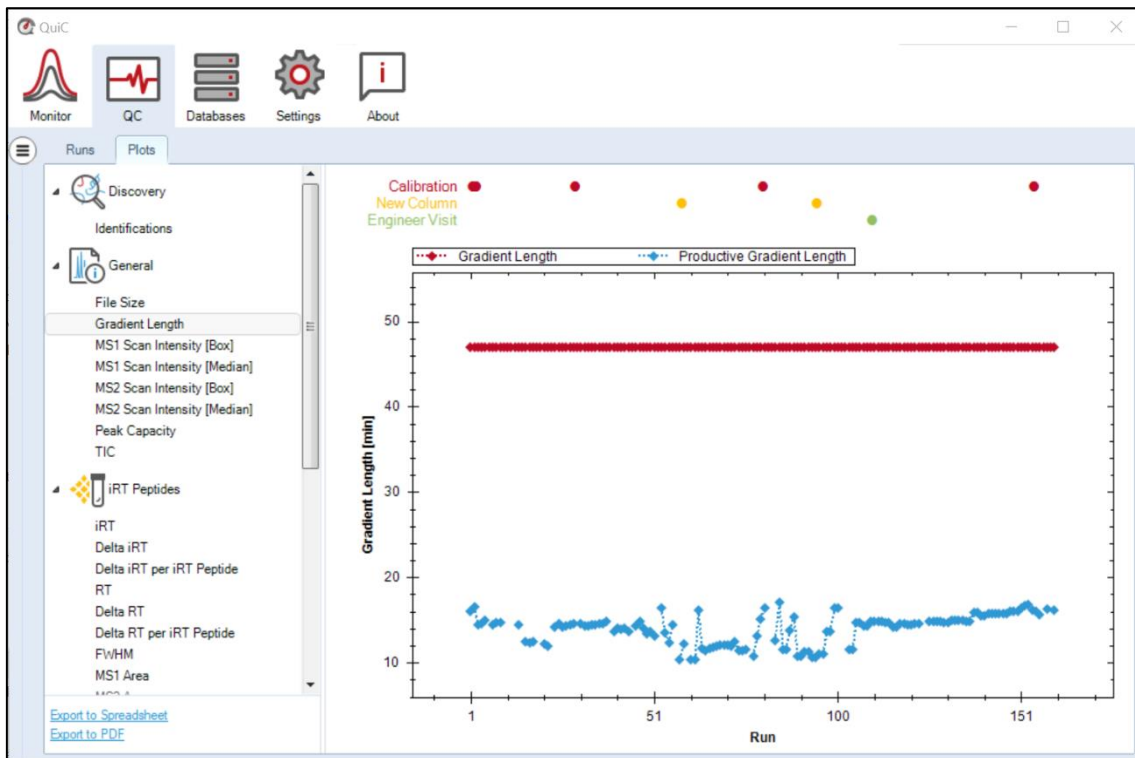


Figure 3.17. The Gradient Length plot shows the gradient duration [min] across selected runs. This plot informs if the method was correctly set. For instance, if the same gradient was used across runs or if washing runs were included. It is recommended to sort the x-axis by the “Acquisition Index”.

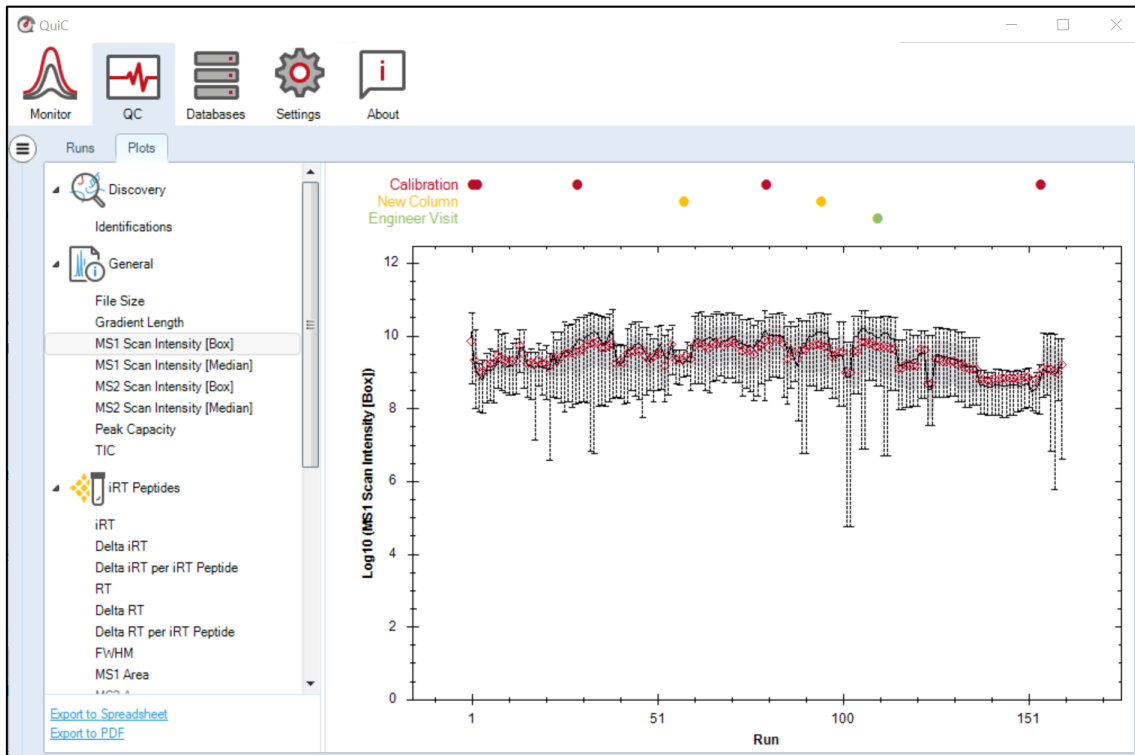


Figure 3.18. The MS1 Scan Intensity [Box] plot shows the intensity of the MS1 scans on a log10 scale across runs. The same plot is available at the MS2 level. The box plot is computed from the “productive range,”

which in MS1 considers the scans for which peptides eluted. The Scan Intensity plots display intensity fluctuations. The red diamonds represent the mean intensity values in each run, the lower and the upper edges of the gray area represent the first (Q1) and third (Q3) quantiles, respectively. The black line shows the median intensity across runs.

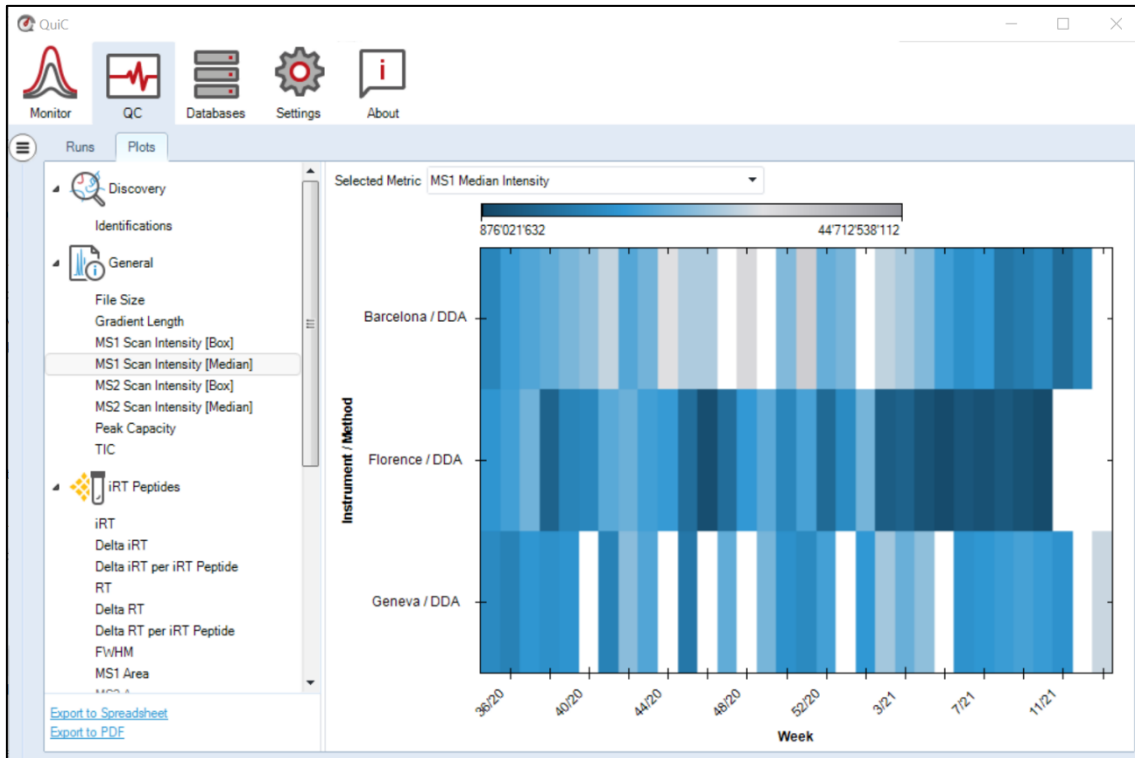


Figure 3.19. The MS1 Scan Intensity [Median] plot for more instruments shows the intensity of the MS1 scans on a log10 scale across instruments/methods. The same plot is available for all the MS1 and MS2 scan intensities ([Box] and [Median]). The Scan Intensity plots display intensity fluctuations and therefore instruments stability over time (in this example, Weeks).

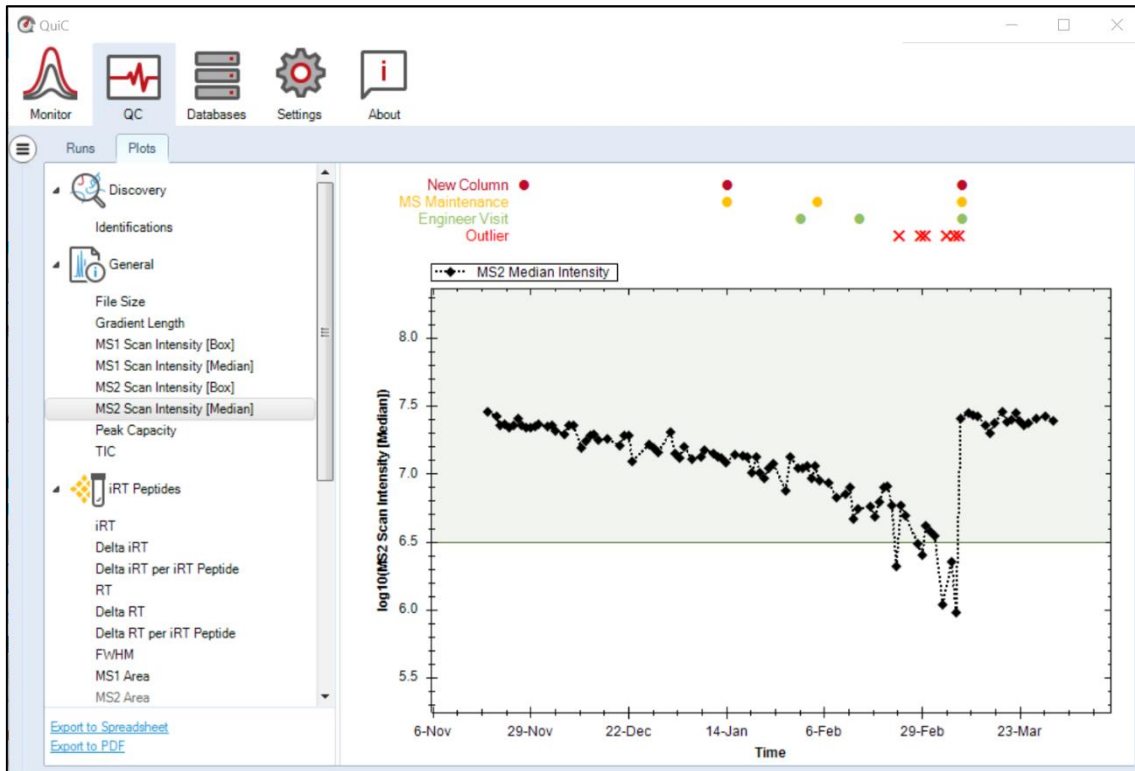


Figure 3.20. The MS2 Scan Intensity [Median] plot shows the median intensity of the MS2 scans on a log10 scale across runs. The same plot exists at the MS1 level. The median is computed from the “productive range”, which in MS2 considers the range between the iRT standard peptides b and l, namely the second and the last eluting peptides. The Scan Intensity plots display intensity fluctuations attributable, for instance, to system calibration, LC column cleaning or replacement, or lens cleaning. Event Tags annotations (i.e., MS Maintenance, New Column, Engineer Visit) and outlier runs are noted above the plot.

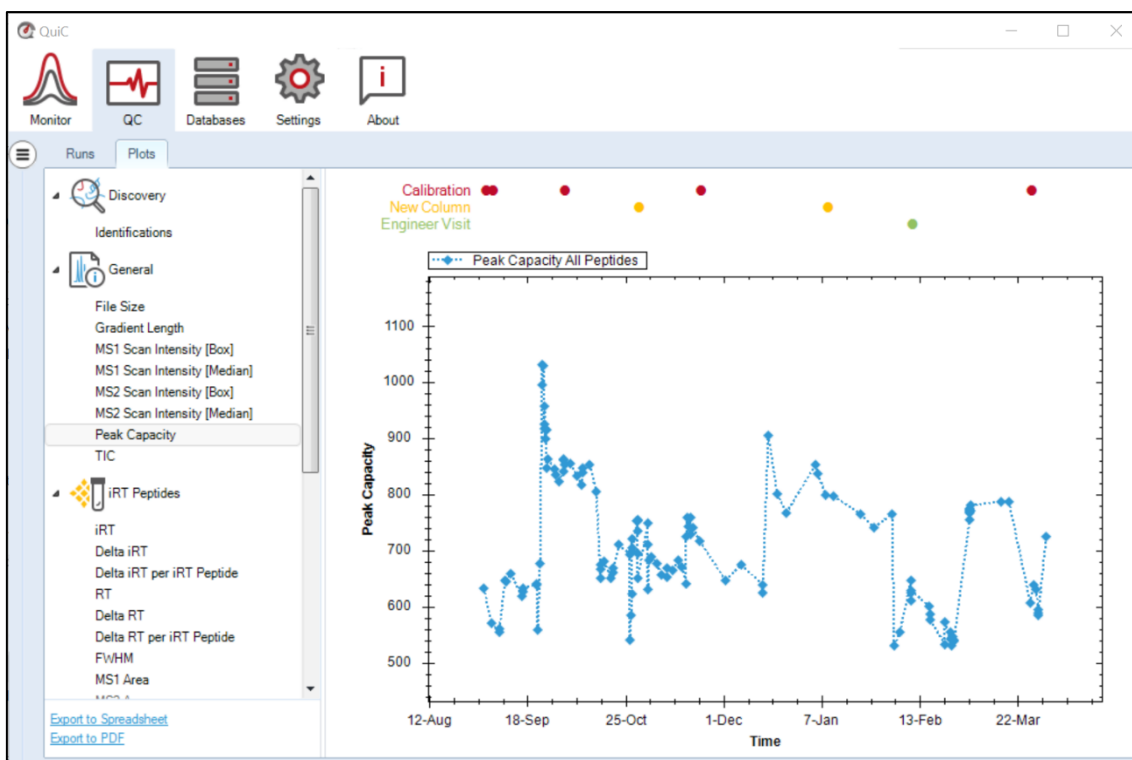


Figure 3.21. The Peak Capacity Plot displays the efficiency of the Ultra-Performance Liquid Chromatography (UPLC) column at separating peptides. Higher peak capacity represents higher and narrower chromatographic peaks, thus better peptides separation. The peak capacity is calculated by dividing the length of the linear portion of the gradient by the average full width at half maximum (FWHM) for all peptide precursors with a q-value < 0.01

3.5.2.3 iRT peptides parameters

This section summarizes QC data based on the iRT standard peptides (iRT kit) identified in the runs. For most workflows, this section is sufficient at assessing the LC-MS setup.

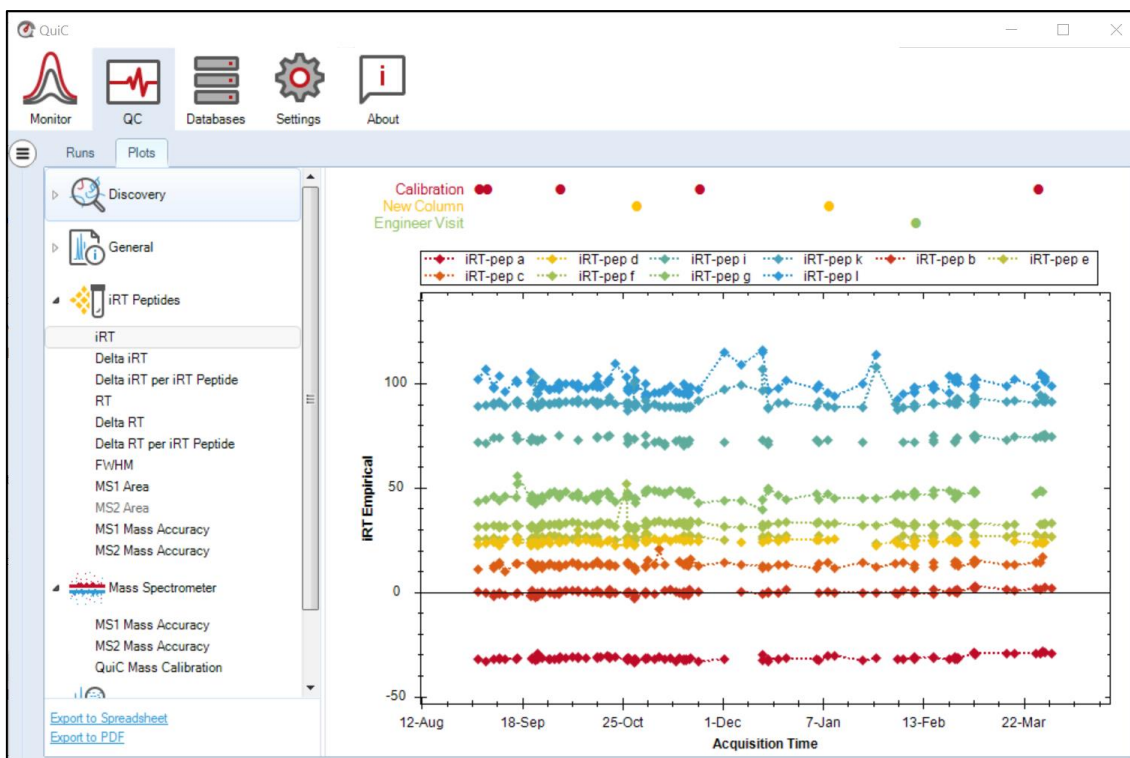


Figure 3.22. The iRT plot shows the normalized RT for the 11 iRT standard peptides spiked in the runs. The iRT standard peptides are evenly spread over the retention time with the second peptide (iRT-pep b) being defined as iRT=0, and the last peptide (iRT-pep l) as iRT=100.

Delta iRT

The Delta iRT is the difference between a peptide theoretical and empirical iRT, computed from the linear iRT/RT regression function (Escher *et al.*, 2012). The bigger the Delta iRT, the further away the empirical from the theoretical iRT is.

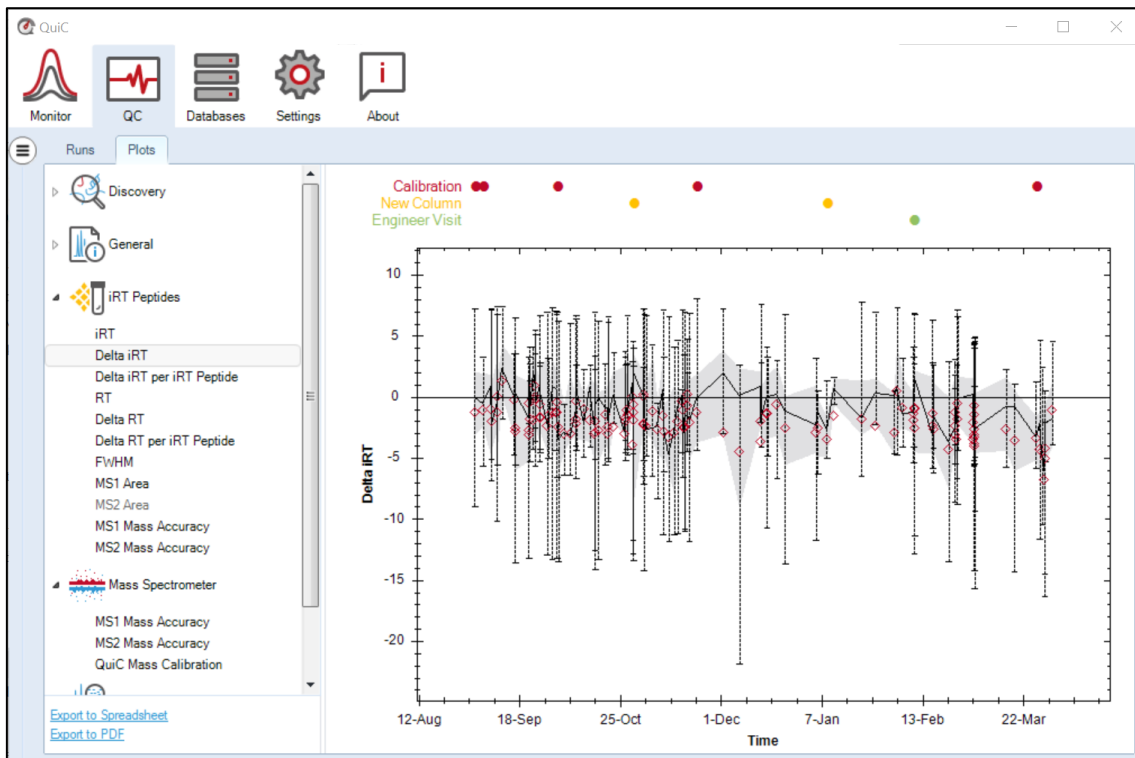


Figure 3.23. The Delta iRT plot represents the difference of the empirical from the theoretical iRT value within and across runs. The red diamonds represent the mean iRT values in each run, the lower and the upper edges of the gray area represent the first (Q1) and third (Q3) quantiles, respectively. The black line shows the median Delta iRT across runs.

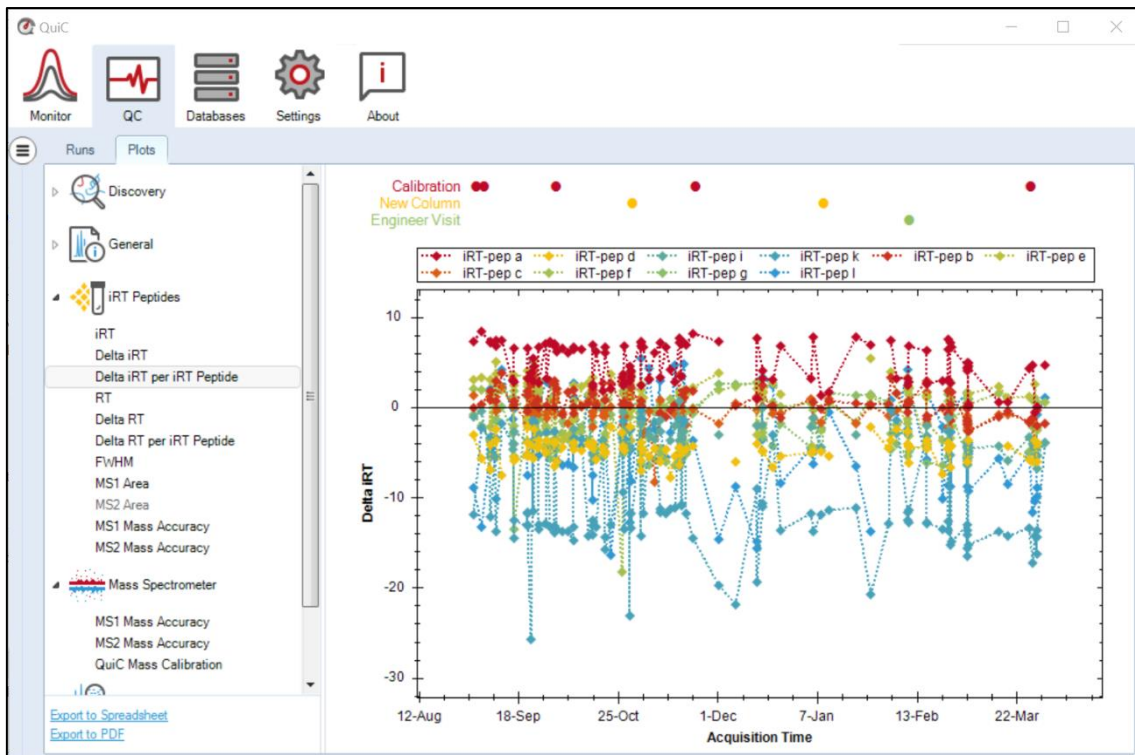


Figure 3.24. The Delta iRT per iRT peptide plot represents the difference of the empirical from the theoretical

iRT value within and across runs. Instead of summarizing over all iRT peptides, the raw individual values are shown.

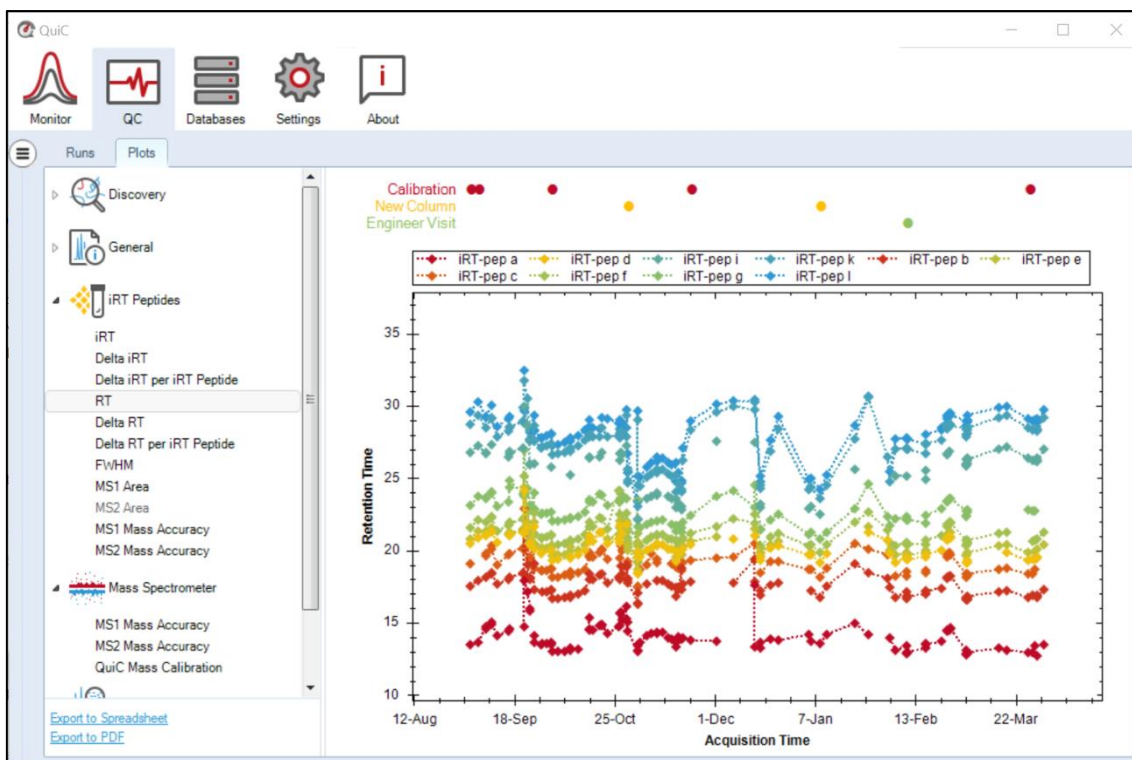


Figure 3.25. The RT plot shows the actual retention time (non-normalized) of the 11 iRT standard peptides across runs. Missing data points represent not identified peptides. This is more common in DDA due to semi-stochastic sampling. Variation in the retention time across samples, as well as lack of their identification, can also occur with different LC gradients and columns, high sample complexity, or low standard peptides concentrations.

MS1 and MS2 Mass Accuracy

These plots display the mass accuracy for all identified iRT standard peptides at the precursor (MS1) and fragment ions (MS2) level. Mass accuracy is critical for ion chromatogram extraction and scoring.

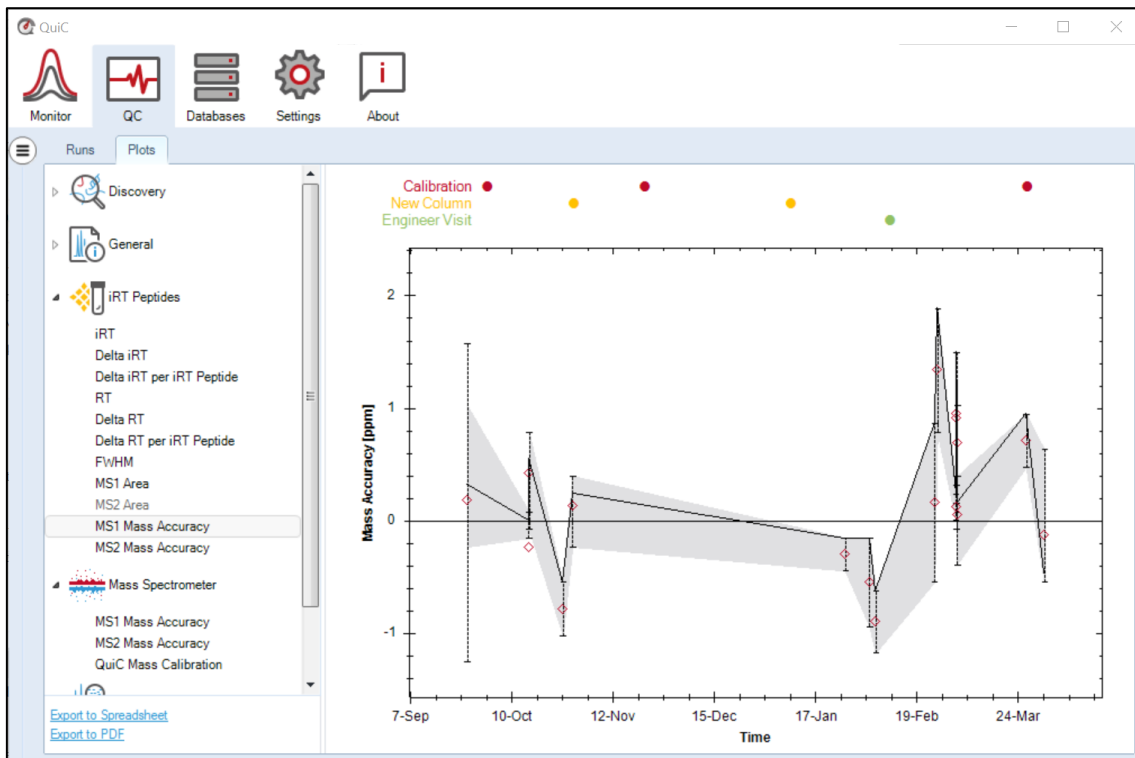


Figure 3.26. The MS1 Mass Accuracy plot represents the mass errors for all identified iRT peptide precursor ions in ppm. The same plot is available at the MS2 level, which usually appears less accurate than MS1 due to higher complexity. The red diamonds represent the mean accuracy in each run, the lower and the upper edges of the gray area represent the first (Q1) and third (Q3) quantiles, respectively. The black line shows the median mass accuracy across runs.

3.5.2.4 Mass Spectrometer parameters

This section summarizes data based on all the peptides identified across runs. This section is useful for more advanced

MS1 and MS2 Mass Accuracy

These plots display the mass accuracy for all identified peptides at the precursor (MS1) and fragment ions (MS2) level. Mass accuracy is critical for ion chromatogram extraction and scoring.

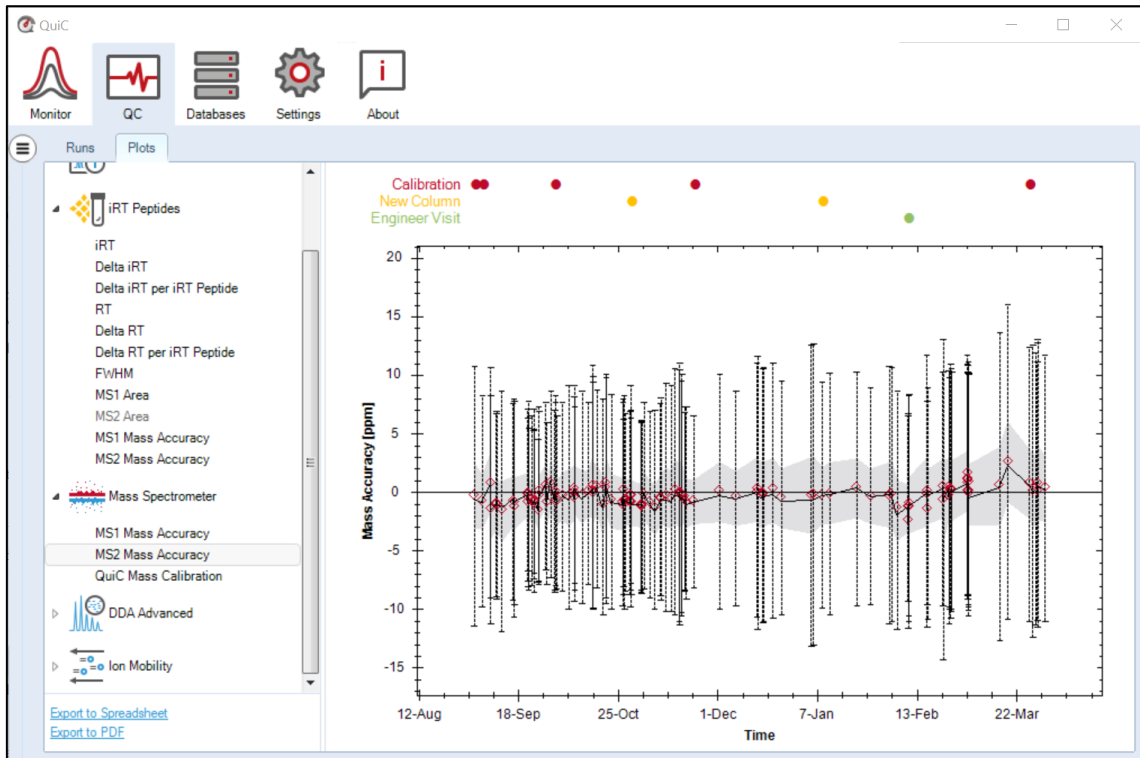


Figure 3.27. The MS1 and MS2 Mass Accuracy plots represent the accuracy of the precursor or fragment ions mass relative to the targeted ion mass. The red diamonds represent the mean accuracy in each run, the lower and the upper edges of the gray area represent the first (Q1) and third (Q3) quantiles, respectively. The black line shows the median mass accuracy across runs.

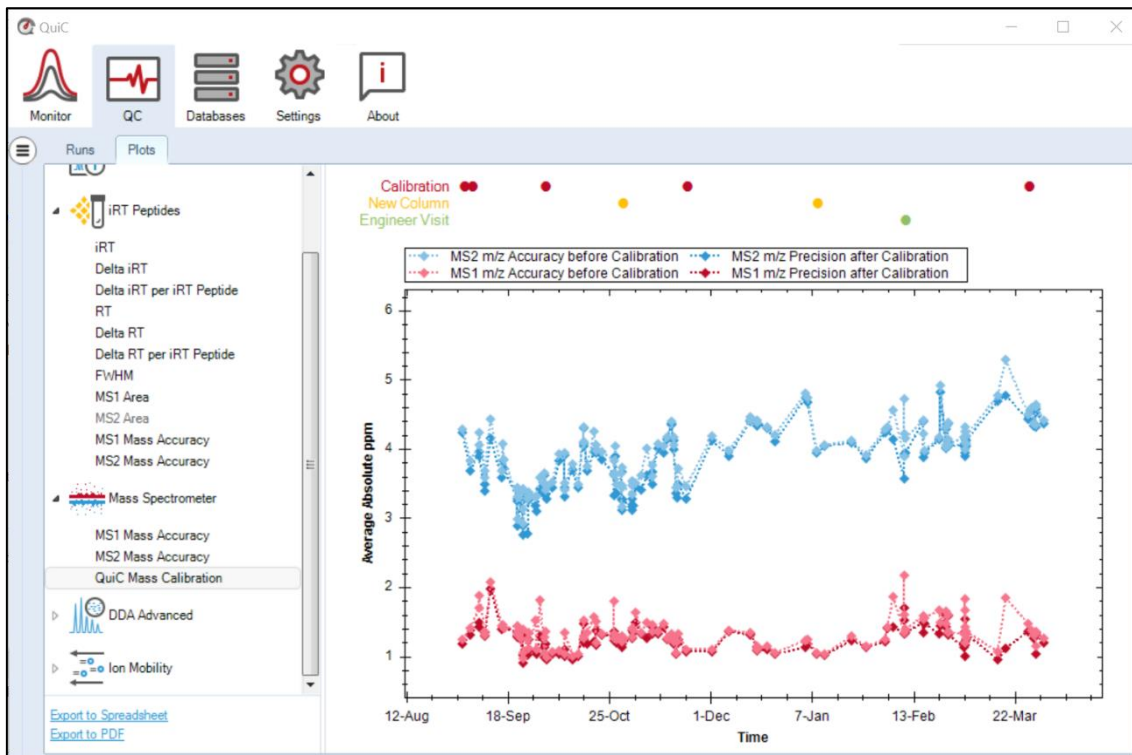


Figure 3.28. The graph shows how the average absolute ppm is corrected by QuiC. Indeed, QuiC handles mass drifts in different acquisition methods (DDA, DIA, PRM) by performing extensive MS1 and MS2 mass calibration.

3.5.2.5 DDA Advanced

These plots pertain only to DDA data and were first presented in (Ma *et al.*, 2012).

Scans

The number of MS1 (MS2) scans that were collected.

Max Scan Frequency

The fastest frequency [scans per minute] for MS1 (MS2) collection in any minute.

PSM Charge Profile

A breakdown of all of the peptide spectrum matches (PSMs) with a clear feature having charges 1, 2, 3, 4, 5, >5. Also considered are peaks originating from the precursor window that lack known charge but appear to have charge 1 or charge >1. It is recommended to sort the x-axis by the "Acquisition Index".

3.5.2.6 Ion Mobility

These plots pertain only to PASEF or diaPASEF data, and show the quality of your Bruker timsTOF runs.

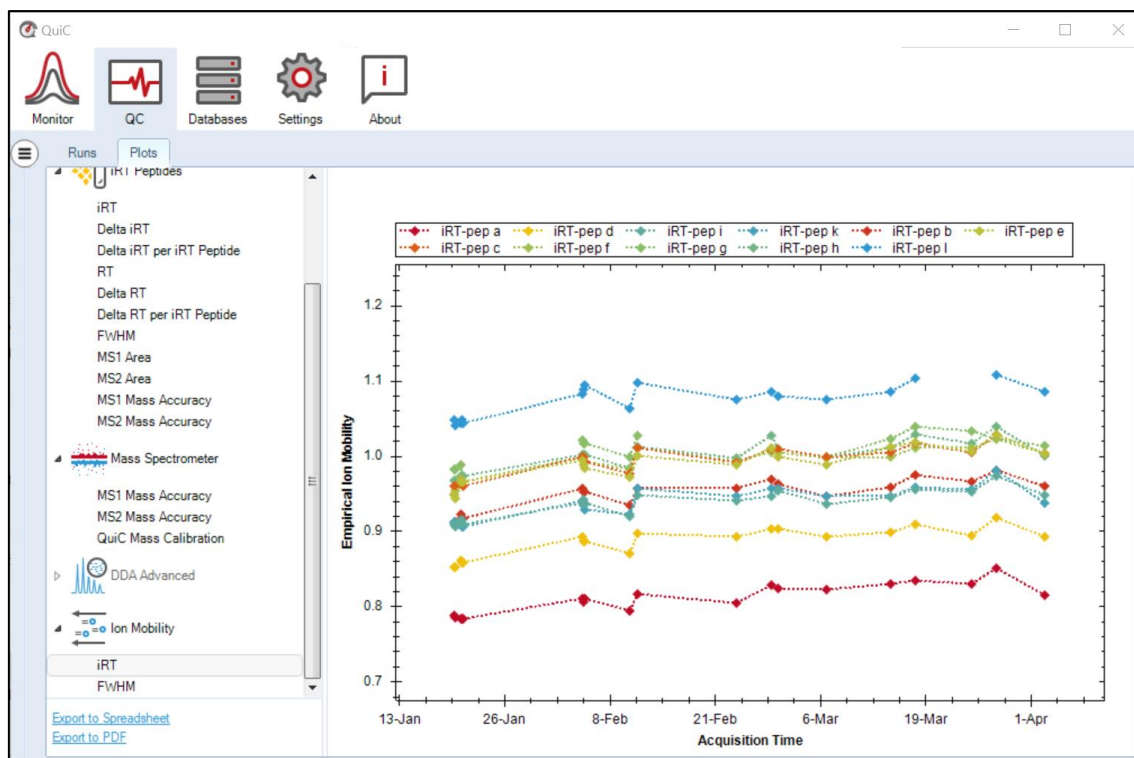


Figure 3.29. The IM-iRT plot shows the Ion Mobility value for the 11 iRT standard peptides spiked in the runs.

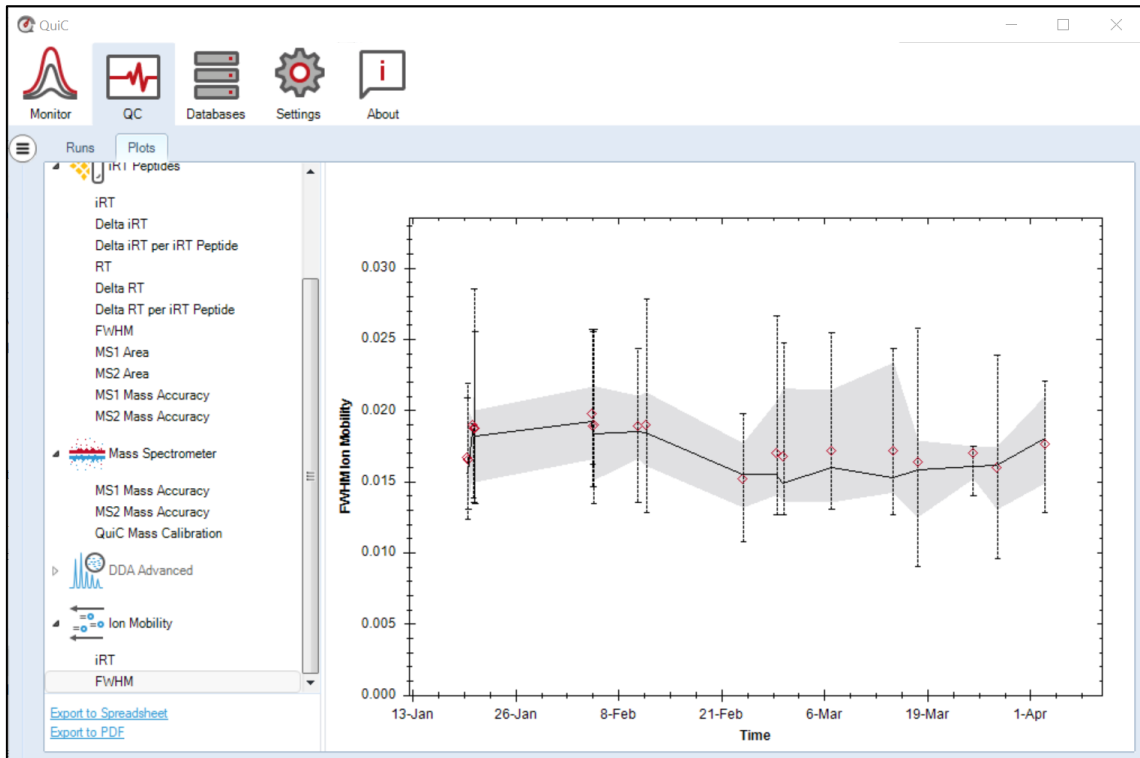


Figure 3.30. The FWHM is the width of a peak at half height. The FWHM plot represents the full width at half maximum of the Ion Mobility values, summed up for each iRT peptide.

3.5.3 Events

The Events tab allows for the management of event tags (Figure 3.31). Here you will find event tags that have been parsed from the run file names (see section 3.2.1). Please be aware that QuiC will only associate the first ten tags to all instruments automatically. The rest must manually be assigned in the Instrument tab (this is to prevent clutter in the plots).

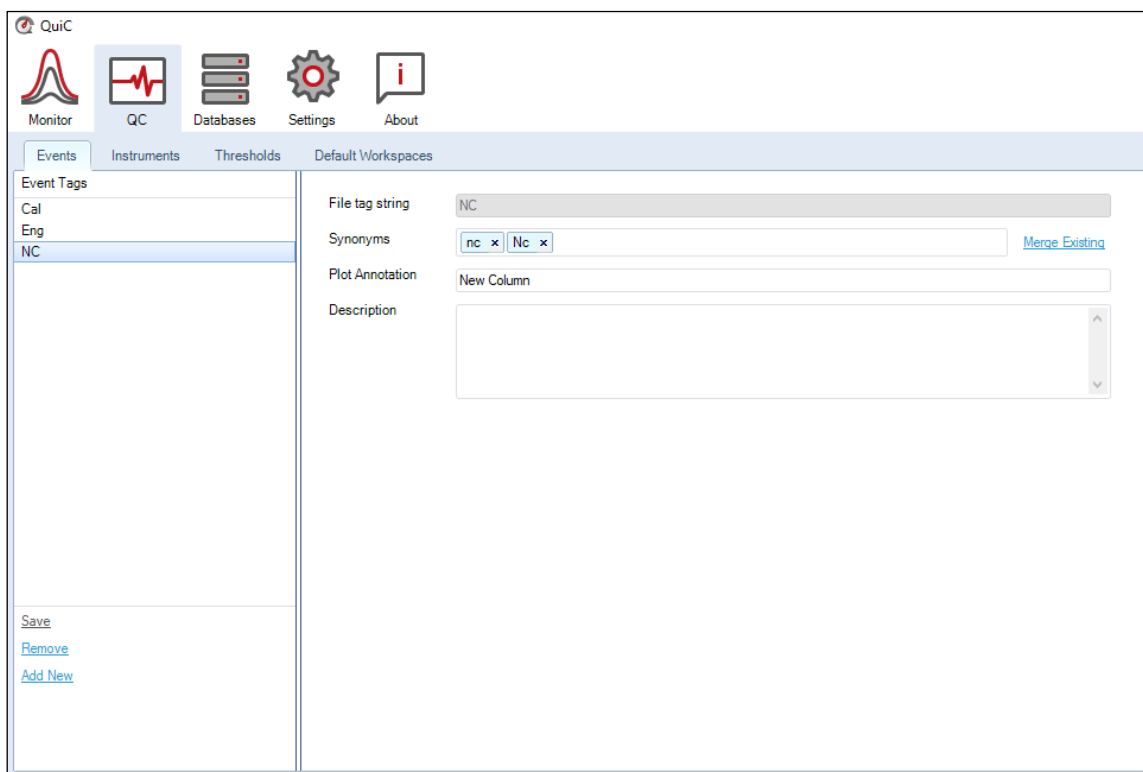


Figure 3.31. Event Tags can be parsed from file names according to the schema specified in the Global Settings. For instance, if the file name contains information on a new column (e.g., FILENAME_NC_*), the event tag “NC” can be created and added as annotation “New Column” to plots.

It is common to have typographical errors in the file name, that might not be obvious to the human user, but are significant for the parser. One very common one is the case-sensitivity of the event tag. As an example, the tags “NC”, “nc”, and “Nc” belong to the same event. It is now possible to manually correct errors after parsing, by grouping events into a correct one (Figure 3.31). This is done, by selecting the event tag you want to keep and by adding the redundant tags as "Synonyms". Another possibility is to click on "Merge Existing". In this way, a list of available tags appears, and the tags of interest can be merged by double-click.

3.5.4 Instruments

The Instruments tab allows for the management of instruments. On the left panel, you will find the instrument IDs that have been parsed from the metadata in each run file. The instrument ID should be unique and tied to the physical mass spectrometer (e.g., Exactive Series slot #xyzwz_QExactiveHFXOrbitrap). However, it happens that instrument configuration errors generate more than one ID, although no other change to the instrument has occurred. In this tab, the user can assign the same name to different instrument IDs. There are no restrictions on instrument names. However, if you assign

the same instrument name to multiple instrument IDs, the runs coming from these IDs will be displayed as one instrument in the QC plots.

Finally, in this tab, the event tags can be assigned to individual instruments. Please be aware that only a full match with an existing event tag, including the case of the word, is needed.

3.5.5 Thresholds

QuiC requires manual inspection of the QC metrics to be able to determine the state of an instrument. In this tab, the user can set thresholds to help to visualize outliers on the QC plots.

You can define the thresholds for each instrument (by selecting the appropriate instrument node) and the acquisition method (DDA, DIA, MRM, PRM), or define acceptable thresholds across methods (Figure 3.32). Assuming you would expect to identify 15,000 +/- 1,000 peptides and at least 9,000 protein groups per QC run for instrument "Earth". You can configure the thresholds for this instrument by setting Discovery -> Identifications -> Identified Precursors to $14,000 \leq x \leq 16,000$ and Identified Protein Groups to $9,000 \leq x$. As soon as a QC run violates the thresholds, this will be flagged as an outlier in the identification plot (Figure 3.14). Thresholds are also colored directly in the graph for visual inspection.

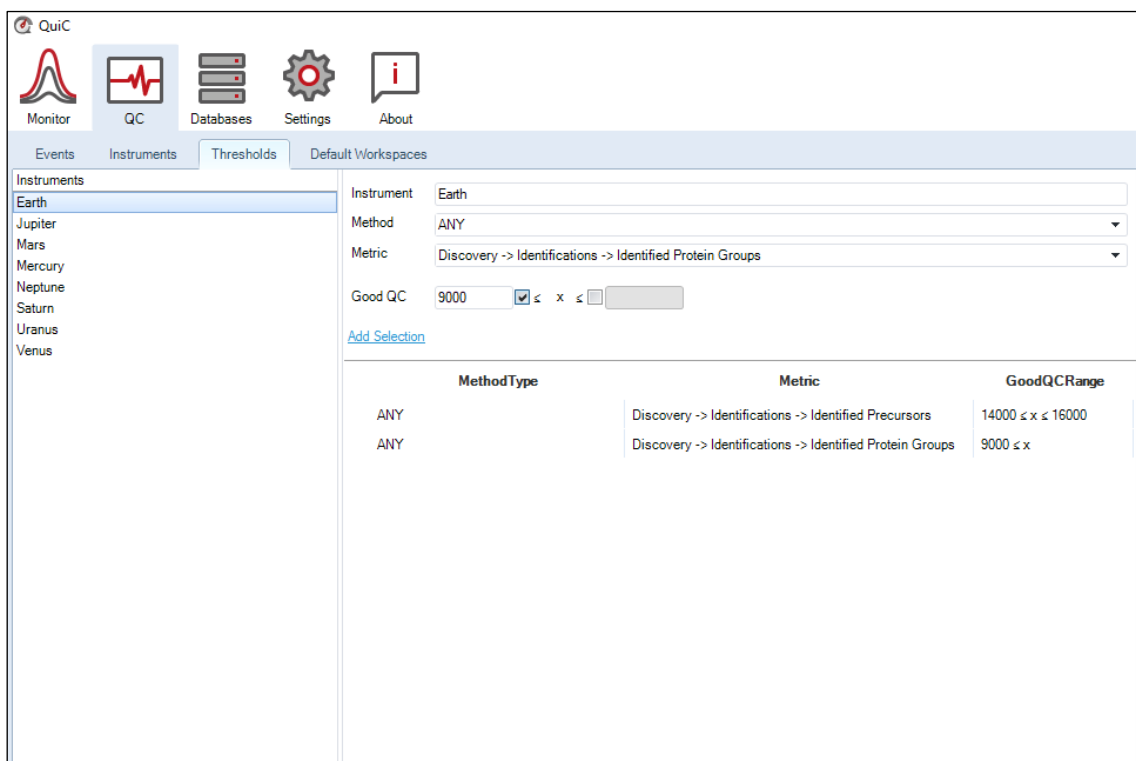


Figure 3.32. Thresholds can be set for individual or across instruments and acquisition methods. Once thresholds are set for a metric, the outlier runs will be flagged in the respective QC plot. Active thresholds can be deleted by right-clicking on them.

3.5.6 Default Workspaces

To ease the visualization of selected instruments or acquisition methods, the Default Workspaces tab is automatically populated with preconfigured filter settings applied to the Runs tab. By clicking on an instrument node, the list of entries in the Runs tab shrinks to the runs acquired with that instrument irrespective of the acquisition method (Figure 3.33). When expanding the instrument node, all the acquisition methods (i.e., DDA, DIA, MRM, and PRM) appear. Choosing an acquisition method shrinks the runs list (in the Runs tab) to that instrument/method combination. Be aware that clicking on an instrument/method changes previous filtering settings. Therefore, if necessary, first save the workspace (see section 3.5.1).

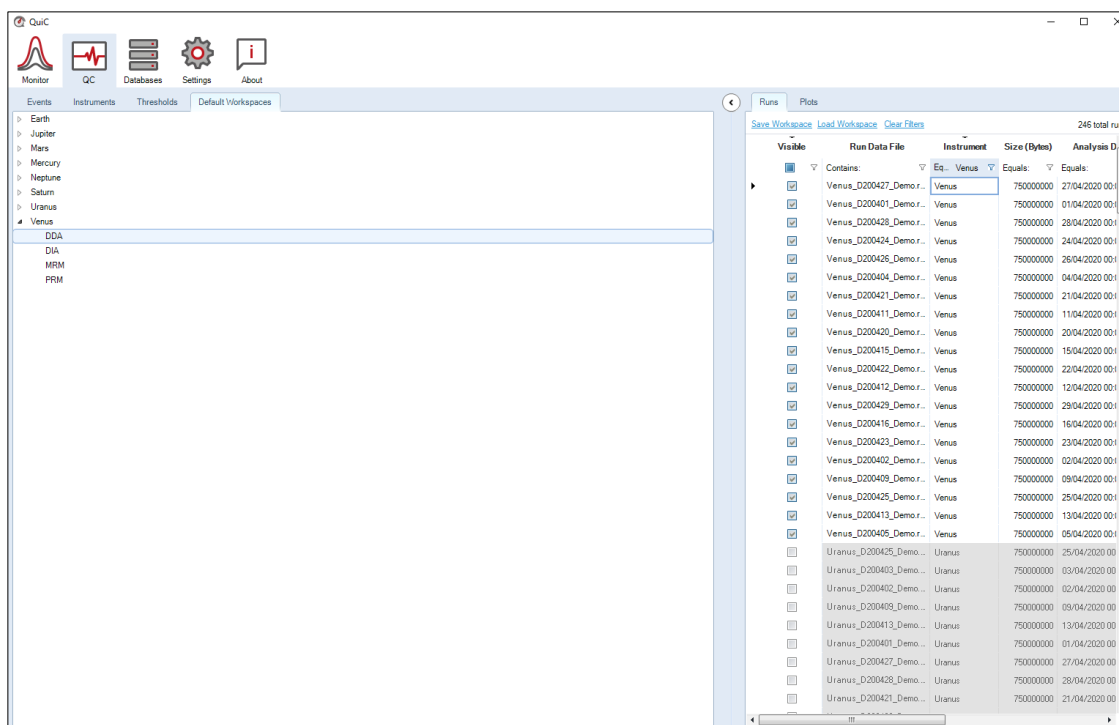


Figure 3.33. The run files are ordered per instrument and acquisition method in the tab Default Workspaces. This simplifies the search of specific runs. For example, if one is only interested in the DDA runs acquired with the MS instrument “Venus”, the instrument and method nodes can be selected in the Default Workspaces, and the selection will directly apply to the Runs and Plots tabs.

3.6 About Perspective

This perspective contains information on the QuIC version installed, the activation key of the license in use, the history of error logs, and this user manual.

4 References

- Escher, C., Reiter, L., MacLean, B., Ossola, r., Herzog, F., Chilton, J., . . . Oliver, R. (2012). Using iRT, a normalized retention time for more targeted measurement of peptides. *Proteomics*, 1111-1121.
- Ma, Z. Q. (2012). QuaMeter: multivendor performance metrics for LC–MS/MS proteomics instrumentation. *Analytical chemistry*, 5845-5850.

5 Appendixes

5.1 Quality control parameters monitored by QuiC

Parameter	Description
Identifications	Number of identified precursors and protein groups
Gradient Length	The length of the LC gradient estimated from the data
MS1 Scan intensity	Distribution of MS1 precursor ion intensities per scan
MS2 Scan intensity	Distribution of MS2 fragment ion intensities per scan
Peak Capacity	Separation efficiency calculated by dividing the length of the linear portion of the gradient by the average full width at half maximum (FWHM) for all peptide precursors with a q-value < 0.01
TIC	Total Ion Current. Sum of all ion current intensities in RT
Delta iRT	iRT difference of the iRT Kit peptides between predicted and observed. The predicted iRT is an arbitrary value based on the gradient length.
Delta RT	Peptide retention time difference between the predicted and observed RT. The predicted RT is computed from an iRT/RT regression
FWHM	Full width at half maximum. Used to determine the resolving power by the ratio $m/z / FWHM$
iRT	Retention time monitoring of the 11 iRT Kit standard peptides
MS1 Area	Average MS1 precursor response
MS1 Mass Accuracy	$(\text{observed } m/z - \text{theoretical } m/z) / \text{theoretical } m/z$ (in ppm) – on precursor ions
MS2 Area	Average MS2 fragment response

Parameter	Description
MS2 Mass Accuracy	(observed m/z-theoretical m/z) / theoretical m/z (ppm) – on fragment ions
RT	Observed retention times of the 11 iRT Kit peptides
QuiC Mass Calibration	Plot of Mass Accuracy and Precision before and after Calibration (Average Absolute ppm)
Max Scan Frequency	The fastest frequency for MS1 (MS2) data collection per minute.
PSM charge profile	The distribution of all the PSM charge states for the same precursor.
Scans	Number of scans that were collected
Ion Mobility	The ability of ions to move through a medium in response to an electric field that is pulling them. The separation of ions according to their mobility in gas phase is called ion mobility spectrometry.

5.2 Library Generation Settings

Configure the library generation settings. Most settings are described below. For further information, there is also helpful text hovers directly in the software.

Tolerances

QuiC 4 will, by default, calculate the ideal mass tolerances to generate the library. QuiC 4 performs two calibration searches: based on the first-pass calibration (rough calibration), the ideal tolerance for the second-pass calibration is defined; based on the second-pass calibration (finer calibration), the ideal tolerance for the main search is defined. QuiC 4 will do this under default settings (Dynamic).

However, QuiC 4 allows you to set your preferred tolerances for the different MS instruments (Thermo Ion Trap, Thermo Orbitrap, TOF). Hence, for both the calibration search (second-pass, finer calibration) and the main search, you can define your tolerances:

- **Dynamic:** determined by Spectronaut based on the precedent search (default). You can set a correction factor for MS1 and MS2 levels (default is no correction = 1)
- **Relative:** set a relative mass tolerance in ppm for both MS1 and MS2 levels
- **Static:** set a fix mass tolerance in Thomson for both MS1 and MS2 levels

Protein Inference

You can have this option activated or deactivated. If you let Spectronaut do your proteins inference, you can refine your sequence settings further in these settings:

Enzyme/Cleavage Rules	Proteases used to <i>in silico</i> digest the proteins from the protein database(s). Defined in Databases → Cleavage Rules
Digest Type	Specific: both N- and C-terminus follow the specified digest rules Semi-specific: only of the termini follows the specified digest rules Unspecific: no digest rules
Toggle N-terminal M	Pre-processing of the protein database by toggling (processing both with and without) the protein N-terminal methionine (when there is one) to account for N-terminal methionine excision.

Spectral Library Filters

You have a number of options to filter the search engine results for library generation. There are filters at the level of fragment ion and at the level of precursor. The filters are quite self-explanatory. Please, use the hover text-tools if you need more information. Find below some of the most relevant.

Fragment Ions	<p>Filter peptides not fulfilling the conditions specified regarding fragment ions. Find more details by hovering over the option in the software. You can specify your defined criteria for:</p> <ul style="list-style-type: none">• Amino acid length• Ion charge• Ion type (y, b)• m/z window• Relative intensity (%)
Precursors	<p>Amino Acids: Filter peptides containing specified amino acids</p> <p>Best N fragments per Peptide: specify the range in number of fragment ions based on response</p> <p>Best N Peptides per Protein Group: keep only the N most abundant peptides per protein</p> <p>Channel Count: keep peptides that are observed in a range of channels</p> <p>FASTA matched: keep only peptide that are found in a user-specified FASTA sequence database and abide the digest rules. Only available if protein inference is selected.</p> <p>Missed Cleavage: keep peptides with N or less missed cleavages</p> <p>Modifications: Filter peptides according to modifications. Find more details by hovering over the option in the software</p> <p>Peptide Charge: keep peptides with</p> <p>Proteotypicity: If a peptide has a unique protein mapping (matched to a single FASTA entry from the sequence database).</p>

iRT Calibration

Set your preferences for iRT calibration:

iRT Reference Strategy	<p>Define how the reference iRT is derived for iRT calibration:</p> <ul style="list-style-type: none">• Empirical iRT Database. Use Spectronaut's internal empirical iRT reference database of more than 100.000 iRT reference peptides from multiple sources.• Deep Learning Assisted iRT Regression. Use the new Deep Learning algorithm to generate the iRT reference set. This is useful when working with non-model organisms hardly covered in Spectronaut's internal empirical iRT reference dataset.• Use RT as iRT. No iRT calibration will be performed. It should only be used if the peptide separation method is very stable, homogeneous and non-standard, such as capillary zone electrophoresis (CZE).
Minimum Rsquare	<p>Choose how strict you want to be to accept the iRT calibration of your data by specifying the minimum coefficient of determination (R^2) allowed.</p>
Use RT as iRT	<p>Use empirical RT as iRT in case the run doesn't match enough iRT reference peptides. If not selected and if less than 3 iRT reference peptides were found, the run processing will be aborted.</p>
Use Stripped Sequence to Identify Reference Peptides	<p>If selected, will ignore any modifications of the iRT reference peptides when identifying them for calibration.</p>

Workflow

Fragment Ion Selection Strategy: defines the strategy to be used for selecting the top N fragment ions per peptide precursor

- **Intensity Based:** Prioritizes fragment ions by their intensity in the consensus spectra
- **Evidence Based:** Prioritizes fragment ions by how often they have been observed in the experiment for the same precursor
- **Maximize sequence coverage:** Groups fragment ions by type and position and ranks them based on the best fragment ion per group (either by intensity or by evidence) in an iterative manner.

In-Silico Generate Missing Channels: if generating a library for an isotopically labeled sample, you can activate this option to *in-silico* generate an assay for a missing label pair. For example, if you have a sample of only spiked-in heavy aqua peptides, it will make a light/heavy library where the label-free channel is generated *in-silico* based on the heavy channel and with a consistent fragment ion selection. This option works with n-channel search results as long as you are working with isotopic labels. The workflow options are:

Spike in workflow: will create a light channel for all heavy (SIS) peptides that are identified without a light counterpart.

Labeled workflow: will detect the labeling setup of an experiment and add the channels that are missing for a given peptide

Inverted spike in: will create a heavy channel for all light peptides that are identified.

5.3 Supplementary plots

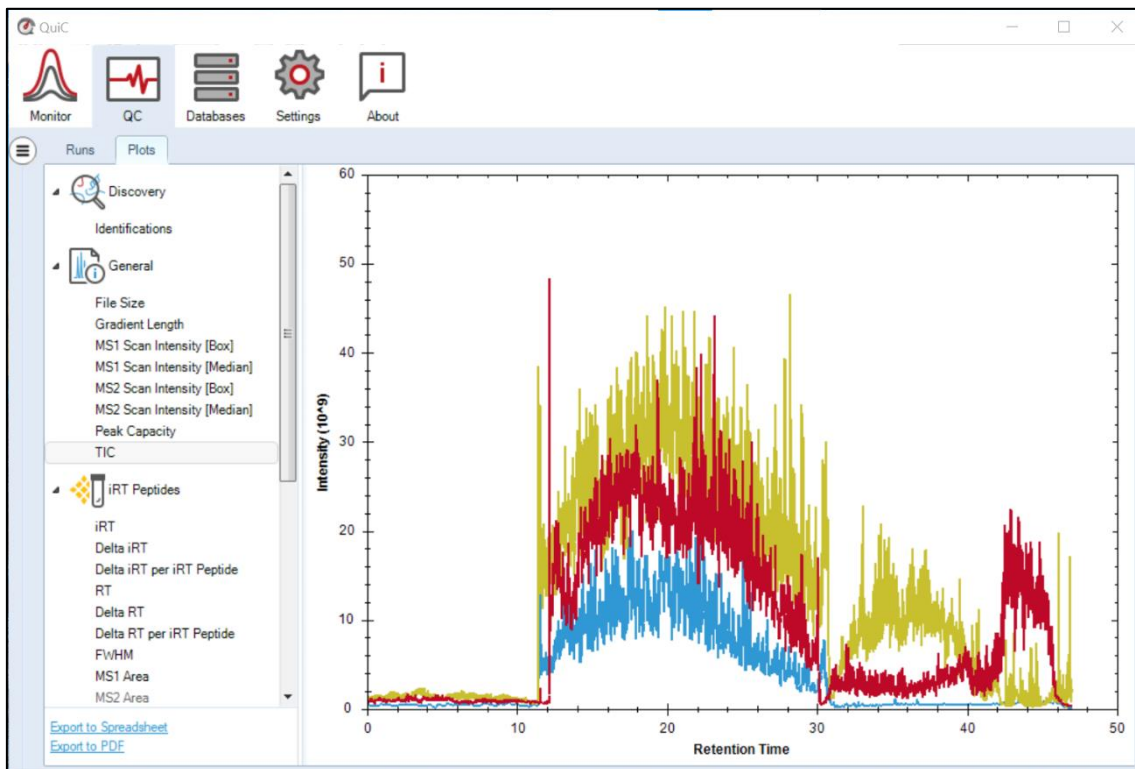


Figure S1. The Total Ion Current (TIC) chromatogram shows the sum of all the mass peak intensities belonging to the same scan. When making runs' comparisons, the scan range used for data acquisition should be considered. For visualization purposes, we recommend selecting only a few runs simultaneously.

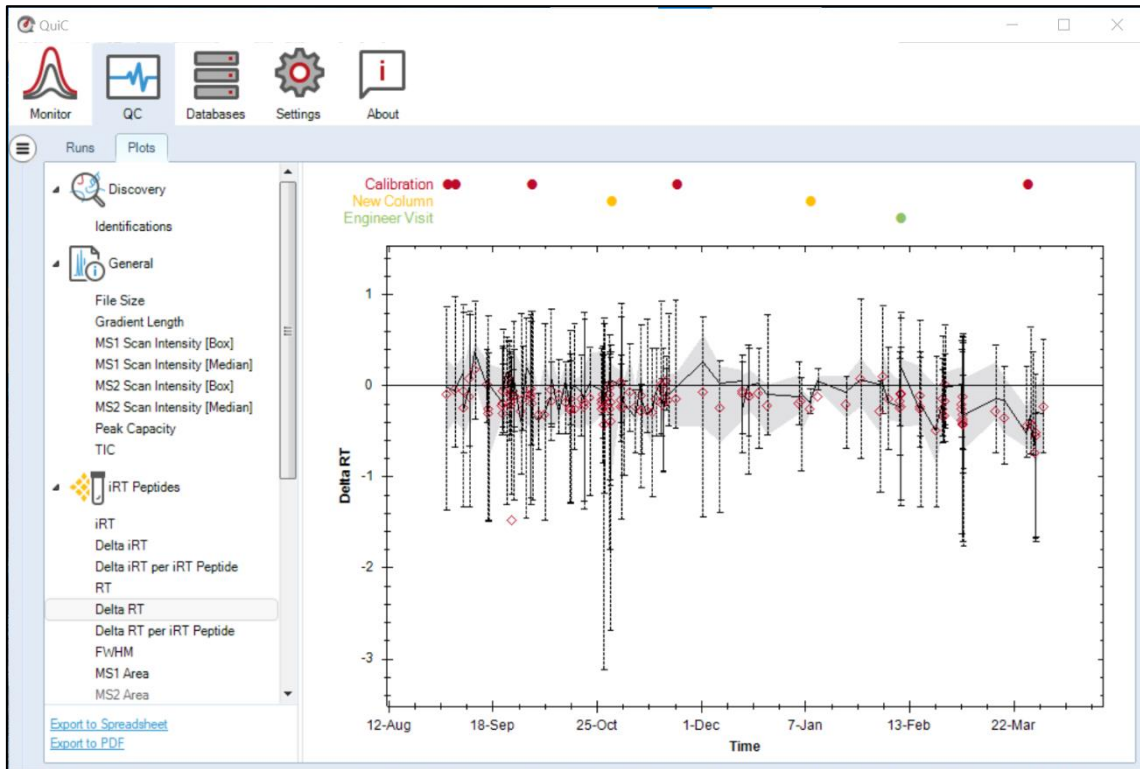


Figure S2. The Delta RT plot represents the difference between the predicted and observed RT (not normalized) for the iRT standard peptides. The Delta RT is expressed in [min].

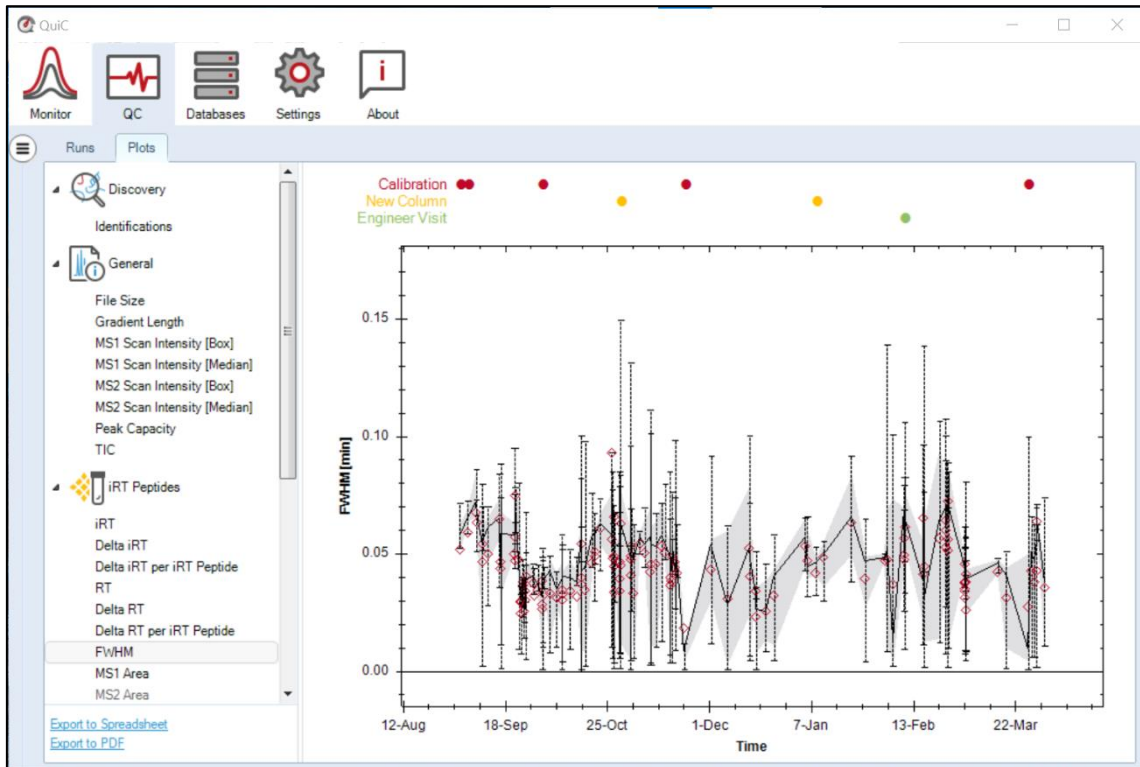


Figure S3. The FWHM is the width of a peak at half height. The FWHM plot represents the full width at half maximum of the MS2 fragment ion intensities (summed up for each precursor peptide). The FWHM is generally used to determine the peak capacity by the ratio: m/z over FWHM. The FWHM is, therefore, inversely proportional to the peak capacity of a peptide. No peak capacity plot is available for the 11 iRT standard peptides, as this would have few data points.

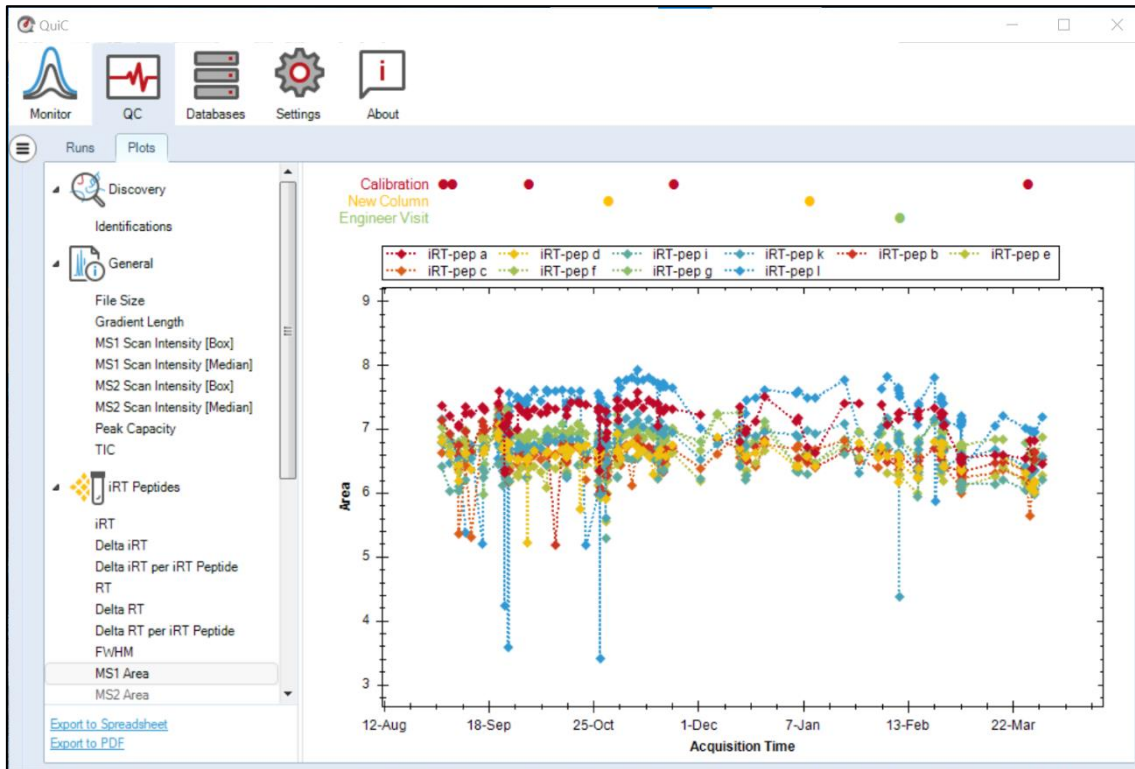


Figure S4. The MS1 Area is measured as the \log_{10} of the area under the precursors' peaks for the iRT standard peptides. While in DDA, only the MS1 Area plot is available (no MS2 quantification), DIA data also include the MS2 Area plot. The MS1 and MS2 Area plots represent the quantitative stability of the iRT standard peptides across runs.

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