Tutorial

This tutorials explains how to use the **QuantyFey** application, step by step. It covers everything you need to get started and use it efficiently. QuantyFey is designed to quantify targeted LC-MS/MS data using external calibration, but can also be used with other data formats that include intensity and retention time values.

Installation

The standalone version of this application runs on **Windows** and **Linux**. You can also run it directly from **R**, **RStudio**, or **VS Code**, which makes it compatible with **macOS** as well.

Prerequisites

Windows

- **RTools 4.2** is required:
  - **Option A**: Install from the official CRAN page.
  - **Option B**: Use the included Portable R:
    - Navigate to `R-portable/bin/` and launch `R.exe`
    - Run the following in the R console:

```
install.packages("installr", repos = "https://cloud.r-
project.org/")
installr::install.Rtools()
```

    - Do not update the R version if prompted.
    - Follow the installer instructions. You can ignore any non-critical error during the final steps.

Linux

Make sure all system dependencies are installed:

```
sudo apt install -y cmake libcurl4-openssl-dev libssl-dev libfontconfig1-dev
libfreetype6-dev \
libharfbuzz-dev libfribidi-dev libpng-dev libjpeg-dev libtiff5-dev default-jdk
libtirpc-dev \
build-essential pkg-config

sudo R CMD javareconf
```

Standalone Installation

- **Download** the latest version of QuantyFey
- **Unzip** the folder to a destination of your choice.

- **Run** the batch (Windows) or shell (Linux) file to start the App.

> **Note:** you may need to approve the execution of the script on Windows*

- A console will open and first all requirec packages will be installed automatically

Installation for launching the app using RStudio, VS Code etc.

- **Download** the GitHub repository.
- **Unzip** the files to a destination of your choosing.
- **Install** RStudio or VS Code
- **Install** pandoc
- **Install** prerequisites as mentioned above.
- **Open** `app.R` and **run** run the script

> *Note: On first launch, the app uses the `renv` package to restore the required R package environment. This enusres compatibility by installing the correct package versions. This process may take up to 20 minutes.*

Notes before using it on your own data

The application comes with several test datasets based on LC-MS/MS data. These are included to help you get started and explore the app's features.

> **Important:** The app is preconfigured to work with these test datasets. This includes predefined **Calibration Standard Names** and their **Concentrations**. Before using your own data, you'll need to update this setup.

To adjust the configuration:

1. open the file at `Dependencies/templates.xlsx`
2. Add a new sheet or edit an existig one to define your own analysis setup.

Template Structure Requirements The template must follow this structure exactly:

- `Cal.Name`: The first column must be named `Cal.Name` (spelling and case-sensitive). It should list the **names of the Calibration Standards** used in your sequence.
- `Concentration`: The second column should specify the **concentration** for each corresponding **Calibration Standard**. You may also define concentrations at the **transition level** (e.g., different concentrations for every single transition). In that case:
- Every single **transition name must be included in the template**.

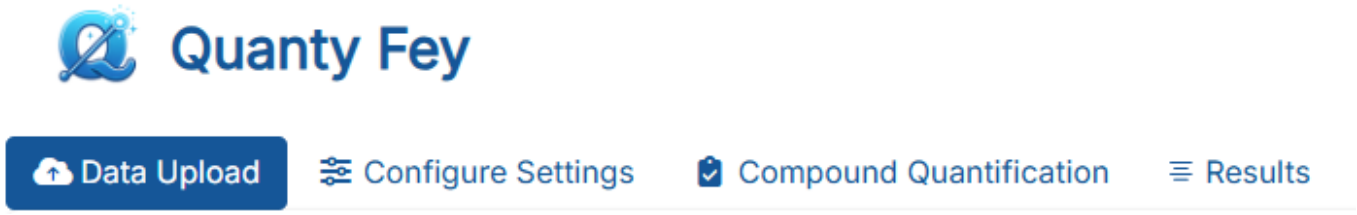
The app uses this file to **map the concentrations** of the Calibration standards to their corresponding names in the dataset. The concentrations are used for compound quantification.

> **Note:** Standard names in the template **must exactly** match those in the sequence (**case-sensitive**).
> **Note:** if any standard that is defined in your data (**Sample.Type column**) is not included in this template, it will be appended with a concentration of **0** - and will not be included in the quantification process.

	A	B
1	Cal.Name	Concentration
2	Cal 7	0.001
3	Cal 6	0.003
4	Cal 5	0.01
5	Cal 4	0.03
6	Cal 3	0.1
7	Cal 2	0.3
8	Cal 1	1

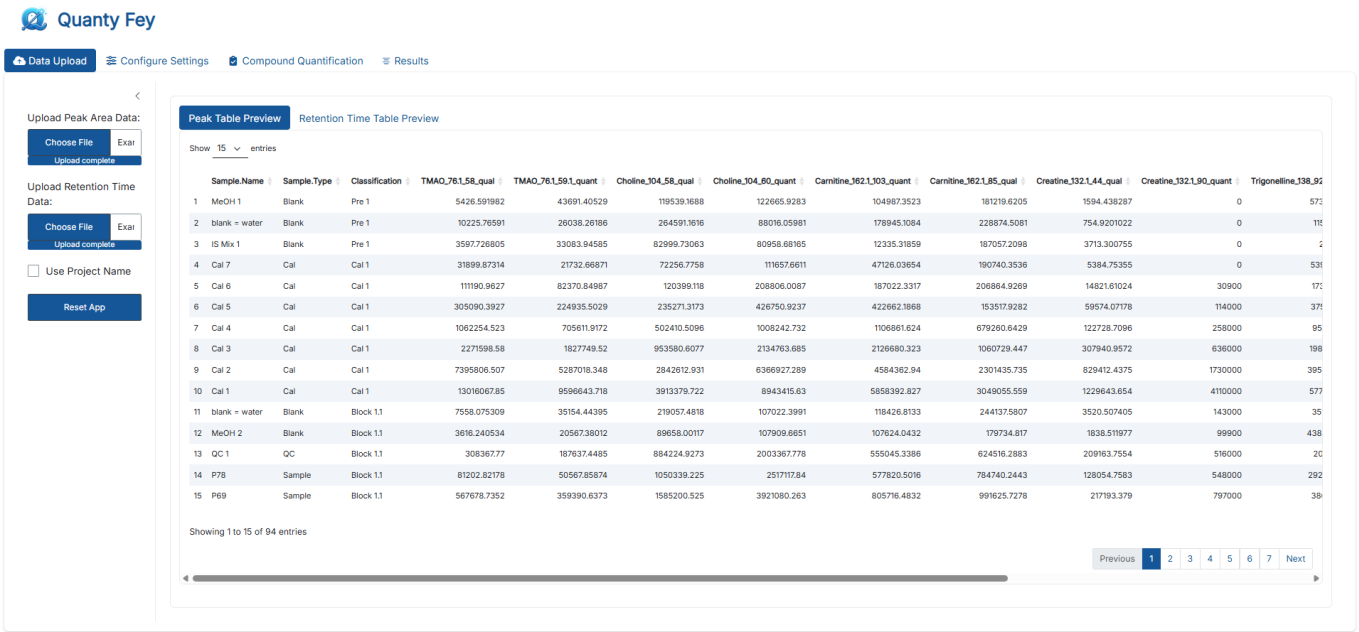
Application Structure

The app is divided into multiple tabs, each with a specific function. This layout helps guide users through the analysis process step by step.



Data Upload

The **Data Upload** tab lets you import the two required data files for analysis. Two files must be uploaded in **csv**, **txt**, or **xlsx** format using standard delimiters (`,`, `;`, `:`, or tab `\t`):



1. Peak Table

This file contains **peak intensity data** (e.g., *Peak Areas* or *Peak Heights*) for the measured compounds. The required structure is as follows:

- `Sample.Name`: Identifier for each sample.

- `Sample.Type`: Must be one of the following (case-sensitive):
  - `Sample` – Experimental samples.
  - `Standard` or `Cal` – Calibration standards.
  - `Blank` – Blank samples for background correction.
  - `QC` – Quality control samples.
- `Classification` *(optional)*: Defines distinct sequence blocks for **custom bracketing analysis**.
  - Calibration standards must follow the pattern `Cal n` (e.g., `Cal 1`, `Cal 2`, ...).
  - Sample blocks may be named freely (e.g., `Sample Block 1`, `Block A`).

> **Note**: If this column is missing, it will be auto-generated:
>
> - Calibration curves are identified as three or more consecutive standards (as defined in `Sample.Type` column).
> - Sample blocks are defined between these curves.
> - Samples before the first calibration are labeled `Pre 1`.

> **Important**
>
> - Missing or incorrectly formatted columns will lead to an error massege and upload will be halted.
> - If internal standards are not recognized by the default pattern, a warning message will appear and internal standard correction features will be disabled. By updating the internal standard pattern these features can be restored.

Example Table: Peak Table Format

Example file: `Example_Datasets/Example1_Drift_Areas.csv`

	A	B	C	D	E	F	G	H	I	J
1	Sample.Nam	Sample.Type	Classificatio	TMAO_76.1_	TMAO_76.1_	Choline_104	Choline_104	Carnitine_16	Carnitine_16	Creatine_13
2	MeOH 1	Blank	Pre 1	5426.59198	43691.4053	119539.169	122665.928	104987.352	181219.621	1594.43829
3	blank = wate	Blank	Pre 1	10225.7659	26038.2619	264591.162	88016.0598	178945.108	228874.508	754.920102
4	IS Mix 1	Blank	Pre 1	3597.72681	33083.9459	82999.7306	80958.6817	12335.3186	187057.21	3713.30076
5	Cal 7	Standard	Cal 1	31899.8731	21732.6687	72256.7758	111657.661	47126.0365	190740.354	5384.75355
6	Cal 6	Standard	Cal 1	111190.963	82370.8499	120399.118	208806.009	187022.332	206864.927	14821.6102
7	Cal 5	Standard	Cal 1	305090.393	224935.503	235271.317	426750.924	422662.187	153517.928	59574.0718
8	Cal 4	Standard	Cal 1	1062254.52	705611.917	502410.51	1008242.73	1106861.62	679260.643	122728.71
9	Cal 3	Standard	Cal 1	2271598.58	1827749.52	953580.608	2134763.69	2126680.32	1060729.45	307940.957
10	Cal 2	Standard	Cal 1	7395806.51	5287018.35	2842612.93	6366927.29	4584362.94	2301435.74	829412.438
11	Cal 1	Standard	Cal 1	13016067.9	9596643.72	3913379.72	8943415.63	5858392.83	3049055.56	1229643.65
12	blank = wate	Blank	Block 1.1	7558.07531	35154.444	219057.482	107022.399	118426.813	244137.581	3520.50741
13	MeOH 2	Blank	Block 1.1	3616.24053	20567.3801	89658.0012	107909.665	107624.043	179734.817	1838.51198
14	QC 1	QC	Block 1.1	308367.77	187637.449	884224.927	2003367.78	555045.339	624516.288	209163.755
15	P78	Sample	Block 1.1	81202.8218	50567.8587	1050339.23	2517117.84	577820.502	784740.244	128054.758
16	P69	Sample	Block 1.1	567678.735	359390.637	1585200.53	3921080.26	805716.483	991625.728	217193.379
17	P40	Sample	Block 1.1	235778.611	132486.92	822083.694	2086563.43	1239872.78	942693.838	295224.428
18	P64	Sample	Block 1.1	58355.8542	22917.1177	516579.091	1373097.06	650072.066	803060.691	65862.4721
19	P41	Sample	Block 1.1	588918.429	412641.997	785606.712	1914433.95	1395121.74	982263.684	236047.385
20	P79	Sample	Block 1.1	352120.909	191910.208	851669.82	2070310.87	466656.197	684900.798	145003.954
21	P43	Sample	Block 1.1	446134.004	359386.363	675663.578	1768525.42	852293.779	830458.442	200303.028
22	P72	Sample	Block 1.1	429402.408	284692.865	973791.773	2183400.1	650110.65	806834.235	160469.291
23	Cal 3	Standard	Block 1.2	1824967.15	1222004.31	850861.897	1951470.47	1695841.44	996697.311	183257.571
24	IS Mix 2	Blank	Block 1.2	N/A	19913.9868	45541.3898	51240.4607	98113.03	122386.675	4733.77081
25	P77	Sample	Block 1.2	359183.028	255466.264	832192.001	2014077.54	456499.19	599997.157	84184.7826
26	P52	Sample	Block 1.2	94931.4427	74001.3363	1030243.92	2400687.81	789419.289	877234.765	293224.153
27	P60	Sample	Block 1.2	34268.6446	9819.30281	813590.863	1932327.61	1057157.53	1018325.52	276193.506
28	P73	Sample	Block 1.2	116288.951	67118.6296	983095.873	2566516.54	567782.352	939219.159	111591.711
29	P53	Sample	Block 1.2	58286.1371	36953.8689	570835.149	1413030.4	573471.426	554580.23	282563.764
30	P68	Sample	Block 1.2	223913.326	110380.734	705293.224	1697903.17	404404.021	713885.943	126609.026
31	P66	Sample	Block 1.2	153714.067	100077.969	651583.17	1697976.89	800781.546	982154.782	81247.0534
32	P82	Sample	Block 1.2	122620.964	62419.8588	881124.688	2091096.07	499168.2	639242.932	140300.843
33	Cal 3	Standard	Block 1.3	1544396.39	966874.039	715776.412	1637662.05	1667292.72	974430.988	149841.874
34	IS Mix 2	Blank	Block 1.3	N/A	66587.6368	32342.0169	31641.141	63212.5597	24079.53	1544.77024
35	P46	Sample	Block 1.3	625209.773	363949.119	831531.593	1939319.98	547347.016	618875.273	111322.221
36	P65	Sample	Block 1.3	365502.285	221006.719	726236.573	1834655.84	535918.577	699238.566	33988.3225
37	P49	Sample	Block 1.3	376803.897	232575.025	944099.058	2218000.27	395076.547	742029.334	95115.9248
38	P56	Sample	Block 1.3	159109.113	95568.5002	677769.854	1684608.48	508361.727	590160.122	97148.4098

2. Retention Time Table

This file provides **retention time (RT)** data for the compounds.

> **Note:** The upload button will only appear after the **Peak Table** was uploaded.

- Must include a `Sample.Name` column identical to that in the Peak Table.
- Only compounds present in the Peak Table will be considered.
- Upload the RT Table **after** the Peak Table.

> **Note:** the app will ignore any additional columns that aren't needed.

Example Table: Retention Time Table Format

Example file: `Example_Datasets/Example1_Drift_RT.csv`

	A	B	C	D	E	F	G	H	I
1	Sample.Nam	Sample.Type	TMAO_76.1_	TMAO_76.1_	Choline_104	Choline_104	Carnitine_16	Carnitine_16	Creatine_13
2	MeOH 1	Unknown	0.16684883	0.17938056	0.25194086	0.26006593	0.31327645	0.18960175	0.26517553
3	blank = wate	Unknown	0.20626313	0.66467404	0.24812574	0.260908	0.24574482	0.22623179	0.25715926
4	IS Mix 1	Unknown	0.151439	0.19140657	0.23260446	0.26212561	0.23413321	0.18594254	0.27096674
5	Cal 7	Unknown	0.25991771	0.26471666	0.24218616	0.25898092	0.26498574	0.18962964	0.27029683
6	Cal 6	Unknown	0.26368089	0.26336981	0.25341495	0.25784875	0.26260091	0.20107735	0.2644588
7	Cal 5	Unknown	0.26279085	0.26494092	0.25598036	0.25720266	0.26166795	0.25724511	0.26642379
8	Cal 4	Unknown	0.26324391	0.26202178	0.25759801	0.25677195	0.26175561	0.25756276	0.26481418
9	Cal 3	Unknown	0.26274974	0.26388911	0.25720775	0.25622964	0.26139244	0.25989211	0.26340305
10	Cal 2	Unknown	0.26305417	0.26361763	0.25900487	0.25873283	0.26242215	0.26192019	0.26556554
11	Cal 1	Unknown	0.26486155	0.26525941	0.25504898	0.25621501	0.26403694	0.26247741	0.2670949
12	blank = wate	Unknown	0.25472888	0.16397883	0.25042417	0.25949031	0.24576366	0.23233072	0.27345922
13	MeOH 2	Unknown	0.14628303	0.15966025	0.25127115	0.26175915	0.3156593	0.17671761	0.29960049
14	QC 1	Unknown	0.27094407	0.26978924	0.26104842	0.25918592	0.27286173	0.22569448	0.27208678
15	P78	Unknown	0.27221292	0.27172804	0.26206756	0.26119175	0.27333577	0.22620418	0.27617971
16	P69	Unknown	0.27239636	0.271831	0.26424422	0.26329975	0.27377269	0.23076506	0.27148056
17	P40	Unknown	0.2716874	0.26891411	0.26195348	0.26182025	0.27287303	0.24725656	0.27383473
18	P64	Unknown	0.26707984	0.27217436	0.25941144	0.25876215	0.27097281	0.23297653	0.27326961
19	P41	Unknown	0.2699452	0.26931156	0.25984859	0.25797059	0.27106269	0.25907917	0.27069395
20	P79	Unknown	0.27086952	0.27043151	0.26097035	0.26067037	0.27206649	0.22592024	0.2717832
21	P43	Unknown	0.26915383	0.27066371	0.26162247	0.26196603	0.2722199	0.24207707	0.27283093
22	P72	Unknown	0.26868131	0.26849529	0.2637931	0.26245405	0.27117973	0.22644887	0.27312813
23	Cal 3	Unknown	0.26330201	0.26391545	0.25802852	0.25657334	0.26093516	0.26181014	0.26506507
24	IS Mix 2	Unknown	N/A	0.16886235	0.26046773	0.25680234	0.31738253	0.17651956	0.27165468
25	P77	Unknown	0.27008585	0.27117233	0.26267901	0.26253108	0.2739091	0.22327992	0.27409615
26	P52	Unknown	0.27086855	0.26999416	0.26225365	0.26153303	0.27311191	0.2261214	0.27235793
27	P60	Unknown	0.27032375	0.26485716	0.26270543	0.26183563	0.27235402	0.24742279	0.27479082
28	P73	Unknown	0.27041182	0.26907487	0.26342665	0.26224204	0.27270153	0.22814576	0.27476709
29	P53	Unknown	0.27016662	0.26981335	0.26261763	0.26219015	0.27382903	0.23238029	0.2704658
30	P68	Unknown	0.26910705	0.27016214	0.26098825	0.26034048	0.27223231	0.22734317	0.2723728
31	P66	Unknown	0.27201638	0.27055547	0.26200397	0.26286402	0.27215374	0.22898909	0.27697323
32	P82	Unknown	0.26994948	0.27056762	0.2638745	0.26575546	0.27435975	0.22501013	0.27076162
33	Cal 3	Unknown	0.26474762	0.26495237	0.25691818	0.25839228	0.26405727	0.26305454	0.26545915
34	IS Mix 2	Unknown	N/A	0.39821371	0.24143886	0.26104997	0.30418798	0.20195572	0.26629609
35	P46	Unknown	0.27064271	0.27096464	0.26206566	0.26095903	0.2724883	0.22750591	0.27408238
36	P65	Unknown	0.27166675	0.27041949	0.26376297	0.26356584	0.27066031	0.23920818	0.27221986
37	P49	Unknown	0.26982416	0.26922256	0.26220831	0.26128237	0.27253975	0.22331139	0.27481717
38	P56	Unknown	0.26959978	0.26833719	0.26239238	0.262673	0.26924848	0.24144714	0.27038089

3. Project Name (optional)

You may optionally enter a **Project Name**, which will be used to label output folders.

- Output will be saved in the user's **Documents** folder under:
  `Documents/QuantyFey/<ProjectName>/`

4. Reset the Application

Click **Reset App** to restart the session and clear all uploaded data — useful if the wrong files were selected or you want to begin a new analysis.

Summary: Minimum Upload Requirements

File	Required Columns	Notes
Peak Table	`Sample.Name`, `Sample.Type`	Optional: `Classification`; supports multiple transitions.
RT Table	`Sample.Name`, transitions from Peak Table	Must match Peak Table sample names.

Configure Settings:

Selecting Quantification Template

To perform quantification, you need to select the appropriate template. Use the dropdown menu in the app to choose from available sheets in teh `templates.xlsx` file. Each sheet represents a separate template. When selected, the left pane will be updated with the respective template. If the template encompasses all transition names, it will show the concentrations of the selected compound.

> **Note:** Make sure the correct template is selected - quantification will not work if the template does not match your data or the wrong sheet is chosen.

Change patterns

QuantyFey automatically detects **Quantifier**, **Qualifier**, and **Internal Standard (IS)** transitions by searching for predefined patterns in the column names of the Peak Table. These patterns can be customized to match your dataset's naming. Patterns can be changed by selecting the `Change Patterns` Checkbox. Then three inputs will be shown, that can be adjusted.

> **Tip:** You can set default values by modifying the `default_settings.R` file, or override them directly within the app interface.

Default Pattern Setup (*in `default_settings.R`*)

Below are the default settings used by the app. You can change them in the `default_settings.R` file.

Setting	Default Value	Description
`quant_pattern`	`_quant`	Identifies quantifier transition columns
`qual_pattern`	`_qual`	Identifies qualifier transition columns
`IS_pattern`	`IS`	Identifies internal standard transition columns
`conc_unit`*	`µg/mL`	Unit used for concentration in plots and tables
`int_unit`*	`NULL`	Unit used for intensity values in outputs
`rt_unit`*	`min`	Unit used for retention time
`Template_name`	`Example1`	Default name of the template used for quantification setup

> * These unit values can only be adjusted in the default_settings.R file and are not editable from within the app interface.

Code (as seen in `default_settings.R`)

```
## Setup Default Settings for QuantyFey

# Default Template name
Template_name = "Example1"
# Pattern for Quant Transition:
quant_pattern = "_quant"
# Pattern for Qual Transition:
qual_pattern = "_qual"
# Pattern for IS Transition:
IS_pattern = "IS"

#### ---- Units ---- ####
# Set to NULL if you don't want to show the unit in the plot
# Concentration
conc_unit = "µg/mL"
# Intensity Unit
int_unit = NULL
# RT Unit
rt_unit = "min"
```

> **Note:** Updating the units helps ensure consistency between your dataset and the visualization/output displays in the app.

Pattern for Quantifier Transitions

This pattern identifies **quantifier** transitions from Peak Table column names.

- Supports **regular expressions** (*Regex*)
- Only columns matching this pattern (and **not** matching the IS pattern) will be used for quantification.

Examples:

- `^Compound1_` → Columns starting with `Compound1_`
- `_quant$` → Columns ending with `_quant`
- `Compound[0-9]+_` → Matches names like `Compound1_`, `Compound2_`, etc.
- `.*_qual` → Columns containing `_qual`
- `^Cal.*ppb$` → Columns starting with `Cal` and ending with `ppb`

> **Important: Understanding Regular Expressions**
>
> - `.` matches any single character.
> - `*` means zero or more of the previous character.
> - `^` anchors the match to the start of the text.

> - `$` anchors the match to the end of the text.
> - `.*` matches any number of any character.
> - To match a literal `.` or `*`, escape it with a backlash: `\.` or `\*`.
> - For more information check out this Regex Quick Reference.

Pattern for Qualifier Transitions

Defines how **qualifier** transitions are detected.

- Also uses **regular expressions**
- Matches are determined based on the **prefix** of the corresponding quantifier transition.

Prefix Matching Logic:

- The **prefix** is the string before the first underscore (_)
- Example formats:
  - `CompoundID_Q1_Q3_CE_quant`
  - `CompoundID.Q1_Q3_CE_qual`

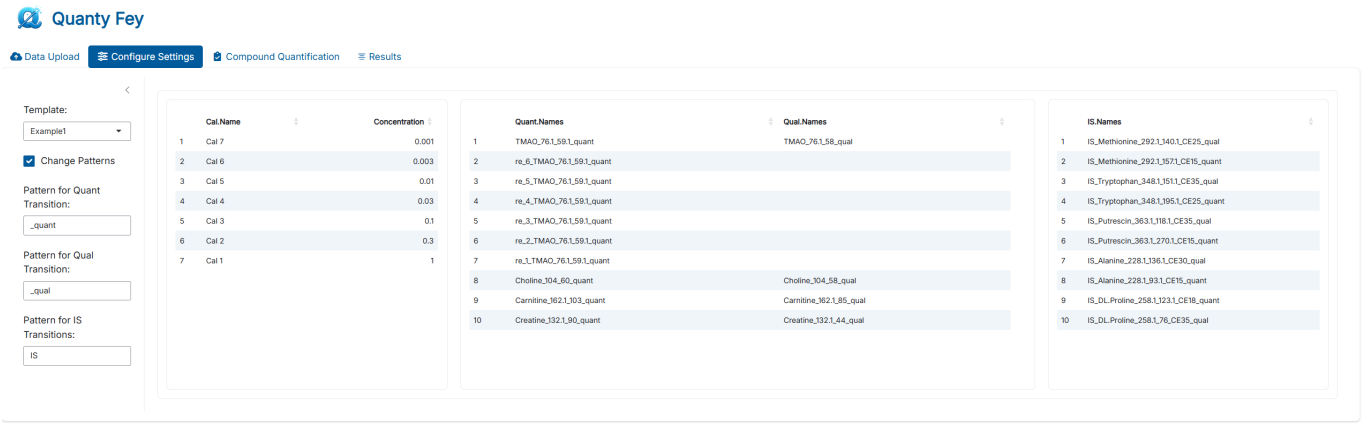
> If no matching qualifier transitions are found, the **Qualifier/Quantifier Ion Ratio Analysis** tab will be hidden.

Pattern for IS Transitions

This pattern identifies **internal standard** transitions.

- Supports **regular expressions**
- Matching columns will:
  - be **excluded** from quantification
  - be **used** for IS correction (if enabled)

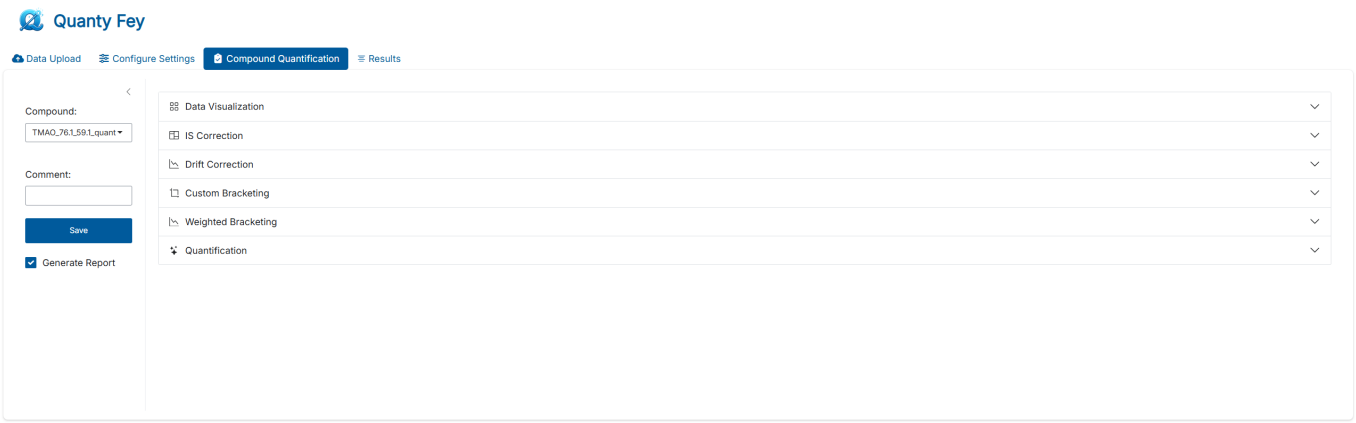
> If no IS transitions are detected, IS correction will be disabled automatically and a warning message will appear.



Compound Quantification

The **Compound Quantification** tab is the main workspace for **visualization**, **drift correction**, **regression model optimization**, and **quantification**.

Setup



In the left-side panel, users can configure the following:

- **Compound**: Select the quantification transition.
- **Comment**: Add notes for the quantification process.
- **Save**:
    - Ouput is saved to users **Documents** folder
    - If the default path fails (e.g., due to permissions), results are saved to the local QuantyFey directory.
    - Files are placed inside a folder names `Results_<date>` unless a different name is specified as **Project Name** in the **Data Upload** tab.
    - Two output files are created and updated with each save:
    - A **concentration table** (`Results_quant.csv`) per compound.
    - A **summary file** (`Quant_summary.xlsx`) listing all parameters and settings, with one sheet per save.
- **Generate Report**: Optional checkbox. If enabled, a PDF report will be created containing all plots and a summary of the quantification.

> **Note on File Management:** To avoid overwriting while limiting file clutter:
>
> - Concentration tables: The app checks for **differences** between the **existing file** and the **new data**. **If all data** from the existing file **matches** data from the new data, the file is **overwritten**. If **differences** exist, a **new file** is created with a **time stamp**.
> - Summary File: With every save, data is **appended** as **new worksheet** to the file.
> - PDF Report: Reports may be overwritten if the app is restarted and outputs are saved to the same directory. **Suggestion:** After launching the app, create a new output folder by renaming the **Project Name** in the **Data Upload** tab. This helps prevent conflicts and ensures all outputs are saved realiably.

Main Tabs

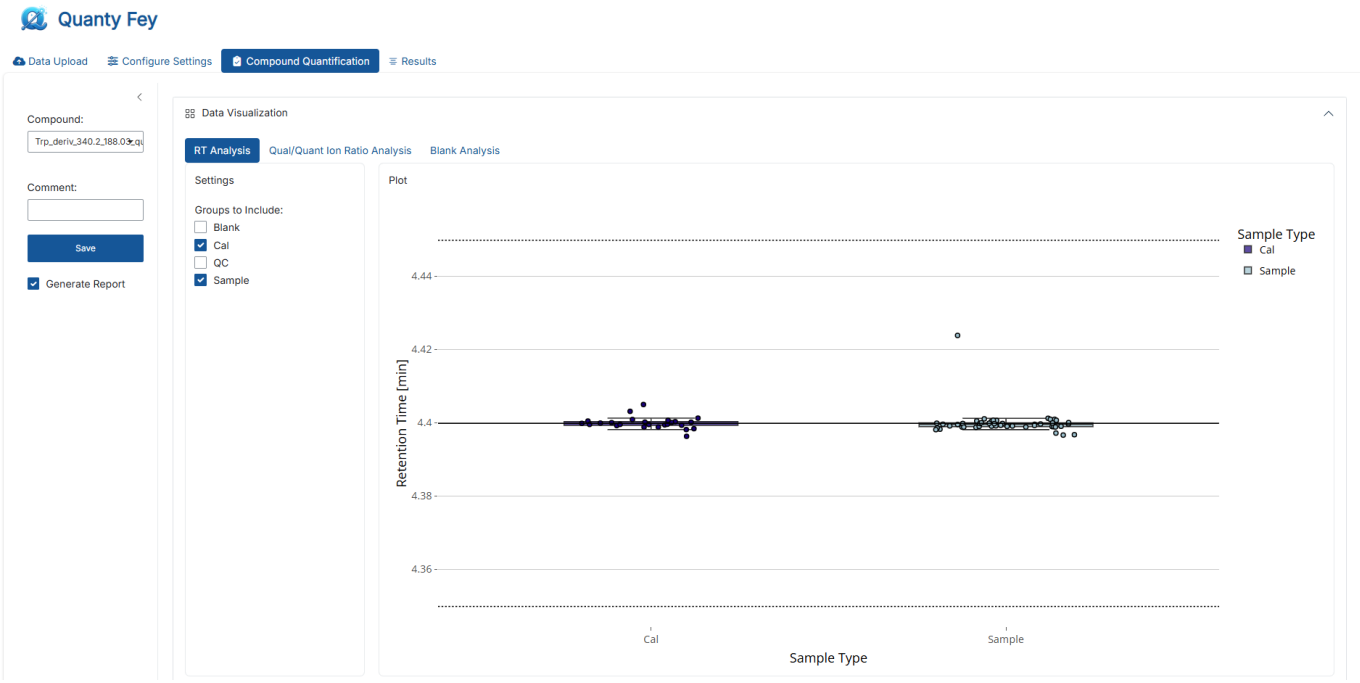
The main panel contains six tabs:

- Data Visualization

- IS Correction
- Drift Correction
- Custom Bracketing
- Weighted Bracketing
- Quantification

Each tab will be explained in detail in the following section.

Data Visualization



This tab provides an overview of the data for the selected transitions, including **Retention Time (RT)**, **Ion Ratio Analysis**, and **Blank Analysis**.

- **Retention Time**: Interactive plot of RT values for selected sample types. Hover over any point to view sample-specific details.
- **Ion Ratio Analysis**: Interactive plot of Qualifier-to-Quantifier Ion ratios for selected sample types. Hover over any point to view sample-specific details.

  > Note: This section is only visible if a matching Qulaifier was found for the selected Quantifier transition.

- **Blank Analysis**: Boxplots comparing blank and sample signal ratios. Blank ratios are calculated as: `sample intensity / mean blank intensity` This helps distinguish background noise from true signals.

This visualization tab is designed to help **verify compound identity** by comparing **RT**, and **Ion Ratios** between Samples and Standards and to compare signal intensities from Samples to Blanks to assess **carry over** and **background signals**.

Correcting for Intensity Drift

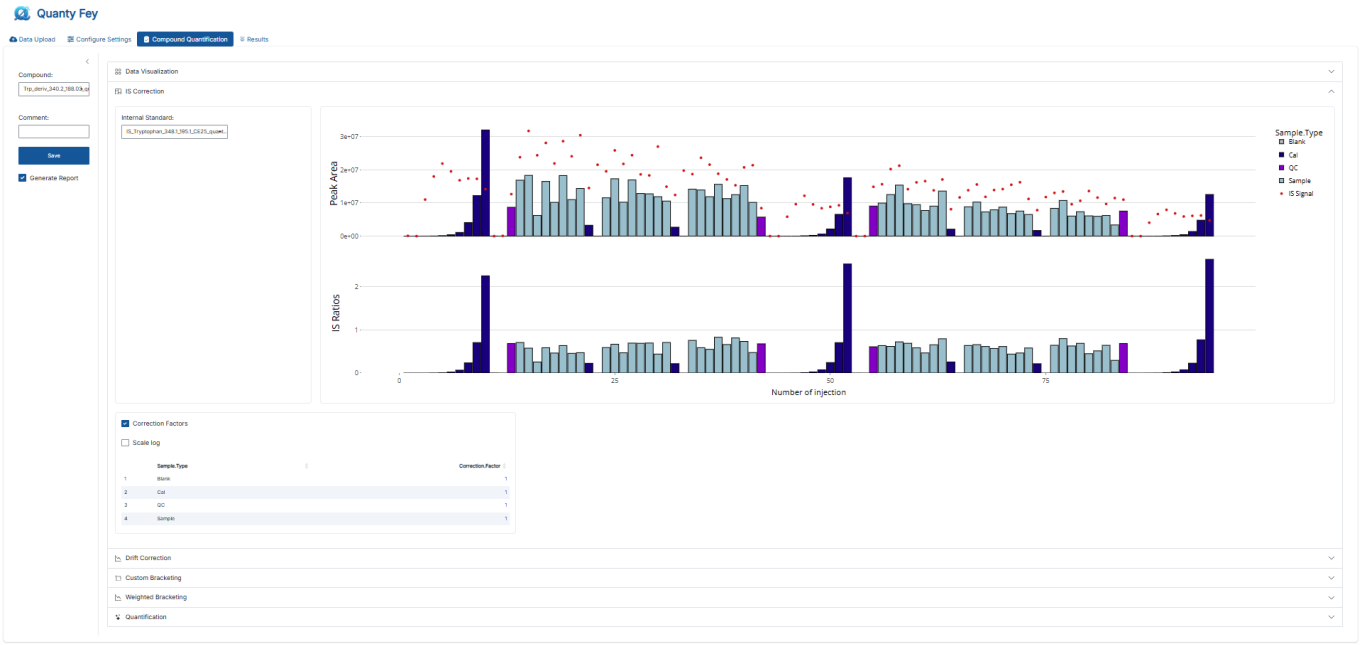
The following four tabs are used to set up different **drift correction strategies**. For best results, it's recommended to configure all applicable methods **before** performing quantification.

IS Correction

This module performs internal standard correction by calculating intensity ratios:

> **IS ratio = Peak Area of Quantifier / Peak Area of IS**

These ratios are then used instead of raw intensities for both the regression model and concentration calculations.

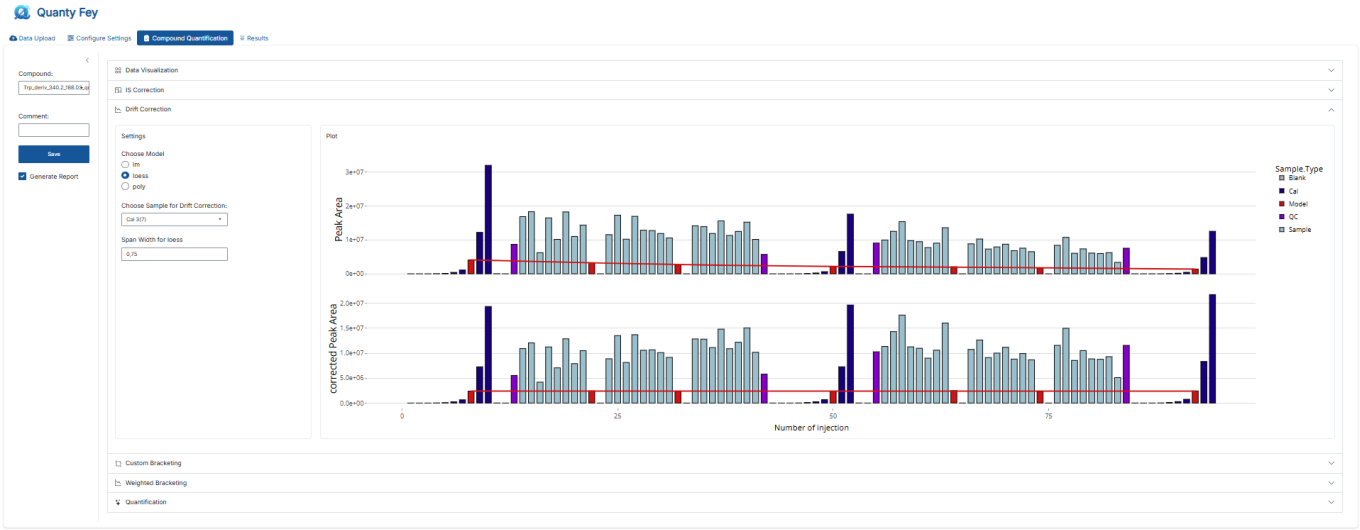


- **Plots:**
  - **Raw Intensity**: Bars indicate raw intensities; red dots indicate IS intensities.
  - **IS Ratios**: Displayes IS ratios.

Note: For IS intensites falling below 0.1 % of the median IS value, the median value is used for correction.

Drift Correction

This module corrects signal drift based on QC samples or other repeated injections. It builds a model based on the injection order and intensity values. This model is used to correct for intensity drift by normalizing intensities to the model.



- **Models**:
  - **Linear Model (lm)**: Simple linear regression.
  - **Loess**: Non-linear locally estimated scatterplot smoothing.
  - **Poly**:Polynomial fit.
- **Sample for Drift Correction**:
  - Select a sample (e.g., QC) injected regularly throughout the sequence. Only non-blank samples injected **3+ times** can be selected.
- **Span Width** (for loess only): Controls smoothness of the loess fit.
- **Degree** (for poly only): Specifies the degree of the spline function.

> **Note:** For **Span Width** only use values > 0.4. For **Degree** only values smaller than the number of datapoints (in this case injections of the sample) work. If the model was not generated correctly, an error message will show. Adjust the model and only use **Drift Correction** if the model was correctly generated.

The main panel displays:

- **Raw Intensity Plot**: Intensity values before correction.
- **Corrected Intensity Plot**: Intensity values after drift correction.

> **Note:** Loess models cannot extrapolate: Edge values are estimated using the nearest available data point. Span width must be above 0.4. Smaller values may cause errors. If an error appears, adjust the parameters and retry. Span width cahnges are only applied after clicking outisde the input box.

Custom Bracketing

This tab allows you to manually assign **calibration blocks** to specific sections of your sequence. These are derived from the `Classification` column in the **Peak Table**.

Bracketing Table:

- Rows = All blocks (from `Classification`)
- Columns = Calibration blocks (also from `Classification`)
- Checkboxes let you assign the calibration data to the respective blocks.

> **Note**: Every sample block must be linked to **at least one** calibration block for bracketing to be used in quantification.

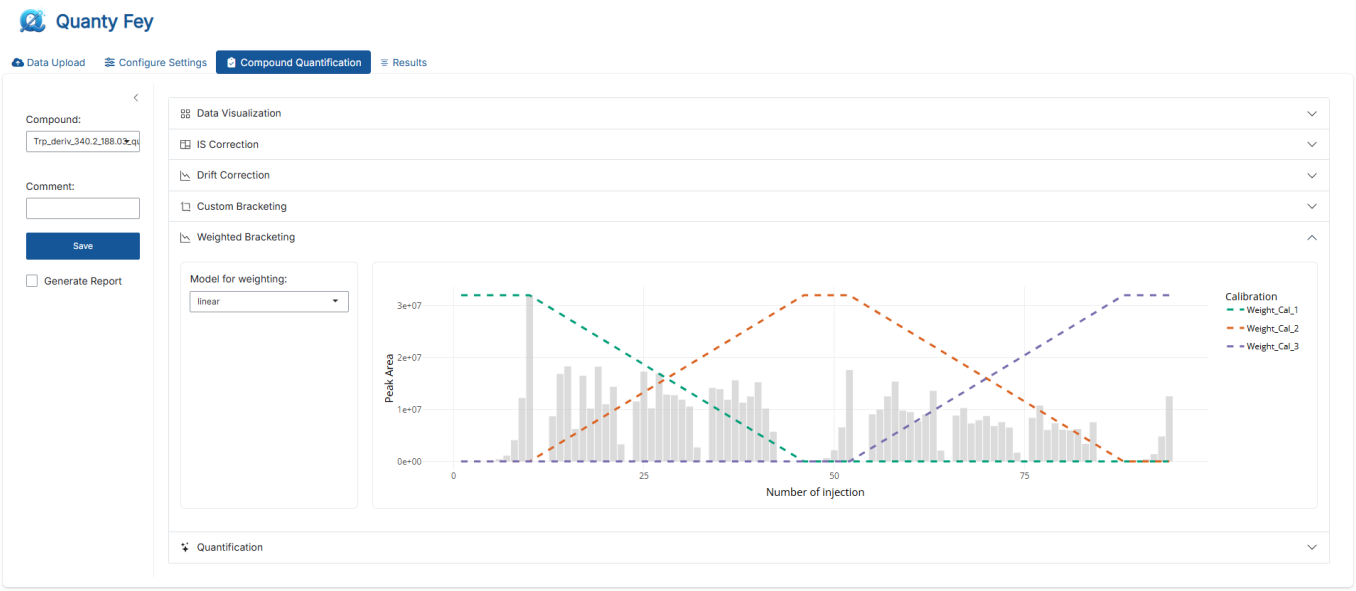
Weighted Bracketing

This module builds a separate **regression model for each injection**, based on its position between two calibration blocks. The weight assigned to each calibration point is determined by the selected model type:

- In the **linear model**, weights increase or decrease **linearly** across the injection sequence.
- In the **non-linear models** (requires technical replicates over the sequence), a model is fitted to their intensities over the measurement sequence. The **derivative** of this model is then used to define how the weights change across the sequence.

Settings:

- **Model Type**
  - `linear` - linear weight increase/decrease (*does not require QC samples*)
  - `non-linear` - use of technical replicates.
    - loess: adjustable **span width**
    - poly: adjustable **degree**

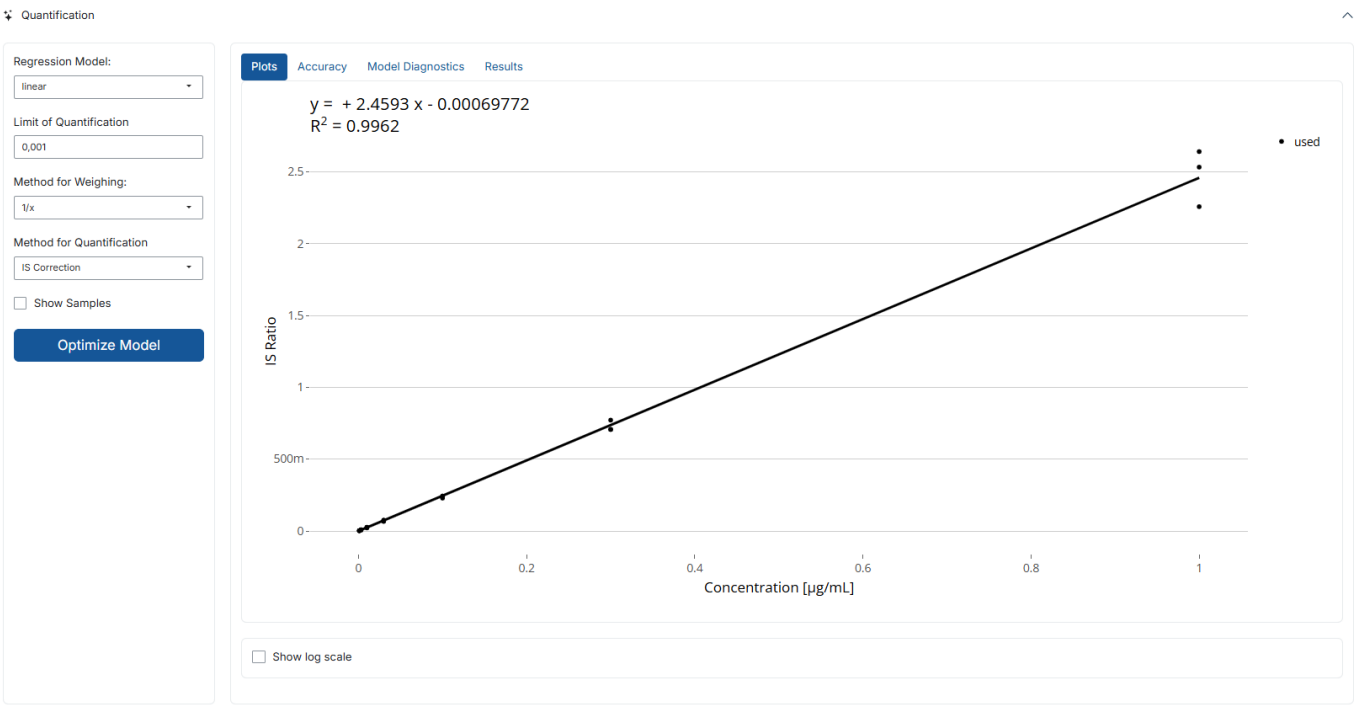


> **Note**: Weights using the linear model are just increasing/decreasing weights from one to the next calibration block. They dont require QC injections Non-linear models require QC samples.

Quantification

The **Quantification** tab is where the actual quantification of the selected transition takes place. The left panel allows configuration of key model parameters, while the main area provides tools for visualization, diagnostics, and results review.

Parameters



- **Regression Model**: Choose between `linear` and `quadratic` regression models.
- **Limit of Quantification (LLOQ)**: Defines the lower reporting threshold for concentration values.
  - Defaults to the lowest calibration standard.
  - Does not affect the plot, but any value below the LLOQ will be marked as "< LLOQ" in the output.
- **Weighting Method**: Specifies the regression weighting:

    - `1/x`: Weight = 1 / Concentration.
    - `1/x2`: Weight = 1 / Concentration^2.
    - `1/y`: Weight = 1 / PeakArea
    - `1/y2`: Weight = 1 / PeakArea^2
    - `1/x force 0`: Weight = 1 / Concentration and goes through 0|0
    - `1/y force 0`: Weight = 1 / PeakArea and goes through 0|0
    - `None`: No weighting applied.

      > **Note**: Values with PeakArea = 0 will automatically hava a weight of 0.

- **Quantitation Method**: Selects the quantification approach:
    - `IS Correction`: One regression over all calibration blocks using IS ratios
    - `Drift Correction`: One regression over all calibration blocks using drift corrected peak areas.
    - `Custom Bracketing`: Individual regressions over the respective calibration data assigned to each block.
    - `Weighted Bracketing`: Individual regressions for each injection weighted according to the position in the sequence.
    - `Default Bracketing`: One regression over all calibration blocks.
- **Show Samples**: Toggles sample visibility in plots.

Bracketing specific settings

(Visible only if **Custom Bracketing** or **Weighted Bracketing** is selected as the quantitation method)

- **Block to Visualize**: Select which classification block to display in the plot.

  > **Note**: in Weighted Bracketing, each sample is treated as its own block.

- **Apply Cal Levels to All**: Apply the currently selected calibration level settings to **all blocks**.

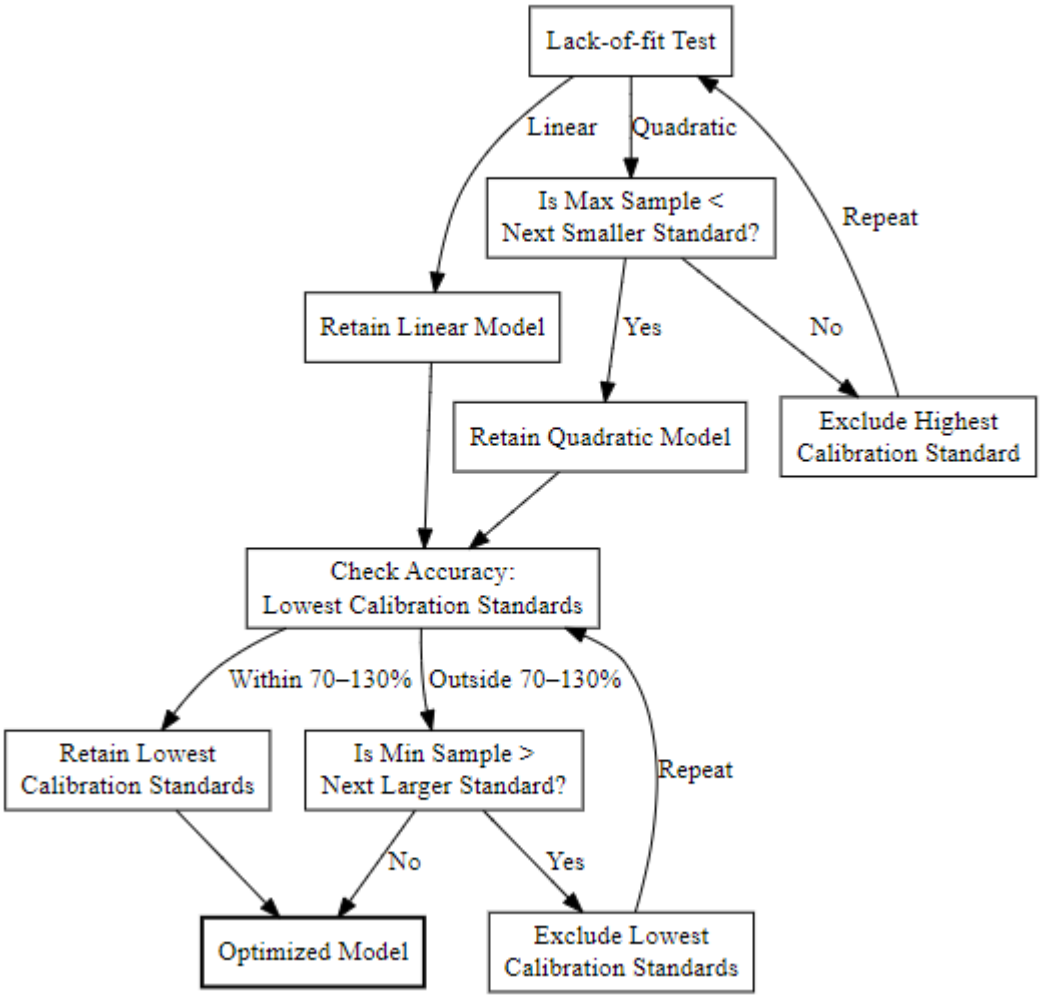
  > **Note**: If a calibration level has been removed in one block, this action will remove that level across all blocks.

- **Apply LLOQ to All**: Applies the defined LLOQ settings to all blocks.

An automatic optimization Button allows the user to do a generic optimization of the regression model.

- **Optimize Model**: Automates model optimization:
    - Removes higher standards for quadratic models if samples are lower.
    - Removes lower standards if accuracy falls outside 70–130% and samples are higher.
    - Selects linear or quadratic models based on a lack-of-fit test.

> **Note**: Optimization may fail or produce poor fits for low-quality data. Always verify the model manually before saving. For **Custom Bracketing** or **Weighted Bracketing**: Use `Apply LLOQ to All` after `Optimize Model` to make sure, a value is saved for LLOQ, as for some individual "Blocks" there might not be a suitable value due to poor accuracy.

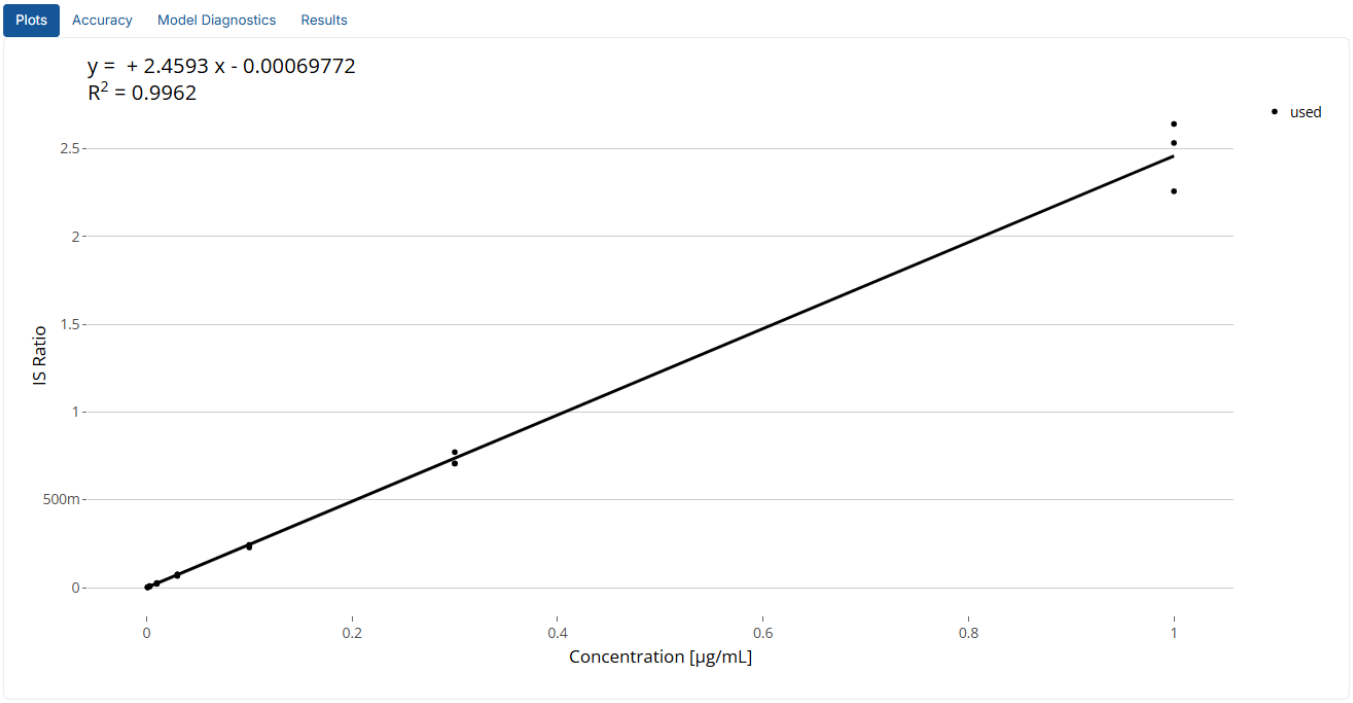


Interactive Features

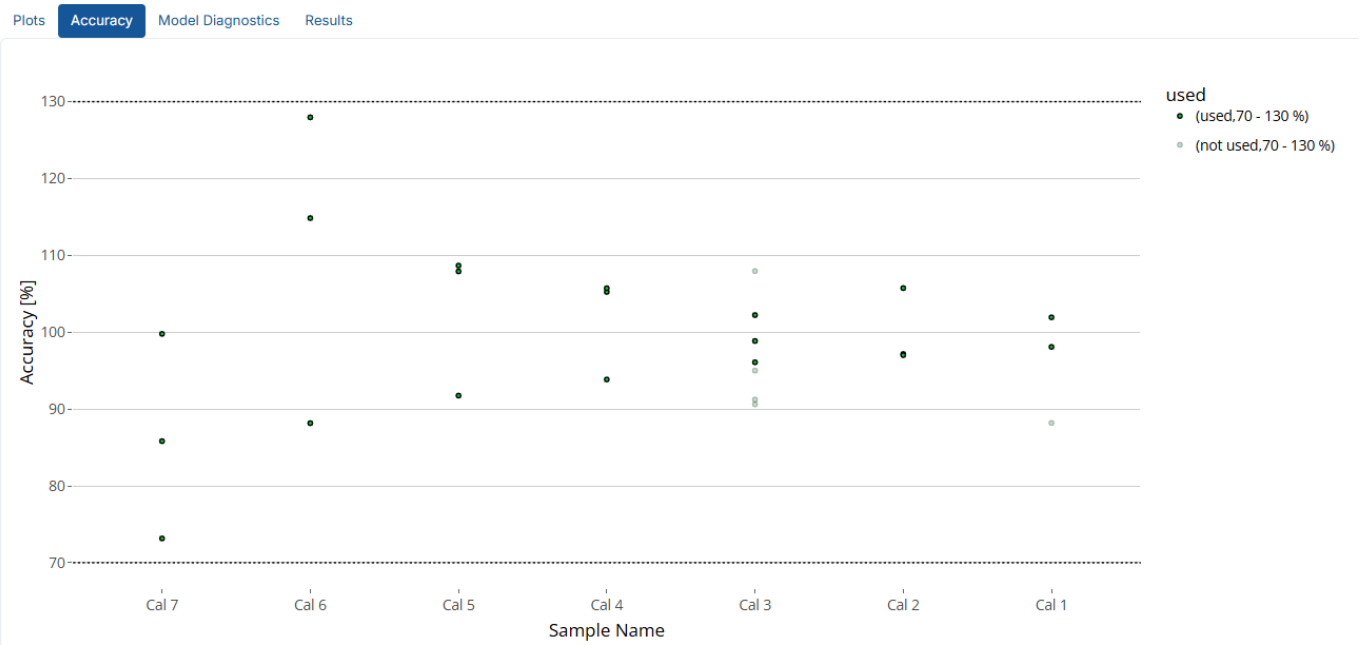
- **Exclude Standards**: Left-click on a standard to exclude it from the calibration. Click again to restore.
- **Toggle Standards**: By using one of the select tools in the upper corner, multiple standards can be removed/added to the model by selecting them and approving the message.

Main Tabs

1. **Plots**: Interactive regression plot. Calibration points can be excluded/restored in real time.

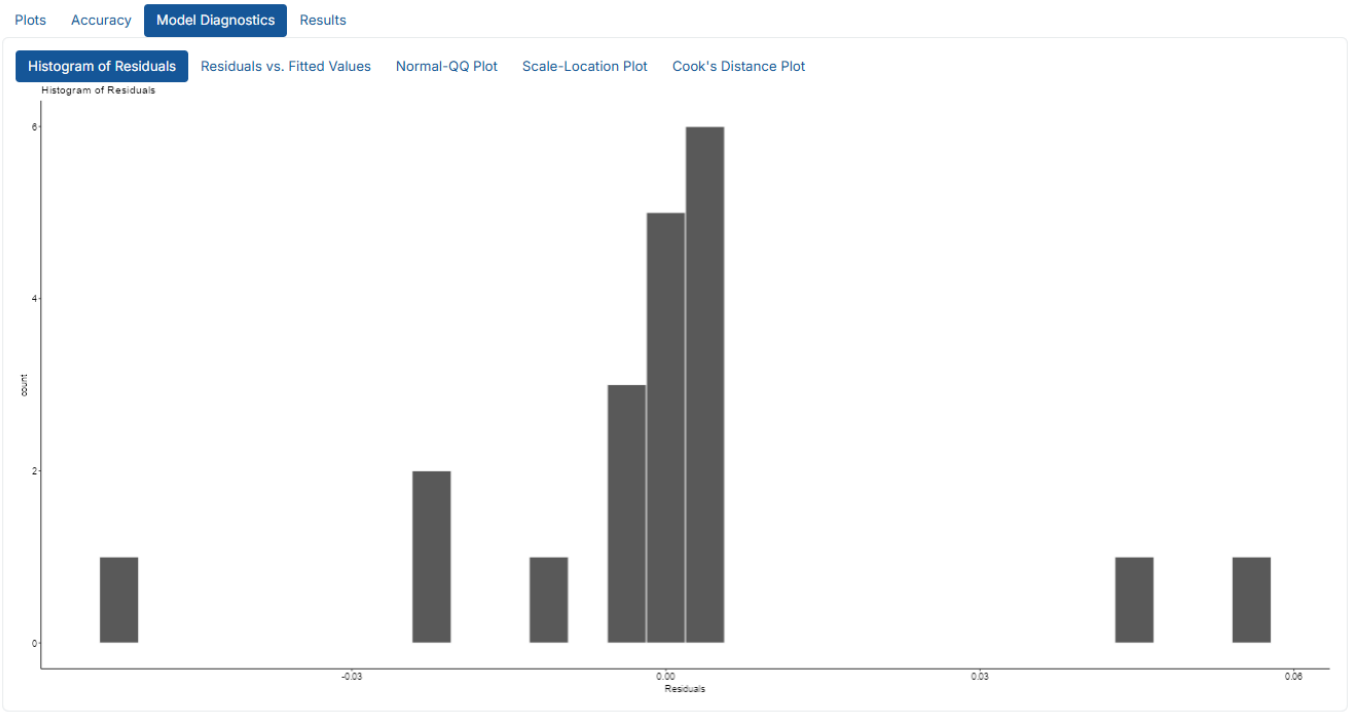


2. **Accuracy**: Visual and numeric summary of model accuracy. Points can also be removed here by clicking on them.

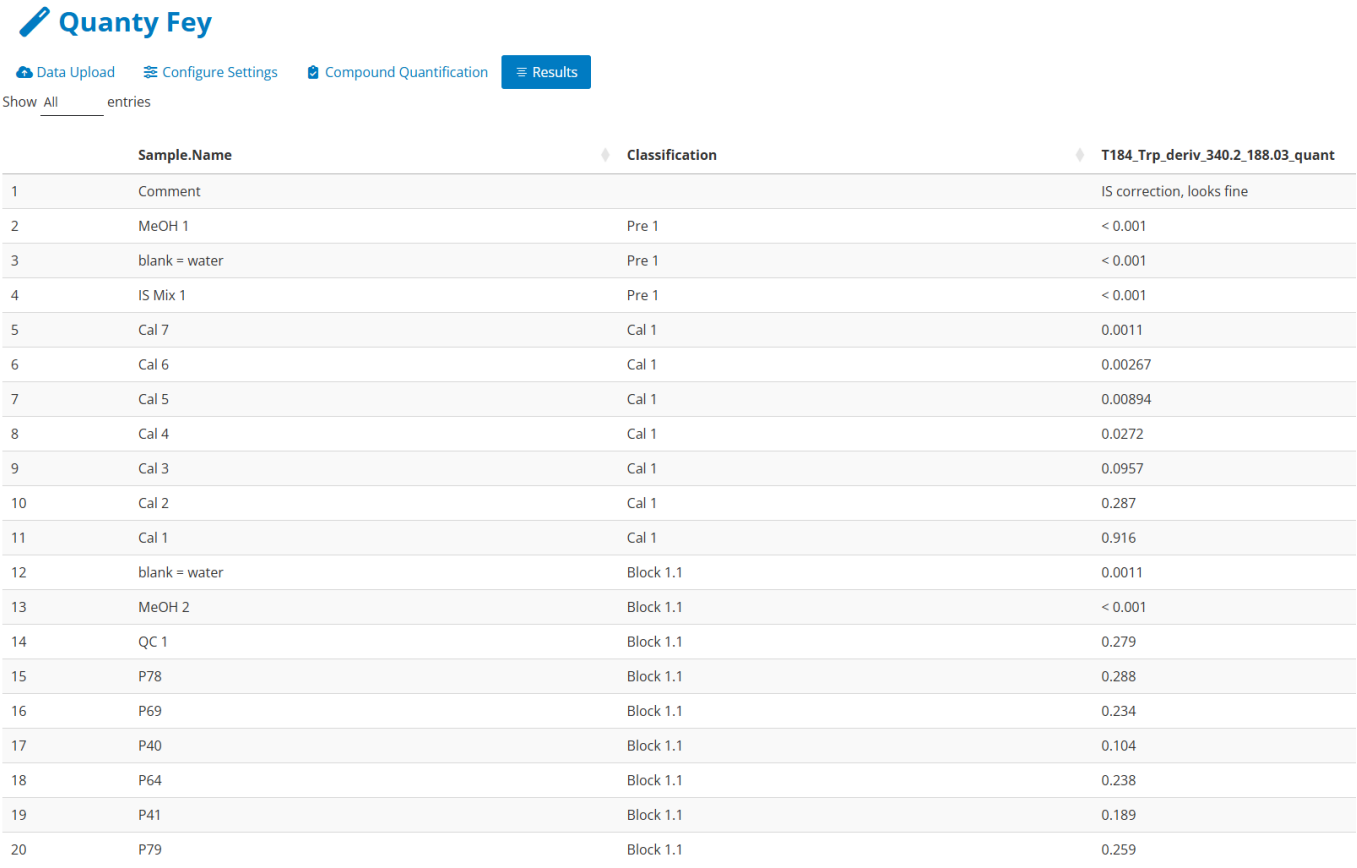


3. **Model Diagnostics**: Provides diagnostic plots (via `lindia` package):
    - Histogram of Residuals
    - Residuals vs. Fitted Values
    - Normal Q-Q Plot
    - Scale-Location Plot
    - Residuals vs. Leverage
    - Cook's Distance

> **Note**: For more information please look at the documentation/vignette fo the `lindia` package.



4. **Results**: Displays quantification results for the selected transition.



	Sample.Name	Classification	T184_Trp_deriv_340.2_188.03_quant
1	Comment		IS correction, looks fine
2	MeOH 1	Pre 1	< 0.001
3	blank = water	Pre 1	< 0.001
4	IS Mix 1	Pre 1	< 0.001
5	Cal 7	Cal 1	0.0011
6	Cal 6	Cal 1	0.00267
7	Cal 5	Cal 1	0.00894
8	Cal 4	Cal 1	0.0272
9	Cal 3	Cal 1	0.0957
10	Cal 2	Cal 1	0.287
11	Cal 1	Cal 1	0.916
12	blank = water	Block 1.1	0.0011
13	MeOH 2	Block 1.1	< 0.001
14	QC 1	Block 1.1	0.279
15	P78	Block 1.1	0.288
16	P69	Block 1.1	0.234
17	P40	Block 1.1	0.104
18	P64	Block 1.1	0.238
19	P41	Block 1.1	0.189
20	P79	Block 1.1	0.259

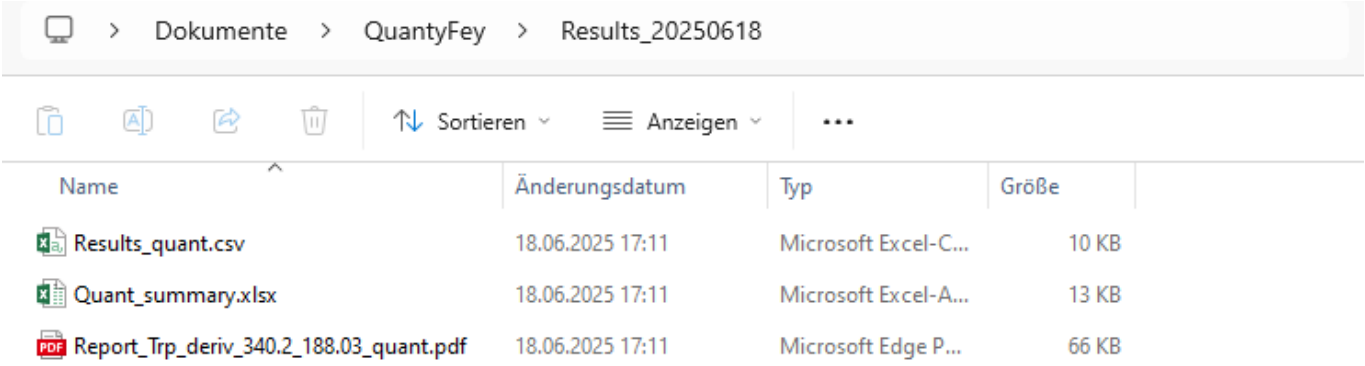
Saving Results

Once the quantitation method is selected, and the model is optimized, results can be saved by clicking on the save button:

- **Comment**: Add notes for the compound.

- **Save**: Saves the data and generates in the documents folder `Documents/QuantyFey/Results_<date>/` (*folder name can be customized by setting a **Project Name** in the **Data Upload** tab*):
    - `Results_quant.csv`: Contains concentrations for all quantified compounds. Values below LLOQ are labeled as "< <LLOQ>".
    - `Quant_summary.xlsx`: Summarizes all parameters and results for each saved compound, with one worksheet per quantification.
    - **Generate Report** (optional): Creates a PDF report with relevant plots and details.

> **Notes**: To prevent overwriting, files are timestamped if duplicates exist. Reports may be overwritten - when the app is restarted with the same project name; rename or move them to avoid conflicts. All files are saved in the `QuantyFey/` folder in the user's **Documents** directory.



Quant_summary.xlsx

This excel file contains all information necessary for the repetition of the exact concentrations calculated for the quantified compounds. For each saved transition, a new sheet is apended.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	Sample.Name	Classification	PeakArea	IS_PeakArea	IS_Ratio	Retention Time	Concentration		info_label	info_value		Sample.Name	Classification	PeakArea	Concentration	weights	used	predicted. Concentration	Accuracy
2	MeOH 1	all	3457.959	93316.50492	0.000249	4.1496157	0.000269		Function	y = -0.0003855028 + 2.362587 * x + 0.2244043 * x^2		Cal 7	Cal 1	0.00197	0.001	1000.0000	✓	0.000998	99.78
3	blank = water	all	2412.538	1582.168792	0.000174	4.0355212	0.000237		R2	0.998948957818493		Cal 6	Cal 1	0.00586	0.003	333.3333	✓	0.002640	88.16
4	IS Mix 1	all	3212.359	11025863.26	0.000291	3.9633051	0.000286		LLOQ	0.001		Cal 5	Cal 1	0.02130	0.010	100.0000	✓	0.009170	91.75
5	Cal 7	Cal 1	35495.71	17998776.91	0.001972	4.3962489	0.000998		IS Compound	IS_Tryptophan_348.1_195.1_CE25_quant		Cal 4	Cal 1	0.06630	0.030	33.3333	✓	0.028200	93.84
6	Cal 6	Cal 1	128367	21887578.53	0.005865	4.3997893	0.00264		Correction Factors	Blank = 1, Cal =1 , QC = 1, Sample = 1		Cal 3	Cal 1	0.23500	0.100	10.0000	✓	0.098900	98.86
7	Cal 5	Cal 1	416216.6	19532393.15	0.021309	4.3993673	0.00917					Cal 2	Cal 1	0.70700	0.300	3.3333	✓	0.291000	97.16
8	Cal 4	Cal 1	1116937	16845003.87	0.066307	4.3991711	0.0282					Cal 1	Cal 1	2.26000	1.000	1.0000		0.882000	88.19
9	Cal 3	Cal 1	4093655	17393034.09	0.235362	4.4008333	0.0989					Cal 7	Cal 2	0.00134	0.001	1000.0000	✓	0.000732	73.16
10	Cal 2	Cal 1	12224889	17282684.66	0.707349	4.3994789	0.291					Cal 6	Cal 2	0.00776	0.003	333.3333	✓	0.003450	114.85
11	Cal 1	Cal 1	32019578	14182609.51	2.257665	4.4004171	0.882					Cal 5	Cal 2	0.02510	0.010	100.0000	✓	0.010800	107.91
12	blank = water	all	27579.27	11467.37668	0.001988	4.3965593	0.001					Cal 4	Cal 2	0.07440	0.030	33.3333	✓	0.031600	105.24
13	MeOH 2	all	5146.321	85769.67915	0.000371	4.3971508	0.00032					Cal 3	Cal 2	0.24300	0.100	10.0000	✓	0.102000	102.23
14	QC 1	all	8699122	12672229.62	0.686471	4.4015441	0.283					Cal 2	Cal 2	0.70600	0.300	3.3333	✓	0.291000	97.01
15	P78	all	16863758	23803112.19	0.708469	4.4005832	0.292					Cal 1	Cal 2	2.53000	1.000	1.0000	✓	0.981000	98.09
16	P69	all	18320426	31727461.34	0.577431	4.4003369	0.239					Cal 7	Cal 3	0.00164	0.001	1000.0000	✓	0.000858	85.82
17	P40	all	6244846	24386966.52	0.256073	4.3981911	0.107					Cal 6	Cal 3	0.00869	0.003	333.3333	✓	0.003840	127.96
18	P64	all	16507081	28111029.32	0.58721	4.400444	0.243					Cal 5	Cal 3	0.02530	0.010	100.0000	✓	0.010900	108.67
19	P41	all	10179213	21927485.01	0.464222	4.4006291	0.193					Cal 4	Cal 3	0.07480	0.030	33.3333	✓	0.031700	105.72
20	P79	all	18262339	28644851.92	0.637544	4.399837	0.263					Cal 3	Cal 3	0.22900	0.100	10.0000	✓	0.096100	96.08
21	P43	all	11013702	24079089.21	0.457397	4.3987729	0.19					Cal 2	Cal 3	0.77200	0.300	3.3333	✓	0.317000	105.74
22	P72	all	14383311	30449195.59	0.472371	4.3996139	0.196					Cal 1	Cal 3	2.64000	1.000	1.0000	✓	1.020000	101.93
23	Cal 3	all	3282470	14517698.93	0.226101	4.4002502	0.095					Cal 3	all	0.22600	0.100	0.0000		0.095000	95.01
24	IS Mix 2	all	4077.075	21530817.12	0.000189	4.3970871	0.000243					Cal 3	all	0.21700	0.100	0.0000		0.091200	91.23
25	P77	all	11556223	19558994.8	0.590839	4.3990975	0.245					Cal 3	all	0.25700	0.100	0.0000		0.108000	107.95
26	P52	all	17303256	25857595.22	0.669175	4.39937	0.276					Cal 3	all	0.21600	0.100	0.0000		0.090600	90.61
27	P60	all	10235174	21786889.07	0.469786	4.3986985	0.195												
28	P73	all	16955275	24406412.79	0.694706	4.3991163	0.286												
29	P53	all	12867833	18633194.07	0.690587	4.4008975	0.285												

Results_quant.csv

This csv file contains the concentrations of all quantified compounds in the current session. It is frequently apended after every save, and is represented by the results tab.

	A	B	C	D	E
1	Sample.Nam	Classificatior	Trp_deriv_340.2_188.03_quant		
2	Comment				
3	MeOH 1	Pre 1	< 0.001		
4	blank = wate	Pre 1	< 0.001		
5	IS Mix 1	Pre 1	< 0.001		
6	Cal 7	Cal 1	< 0.001		
7	Cal 6	Cal 1	0.00264		
8	Cal 5	Cal 1	0.00917		
9	Cal 4	Cal 1	0.0282		
10	Cal 3	Cal 1	0.0989		
11	Cal 2	Cal 1	0.291		
12	Cal 1	Cal 1	0.882		
13	blank = wate	Block 1.1	0.001		
14	MeOH 2	Block 1.1	< 0.001		
15	QC 1	Block 1.1	0.283		
16	P78	Block 1.1	0.292		
17	P69	Block 1.1	0.239		
18	P40	Block 1.1	0.107		
19	P64	Block 1.1	0.243		
20	P41	Block 1.1	0.193		
21	P79	Block 1.1	0.263		
22	P43	Block 1.1	0.19		
23	P72	Block 1.1	0.196		
24	Cal 3	Block 1.2	0.095		
25	IS Mix 2	Block 1.2	< 0.001		
26	P77	Block 1.2	0.245		
27	P52	Block 1.2	0.276		
28	P60	Block 1.2	0.195		
29	P73	Block 1.2	0.286		
30	P53	Block 1.2	0.285		
31	P68	Block 1.2	0.287		
32	P66	Block 1.2	0.183		
33	P82	Block 1.2	0.292		
34	Cal 3	Block 1.3	0.0912		

Report

The **report** can be generated by ticking the **generate report** before saving the quantitation results. This will generate a pdf report with all plots, and information about the quantification.

QuantyFey - Report

markus_aig

2025-06-18

Quantitation Summary for Trp_deriv_340.2_188.03_quant

This report provides a detailed summary of the quantification results for Trp_deriv_340.2_188.03_quant. It includes all plots from the Overview tab, a description of the quantitation method applied, individual plots per method, calibration function summaries, and relevant parameter settings.

Overview of the Data

Retention Time Analysis

The plot below illustrates the retention time (RT) of the compound across different sample types. This helps to assess consistency and identify any retention shifts that may indicate anomalies in chromatography.

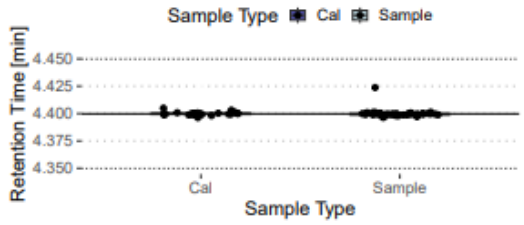


Figure 1: overviewview of Retention Time per Sample Type

1

Qualifier and Quantifier Analysis

This section shows the ion ratio between the Quantifier and Qualifier transitions (Q/Q) for each sample type. A stable ion ratio is important for confirming compound identity and method reliability.

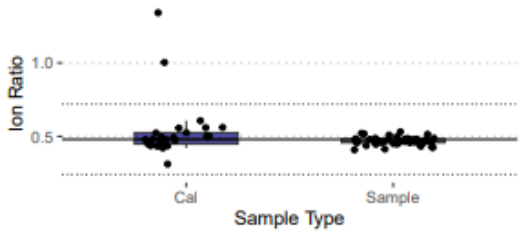


Figure 2: overviewview of ion ratio per Sample Type

Blank Analysis

The following plot compares compound intensity in blank samples against the corresponding measurements in actual samples. This is useful to detect potential contamination or carryover.

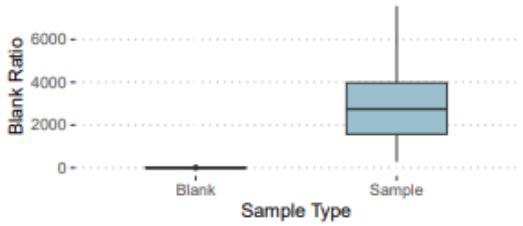


Figure 3: Overview of Sample to Blank

2

Method for Quantification

For Trp_deriv_340.2_188.03_quant, the quantitation was performed using the **IS Correction** method.

IS correction was used for this analyte. In the following plot the red dots mark the Peak Areas of the Internal Standard compounds and the Bars represent the Peak Areas of the Compound over the whole Measurement Sequence. The lower plot represents the ratios from the respective Sample after each Peak Area from the Compound was divided by the Peak Area from the selected Internal Standard.

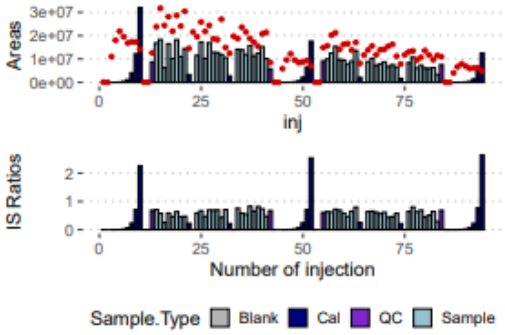


Figure 4: Overview of internal standard correction

The following table shows the correction factors used for the specific Sample Types. This is useful if different dilutions were used for the analysis.
Table 1: Information about the correction factors for each sample type

Sample.Type	Correction.Factor
Blank	1
Cal	1
QC	1
Sample	1

3

Quantitation Summary

Summary of Calibration Data

The following plots display the generated calibration curves, in combination with further information about the calibration data.

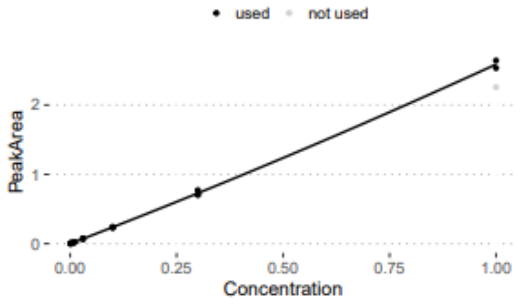


Figure 4: Calibration Plot for Block: all

Table 1: Concentration levels

Cal.Name	Concentration
Cal 7	0.001
Cal 6	0.003
Cal 5	0.010
Cal 4	0.030
Cal 3	0.100
Cal 2	0.300
Cal 1	1.000

Summary of Settings:

Model used: quadratic
Quantitation Method used: IS Correction
Lower Limit of Quantification: 0.001
Weighting Method: 1/x
IS Compound for Correction:
IS-Tryptophan-340.1-195.1-CE25-quant
Comment added to this Compound:

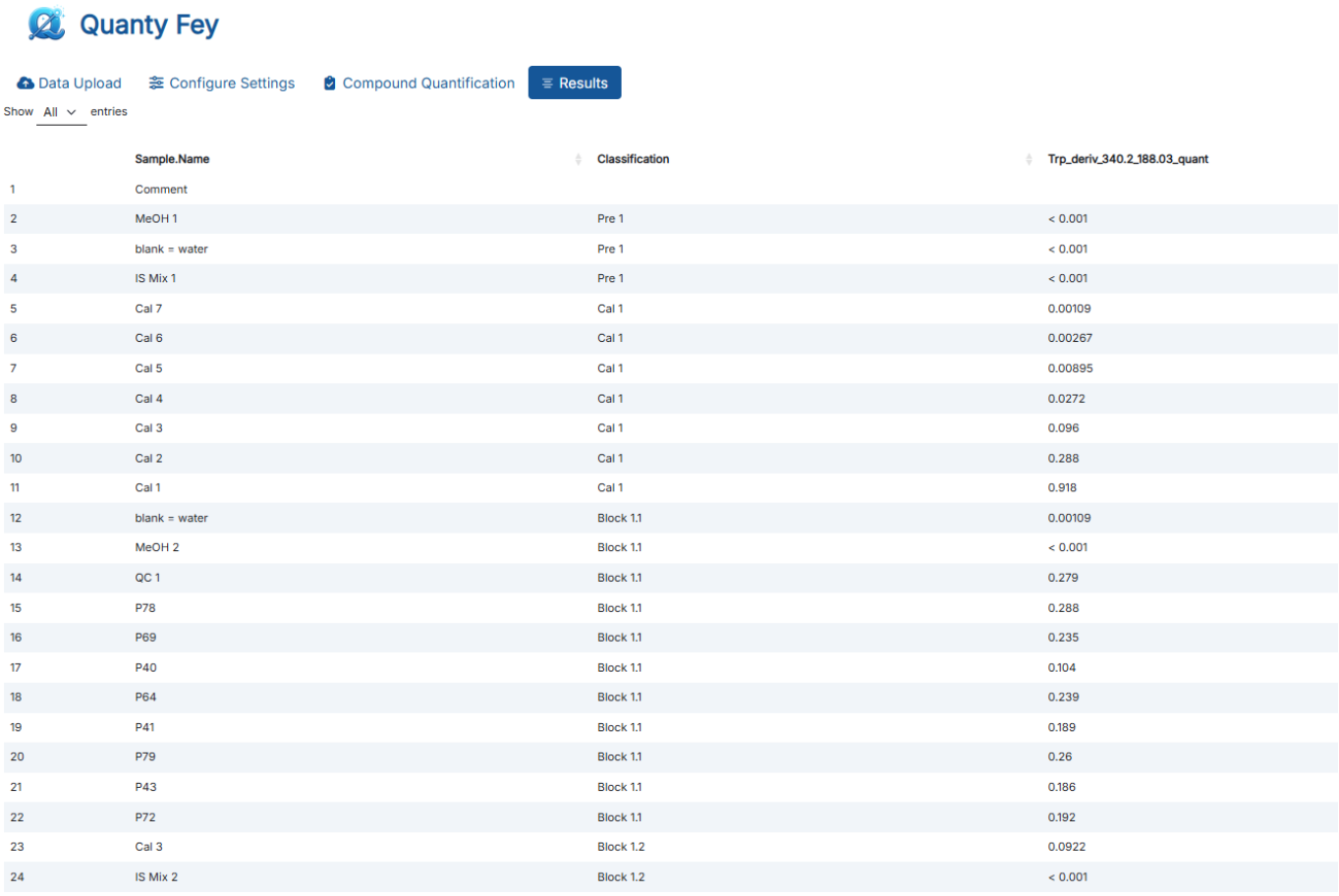
4

Results

This tab becomes active once **at least one compound** has been quantified and saved. It provides the overview of the concentration table and comments for the analysis - it reflects the contents of the `results_quant.csv` file.

> **Note:** You can manually edit cells in this tab - for example, to change a **comment** - but **these changes will only take effect after the next** `save` The update will not appear in the **PDF Report**. If you save a compound twice, it will add a prefix to it, and save it as an additional transition - a new column in `Quant_results.csv` will be added with the new transition name (e.g., re_1_TransitionName), a new sheet, and a new report with the adjusted name will be generated.

> **Recommendation:** When saving the same compound multiple times, make sure to use clear, informative comments. This helps you identifying which version you want to keep or report later on. All saved versions are kept - **nothing is overwritten** - but without good labeling, it can get confusing.



	Sample.Name	Classification	Trp_deriv_340.2_188.03_quant
1	Comment		
2	MeOH 1	Pre 1	< 0.001
3	blank = water	Pre 1	< 0.001
4	IS Mix 1	Pre 1	< 0.001
5	Cal 7	Cal 1	0.00109
6	Cal 6	Cal 1	0.00267
7	Cal 5	Cal 1	0.00895
8	Cal 4	Cal 1	0.0272
9	Cal 3	Cal 1	0.096
10	Cal 2	Cal 1	0.288
11	Cal 1	Cal 1	0.918
12	blank = water	Block 1.1	0.00109
13	MeOH 2	Block 1.1	< 0.001
14	QC 1	Block 1.1	0.279
15	P78	Block 1.1	0.288
16	P69	Block 1.1	0.235
17	P40	Block 1.1	0.104
18	P64	Block 1.1	0.239
19	P41	Block 1.1	0.189
20	P79	Block 1.1	0.26
21	P43	Block 1.1	0.186
22	P72	Block 1.1	0.192
23	Cal 3	Block 1.2	0.0922
24	IS Mix 2	Block 1.2	< 0.001

Summary

Once all settings are configured, the application supports a full evaluation of data quality and compound identification:

- **RT** and **Ion Ratios** help verify correct compound identification.
- **Blank Analysis** helps in distinguishing true signals from background noise.

Before quantification, users can apply **drift correction** strategies using the following methods:

- Internal standard correction
- QC-based drift correction
- Custom Bracketing
- Weighted Bracketing

Each method can be configured before quantification, and then methods can be compared, and the most appropriate method can be selected. The quantification interface includes tools for **interactive regression**

model optimization, allowing users to:

- Adjust model parameters
- View diagnostic plots
- Assess model fit

An **automatic optimization feature** is available to suggest a possible regression fit based on a simple algorithm, but revision of the optimization is advised.

Overwriting safeguards are in place to avoid accidental overwriting, while reducing file clutter.

Troubleshooting

- **Package Installation Failure**:
    - Ensure RTools 4.2 is correctly installed.
- **Console Does Not Open**:
    - Relocate the application folder to a different directory.
    - Avoid running the application from the "Downloads" folder, as this may cause issues.