Setup of the LIMS for Lasers Add-On tool

1. Introductory remarks

- This software tool is unsupported.
- The tool was developed in a Microsoft® Windows 10 64-bit environment with a 32-bit Office-365 version. Other versions may be functional but are not tested.
- Several functionalities use simplistic programming routines which are prone to unexpected behaviors or database inconsistencies. Note that all functions triggering a "write" action will write to the LIMS backend database immediately; there is no "undo" function.
- Whilst most of the bugs are eliminated, the software has not undergone professional and security testing.
- This software lacks entirely a sample registration facility. To add new samples and projects, use the LIMS9 or LIMS for Lasers frontends, supplied e.g., here: https://nucleus.iaea.org/sites/ihn/Pages/Homepage.aspx (IAEA-NUCLEUS user login required)
- This version is intended for offline use and cannot be intermingled with existing LIMS9 or LIMS for Lasers databases. The tool modifies data in the LIMS backend with the above constraints, and we caution users not to connect it to an established LIMS production backend used by others.
- The exercises and test datasets supplied were designed to work with this demo version.
- The tool computes $\Delta'^{17}O$ in per meg (while in the manuscript, a ‰ annotation is used).

2. Setup

We recommend setting up the tool in a single folder. This folder needs to contain the following files:

- LIMS_Addon_FE.accdb (the interface frontend)
- LIMS_Addon_BE.accdb (the backend for additional data not storable in LIMS)
- LIMS Backend Database.accdb (the LIMS backend)
- LIMS_Addon_Config.xml (this file configures the file paths to the data tables)

The config file can be edited in Notepad or WordPad or similar applications able to read and edit plain text only. Do not use Office word processing software to edit file paths as needed. The config file must read like:

```
<?xml version="1.0" encoding="UTF-8"?>
<dataroot xmlns:od="urn:schemas-microsoft-com:officedata" generated="2022-08-
12T20:25:07">
	<tmpLocalSettings>
		<strTokenName>strLimsDbPath</strTokenName>
		<strTokenValue> c:\LIMS_Addon\LIMS Backend DB.accdb </strTokenValue>
	</tmpLocalSettings>
	<tmpLocalSettings>
		<strTokenName>strLimsAddonDbPath</strTokenName>
		<strTokenValue>c:\LIMS_Addon\LIMS_Addon_BE.accdb</strTokenValue>
	</tmpLocalSettings>
</dataroot>
```

If the file paths are not set correctly or connections are unavailable, LIMS-Addon will prompt you to specify the correct paths; otherwise, it will not start.

3. Parameterizations in addition to LIMS

3.1. "Service"

The LIMS-Addon uses the concept of a lab "service" to define the number of repetitions and acceptance criteria. For example, one service called "Dual isotope two assays" is defined as:

Measurable	1/#Assays*	Max. Unc.	Max. SD	σ_P
$\delta^{2}H$	0.5		1.6	0.8
$\delta^{18}O$	0.5		0.2	0.1

Note that the number of assays (*) is expressed for computational logic reasons as 1/n; hence two assays need to be annotated as 0.5; three such as 0.333 etc.

Services can also be defined including derivative parameters like deuterium excess or $\Delta'^{17}O$:

Measurable	1/#Assays*	Max. Unc.	Max. SD	σ_P
$\delta^{2}H$	0.33333	0.8	1.2	0.4
$\delta^{18}O$	0.33333	0.09	0.06	0.03
$\delta^{17}O$	0.33333	0.045	0.03	0.015
d-excess	0.33333	0.8	1.2	0.8
$\Delta'^{17}O$	0.33333		20**	10**

(**) Note that the LIMS-Addon uses the per meg annotation.

The LIMS-Addon does not conduct a validation check of what has been entered.

You can define your "services" in the "Settings" menu using the GUI. Click "New service" to create a new one and set attributes through the GUI (Figure 1). The GUI window also allows associating instrument templates to a given workflow.

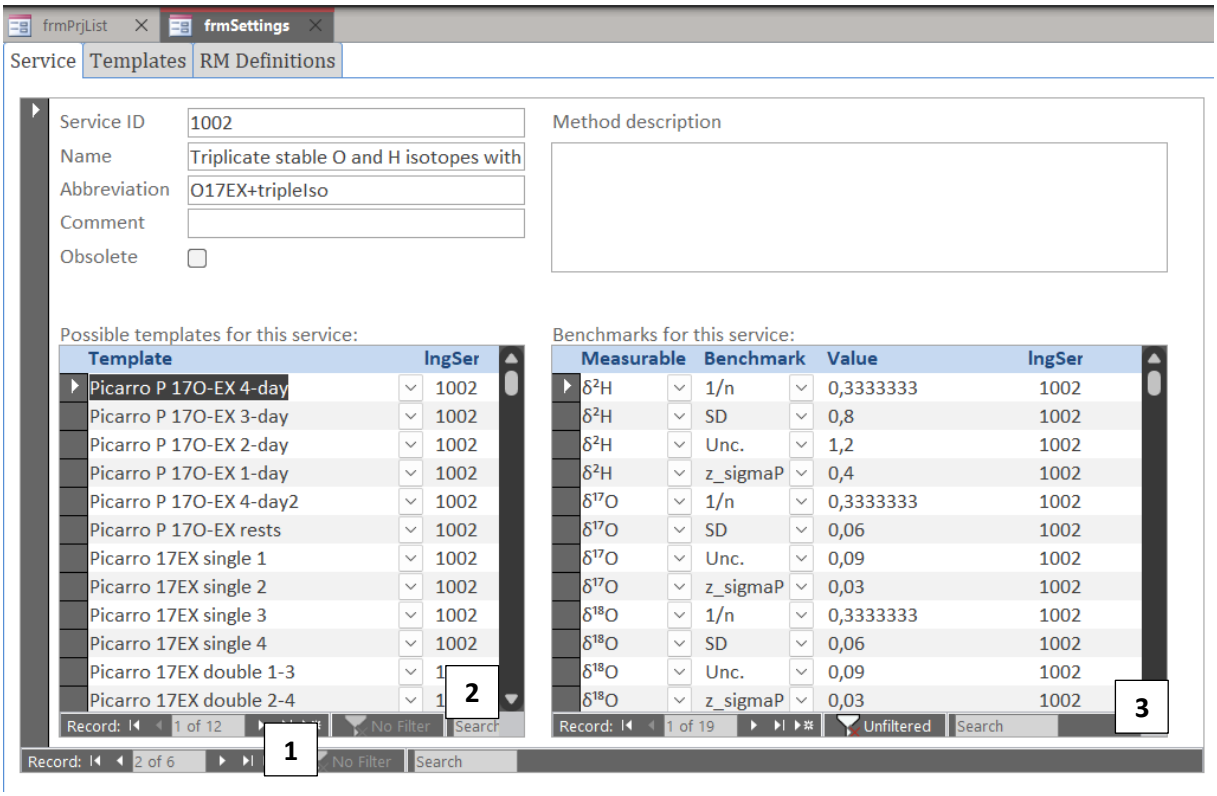


Figure 1: "Services" menu in settings: (1) click navigation buttons to go to next "service" in list; (2) go to new line (*) to link an additional template; (3) go to new line (*) to create a new benchmark criterion.

3.2. Advanced run template properties

Use the advanced run template property menu to specify features unavailable in LIMS:

- On the template level, you can specify to:
  - Randomize samples within a normalization subrange,
  - Randomize controls within a normalization subrange, and
  - How many injections by default a template uses.
- On the sample position level, you can define
  - Normalization subranges (from 1 to n; this is needed for the randomization commands)
  - The function of a sample (Unknown, control, enriched or depleted RM, drift control, linearity control or wash sample), and
  - The actual number of injections for this position. (For Picarro analysers less relevant, because this is defined in the autosampler setup.)

When creating a new template, it is mandatory to

1. Create a new template using LIMS
2. Populate its positions either in the LIMS GUI or by uploading them from a spreadsheet. Mind the page/slot order.
3. Change to the LIMS-Addon GUI and define the additional properties (see window below).

Note: When changing a template; this has to be done through LIMS first to maintain compatibility. The LIMS-Addon has no viewer capable of editing the LIMS backend tables themselves. In this case, perform the edits in LIMS, and in the third step, use either the "Clean up additional info" or "Delete all additional info": The former deletes only superfluous positions. The latter deletes all additional

information, and you can set those instructions again. Figure 2 shows a screenshot of the GUI. The menu further allows associating the template with one or more services.

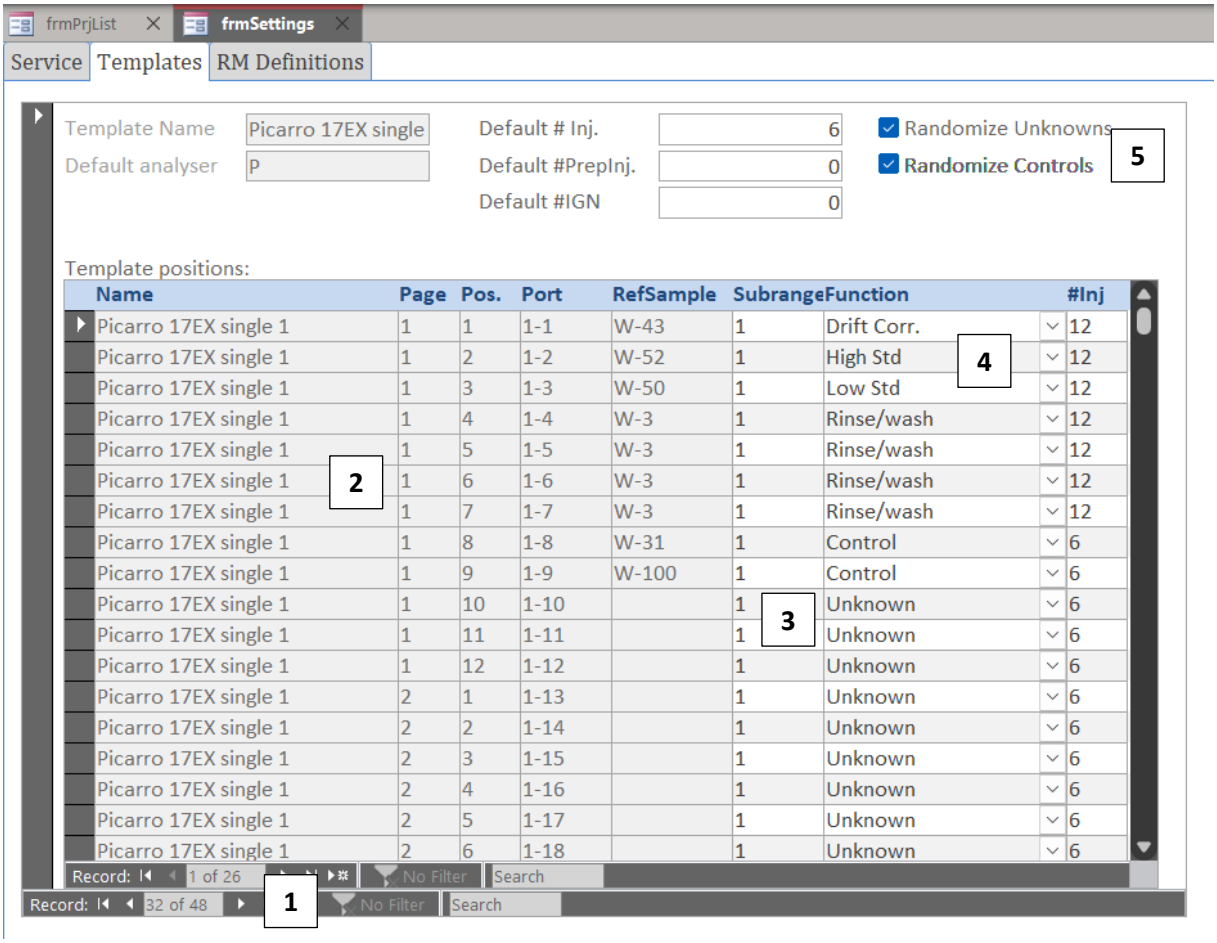


Figure 2: Additional template properties in the settings menu. (1) use navigation buttons to move to next template. (2) greyed-out properties are to be set in LIMS for Lasers. (3) specify subranges if your run design includes multiple. (4) specify sample function. (5) click checkboxes to randomize unknowns and/or controls across a subrange. Note that the sequence is governed by the autosampler control; hence wash positions 4-7 may not come to use.

3.3. RM definitions

The RM definitions module was foreseen to be able to assign revised values of the same RM to the unknowns analysed in the run concerned. This feature was intended with a view to a possibly changing RM definition for $\delta^{17}O$ because SLAP2 is provisional. In its current state, the LIMS-Addon fetches the most recent non-expired definition of an RM. It is possible to assign reference values to normalization anchors and controls, and also to δ values themselves but also to the derivative parameters deuterium excess and $\Delta'^{17}O$. Figure 3 shows a screenshot of the GUI window.

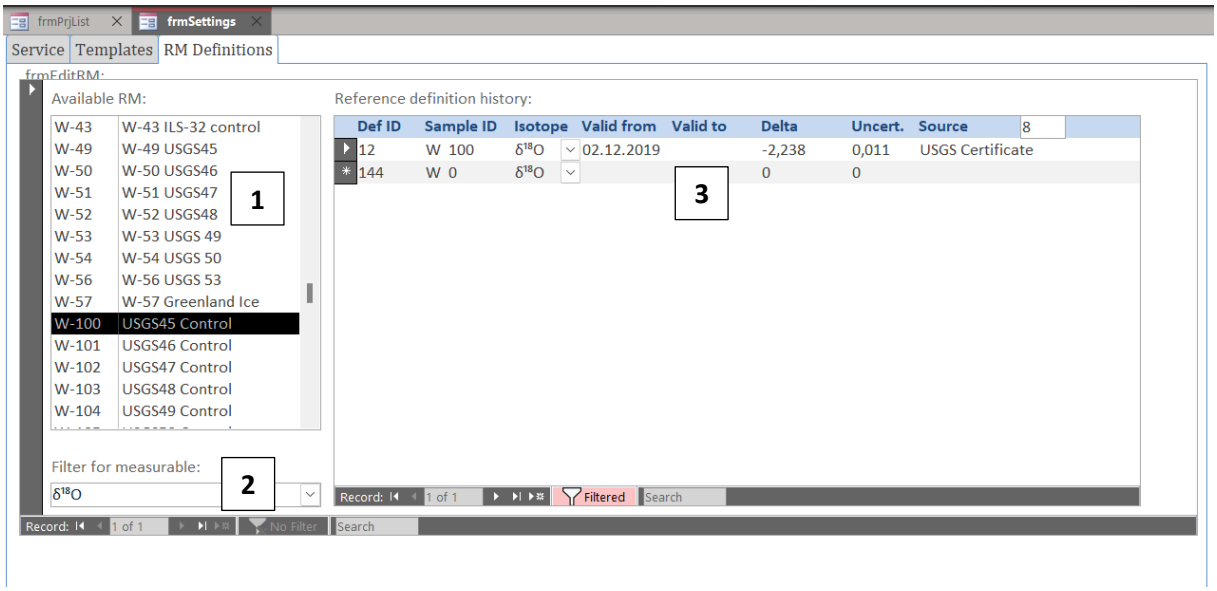


Figure 3: RM Definitions menu in the settings menu. (1) select RM to define, (2) filter for isotope or derivable, (3) type in line with (*) to add a new definition, or in the corresponding line to edit an existing one.

4. Handling projects and samples

4.1. Projects' list and filters

The main project window allows a user to filter for some LIMS core and expanded attributes. Text-based filter criteria always select for where the search string is contained. The filter menu also includes lookup-based selection criteria, such as filtering for a certain service, or a project status. After making selections, click on "apply filters". A double-click on a line in the project list opens the project properties (Figure 4).

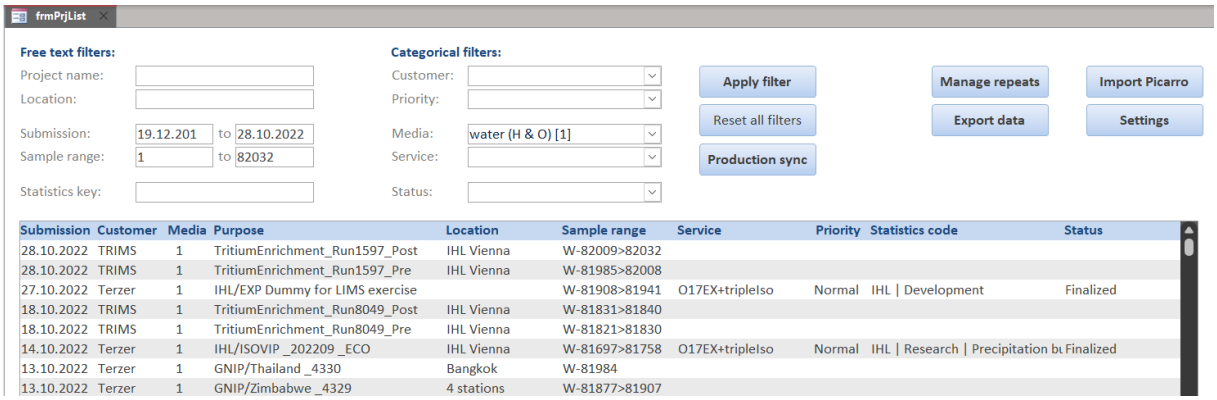


Figure 4: Project main menu

4.2. Additional project properties

An essential step in the LIMS-Addon work is to assign the additional project and sample attributes to an existing LIMS project (Figure 5). To initiate this process, click on the project dropdown list (it has only one entry here) and confirm the message following. This function is necessary to also zero the project and sample statuses. Once this is initiated, you can set the workflow, statistics, priority, etc.

settings. All sample and project statuses can be recalculated by pressing the “Validate statuses” button. (Note: At the current stage of LIMS-Addon, it is necessary to conduct this step manually.)

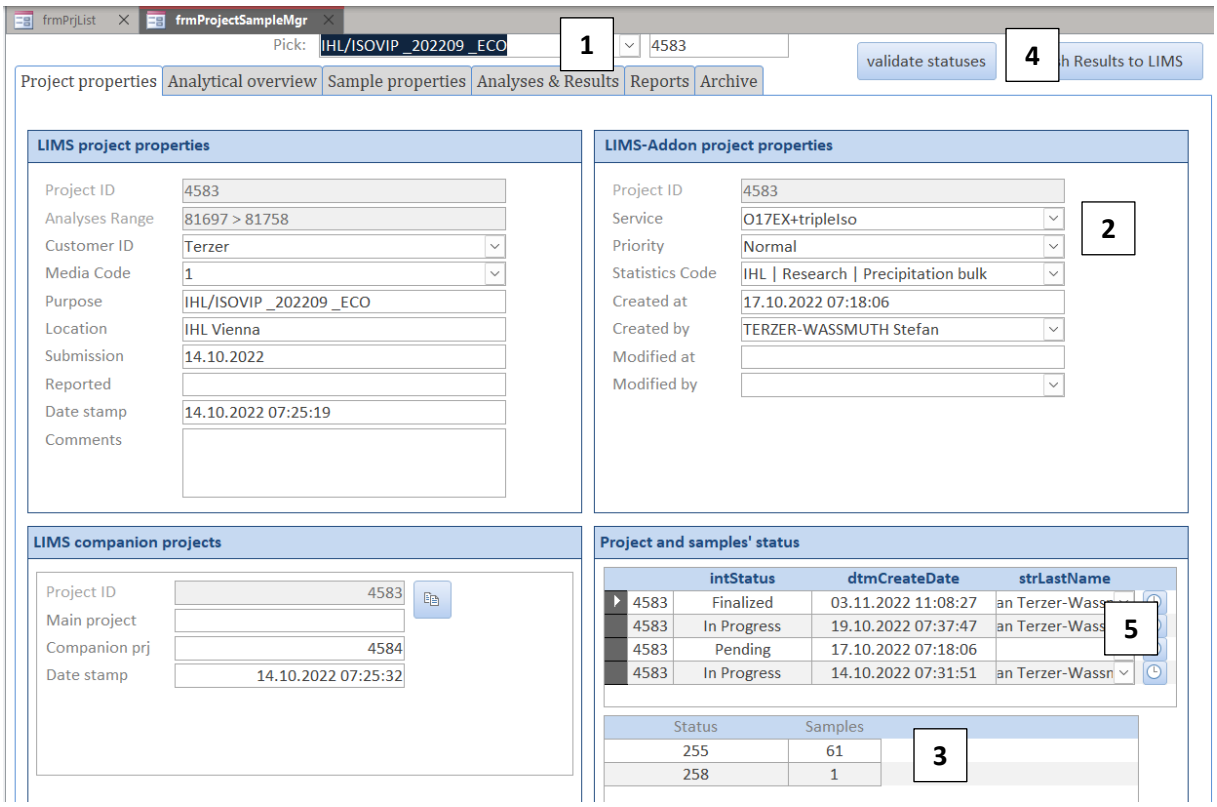


Figure 5: Additional project properties menu. (1) click on the project name in the drop-down list to create the additional properties’ record. (2) set additional attributes. (3) sample statuses (255=finalized for 61 samples). (4) click “validate statuses” to recalculate the project’s and samples’ statuses. (5) status history of the project.

4.3. Sample properties: Status and Tags

Click on the “Samples” tab to edit samples’ additional properties:

- You can cancel samples’ analyses (Note: This action cannot be undone through the addon GUI.) – this sets their status to “Cancelled” and they will not be considered for further analysis. You can use the complex multi-select function (Ctrl+Shift+click) to select any combination of samples for this action.
- You can assign tags to samples, e.g., when samples from a common area span over several LIMS projects but you wish to query across them. It is possible to assign multiple tags to a sample, but also you can choose multiple samples (again through complex multi-select) for the same tag. Tags do not have a dictionary (lookup) in the background, but the dropdown selection allows to reuse tags used before (Figure 6).

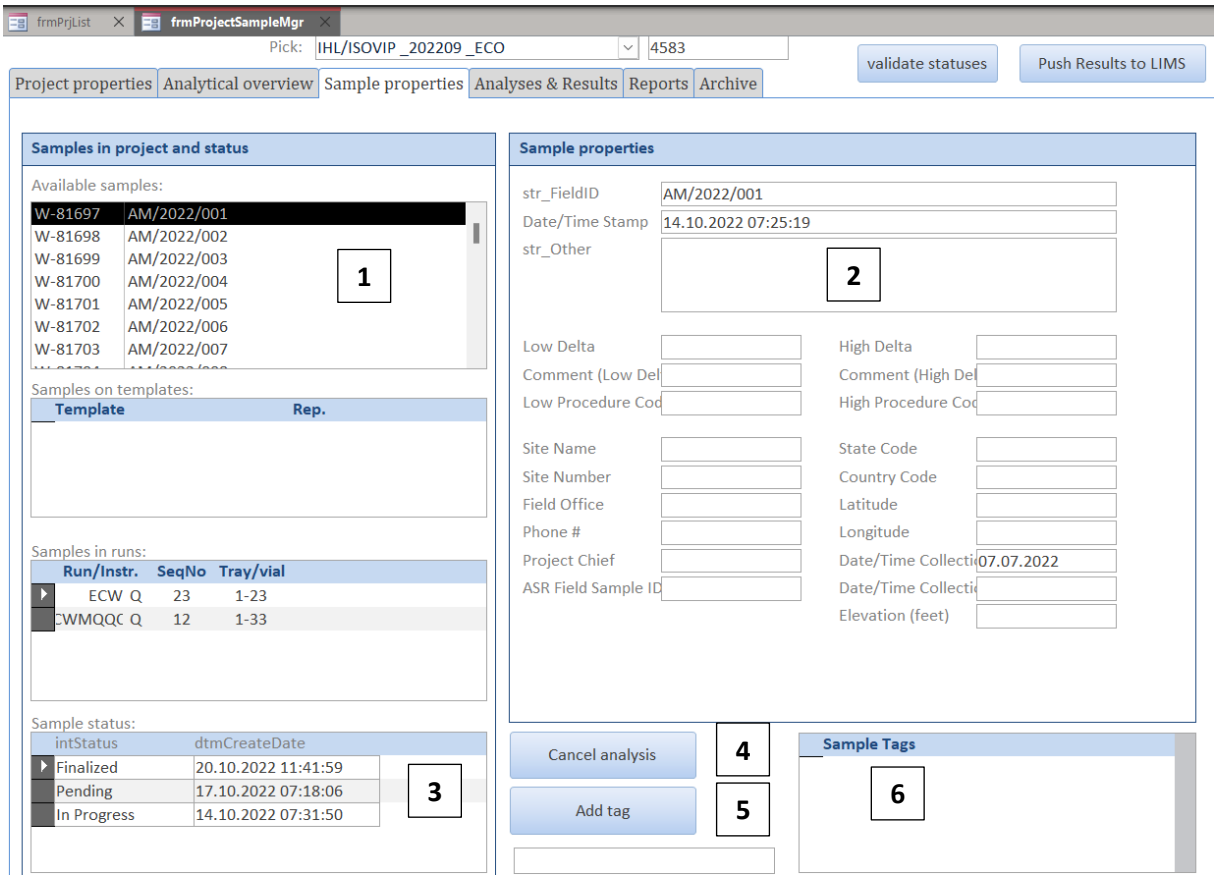


Figure 6: Additional sample properties. (1) list of samples in the project, (2) sample's LIMS properties, (3) sample statuis history, (4) click to cancel (takes it out of "in progress", (5) click to add tags, (6) tags assigned to this sample.

4.4. Analyses and results; assigning samples to templates

The LIMS-Addon has an evaluation menu that works on the project and run level (i.e., the same menu serves at two different steps of the post-processing). The menu displays the current measurement results and benchmarks them against a service's performance criteria:

- The checkbox "only show pending" shows samples that are not yet finalized (be it for not meeting the repetition/uncertainty targets or for not yet having been set to finalized).
- The "cancel" buttons set all (selected) samples' status to "canceled".
- The "finalize" buttons set all (selected) samples' status to "finalized". The "finalize selected" button also allows overriding if the specifications are not met.
- The "repeat" buttons place all (selected) samples onto the template specified in the dropdown menu. Only those templates that are available for a given service are shown in the list.
- The lower part of the menu shows individual analyses. If you wish to (un-)ignore assays, click the pencil next to the "IGN" checkbox.
- See also chapter 5.7 for further information.

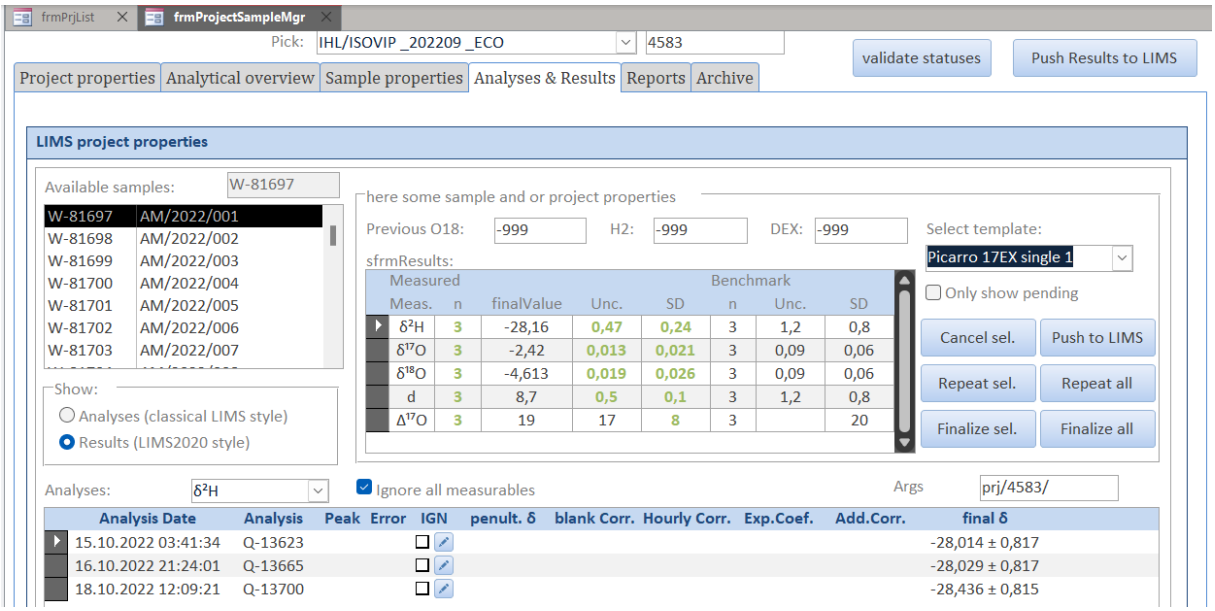


Figure 7: Results evaluation window.

5. Importing CRDS analyses

5.1. Step 1: Import dialogue box

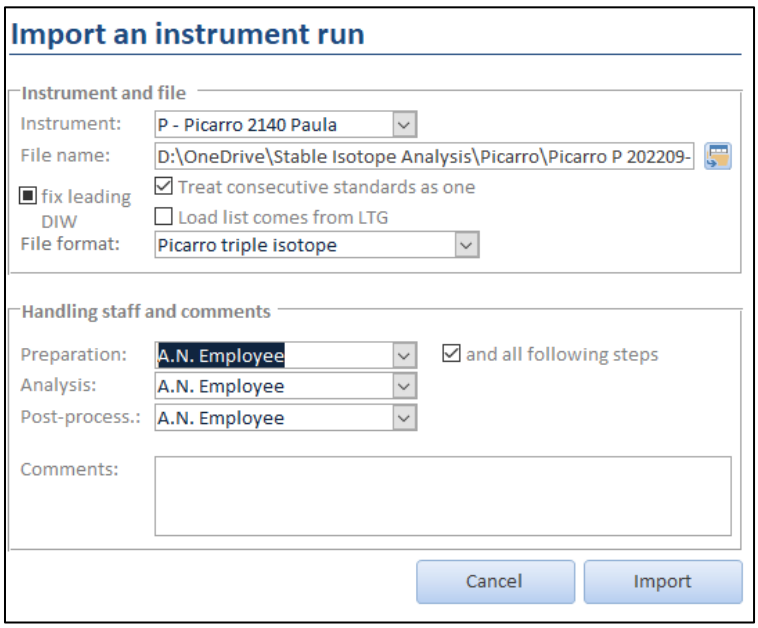


Figure 8: Picarro data import main screen.

In this dialogue, the user must specify:

- the file path, instruments, the user in charge of the post-processing and
- the file format (Picarro dual- or triple isotope),
- and whether consecutive standard vials are treated as one sequence – the latter ensures compatibility with LIMS for Lasers which requires two vials per standard at the usual number of injections, while LIMS-Addon uses one single vial each with twice the number of injections)

5.2. Step 2: Define normalization subranges

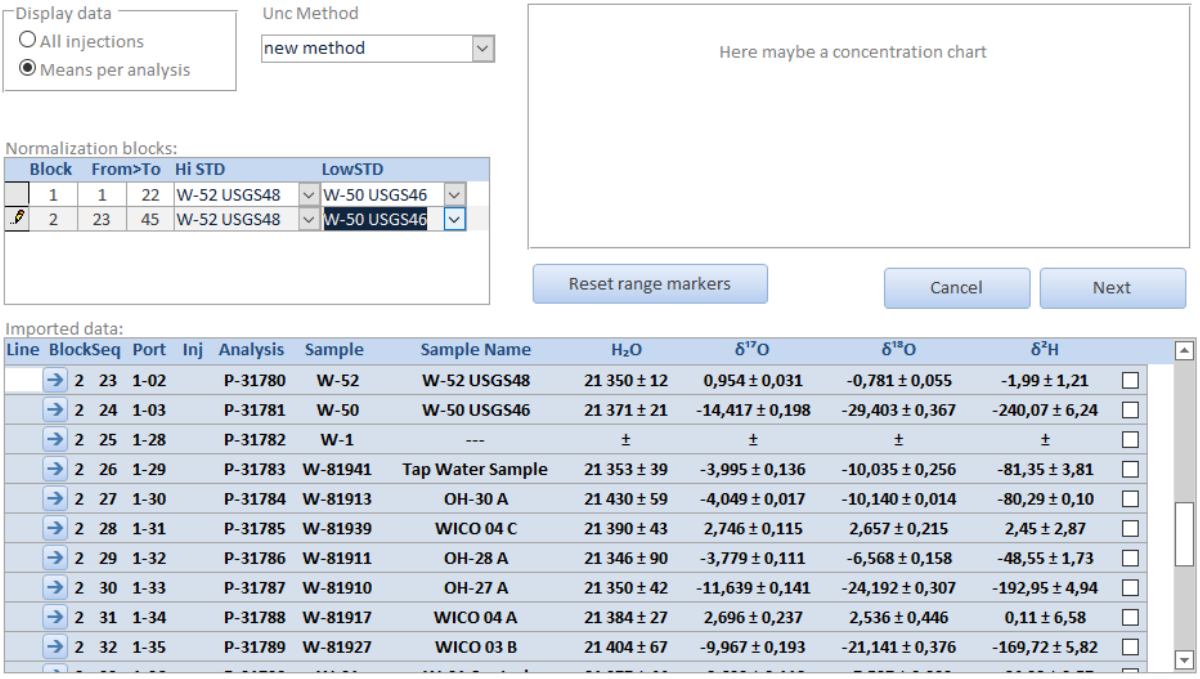
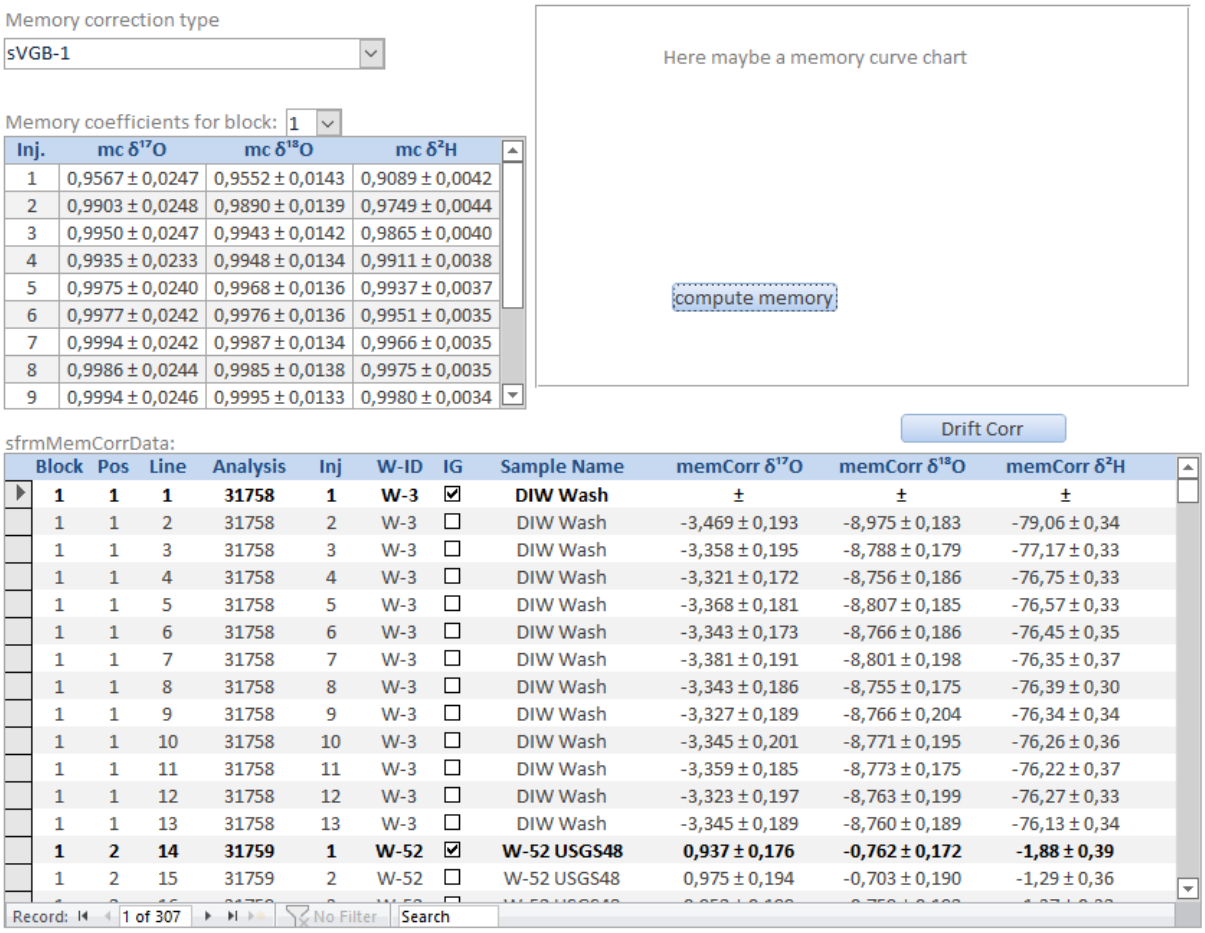


Figure 9: Step 1: Definition of normalization subranges and specification of high and low RMs.

- The user can toggle between all injections displayed, or only means (note – means of the raw data)
- By clicking on the ➔ button, a new normalization subrange is established, going from the analysis clicked on to the end, whereby clicking on a later sample redefines the end of the previous subrange. In the example above, we clicked positions 1 (getting first 1-68), then 24 (creating 1-23, 24-68) and then 46 (getting 1-23, 24-45 and 46-68).
- In case of a mistake, the whole range marking is nullified by clicking the “Reset range markers” button and starting over.
- For each subrange, the high and the low standards are chosen, in our case USGS48 as high standard and USGS46 as low standard.
- The user can ignore individual injections or a whole sample by clicking the “ignore” checkbox to the right of the δ^2H column.
- When done with settings, click “next” to move to the next step: Memory correction.

5.3. Step 3: Memory correction

This current version supports one memory correction method, which is a modified version of the van Geldern and Barth (2011) calculations but ignoring the first injection.



Inj.	mc $\delta^{17}O$	mc $\delta^{18}O$	mc $\delta^{2}H$
1	0,9567 ± 0,0247	0,9552 ± 0,0143	0,9089 ± 0,0042
2	0,9903 ± 0,0248	0,9890 ± 0,0139	0,9749 ± 0,0044
3	0,9950 ± 0,0247	0,9943 ± 0,0142	0,9865 ± 0,0040
4	0,9935 ± 0,0233	0,9948 ± 0,0134	0,9911 ± 0,0038
5	0,9975 ± 0,0240	0,9968 ± 0,0136	0,9937 ± 0,0037
6	0,9977 ± 0,0242	0,9976 ± 0,0136	0,9951 ± 0,0035
7	0,9994 ± 0,0242	0,9987 ± 0,0134	0,9966 ± 0,0035
8	0,9986 ± 0,0244	0,9985 ± 0,0138	0,9975 ± 0,0035
9	0,9994 ± 0,0246	0,9995 ± 0,0133	0,9980 ± 0,0034

Block	Pos	Line	Analysis	Inj	W-ID	IG	Sample Name	memCorr $\delta^{17}O$	memCorr $\delta^{18}O$	memCorr $\delta^{2}H$
1	**1**	**1**	**31758**	**1**	**W-3**	☑	**DIW Wash**	**±**	**±**	**±**
1	1	2	31758	2	W-3	☐	DIW Wash	-3,469 ± 0,193	-8,975 ± 0,183	-79,06 ± 0,34
1	1	3	31758	3	W-3	☐	DIW Wash	-3,358 ± 0,195	-8,788 ± 0,179	-77,17 ± 0,33
1	1	4	31758	4	W-3	☐	DIW Wash	-3,321 ± 0,172	-8,756 ± 0,186	-76,75 ± 0,33
1	1	5	31758	5	W-3	☐	DIW Wash	-3,368 ± 0,181	-8,807 ± 0,185	-76,57 ± 0,33
1	1	6	31758	6	W-3	☐	DIW Wash	-3,343 ± 0,173	-8,766 ± 0,186	-76,45 ± 0,35
1	1	7	31758	7	W-3	☐	DIW Wash	-3,381 ± 0,191	-8,801 ± 0,198	-76,35 ± 0,37
1	1	8	31758	8	W-3	☐	DIW Wash	-3,343 ± 0,186	-8,755 ± 0,175	-76,39 ± 0,30
1	1	9	31758	9	W-3	☐	DIW Wash	-3,327 ± 0,189	-8,766 ± 0,204	-76,34 ± 0,34
1	1	10	31758	10	W-3	☐	DIW Wash	-3,345 ± 0,201	-8,771 ± 0,195	-76,26 ± 0,36
1	1	11	31758	11	W-3	☐	DIW Wash	-3,359 ± 0,185	-8,773 ± 0,175	-76,22 ± 0,37
1	1	12	31758	12	W-3	☐	DIW Wash	-3,323 ± 0,197	-8,763 ± 0,199	-76,27 ± 0,33
1	1	13	31758	13	W-3	☐	DIW Wash	-3,345 ± 0,189	-8,760 ± 0,189	-76,13 ± 0,34
1	**2**	**14**	**31759**	**1**	**W-52**	☑	**W-52 USGS48**	**0,937 ± 0,176**	**-0,762 ± 0,172**	**-1,88 ± 0,39**
1	2	15	31759	2	W-52	☐	W-52 USGS48	0,975 ± 0,194	-0,703 ± 0,190	-1,29 ± 0,36

Record: 1 of 307 No Filter Search

Figure 10: Memory coefficient determination and run computation.

- For each block, the memory coefficients from high to low standard are calculated.
- Click “compute memory” to apply the memory coefficients and proceed to the drift correction menu.

5.4. Step 4: Drift correction

While both van Geldern and Barth (2011) and Pierchala et al. (2019) recommend a drift correction based on interspersed samples of deionized water, this step was skipped in the processing after ca. 3 months. Nevertheless, the LIMS-Addon program can do a drift correction on high and low standards, on dedicated drift samples or no correction.

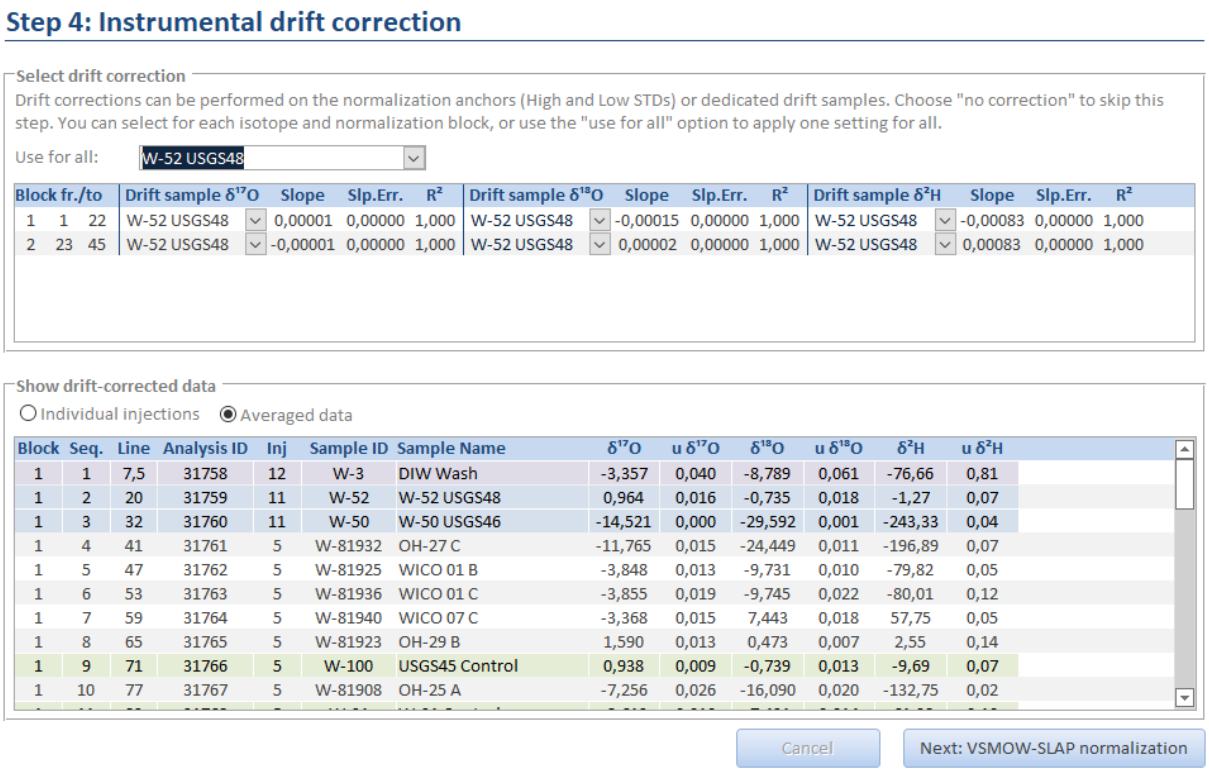
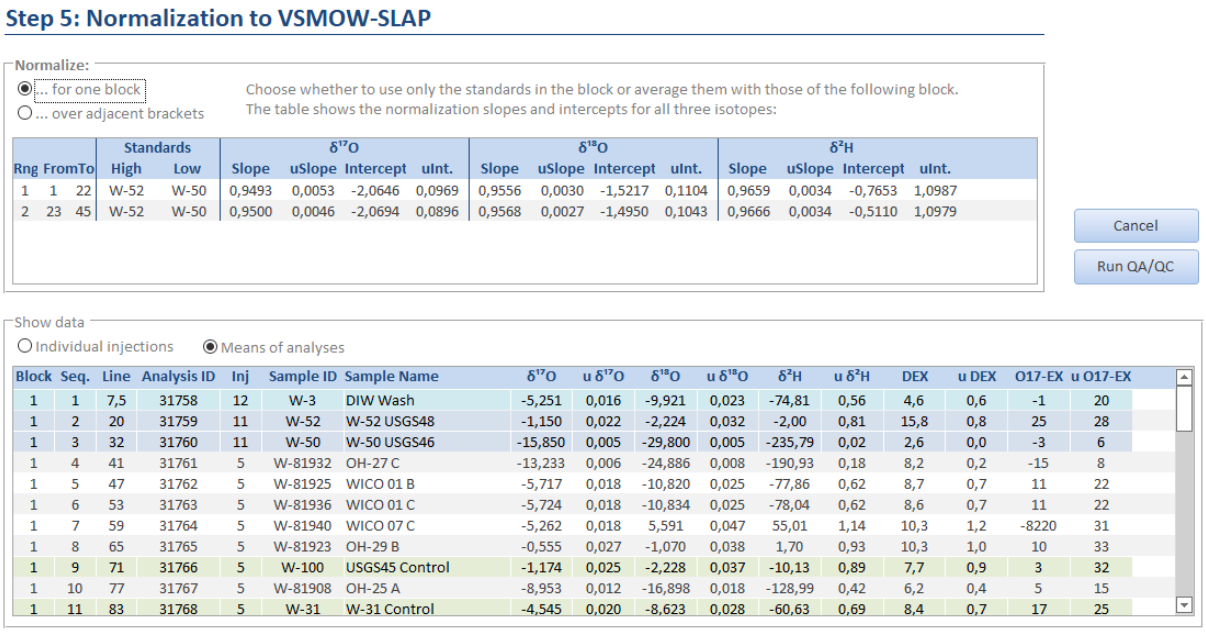


Figure 11: Drift correction

- By using the combo box on top, one drift sample can be specified for the whole run and all isotopes.
- For finer details, any subrange's drift can be set individually.
- If there is no closing bracket (or adjacent opening bracket), no drift can be calculated for a subrange.
- LIMS-Addon displays "function samples" in different colours: standards in blue, controls in green, drift samples in purple.
- After making all settings, click "VSMOW-SLAP normalization" to complete the last step of the post-processing.

5.5. Step 5: Normalization to the VSMOW-SLAP range



Rng	From	To	Standards High	Standards Low	δ17O Slope	δ17O uSlope	δ17O Intercept	δ17O uInt.	δ18O Slope	δ18O uSlope	δ18O Intercept	δ18O uInt.	δ2H Slope	δ2H uSlope	δ2H Intercept	δ2H uInt.
1	1	22	W-52	W-50	0,9493	0,0053	-2,0646	0,0969	0,9556	0,0030	-1,5217	0,1104	0,9659	0,0034	-0,7653	1,0987
2	23	45	W-52	W-50	0,9500	0,0046	-2,0694	0,0896	0,9568	0,0027	-1,4950	0,1043	0,9666	0,0034	-0,5110	1,0979

Block	Seq.	Line	Analysis ID	Inj	Sample ID	Sample Name	δ17O	u δ17O	δ18O	u δ18O	δ2H	u δ2H	DEX	u DEX	O17-EX	u O17-EX
1	1	7,5	31758	12	W-3	DIW Wash	-5,251	0,016	-9,921	0,023	-74,81	0,56	4,6	0,6	-1	20
1	2	20	31759	11	W-52	W-52 USGS48	-1,150	0,022	-2,224	0,032	-2,00	0,81	15,8	0,8	25	28
1	3	32	31760	11	W-50	W-50 USGS46	-15,850	0,005	-29,800	0,005	-235,79	0,02	2,6	0,0	-3	6
1	4	41	31761	5	W-81932	OH-27 C	-13,233	0,006	-24,886	0,008	-190,93	0,18	8,2	0,2	-15	8
1	5	47	31762	5	W-81925	WICO 01 B	-5,717	0,018	-10,820	0,025	-77,86	0,62	8,7	0,7	11	22
1	6	53	31763	5	W-81936	WICO 01 C	-5,724	0,018	-10,834	0,025	-78,04	0,62	8,6	0,7	11	22
1	7	59	31764	5	W-81940	WICO 07 C	-5,262	0,018	5,591	0,047	55,01	1,14	10,3	1,2	-8220	31
1	8	65	31765	5	W-81923	OH-29 B	-0,555	0,027	-1,070	0,038	1,70	0,93	10,3	1,0	10	33
1	9	71	31766	5	W-100	USGS45 Control	-1,174	0,025	-2,228	0,037	-10,13	0,89	7,7	0,9	3	32
1	10	77	31767	5	W-81908	OH-25 A	-8,953	0,012	-16,898	0,018	-128,99	0,42	6,2	0,4	5	15
1	11	83	31768	5	W-31	W-31 Control	-4,545	0,020	-8,623	0,028	-60,63	0,69	8,4	0,7	17	25

Figure 12: VSMOW-SLAP normalization

- The user has the option to normalize based on the starting bracket only (default) or using opening and closing bracket (mathematically a mean of the two).
- LIMS-Addon displays the normalization slopes and intercepts for each subrange in the top window.
- In the bottom window, the normalized data are displayed (with the same colour coding as shown before).
- By clicking “Run QA/QC” the user moves to the final step of the run evaluation.

5.6. Step 6: Run QA/QC

In this window, the user can evaluate subranges based on the controls included. LIMS-Addon uses z- and ζ-scores against the controls' known values to validate the goodness of the run. For $\delta^{17}O$ and $\Delta'^{17}O$ analysis, the thresholds specified in the service (cf. chapter 3.1) were used.

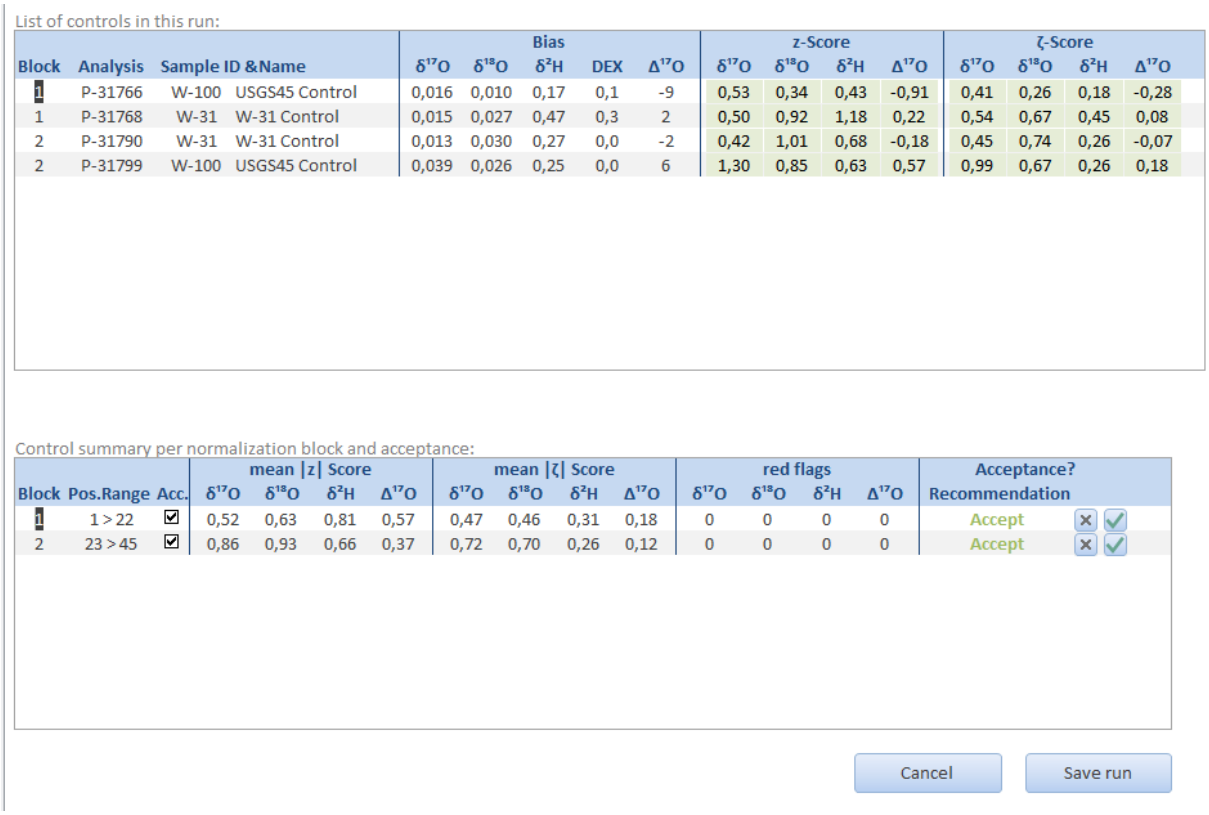
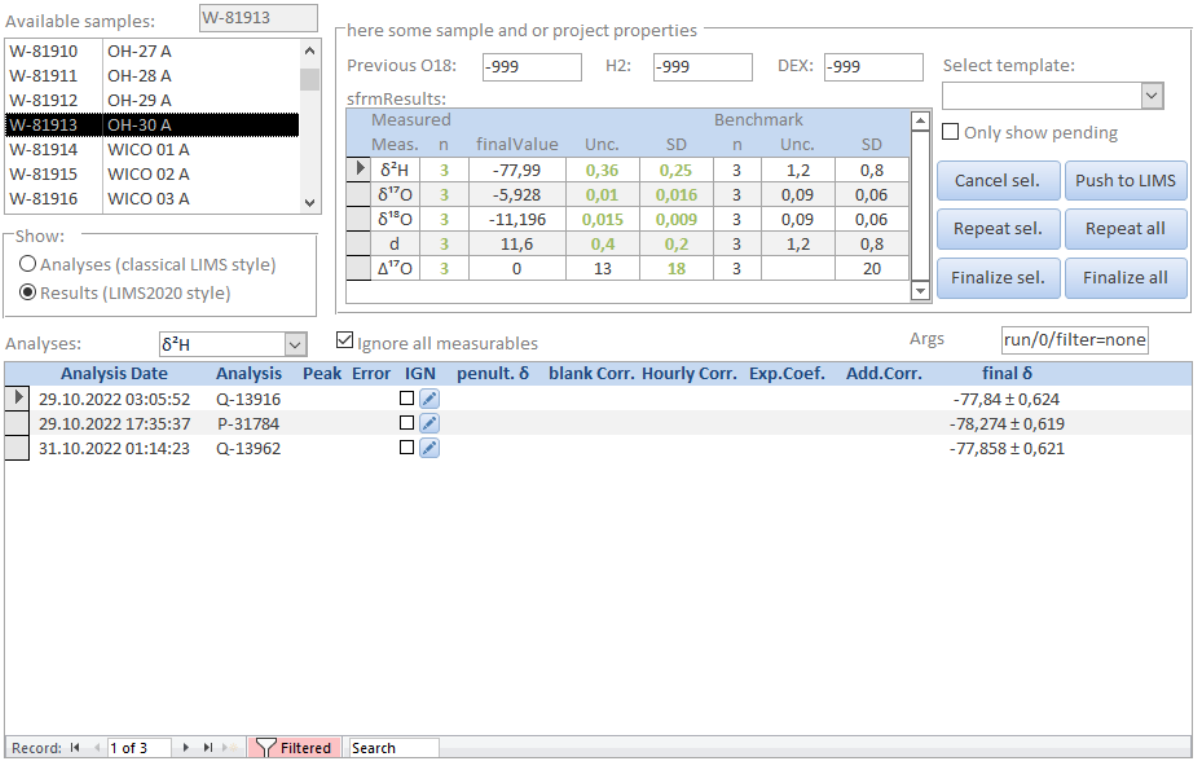


Figure 13: QA/QC check

- All acceptable values are in green, questionable in yellow and unacceptable controls are in orange.
- Based on the z and ζ-scores, LIMS-Addon determines a recommendation to accept or reject a subrange and its samples. If no control is included, a subrange is rejected by default.
- The user can manually override acceptance (e.g., if it becomes clear that the control vial itself has been compromised); however, there is also the option to un-ignore individual analyses in step 7. This is however a practice to be used with extreme caution!

5.7. Step 7/8: Evaluate the results

LIMS-Addon allows evaluation of all samples of a run, but also the samples' project.



Measured						Benchmark		
	Meas.	n	finalValue	Unc.	SD	n	Unc.	SD
$\delta^{2}H$	3	-77,99	0,36	0,25	3	1,2	0,8
$\delta^{17}O$	3	-5,928	0,01	0,016	3	0,09	0,06
$\delta^{18}O$	3	-11,196	0,015	0,009	3	0,09	0,06
d	3	11,6	0,4	0,2	3	1,2	0,8
$\Delta^{17}O$	3	0	13	18	3		20

Analysis Date	Analysis	Peak	Error	IGN	penult. δ	blank Corr.	Hourly Corr.	Exp.Coef.	Add.Corr.	final δ
29.10.2022 03:05:52	Q-13916			☐						-77,84 ± 0,624
29.10.2022 17:35:37	P-31784			☐						-78,274 ± 0,619
31.10.2022 01:14:23	Q-13962			☐						-77,858 ± 0,621

Figure 14: Analysis results evaluation per sample

- In the top left list box, the user selects a sample of the run or project.
- The centre top window shoes the current and previous (from LIMS) measurements.
    - Results are shown against the benchmark previously specified for an "analytical service" and are red when violating the benchmark and green if passing.
    - For example, the sample shown meets the $\delta^{17}O$ criteria for propagated uncertainty (0.082 < 0.09), repeatability (SD 0.021 < 0.06) but fails the number of repetitions (2 done < 3 needed). Had the requirements been to repeat only twice, it would have passed.
- In the bottom window, the analyses are displayed. The user can ignore analyses manually – it is best to keep the "ignore all measurables" option checked, so that ignoring one measured parameter ignores all.
- In the upper right area, actions can be set:
    - A Picarro template for repeats can be selected.
    - The samples displayed can be constrained to those pending.
    - The user can cancel a sample (e.g., if the repeated run is far off the initial value)
    - The user can book the selected sample (or all in the samples' list) onto a template to repeat it.
    - The user can set all samples matching the benchmark criteria to "finalized", or only one (setting one individual sample also allows to override if the criteria are not met).

6. Export functions

6.1. Export results

The export menu allows the user to export data by LIMS project, or by tag across projects, to a Microsoft® Excel spreadsheet. You can access it from the main screen by clicking the “export” button. The LIMS-Addon software will prompt you for a filename /-location.

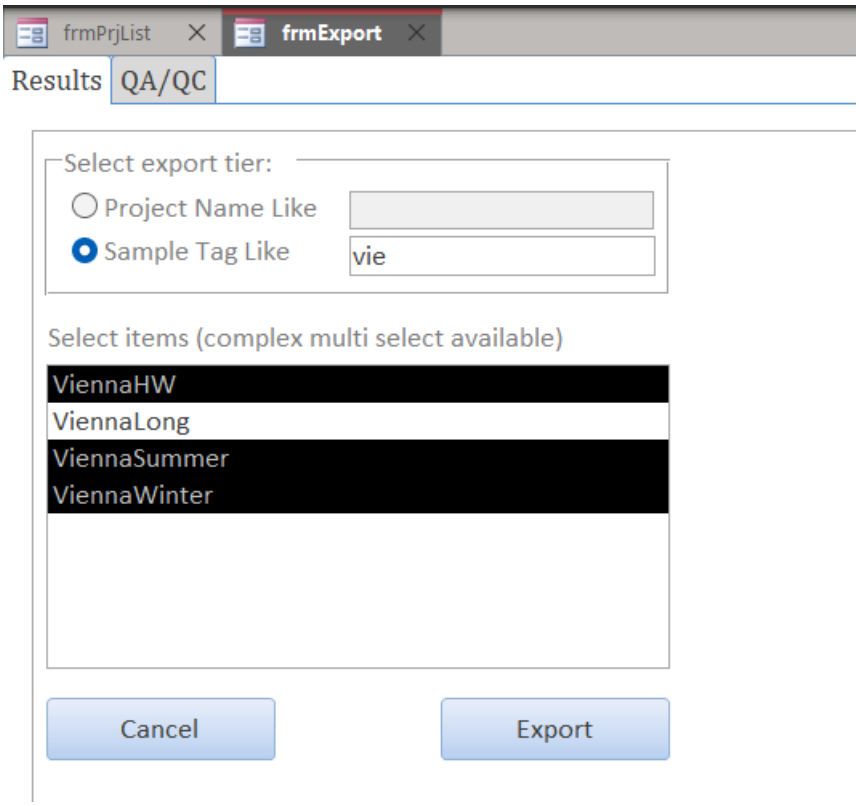


Figure 15: Available tags filtered for those containing “Vie”, then using Ctrl+Shift+Click to select all samples with the tags “ViennaHW”, “ViennaSummer”, “ViennaWinter” for export.

6.2. Export QA/QC

The QA/QC menu exports the quality control record (separated by control sample ID, analysis date, and instrument) to a Microsoft® Excel spreadsheet. The LIMS-Addon software will prompt you for a filename /-location (Figure 16).

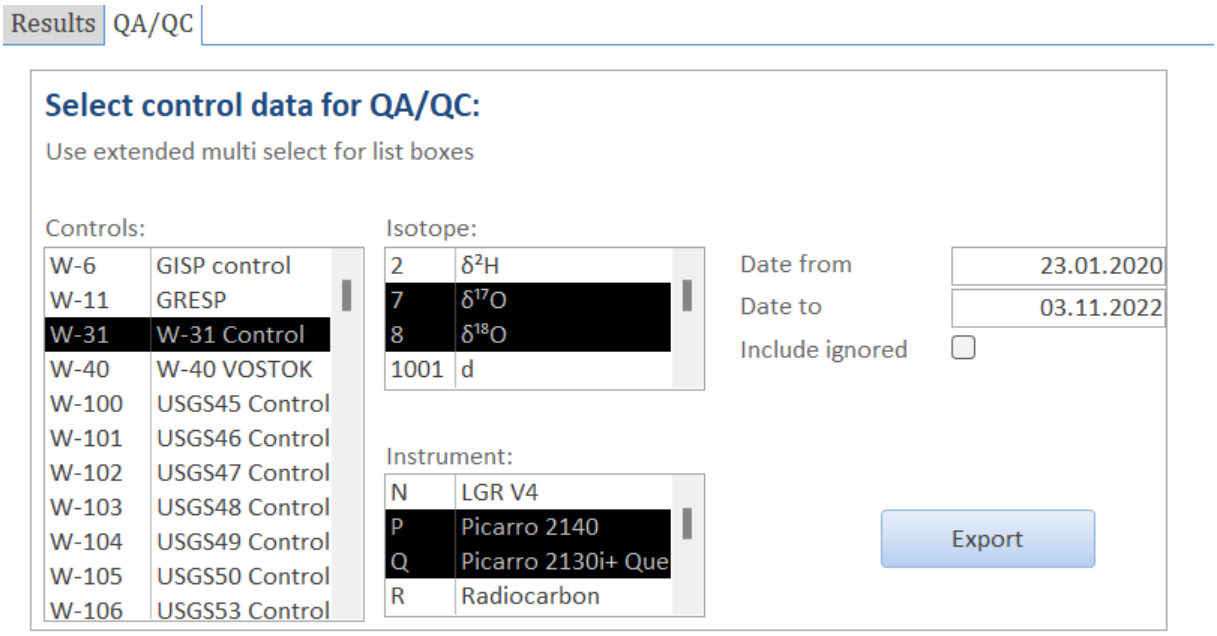


Figure 16: Export facility for QA/QC data. Example exports all available results for W-31 $\delta^{17}O$ and $\delta^{18}O$ results measured on Picarro analyzers P and Q.

7. Additional figures

7.1. Tray design chart

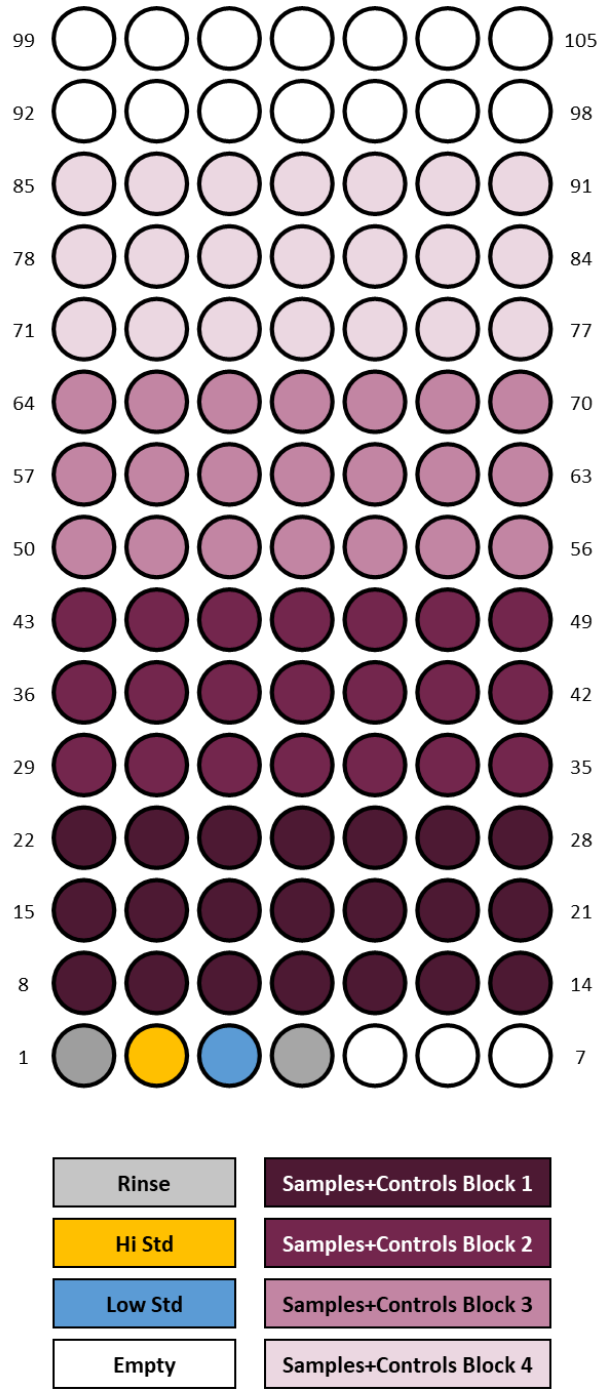


Figure 17: Liquid autosampler tray layout sequence for multi-day triple-isotope runs. The rinse sample at position 4 is optional.

7.2. STD06 QA/QC charts

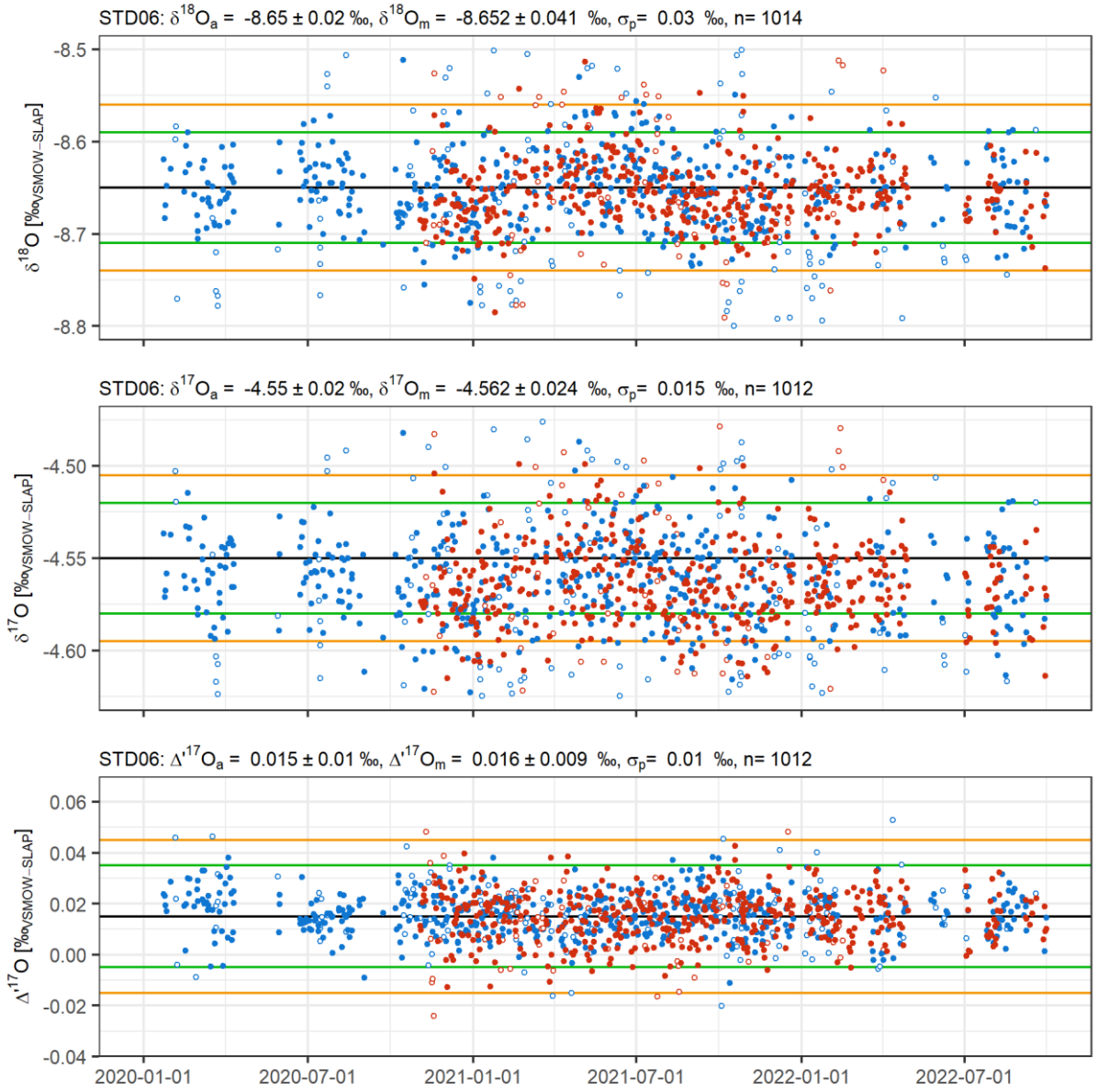


Figure 18: Protocol QA/QC chart of the control standard STD06. δ_a is assigned value; δ_m measured value; σ_p accuracy benchmark (2 σ_p and 3 σ_p denoted by green and orange lines)