Technical note: A fast and objective autosampler for direct vapor equilibration isotope measurements

Jonas Pyschik[1], Stefan Seeger[2,1], Barbara Herbstritt[1], and Markus Weiler[1]

[1]Hydrology, Faculty of Environment and Natural Resources, University of Freiburg, Freiburg im Breisgau, Germany
[2]Division of Soil Physics, Department of Crop Sciences, Georg-August University of Göttingen

Correspondence: Jonas Pyschik (jonas.pyschik@hydrology.uni-freiburg.de)

Abstract.

To investigate water movement in environmental systems, stable water isotopes (^{2}H and ^{18}O) are commonly used tracers. Analyzing the isotopic composition of water bonded to substances like soil or plant tissue necessitates its extraction prior to analysis. One such method, direct vapor equilibration, is popular due to its cost-effectiveness and straightforward sample

5 processing. However, sample analysis requires a significant manual labor, thereby limiting the number of samples that can be analyzed. This limitation is compounded by the fact that stored samples undergo isotopic composition changes over time, and in addition manual measurements may lack objectivity. To address these challenges, we have developed a device that automates the analysis process. Our vapor autosampler for vapour samples, named VapAuSa, features a modular design with up to 350 ports for direct vapor equilibration samples. These ports sequentially connect the prepared samples to the analyzer,

10 enabling continuous automatic measurements. Within the accompanying software, measurement criteria can be specified, facilitating objective analysis. VapAuSa measurements have a similar uncertainty compared to manual analysis of identical samples (VapAuSa δ^2H= +/- 4.5 ‰ δ^{18}O = +/- 0.58 ‰ vs. manual δ^2H= +/- 5.7 ‰ δ^{18}O = +/- 0.37 ‰). However, the increased sample throughput minimizes storage-induced isotopic changes. Moreover, VapAuSa triples sample throughput per week while reducing direct labor time to just 10 % of manual processing.

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1 Introduction

Stable water isotopes (δ^{18}O and δ^2H) have found widespread application as tracers in earth and environmental system sciences. They are applied in many studies to elucidate storage and redistribution processes of water in various compartments. In soils, stable water isotope analysis revealed different flow-process along hillslopes like lateral- and preferential-flow as well as mixing

20 (Garvelmann et al., 2012; Thomas et al., 2013; Peralta-Tapia et al., 2015). They were also used to obtain soil properties by inversely modeling soil-water isotope profiles, better representing the soil than traditional pedotransfer functions (Sprenger et al., 2016). In addition, the tracking of vertical infiltration using soil isotope profiles has allowed for the quantification

of groundwater recharge rates (Filippini et al., 2015; Chesnaux and Stumpp, 2018; Boumaiza et al., 2020). Analyzing the
distribution of stable water isotopes in groundwater has revealed hydrogeological differences and solute transport mechanisms
(Hendry and Wassenaar, 2009; Hendry et al., 2011a; Hendry and Wassenaar, 2011; Stumpp and Hendry, 2012). Moreover,
the use of stable water isotopes in plants has shed light on plant water uptake, water transit times, and partitioning effects,
providing valuable insights into which sources of water plants utilize for various purposes (Bertrand et al., 2014; Smith et al.,
2020; Kuhlemann et al., 2020).

While most isotope analyzers are build to measure the isotopic composition of liquid water, extracting water bound to
substances or tissues, such as soil, sediment or plant material, is still not directly possible. The most popular extraction methods
are direct vapor equilibration (DVE-LS) (Wassenaar et al., 2008) and cryogenic vacuum distillation (CVD). For CVD the
sample is heated inside a vial so all water evaporates. Next, another vial, which is cooled and contains a vacuum, is connected
to the first. This causes the vapor to transfer to the cooled vial and condense (Ingraham and Shadel, 1992). The condensate can
then be analysed.

By contrast, in DVE-LS, the water vapour of a samples is measured and recalculated to its liquid composition using calibra-
tion standards. The process works as follows: (1) The sample (soil, plant, etc.) is placed in an inflatable, sealable and diffusion
tight bag. Many different bag-materials are used, however most are insufficient due to diffusive losses. These losses can be
minimized with aluminum laminated bags (Gralher et al., 2021). (2) Regarding identical treatment of calibration standards
(Werner and Brand, 2001), liquid water of known isotopic composition is also filled into identical bags. (3) The sample and
calibration standard bags are inflated with dry air and heat sealed. Then, a silicone blot is added to each bag as a septum, needed
for airtight measurements later. (4) Samples are then stored under constant climatic conditions (climatized room where they are
also analyzed) so liquid and gas phase within the bags can isotopically equilibrate. Equilibration time varies between studies
but for aluminum laminated bags, 48 hours are optimal for soil samples (Gralher et al., 2021). (5) After equilibration, the
vapor in the bag's headspace is analyzed using off-axis integrated cavity output spectroscopy (OA-ICOS) or cavity ring-down
spectroscopy (CRDS). To facilitate this analysis, a cannula connected to the analyzer's inlet port is inserted into the septum.
(6) After analysis, the measurements are referenced to the VSMOW-SLAP scale using the co-measured calibration standards
(Craig, 1961).

Advantages of DVE-LS compared to other extraction methods include low technical effort and minimal material require-
ments, making it a cost-effective option (Millar et al., 2018). Additionally, only a small sample volumesimilar to that of CVD
is necessary, allowing for high spatial and temporal resolution sampling (Wassenaar et al., 2008; Garvelmann et al., 2012).
Furthermore, minimal handling is required, reducing the risk of sample damage (Wassenaar et al., 2008). Due to these favor-
able attributes, DVE-LS finds application in various contexts. For instance, all the findings presented in the first paragraph
were obtained through the application of DVE-LS. The popularity of DVE-LS led to numerous methodological improvements,
most focused around container material, equilibration time (Gralher et al., 2021) and the correction of changes in carrier gases
(Gralher et al., 2016, 2018) or co-extracted substances which interfere with the laser spectrometer analysis (Hendry et al.,
2011b; Nehemy et al., 2019).

However, the method still lacks automation in the analysis process. Manual measurement of each sample is time-consuming, imposing limitations on large-scale sampling, as sample storage can alter the isotopic signature (Gralher et al., 2021). Moreover, the current DVE-LS analysis lacks objectivity. There is no standardization for analysis time and measurement stability criteria,
60 leading to potential variations in results among different labs and operators analyzing the same sample. To address these challenges and enhance both the speed and objectivity of the analysis, we introduce our fully autonomous vapor sampler, named "VapAuSa". This innovative system enables high-throughput sample processing with significantly reduced manual labor compared to the prevailing procedure.

2 Design

65 This section provides a summary of the sampler design. More in-depth information such as the list of materials, technical drawings, circuit diagram, code, and manuals, necessary for building the VapAuSa, can be found in the Supplement and on https://gitlab.rz.uni-freiburg.de/hydrology/vapausa (last access: 11 July 2023).

2.1 Hardware

The VapAuSa features a modular design comprising standalone boxes that can also be combined to larger sample units. Each
70 box is equipped with 24 ports, of which 23 are designated for sample bags, and 1 is reserved for flushing the system with ambient air to prevent moisture buildup. The ports consist of valves (Model E3O10A-1W024; Clippard) which regulate vapor flow to the cavity of the ring-down isotope analyzer (in our case a L2130-*i*, Picarro Inc.). The valves are attached to CNC-cut aluminum valve-blocks organized in groups of eight and interconnected by 1/8 inch PTFE tubing (Figure 1). For 23 sample bags are connected with cannulas to the valves through 1/8 inch PTFE tubing. The tubes are secured by super glue into the
75 cannula and linked to the valve block via flangeless fittings (XP-301X; IDEX) (see Figure 1). We experimented with using 1/16 inch tubes and a gas-drying cartridge, but this did not yield improved measurements.

2.2 Circuit board, electronic components and Firmware

The first box of each VapAuSa is controlled by the primary module, while all subsequent boxes are controlled by extension modules. We have designed a circuit board that, depending on the equipped electronic components, can be used to build either
80 of these two. The primary and extension modules share most parts but are equipped with different types of micro-controllers and connectors. This distinction arises because the primary module is the only one directly connected to the power supply and to the Picarro. Each extension module is equipped with a DIP-switch that can be used to assign a unique address between 1 and 16 (the primary module is assigned address 0). In total, this allows for up to 17 boxes in one VapAuSa, capable of accommodating 391 samples simultaneously. The primary module and all extension modules can be daisy-chained one after
85 another, with communication between them facilitated through a RS485-bus system. Communication and power supply for all extensions are achieved using off-the-shelf Ethernet cables.
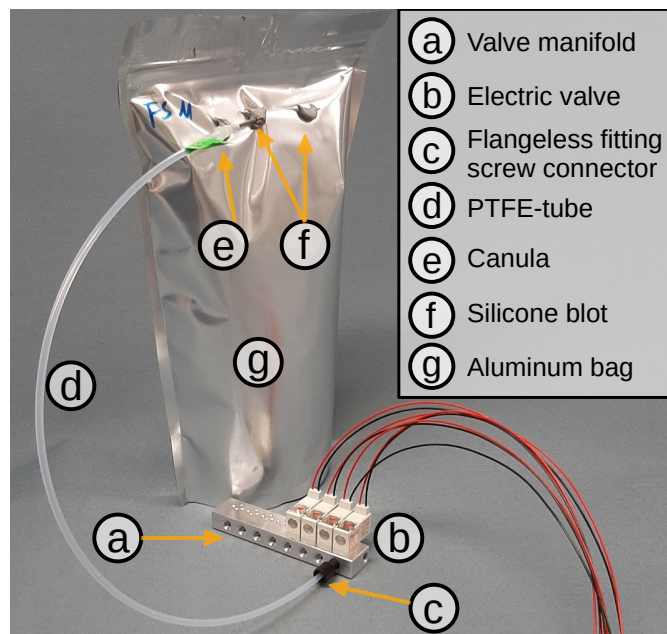
Figure 1. Basic setup of the VapAuSa: A cannula ⓔ is superglued onto a PTFE-tube ⓓ, which is connected to a CNC-cut valve manifold ⓐ via a flangeless fitting connector (XP-301X; IDEX) ⓒ. In this example image, only four of the eight positions on the valve manifold are equipped with electric valves (E3O10A-1W024; Clippard) ⓑ. The cannula is inserted to the laminated aluminum bag ⓖ via a silicone blot ⓕ, which acts as a seal after the bag was been pierced by the cannula.

A detailed part list for the required electronic components to equip the circuit boards can be found in the appendix of this paper. Circuit boards schematics (created with Fritzing) and the firmware for the micro-controllers of primary board and extensions can be found under https://gitlab.rz.uni-freiburg.de/hydrology/vapausa.

2.3 Software

The software used for the VapAuSa is based on software that was developed for in-situ stable water isotope sampling (Seeger & Weiler, 2021). It was designed to operate directly on a Piccaro stable water isotope analyser (model L2120-i, L2130-i or L2140-i) and consists of a collection of modular Python3 (Python-Software-Foundation, 2022) scripts whose purpose is to fulfil two main tasks:

a) Monitoring the measurements: By frequently reading out and parsing the instrument's log-files, the software is aware of the currently measured values of sample gas volumetric vapor content (H_2O), $\delta^{18}O$ and δ^2H (see ④ in Fig. 4). Through a configuration file, the user can define stability criteria, specifying trends and standard deviations of the mentioned measurement parameters over a user-defined time span (⑤ in Fig. 4). This establishes an objective metric for the automated detection of stable plateaus during measurements. This module can operate independently and is potentially suitable for manual DVE-LS
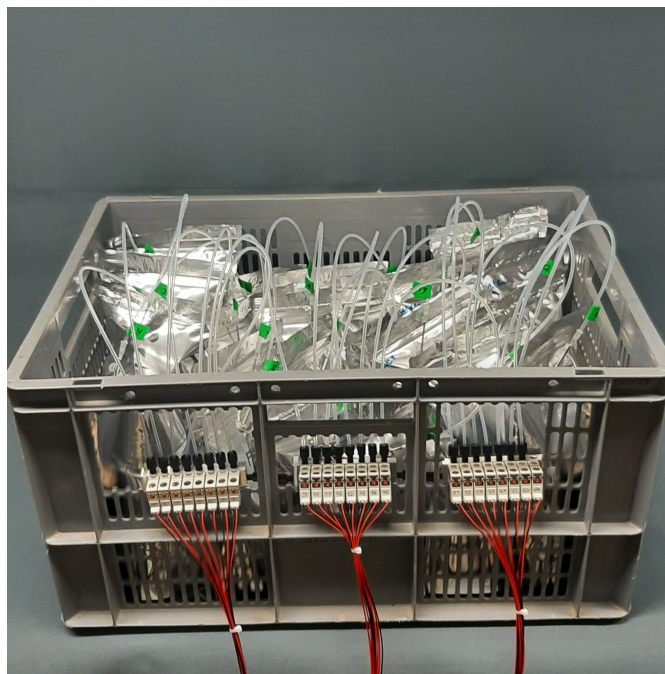
Figure 2. VapAuSa with 24 valves and 23 bag ports

100 measurements, since it provides instant one-click summary statistics for freely-selectable time spans. In contrast, the standard Picarro analyzer GUI often requires tedious zooming in and out to achieve the same purpose.

b) Controlling the valves: Using another configuration file, the user can assign custom names (sample IDs) to specific valve slots. Each valve slot is uniquely adressed by a combination of boxID and slotID (e.g., `2#5` addresses the fifth valve slot of the second extension box). The user has the flexibility to set a maximum sampling time for each valve and determine whether

105 sampling may conclude before that time if all stability criteria are met. Additionally, the user can establish a custom sequence for all defined valve slots, enabling repeated measurements from specific slots (e.g., standards) within a single sequence. Within an active sequence, each measurement phase is preceded by a flush phase during which the valve block of the currently active slot is flushed with ambient air until a customizable H_2O value is undershot or a maximum flush time has been reached. Upon running the main script, a graphical user interface (GUI) is generated, visualizing recent measurements (④ in Fig. 4). This

110 GUI allows the user to open certain valves by clicking on the corresponding buttons (① in Fig. 4). The GUI automatically starts the predefined sequence (③ in Fig. 4) and generates a log file that documents each valve switch and the reason why it occurred (timeout, fulfillment of all stability criteria, or manual click). By combining the analyzer log file with the valve log file, it is possible to automatically aggregate and process the relevant parts of the analyzer's measurements. The software identifies a connected VapAuSa primary module by scanning all available Serial-COM ports and then sends the appropriate

115 `valve boxID#slotID` commands to the primary module (firmware described in Sec. 2.2) in response to user inputs or progression through the predefined sequence.
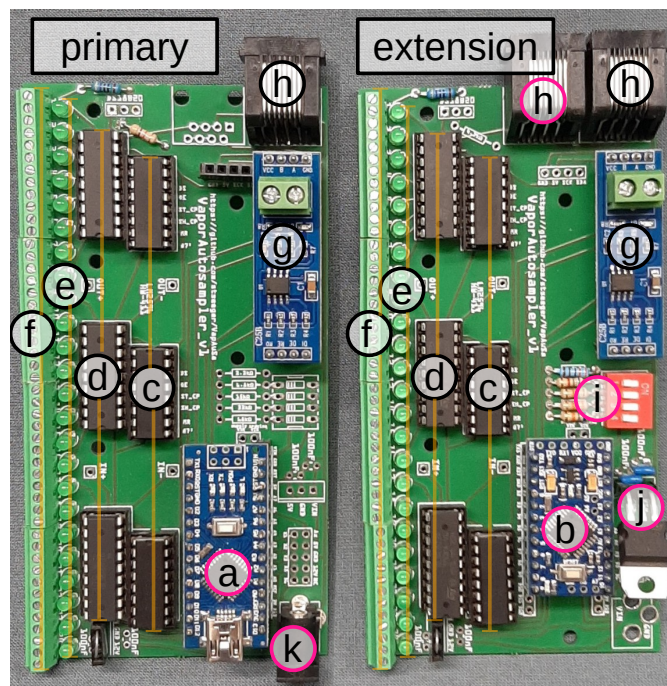
Figure 3. VapAuSa primary module (left) and an extension module (right). Based on identical circuit boards, differences in equipped components (pink circles) lead to the two kinds of modules used in the VapAuSa sytsem. ⓐ Arduino Nano, ⓑ Arduino ProMini, ⓒ Shift-Register, ⓓ ULN2803 TransisorArray, ⓔ Status LEDs, ⓕ Screw terminals, ⓖ RS485-Max communication module, ⓗ Ethernet port, ⓘ Address selection DIP-Switch, ⓙ 5V voltage regulator, ⓚ 12VDC power jack.

An example call of the `valve` command might be `valve 2#5`, which means that the fifth valve of the second extension box should be opened. The receiving primary module would relay this command to all connected extensions. Subsequently, all boxes that are not the second extension would close all of their valves and the second extension would open its fifth valve.

3 Proof of Concept

3.1 Test Setup

We analyzed the accuracy of the VapAuSa by measuring different DVE-LS samples. The test samples were created according to the suggested protocol by Wassenaar et al. (2008) and Gralher et al. (2021). Seven samples (10 ml each) of three isotopically distinct water sources were filled into 1 L aluminum laminated plastic bags. Subsequently, we inflated the bags with dry air, heat-sealed and equipped them with silicone septa. Then the bags were placed in a climate controlled room (20°C +/- 1°C) for 48 hours. Following this period, we randomly distributed the samples in the VapAuSa. To connect the bags to the isotope analyzer (cavity ring-down; L2130i, Picarro Inc.), we inserted the cannulas into the septa, puncturing the bags and ensuring
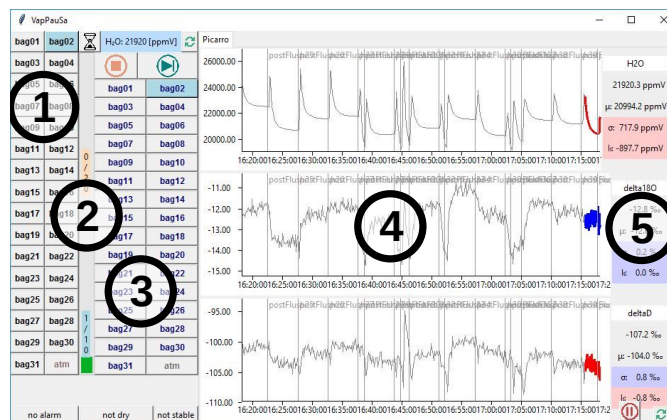
Figure 4. Screenshot of the VapAuSa GUI. ① Buttons for all valves defined in the configuration file, ② Flush and measurement progress bars for currently active valve, ③ Buttons for valve sequence, ④ Recent measurement values for H_2O, $\delta^{18}O$ and δ^2H, ⑤ Fulfilment of stability criteria (red=not fulfilled, blue=fulfilled) for H_2O, $\delta^{18}O$ and δ^2H

Table 1. Stability criteria for the VapAuSa measurements. Only if standard deviation (sd) an trend are all below the given thresholds within evaluation time (eval. time), VapAuSa switches to the next sample.

	sd	trend	eval. time [s]
H_2O	100.0	150.00	120
$\delta^{18}O$	0.2	0.08	120
δ^2H	0.7	0.30	120

airtight vapor flow. The VapAuSa was programmed to activate valves for 10 minutes each, with 5 minutes of system flushing between samples. Stability criteria were defined as shown in table 1.

130   To test a wide isotopic range we selected water samples of 3 different signatures: heavy (sea-water, $\delta^{18}O = 0.7$ ‰ $\delta^2H = 0.3$ ‰), medium (tap-water of Freiburg, $\delta^{18}O = -9.3$ ‰ $\delta^2H = -64.3$ ‰) and light (snow-melt water, $\delta^{18}O = -16.6$ ‰ $\delta^2H = -125.8$ ‰). These compositions were gained by measuring sub-samples on a liquid isotope analyser (L2130-i with attached vaporizer unit A0211, Picarro Inc.) and are considered the samples "true" value throughout the analysis. After VapAuSa analysis, we calibrated the results to these liquid measurements.

135   We determined the measurement uncertainty as one standard deviation (1 σ). To quantify the measurement accuracy associated with the VapAuSa, the results ($\delta^{18}O_a$) were subtracted by the respective liquid analyzer results ($\delta^{18}O_l$):

$$Accuracy\ \delta^{18}O = \delta^{18}O_a - \delta^{18}O_l \tag{1}$$

We assessed the VapAuSa performance by comparing the measurement variability with 230 manual measurements from identical water sources. To calculate the accuracy, we again subtracted the respective liquid measurements from the manual
140   data.

3.2   Results

The VapAuSa measurements are subject to some uncertainty, however, 60% of the measurements stabilized before the switch time threshold. While we programmed the VapAuSa to analyze each sample for 10 minutes, most measurements met the stability criteria (Table 1) within 8 minutes. This reduction in overall runtime from 5 to 4 hours was observed across 21 sample
145   measurements. In comparison to the results from the liquid analyzer, the autosampler exhibits a 1 σ uncertainty of +/- 0.58 ‰ for δ^{18}O and +/- 4.5 ‰ for δ^2H. Across the different water-sources, VapAuSa showed the highest uncertainties for light water with +/- 0.82 ‰ for δ^{18}O and +/- 7.0 ‰ for δ^2H. It best performed in the isotopic range of the medium and high signatures with similar deviations of around +/- 0.47 ‰ for δ^{18}O and +/- 3.2 ‰ for δ^2H (Figure 5).
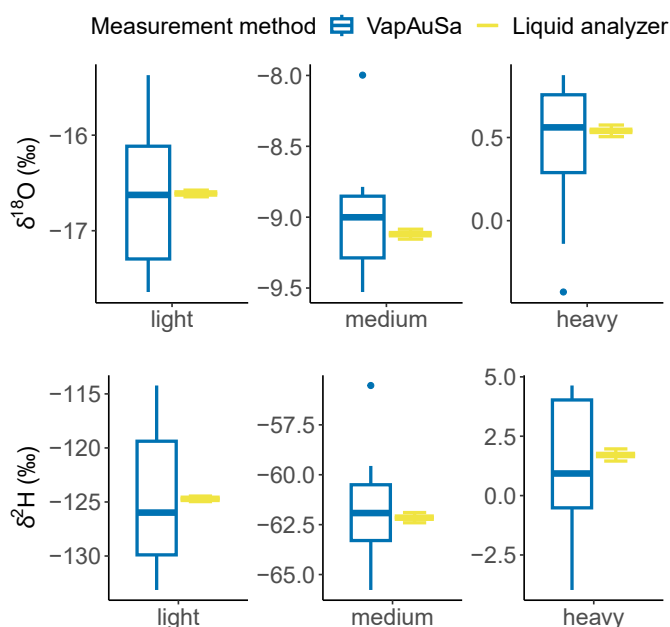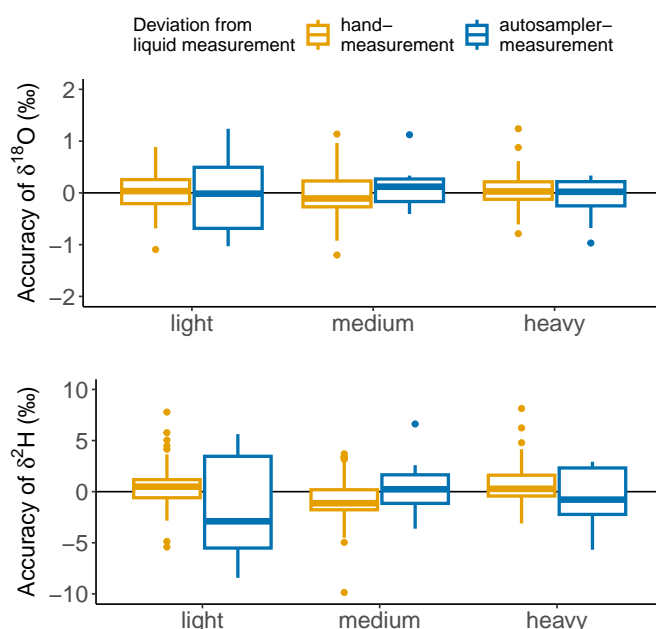

Figure 5. Measurements from 3 water samples of different isotopic composition, analyzed using the VapAuSa and the liquid mode of a Picarro L2130i. Liquid analyzer accuracy: +/- 0.035‰ for δ^{18}O and +/- 0.256 ‰ for δ^2H

Manual analysis had a measurement uncertainty (1 σ) of +/- 0.37 ‰ for δ^{18}O and +/- 5.7 ‰ for δ^2H, being in a similar range
150   as those of the VapAuSa (Results are shown in Figure 6). The high uncertainty of the VapAuSa in the isotopically light range is also visible in manual measurements. While the manual uncertainty is 50 % lower in δ^{18}O than that of the VapAuSa, it is

130 % higher for δ^2H. Therefore this error probably does not stem from the VapAuSa but rather from the DVE-LS extraction method in general.

Figure 6. Deviation of VapAuSa and manual measurements from liquid measurements of 3 water samples of different isotopic composition

4 Discussion

4.1 Accuracy

Sprenger et al. (2015) pointed out in their review that accuracies for different extraction methods vary strongly. Even when comparing the same extraction method among different studies, vastly different accuracy values are named. This comes for two reasons: the chosen extraction medium and the selected accuracy metric.

Oven dried soils spiked with water of known isotopic signatures are often used to examine extraction performance (West et al., 2006; Wassenaar et al., 2008; Volkmann and Weiler, 2014). Accuracies for this method vary between 0.7-1.03 ‰ for δ^2H and 0.15-0.3 ‰ for δ^{18}O for DVE-LS (Wassenaar et al., 2008; Volkmann and Weiler, 2014) and 0.69-3 ‰ for δ^2H and 0.15-0.4 ‰ for δ^{18}O for CVD (West et al., 2006; Koeniger et al., 2011). However, the soil texture can alter the extracted signatures. While isotopic signatures of extracted water from sandy soils is often close to the known composition, a high clay content changes the signatures (Koeniger et al., 2011; Orlowski et al., 2013, 2016; Gaj et al., 2016). This clay-induced error (10 +/-2 ‰ for δ^2H and 2 +/-1 ‰ for δ^{18}O applying CVD) is also extraction-method specific. While DVE-LS depletes signatures, CVD samples are enriched (Orlowski et al., 2016).

Another method to examine a systems performance is the water-to-water extraction, which we also applied in this study. The main benefit is the exclusion of soil as an uncertainty variable, which gives a more precise estimate on the systems accuracy. For CVD, water-to-water extraction has a precision of 0.2 ‰ for δ^{18}O and 0.8 ‰ for δ^2H (Orlowski et al., 2016).

170   Another comparability issue between extraction methods is the applied accuracy metric. While some studies give the accuracy (as in how good a known sample signature can be reproduced after extraction) (Koeniger et al., 2011; Orlowski et al., 2013, 2016), most only give the measurement repeatability (as in how measurements of multiple samples of same signatures deviate from each other)(West et al., 2006; Wassenaar et al., 2008; Mueller et al., 2014; Volkmann and Weiler, 2014). We think repeatability is inferior to accuracy, as it hides the overall measurement inaccuracies. For that reason we chose to present our

175   results as accuracys.

Comparing our results to the water-to-water CVD extraction accuracys stated by (Orlowski et al., 2016) (0.2 ‰ for δ^{18}O and 0.8 ‰ for δ^2H), our DVE-LS extractions perform worse. However, this lower measurement accuracy comes with a higher sample throughput and less manual labor.

4.2   Manual labor in CVD and DVE-LS

180   Assessing sample preparation of CVD and DVE-LS regarding consumables, technical equipment and manual labor, DVE-LS is the least expensive and least time consuming method (Orlowski et al., 2016). However once the samples are prepared, the liquid samples gained through CVD can be autonomously processed by the isotope analyzers liquid autosampler. With the VapAuSa, the formerly manual analysis step is now also fully automated. Therefore, DVE-LS now comes at the same analysis autonomy like liquid CVD samples but at lower sample preparation costs (approx. 3 minutes manual labor per sample).

185   ## 4.3   Storage Time

While in some cases the VapAuSa measurement uncertainty is higher compared to manual analysis, this uncertainty might cancel out due to lower sample storage time. The higher weekly sample throughput leads to quicker analysis, thus causing less isotopic drifts during storage. Over time, these changes appear with every DVE-LS sample container materials. Even aluminum laminated bags sealed by their ziplock (as they would be during storage) have an isotopic drift of 0.042 ‰ per day for δ^{18}O at

190   room-temperature (Gralher et al., 2021). One can assume that even when storing the samples in a fridge the samples isotopic composition also shifts. To minimize these shifts one needs to analyse the samples as quick as possible. Manual DVE-LS sample analysis is limited to about 180 samples per week. However VapAuSa can analyze up to 120 samples per day thus enabling up to 500 sample measurements per week. Relating the throughput to storage unceartainty, analyzing 400 DVE-LS samples adds about 0.6 ‰ storage induced uncertainty to manual δ^{18}O analysis, however only 0.17 ‰ storage uncertainty

195   to VapAuSa δ^{18}O measurements. Therefore, when measuring lots of DVE-LS samples is needed, the basic measurement uncertainty of the VapAuSa quickly cancels out due to minimal storage induced shifts.

4.4 Objectivity

By defining (and documenting!) stability criteria, measurements are less dependent on person specific skills and preferences regarding the detection of "stable" measurements. The subjective influence of the operator diminishes even further, as the system treats all samples equally, without mental fatigue after several hours of measurements. Additionally, the automated measurement eliminates the effect of the operators body heat, which even in an air conditioned lab setting has the potential to warm samples above the temperature at which the sample should have equilibrated for 48 hours. Also, the measurement monitoring part of the VapPauSa-GUI can be used on its own and does not require any other hardware than the isotopic analyzer itself. We think it might even be suited to facilitate and objectify manual DVE-LS measurements, as it provides instant one click summary statistics for freely selectable time spans. Meanwhile the standard Picarro-analyzer GUI requires some tedious zooming in and out for that purpose. On top of that, the possibility to define objective stability criteria should help to reduce the a subjective component of manual DVE-LS measurements.

4.5 Don't buy cheap

To gain the mentioned VapAuSa benefits, we want to stress the importance of using appropriate, vapor capable materials. Even a tiny amount of ambient air leaking into the system can cause major errors with water vapor analysis. The first prototype was build using valves designed for liquids with the connection between valve and cannula only plugged into each other and secured with Parafilm (PARAFILM® M). While this system cost a fraction of our current setup, it eventually developed a leak. This lead to ambient air mixing with the sample vapor, causing wrong measurements which needed correction. Therefore, it is important to use adequate materials like gas-rated valves and secure all connections either by screw-fittings or superglue. Only this can ensure reliable measurements.

5 Conclusions

VapAuSa may be a helpful tool for all disciplines applying DVE-LS. The automatic analysis could also be beneficial for researchers who apply cheaper, more diffusive polyethylene bags for their DVE-LS samples. There, the shorter storage time could be compensated by the increased sample throughput of the VapAuSa. So far we tested the system with liquid and soil samples only, however investigating plant samples (which have an even shorter maximum storage time) would be an interesting next step. Generally we think the VapAuSa is a valuable addition to the tool-sets of isotope geoscientists, enabling large scale, high volume sampling, needed for advancing our understanding of environmental systems.

Code availability. All of the scripts that compose the VapAuSa-GUI can be found under https://gitlab.rz.uni-freiburg.de/hydrology/vapausa.

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