

# Revising The Protocols to Ensure Reliability, Cost Effectiveness and High Sample Throughput for Water Stable Isotope Analyses In Continuous Flow Mode using the Gasbench II.

Abhayanand Singh Maurya, Rajendra Dattataryi Deshpande, Miral Shah, Sushil Kumar Gupta

**ABSTRACT-** The continuous flow isotope ratio mass spectrometer (CF-IRMS) coupled with automated sample preparation device Gasbench II and equilibration system measures oxygen and hydrogen isotope ratios with typical external precision of around  $\pm 0.1$  ‰ for  $\delta^{18}\text{O}$  and  $\pm 1$  ‰ for  $\delta\text{D}$ , although internal analytical precision is better than 0.06 ‰ for oxygen and 0.5 ‰ for hydrogen. In CF-IRMS method, an aliquot of the gas ( $\text{CO}_2/\text{H}_2 + \text{Helium}$ ), equilibrated with water sample, is transferred from the head-space of the exetainer into the mass-spectrometer for isotope ratio measurement. The observed difference between external and internal precision is governed by the operating procedures that influence the online chemistry for equilibration and transfer of the gas in to IRMS. These procedures also govern the sample throughput and long term stability of the machine. With a view to minimize the difference between external and internal precision and to maximize the throughput with high reliability at minimum cost experiments were undertaken. The experiments investigated: (i) optimum duration of equilibration for oxygen analyses, (ii) modes for manual introduction of water sample in the exetainer i.e. before or after flush-filling with equilibrating gas, (iii) effects of the difference between room temperature and sample tray temperature, (iv) effects of septum reuse, and (v) reuse of platinum rod on long term basis. In addition, important maintenance related issue pertaining to analytical needle for the injection and flushing is addressed. The experiments achieved external precision better than 0.07 ‰ for  $\delta^{18}\text{O}$  and 0.8 ‰ for  $\delta\text{D}$  with large sample throughput and long term stability using 300  $\mu\text{l}$  of water sample. Other optimized parameters for oxygen are; equilibration duration of 16 hrs at 32°C, 540 seconds of flushing duration and introduction of water sample after flush-filling. The parameters for hydrogen are 45 minutes and 320 seconds after flush-filling.

**Key Words:** Water Stable Isotope, Protocols, Flush filling, Maintenance.

## I. Introduction

Stable water isotope ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  or  $\delta\text{D}$ ) analyses are routinely used in a wide range of hydrological, environmental and paleoclimatic investigations.

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Continuous flow IRMS is increasingly used in these studies due to simple and streamlined procedures [1,2]. In these analyses, micro volume of water sample is equilibrated with gas mixture ( $\text{He}$  with  $\text{CO}_2$  or  $\text{H}_2$ ) in exetainer in thermostated bath over an optimized duration to achieve the complete equilibration between gas and water sample. The equilibrated gas is then transferred into mass spectrometer using a continuous stream of carrier gas Helium ( $\text{He}$ ). Most commercially available CF-IRMS offer internal precision, 0.06 ‰ for  $\delta^{18}\text{O}$  and 0.5 ‰ for  $\delta\text{D}$ , that depends only on the isotope mass analyses internally in the system. The external precision is also controlled by the operating procedures that influence the online chemistry for equilibration and transfer of the gas in to IRMS, is offered as around  $\pm 0.1$  ‰ for  $\delta^{18}\text{O}$  and  $\pm 1$  ‰ for  $\delta\text{D}$ . The adopted standard operating procedures also affect the throughput of the laboratory.

A new laboratory was set up at Physical Research Laboratory, Ahmedabad (India) as part of a National Programme on Isotope Fingerprinting of Waters of India (IWIN) and is required to analyze oxygen and hydrogen isotopes in about 20,000 water samples over a five year period [3]. This necessitated developing standard operating procedures that ensured a large throughput of measurements with high degree of precision and long term stability. Some of the parameters that affect the precision, throughput of a laboratory and stability of machine are investigated. Duration of equilibration between the water sample and equilibrating gas, particularly in case of oxygen isotope analyses, is an important parameter not only from the point of reliability and reproducibility but also from the point of analytical throughput of a laboratory. Although reduction in equilibration duration can be easily achieved by increasing the equilibration temperature, a large difference between the room and the thermostated tray temperature leads to condensation of water droplets under the rubber septum of the exetainer. This may lead to partial blockage of needle, missing of peaks in the mass spectra and consequently erroneous results. Therefore, minimal duration required for water- $\text{CO}_2$  equilibration should ensure data reproducibility with the least temperature difference between the sample tray and the room temperature to minimize condensation of water droplets below the septum of the exetainer. The sample size is another important issue.

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Reducing the sample size often results in decreasing the molar ratio of oxygen (or hydrogen) between water and equilibration gas (CO<sub>2</sub> or H<sub>2</sub>) necessitating use of a correction factor (CF) for isotopic mass balance during water-gas equilibration. However, in several hydrological applications, e.g. groundwater, surface water or precipitation, the availability of sample volume is not a constraint. Therefore, it may be possible to use slightly larger sample volume for isotopic measurement for avoiding subsequent correction and improving the precision by reducing errors due to minor variations in sample volume and proportion of CO<sub>2</sub> or H<sub>2</sub> in the helium gas mixtures.

The other issue concerns the mode of introducing the water sample into the exetainer, namely, sample introduction before flush-filling (SBFF) or after flush-filling (SAFF). Apart from these some maintenance was investigated to clean the blocked analytical needle with readily available facilities.

A set of experiments were undertaken to optimize; (i) duration of equilibration for oxygen analyses, (ii) modes for manual introduction of water sample in the exetainer i.e. before or after flush-filling with equilibrating gas, (iii) effects of the difference between room and sample tray temperature, (iv) effects of septum reuse; and (v) reuse of platinum rod on long term basis. In addition, some maintenance related issues pertaining to analytical needle (for injection and flushing) were also investigated to economize cost of analyses.

## II. The experiments

As part of IWIN National Programme [3], a new mass spectrometer laboratory was set up with the Delta V Plus IRMS coupled with Gas Bench II, operating in continuous flow mode. For isotopic analyses, water sample is manually introduced in the 12 ml septum-capped exetainer (LABCO) using 1000  $\mu$ l disposable syringe, by piercing the rubber septum. The exetainer is flushed and filled with equilibrating gas mixture (He + CO<sub>2</sub> or He + H<sub>2</sub>) using CTC auto Sampler. The water sample equilibrates with the gas in the exetainer kept inside the thermostated tray. For equilibrating the water samples with CO<sub>2</sub> no catalyst is used whereas for equilibration with H<sub>2</sub> platinum, catalyst rods supplied by Thermo Fisher were used.

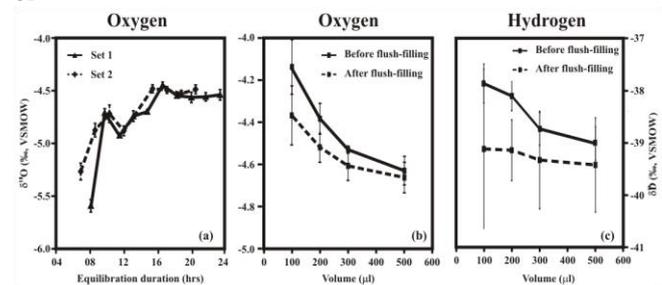
A Gasbench II is an interface for transferring the equilibrated head-space gas contained in the exetainer into the mass spectrometer. The gas is sampled by a specially designed double-holed sampling needle where flow of the He carrier gas pushes the gas mixture through the needle hole into the Valco (a multi-injection loop system). The gas, in turn, pushes through a gas chromatography (GC) column where the gas phases are separated depending on the polarity of the phases and transferred to the inlet of the mass spectrometer for isotope analyses via an active open-split. Any fractionation mechanism within the vial and/ or mixing of even a small ambient laboratory air with head-space gas and the presence of moisture inside the vial or gas are likely to add error to the true isotopic composition of the gas thereby affecting the accuracy of analyses [4].

For all experiments related to oxygen isotopic ratio, initially the secondary laboratory standard (PRL-NARM)

with well established values, were used,  $\delta^{18}\text{O}$  ( $-4.52 \pm 0.06$  ‰) and  $\delta\text{D}$  ( $-35.78 \pm 0.53$  ‰) for evaluating mass spectra [5, 6]. Subsequently, for both oxygen and hydrogen isotopic analyses another secondary laboratory standard (IWIN-1) was used with SMOW-SLAP calibrated value ( $\delta^{18}\text{O}$ :  $-6.54 \pm 0.07$  ‰;  $\delta\text{D}$ :  $-39.75 \pm 0.91$  ‰). The equilibration gas for  $\delta^{18}\text{O}$  analyses was CO<sub>2</sub>+He mixture [0.5% of CO<sub>2</sub> (99.996 %) mixed with helium (99.999 %)]; and for  $\delta\text{D}$  analyses H<sub>2</sub>+He mixture [2% of H<sub>2</sub> (99.996 %) mixed with helium (99.999%)]. The equilibration of water samples with the concerned gas mixture was done in exetainer placed in thermostated sample tray at 32 °C, with ambient room-air temperature of 25 °C.

## III. Result and Discussion

It is observed that a plateau value of  $\delta^{18}\text{O}$  of PRL-NARM, within accepted level of analytical precision ( $\pm 0.1$  ‰), is reached only after 16 hours of equilibration under the above defined temperature conditions (Figure 1a). For gas-water equilibration of less than 16 hours, measured  $\delta^{18}\text{O}$  values are depleted and incorrect. Therefore, 16 hours of



**Figure 1.** (a) A plateau in the  $\delta^{18}\text{O}$  values is obtained only after 16 hours of water-CO<sub>2</sub> equilibration at 32°C. (b) The  $\delta^{18}\text{O}$  values of PRL-NARM water samples obtained during controlled experiments with different sample size and water injection sequence. Sample volume of 200  $\mu$ l was taken as reference for evaluating all the mass spectra. Each data point is an average of 8 aliquots. (c) Same as (b) but for  $\delta\text{D}$  using IWIN-1 secondary standard. equilibration between water sample and the CO<sub>2</sub>-He equilibrating gas, with thermostated tray kept at 32 °C and room temperature maintained at 25 °C, is adopted as standardized protocol for  $\delta^{18}\text{O}$  measurements in our laboratory. Similar experiment for  $\delta\text{D}$  was not performed as the equilibration time is only 45 minutes with catalyst which does not significantly affect the throughput. A clear difference in the  $\delta^{18}\text{O}$  and  $\delta\text{D}$  values obtained by injecting the water sample into exetainer before and after the flush-filling is seen from Figure 1b and Figure 1c. Regardless of the sample volume, a sample injected into exetainer before flush filling gives enriched  $\delta^{18}\text{O}$  values compared to the same sample injected after flush-filling. These results imply that lighter isotopologues of the water sample, when already present into the exetainer, are preferentially flushed out during the process of flush-filling.



Consequently, the water is enriched in heavier isotopologues yielding enriched  $\delta^{18}\text{O}$  values. Therefore, to obtain reliable results, injecting of water samples into exetainer after flush-filling is adopted as a standard protocol.

Further, with increase in sample volume from 100  $\mu\text{l}$  to 500  $\mu\text{l}$ , progressively more depleted values, from  $-4.35\text{‰}$  to  $-4.65\text{‰}$  for  $\delta^{18}\text{O}$  and from  $-37.8\text{‰}$  to  $-39.5\text{‰}$  for  $\delta\text{D}$ , are obtained (Figure 1b and Figure 1c). The plateau value of  $\delta^{18}\text{O}$  is approached with increasing volume of water sample together with relatively enhanced precision of measurement. Between 100  $\mu\text{l}$  to 300  $\mu\text{l}$  there is about 0.3  $\text{‰}$  depletion in  $\delta^{18}\text{O}$  and about 1  $\text{‰}$  depletion in  $\delta\text{D}$ . However, from the 300  $\mu\text{l}$  to 500  $\mu\text{l}$ , the analytical depletion is  $<0.1\text{‰}$  for  $\delta^{18}\text{O}$  and  $<1\text{‰}$  for  $\delta\text{D}$  both for before and after flush-filling. The increase in the sample volume increases the overall moisture content in the system, as reflected in mass 18 peak detected in cup 3. Although there are two Nafion-driers in the system which trap the moisture during analyses, the system background signal increases beyond desirable limits ( $<3000\text{ mv}$ ) after completion of a batch, requiring additional measures for moisture removal such as heating, leaving on standby mode or running standard on-off sequences affecting sample throughput.

Considering these results, 300  $\mu\text{l}$  is adopted as optimum sample volume at which there is no significant increase in the moisture level of the system. The validity of adopting 300  $\mu\text{l}$  as the optimum sample volume is confirmed by calculating applicable isotopic mass balance correction factor for two extreme  $\delta^{18}\text{O}$  values ( $-150\text{‰}$  and  $+150\text{‰}$ ) of equilibrating  $\text{CO}_2$  gas and two extreme  $\delta^{18}\text{O}$  values of equilibrated water ( $-55\text{‰}$  and  $+55\text{‰}$ ), as discussed in the following.

The isotope mass balance correction factor (CF) is the difference in  $\delta$  values between the original water sample before equilibration and the equilibrated water sample. Under optimum operating condition, the volume of water sample, compared to that of equilibrating gas, is expected to be so large that post equilibration, the gas is imparted the isotopic signature of sample water without significantly changing its own isotopic composition [7,8]. Consequently, the isotopic composition of equilibrated water ( $\delta_{\text{ew}}$ ), as calculated from the measured isotopic composition of equilibrated gas ( $\delta_{\text{eg}}$ ), should equal that of sample water ( $\delta_{\text{sw}}$ ), i.e.,  $\delta_{\text{ew}} \approx \delta_{\text{sw}}$ . However, when: (i) the water volume is not large enough compared to equilibrating gas, or (ii) the original isotopic composition of the equilibrating gas ( $\delta_{\text{og}}$ ) is widely separated from  $\delta_{\text{ew}}$ , the isotope mass balance correction factor (CF) such that  $\delta_{\text{sw}} = \delta_{\text{eg}} + \text{CF}$ , need to be applied.

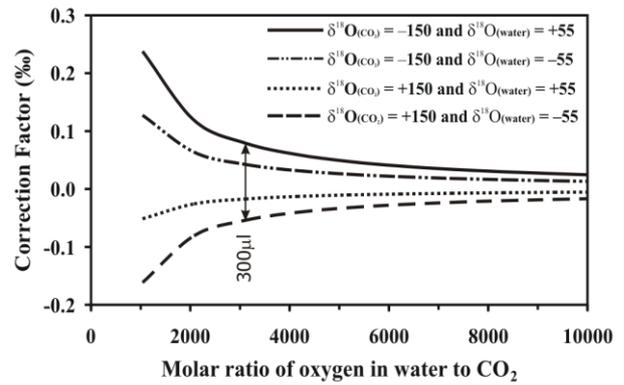


Figure 2. Variation in computed values of correction factor (CF) with increasing molar ratio (K) of oxygen in water- $\text{CO}_2$  equilibration, plotted for two extreme  $\delta^{18}\text{O}$  values of original  $\text{CO}_2$  and water sample.

Isotope mass balance correction factor (CF) for a given volume of water samples and fixed volume (0.06 ml = amount of  $\text{CO}_2$  in 12 ml exetainer) of  $\text{CO}_2$  at equilibration temperature of  $32^\circ\text{C}$  has been computed using:

$$\text{CF} = \left( \frac{\epsilon}{K} \right) - \left( \frac{\delta_{\text{og}}}{K} \right) + \left( \frac{\alpha}{K} \right) \times \delta_{\text{ew}}$$

Where  $\epsilon$  = equilibrium enrichment factor (39.5 $\text{‰}$ ) for  $\text{CO}_2$ - $\text{H}_2\text{O}$  system at  $32^\circ\text{C}$ ; K = molar ratio of oxygen in water sample to  $\text{CO}_2$  in exetainer;  $\alpha$  = equilibrium fractionation factor (1.0395) for  $\text{CO}_2$ - $\text{H}_2\text{O}$  system at  $32^\circ\text{C}$ ;  $\delta_{\text{og}}$  and  $\delta_{\text{ew}}$  represent the  $\delta^{18}\text{O}$  of the original equilibrating gas ( $\text{CO}_2$ ) and equilibrated water sample respectively. Variation in computed value of CF with increasing molar ratio (K) of oxygen in water to  $\text{CO}_2$  is plotted in Figure 2, for two extreme cases,  $\delta_{\text{og}} = +150\text{‰}$  and  $-150\text{‰}$ , and  $\delta_{\text{ew}} = +55\text{‰}$  and  $-55\text{‰}$ . It is observed that magnitude of the correction factor decreases with increasing molar ratio. For a given volume of exetainer and the proportion of  $\text{CO}_2$  in the gas mixture, the molar ratio K depends primarily on the volume of water sample taken. In our experimental conditions 100  $\mu\text{l}$  of water sample corresponds to oxygen molar ratio of 1037. For molar ratio  $>3000$  (corresponding to 300  $\mu\text{l}$  sample water in our case) the CF is within  $\pm 0.1\text{‰}$  and a trade off point between reducing the CF and increasing the ‘mass 18’ background seems to have been reached at  $\sim 300\text{ }\mu\text{l}$  sample volume. As expected, the value of CF is seen to depend on the difference between the  $\delta^{18}\text{O}$  values of  $\text{CO}_2$  and water sample. It is, therefore, evident that using 300  $\mu\text{l}$  of sample volume the application of correction factor is not necessary even for a wide range of combination of water and  $\text{CO}_2$  (300 $\text{‰}$   $\delta^{18}\text{O}$  for  $\text{CO}_2$ ; and 110 $\text{‰}$   $\delta^{18}\text{O}$  for water). Similar calculations for water- $\text{H}_2$  equilibration reveal that application of correction factor is not necessary also in case of hydrogen isotopic analyses.

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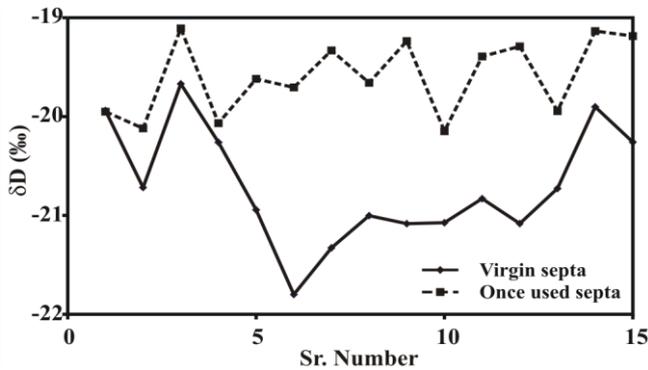


Figure 3 Comparison of measured  $\delta D$  values during a control run using virgin and once used septa for 15 aliquots each of the same sample. The results indicated slightly enriched average value of  $\delta D$  for aliquots in which once used septa were reused.

The performance of bromobutyl rubber septa on repeated use was also examined. During each use, the septum gets pierced thrice (flush-filling, sample injection and analysis). Multiple punctures in the septum weaken the air-tightness which induces diffusive loss, preferentially of lighter gas molecule from the exetainer imparting

undesirable isotope fractionation and resultant enrichment of remaining gas. In one of the experimental runs, 15 exetainers, each of virgin and once-used septum, were employed for analyzing the same sample at similar condition. The results indicated slightly higher average value of  $\delta D$  for aliquots in which once-used septa were reused (Figure 3). Smaller standard deviation observed in case of the reused septa may imply nearly uniform diffusive loss of lighter isotopic molecules from the pressurized exetainers, which masks the random isotopic variations observed in case of virgin septa. Similar observations were also reported by Paul and Skrzypek [4].

With reuse of septa, more frequent blocking of analyses and flushing needles due to fine bromobutyl particles (Figure 4, inset marked G) entering the tiny holes of the needles was noticed. Partial blockage of needle resulted in irregular mass spectra affecting the internal precision and complete blockage resulted in total loss of peaks. This suggested that septum of the exetainer cap is best suited only for a single use.

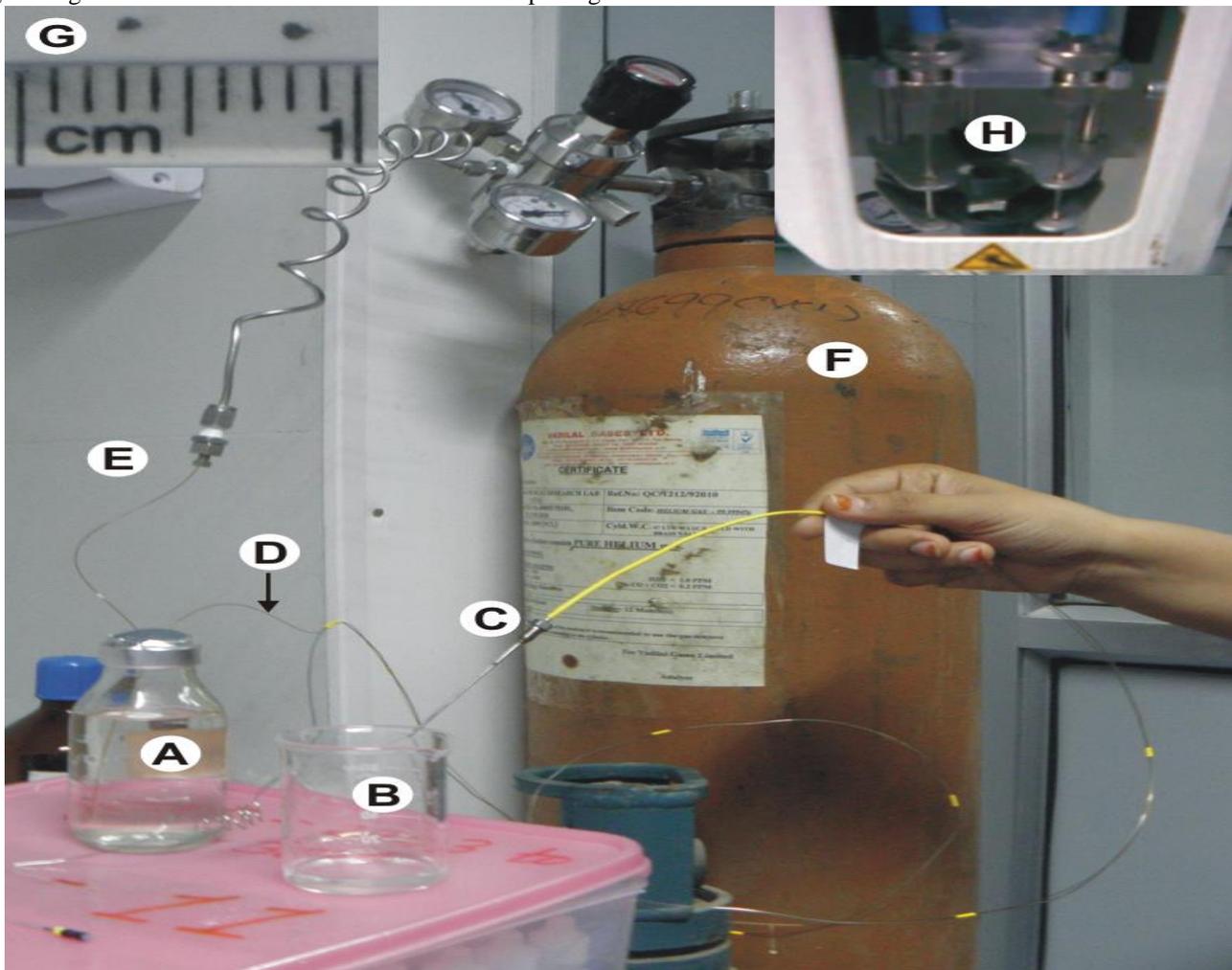


Figure 4. System used for de-clogging the measurement needles. (A) Sealed acetone; (B) Beaker for flowing acetone collection; (C) Measurement needle being de-clogged; (D) Fused silica capillary of the measurement needle being de-clogged; (E) Fused silica coupler; (F) Helium cylinder; (G) Inset showing fine bromobutyl rubber particle recovered

from the de-clogged needle; (H) Inset showing additional needle guide added to auto-sampler.

In most cases, the blockages in the needles could be cleared by forcing out the high purity analytical grade acetone through the fused silica capillary of the needle through its open end. A simple arrangement for de-clogging the needles is shown in Figure 4. A glass bottle (A) was sealed with rubber stub and crimped with aluminum cap. Acetone was introduced in this sealed glass bottle by 100 ml glass syringe. The open end of the fused silica capillary (D) of the needle was inserted right up to the bottom of glass bottle by piercing through a fine hole in the rubber cap. The glass bottle was then pressurized with helium gas (F) using a fused silica coupler (E). In case of fully blocked needle, acetone does not pass through the needle (C), but in the partially blocked needles, it passes drop by drop. The pressure in acetone bottle is increased to a maximum of 4 kg/cm<sup>2</sup>, until a continuous jet of acetone is ejected from the blocked needle. It is also important to flush the de-clogged needle with ultra-high pure helium before its reuse.

Sometimes, the tension chord of the auto-sampler loses elasticity leading to accidental misalignment during piercing the septa and consequent damage to needle. Needle can also get damaged due to material inhomogeneities within septa. An additional needle guide was designed, fabricated and added to auto-sampler for reducing the needle damage (Figure 4, Inset marked H).

Duration of flush filling the extainer concerns the reproducibility and accuracy of the analyses because imperfectly flushed extainer results in a secondary peak of air (mainly nitrogenous gas) preceding the CO<sub>2</sub> peak in the mass spectra. Repeated experiments indicated that 540 sec (≈75 extainer volumes) of flushing gas duration was needed for oxygen analyses. This observation is in conformity with Paul and Skrzypek [4]. Increasing temperature difference between room and thermostated tray indicated tendency of water droplets to condense below the extainer septum and their being dragged into the capillary of the needle used to draw the sample for isotopic analyses, adversely affecting the reproducibility of results. Maintaining this temperature difference between 7±1 °C considerably improved the reproducibility of measurements.

constructed for each batch of analyses and is used to report the corrected values. The insets show the integrated calibration curves based on IAEA certified water standard (VSMOW2, GISP and SLAP2) used to ascertain the values of secondary laboratory standards.

The SMOW-SLAP stretching factor defined by the ratio  $\Delta\delta_{SMOW-SLAP(accepted)} / \Delta\delta_{SMOW-SLAP(measured)}$  [1] is related to compression of measured  $\delta^{18}O$  and  $\delta D$  values of SMOW and SLAP – water standards, that are widely separated in their isotopic composition. The stretching factor is often reported to indicate accuracy of measured values. The closer is the value of the stretching factor to unity, better is the accuracy of the isotopic measurements. In the calibration experiments undertaken following the standardized protocols as above the stretching factor values of 1.0023 for  $\delta^{18}O$  and 1.0056 for  $\delta D$  were obtained indicating negligible compression/ stretching during measurements of widely separated isotopic values.

To monitor the internal and external reproducibility of analytical results, 20 aliquots of three secondary laboratory standards, covering a range from +15‰ to -11‰ for  $\delta^{18}O$ , and +37‰ to -39‰ for  $\delta D$ , are routinely analyzed with every batch of 80 samples. This range of secondary standards was chosen specifically to cover the expected range of natural water samples received from humid tropical and semi-arid regions of India. These secondary laboratory standards have been calibrated with IAEA certified reference materials, applying the integrated calibration curve based on VSMOW2, GISP and SLAP2 measurements, as shown in inset of Figure 5. For reporting the results of routine analyses, an integrated calibration curve (Figure 5) based on secondary laboratory standards, is used. A comparison of measured values of four secondary standards analyzed in our laboratory and those provided by IAEA Isotope Hydrology Laboratory, Vienna is given in Table 1.

**Table 1. A comparison of measured values of four secondary standards analyzed in our laboratory and IAEA Isotope Hydrology Laboratory, Vienna**

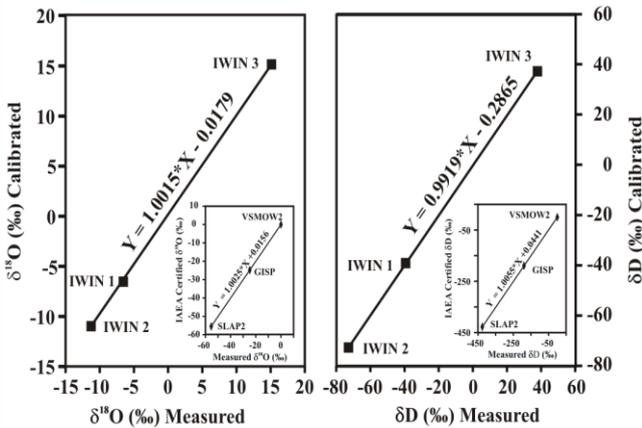


Figure 5 . A typical integrated calibration curves for  $\delta^{18}O$  and  $\delta D$ , based on three secondary laboratory standards covering the expected range of natural water samples from humid tropical and semi-arid parts of India. Each batch of 80 samples contains 10 aliquots of IWIN 1 and 5 aliquots each of IWIN 2 and IWIN 3. A new calibration curve is

Secondary Laboratory Standard	IAEA, Vienna		PRL, Ahmedabad	
	$\delta^{18}O \pm 1$ (‰)	$\delta D \pm 1$ (‰)	$\delta^{18}O \pm 1$ (‰)	$\delta D \pm 1$ (‰)
IWIN-1	-6.57 ± 0.07	-40.5 ± 0.7	-6.54 ± 0.07	-39.8 ± 0.8
IWIN-2	-11.09 ± 0.07	-73.8 ± 0.8	-11.01 ± 0.07	-74.0 ± 0.8
IWIN-3	15.1 ± 0.09	36 ± 0.6	15.07 ± 0.07	36.8 ± 0.8
IWIN-4	-2.29 ± 0.1	-17.6 ± 0.8	-2.21 ± 0.07	-17.3 ± 0.8

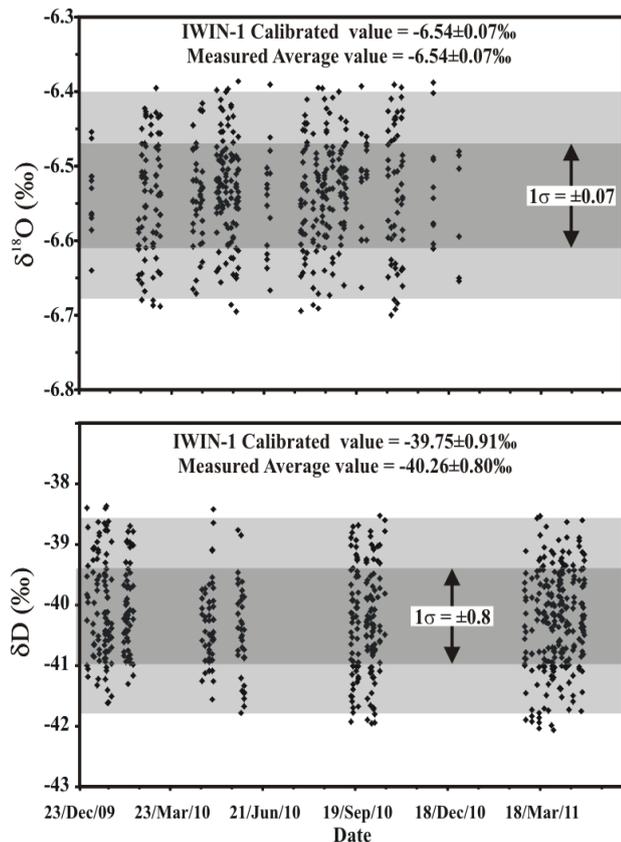


Figure 6. Long term reproducibility of  $\delta^{18}\text{O}$  and  $\delta\text{D}$  of secondary laboratory standard. Most of the analytical results of the secondary laboratory standard fall in  $1\sigma$  band of 0.07 ‰ for  $\delta^{18}\text{O}$  and 0.8 ‰ for  $\delta\text{D}$ . The light gray bands correspond to  $2\sigma$  ranges in both cases.

Following the above mentioned calibration experiments and standardized protocols, long term reproducibility of  $\delta^{18}\text{O}$  and  $\delta\text{D}$  could be achieved even when the system is alternately operated in  $\text{CO}_2$  and  $\text{H}_2$  modes, maintaining a high throughput rate. A typical example of the analytical results of one of the secondary laboratory standard (IWIN-1), routinely used in our laboratory, is shown in Figure 6. It shows that most of the measurements, over a year, have external analytical precision of  $\pm 0.07\%$  for  $\delta^{18}\text{O}$  and  $\pm 0.8\%$  for  $\delta\text{D}$ . Following the standard procedure, we have been able to achieve a throughput of about 2200 samples per year for both oxygen and hydrogen analyses.

#### IV. Summary and Conclusions

Standard operating procedures specified by manufacturers were revisited to ensure large sample throughput with high degree of external precision and long term stability in measurement of oxygen and hydrogen isotope ratios of water samples using continuous flow Isotope Ratio Mass-spectrometer (IRMS) Delta V Plus (Thermo Fisher) using Gas Bench II. The aim was to achieve high throughput of sample analyses with minimum down time and to minimize the error in routine analyses due to the water sample not completely overprinting its isotope composition on the equilibrated gas used for analyses either due to; (i) low molar ratio of oxygen and hydrogen in water sample and the equilibrating gas, or (ii) inadequate duration

of equilibration. The exercise also included investigating the effect of the mode of water sample introduction in the exetainer i.e. before or after flush-filling with equilibrating gas.

The equilibration of water samples with the concerned gas mixture was done in exetainers placed in a thermostated sample tray at  $32^\circ\text{C}$ , with ambient room-air temperature maintained at  $25^\circ\text{C}$ . Under these conditions the optimum equilibration duration for oxygen has been estimated as 16 hours. The optimum molar ratio of oxygen in water- $\text{CO}_2$  has been estimated as 3100 corresponding to 300  $\mu\text{l}$  water in 12 ml exetainer with 0.5%  $\text{CO}_2$  in  $\text{CO}_2$ -He mixture.

Several other experiments revealed; (i) septum of the exetainer cap is best suited only for a single use; (ii) reproducibility is better if the laboratory air temperature is maintained within  $25 \pm 1^\circ\text{C}$ , and (iii) when the difference between room and tray temperatures increases, there is tendency of water droplets condensing below the exetainer septum and their being dragged into the capillary of the needle adversely affecting the reproducibility of results.

#### V. Acknowledgements

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