

# $^{18}\text{O}$ -Equilibration on Water, Fruit Juice and Wine Using Thermo Scientific GasBench II

Andreas W. Hilkert, Hairigh Avak, Thermo Fisher Scientific, Bremen, Germany

## Key Words

- GasBench II
- Continuous Flow
- Isotope Ratio MS
- $^{18}\text{O}$  Water Equilibration

## Introduction

The measurement of the  $^{18}\text{O}/^{16}\text{O}$  ratio of water is one of the most important applications of isotope ratio mass spectrometry. Very diverse applications, ranging from studies of variations in natural abundance in the hydrologic cycle to authenticity control on beverages to the use of  $^{18}\text{O}$  enriched tracers in human and animal metabolic studies, demand a general purpose analytical solution which can provide high precision/high accuracy/high throughput analysis of  $^{18}\text{O}/^{16}\text{O}$  in an enormous range of substrates.

Continuous flow techniques, in which the sample is entrained in a carrier gas, have been shown to allow high throughput analysis from elemental analyzers and gas chromatographs, but for high precision work, the dual inlet system has remained the instrument of choice.

The development of the technique of repetitive loop injection has allowed us to close the gap between the dual inlet system and continuous flow inlet systems. The Thermo Scientific GasBench II is a continuous flow interface with autosampler-assisted loop injection in which the precision attainable with the dual inlet system is realized by modern continuous flow technology. In  $\text{CO}_2$ -water equilibration, a small amount of  $\text{CO}_2$  in the headspace of a sample vial is isotopically equilibrated with the water entrained in a liquid sample. The very small amount of  $\text{CO}_2$  in the headspace ensures complete transfer of the  $^{18}\text{O}$  information during equilibration ( $\text{CO}_2/\text{H}_2\text{O} < 1:3000$ ). With the GasBench II, water samples  $< 200 \mu\text{l}$  can be routinely analyzed to a precision and accuracy of  $< 0.06 \%$ .

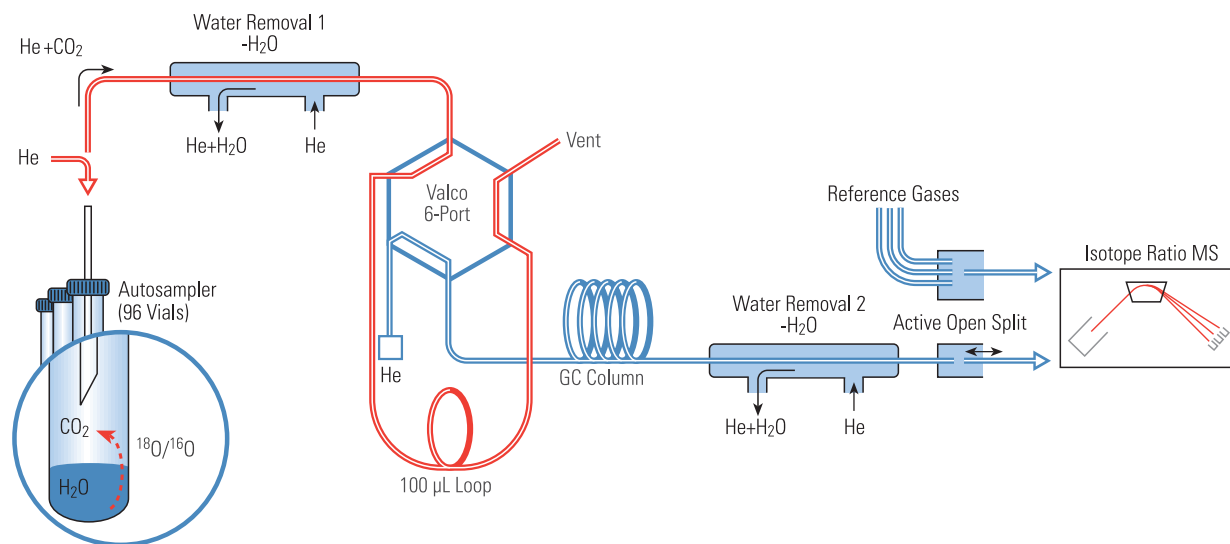


Figure 1: Schematic diagram of the Thermo Scientific GasBench II.

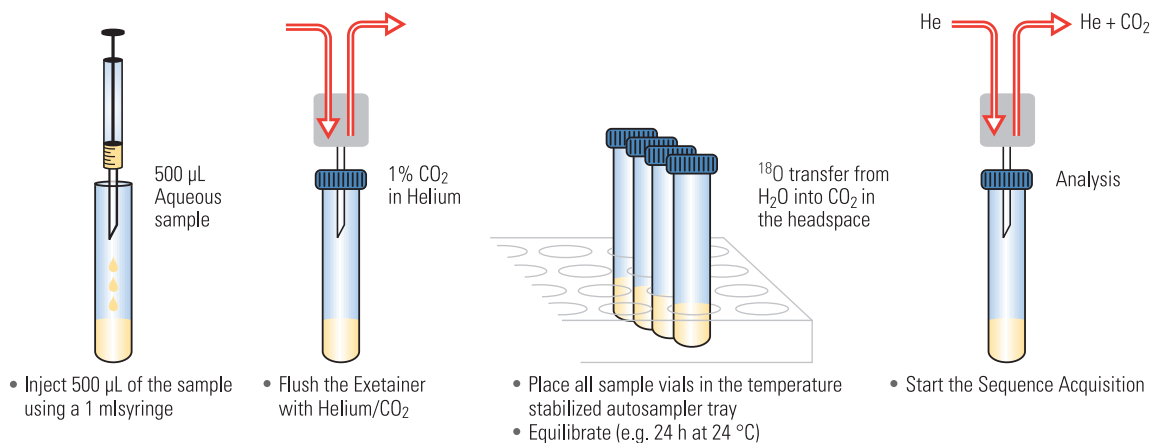


Figure 2: Sample preparation.

### Experimental Conditions

Borosilicate sample bottles are washed with diluted acid, then washed twice with deionized water and dried overnight at 70 °C. After adding 500 µL of sample into the open vials using a disposable syringe they are sealed with new septa. Residual air in the vials is removed from the sample vials by an automated autosampler-assisted flushing procedure, which uses a mixture of 0.3% to 0.5% CO<sub>2</sub> in He. Flushing is carried out at a flow of 100 ml/min for 5 minutes. The 0.3% to 0.5% CO<sub>2</sub> in the flushing He stream is used as equilibration gas.

Measurement is carried out after an equilibration time of 24 h at 24 °C. The temperature stability required for <sup>18</sup>O equilibration is ± 0.1 °C (the temperature dependence is ~ 0.25 ‰ per 1 °C). By helium the sample is moved through the water trap, filling the sample loop and leaving to vent. Water vapor is quantitatively removed on-line from the gas stream. The sampling loop aliquots 100 µl samples of the headspace into an isothermal gas chromatograph, where CO<sub>2</sub> is separated from any other gas species. The use of repetitive loop injection (1-2 min per replicate) allows to approach a precision comparable to that of a dual inlet system. Some of the analysis presented have been prepared by an older, manual method injecting 500 µl of aqueous sample through the septum into 10 ml screwcap Exetainers, prefilled with a commercial mixture of 0.2% up to 1% CO<sub>2</sub> in helium (99.996% purity).

### Referencing to VSMOW

In order to perform VSMOW/VSLAP scaling, required for reporting the data relative to VSMOW, each set of samples includes two reference water samples of different composition. For the samples analyzed here, the scaling corrections are in the range of the analytical error (~ 0.02 ‰).

Sample size:	500 µl of water
CO <sub>2</sub> /He:	1%
Equilibration:	20 h at 24 °C
Sampling loop:	10 µl
Replicates (loop switches):	10
GC column:	PoraPLOT Q 25 m (24 °C)
SMOW/SLAP scaling factor:	0.9946

### Water: Accuracy, Memory, Precision

Three natural water laboratory standards were analyzed on both the GasBench II and on the HDO II water equilibrator. The Thermo Scientific HDO II and its predecessor, the HDO I, are dual inlet peripherals and are the accepted standard for automated high precision water equilibration in 100 laboratories worldwide. Comparison with the HDO II results shows that the two data sets are in very close agreement, with an offset of 0.03 ‰, validating the accuracy of the GasBench II (Table 1).

	GASBENCH II Δ <sup>18</sup> O <sub>VSMOW</sub> (‰)	HDO II Δ <sup>18</sup> O <sub>VSMOW</sub> (‰)	ΔΔ (‰)
PAK	- 37.34	- 37.37	0.03
ANST	- 28.32	- 28.35	0.03
Vostok	- 56.60	- 56.64	0.04

Table 1: Comparison of GasBench II and HDO II water equilibrator.

The Vostok sample, which is significantly depleted in <sup>18</sup>O relative to VSMOW, is used to demonstrate the complete absence of memory effects in the GasBench II (Figure 3).

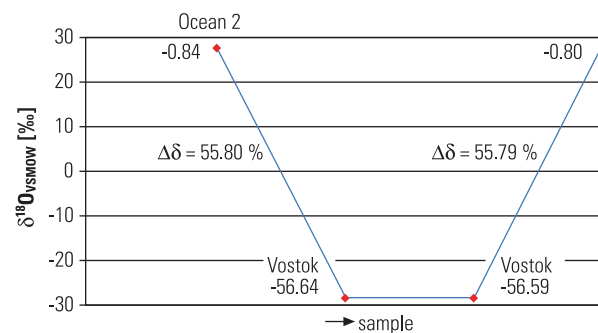


Figure 3: Absence of memory effects.

The average external precision of replicate analyses on the GasBench II was < 0.05 ‰.

## Fruit Juices: Detection of Adulteration

Sample size:	500 µl of fruit juice
CO <sub>2</sub> /He:	0.2%
Equilibration:	20 h at 28 °C
Sampling loop:	100 µl
Replicates (loop switches):	10
GC column:	PoraPLOT Q 25 m (24 °C)
SMOW/SLAP scaling factor:	1.0024

<sup>18</sup>O determination of water in fruit juice is widely used to establish whether a juice has been reconstituted from concentrate using local water. As part of a larger study defining baseline data, apple and orange juices of different origin were analyzed. For purposes of comparison, samples of mineral water and local drinking water were analyzed (Figure 4). Adulteration of orange juice is clearly demonstrated. The average external precision achieved on replicate analyses was < 0.06 ‰.

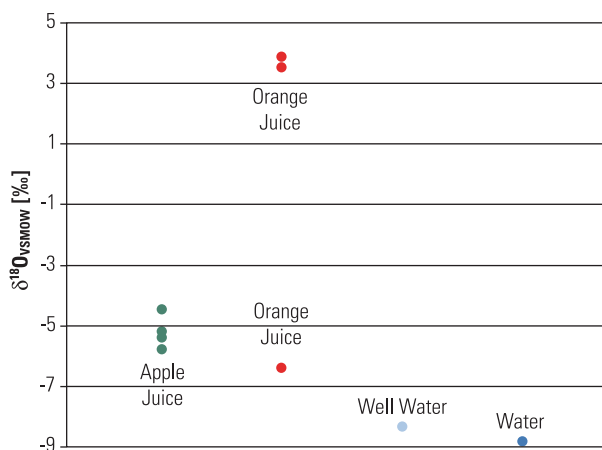


Figure 4: Fruit juices.

## Wine: Authenticity Control

Sample size:	500 µl of wine
CO <sub>2</sub> /He:	1%
Equilibration:	20 h at 24 °C
Sampling loop:	10 µl
Replicates (loop switches):	10
GC column:	PoraPLOT Q 25 m (24 °C)
SMOW/SLAP scaling factor:	0.9980

<sup>18</sup>O isotopic analysis of wine has been a topic of increasing interest, and is now the subject of EU regulation. In order to validate the GasBench II for this application, samples of wine were run on both the GasBench II and the HDO II water equilibrator. The wine samples were analyzed directly, without distillation. Comparison with the results from HDO II (Table 2) shows that the two data sets are in very close agreement within < 0.03 ‰.

	GASBENCH II Δ <sup>18</sup> O <sub>VSMOW</sub> (‰)	HDO II Δ <sup>18</sup> O <sub>VSMOW</sub> (‰)	ΔΔ (‰)
Wine A	0.18 ‰	0.16 ‰	0.02
Wine B	- 1.34 ‰	- 1.37 ‰	0.03
Wine C	1.53 ‰	1.54 ‰	- 0.01

Table 2: Analysis of wine with the GasBench II.

The natural CO<sub>2</sub> in wine was analyzed directly, which opens the possibility to eliminate the addition of CO<sub>2</sub> to the headspace for equilibration. To show the ease and precision of this method, the δ<sup>18</sup>O of Wine C was analyzed with and without addition of CO<sub>2</sub> (Table 3); no difference was seen between the two approaches, and the overall precision was 0.035 ‰.

	Δ <sup>18</sup> O <sub>VSMOW</sub>	EQUILIBRATION	DATE
EQ1a	1.534 ‰	1% CO <sub>2</sub> / He	4 / 27
EQ1b	1.517 ‰	1% CO <sub>2</sub> / He	4 / 27
EQ2	1.494 ‰	CO <sub>2</sub> from wine	4 / 27
EQ3	1.577 ‰	CO <sub>2</sub> from wine	4 / 28
Mean:	1.531 ‰	± 0.035 ‰	

Table 3: Analysis of δ<sup>18</sup>O of wine with the GasBench II without addition of CO<sub>2</sub>.

The high sensitivity of the GasBench II coupled with a Thermo Scientific isotope ratio mass spectrometer DELTA V Advantage, DELTA V Plus and MAT 253 as well as MAT 252 and all DELTA series mass spectrometers opens the possibility of using the CO<sub>2</sub> already present in the beverage as the equilibration gas, which could lead to a significant simplification of sample preparation procedures.

## Conclusions

The GasBench II can be used for highly precise measurements of the oxygen isotopic composition of waters. Analysis of the same samples on the HDO II water equilibrator (a dual inlet system peripheral) shows very close agreement, with a Δδ (GasBench II - HDO II) < 0.03 ‰, serving to validate the technique and the device. Successful analysis of natural waters, fruit juices, and wines shows that the GasBench II can be used for isotope studies on a wide range of water-bearing substrates. Measuring a suite of natural waters which included a very depleted antarctic water establishes that the GasBench II has no memory, and that there is no cross talk between samples as is so often the case for water preparation devices. It is also shown that for samples with natural CO<sub>2</sub> concentration, it is possible to measure the water oxygen composition without any addition of CO<sub>2</sub> for equilibration.

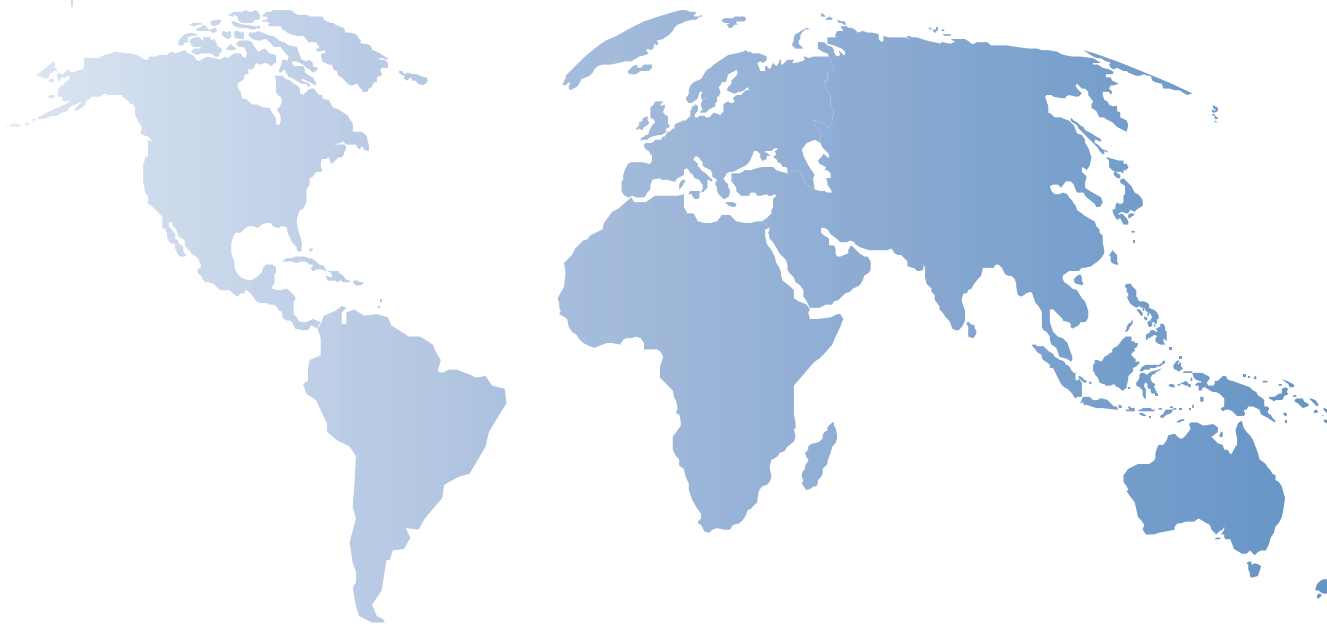
## Literature

- <sup>1</sup> Stephen T. Nelson, Rapid Commun. Mass Spectrom. 14, 1044-1046 (2000)
- <sup>2</sup> Stephen T. Nelson, Rapid Commun. Mass Spectrom. 14, 293-297 (2000)

## Laboratory Solutions Backed by Worldwide Service and Support

Tap our expertise throughout the life of your instrument. Thermo Scientific Services extends its support throughout our worldwide network of highly trained and certified engineers who are experts in laboratory technologies and applications. Put our team of experts to work for you in a range of disciplines – from system installation, training and technical support, to complete asset management and regulatory compliance consulting. Improve your productivity and lower the cost of instrument ownership through our product support services. Maximize uptime while eliminating the uncontrollable cost of unplanned maintenance and repairs. When it's time to enhance your system, we also offer certified parts and a range of accessories and consumables suited to your application.

To learn more about our products and comprehensive service offerings, visit us at [www.thermo.com](http://www.thermo.com).



In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

**Africa**  
+43 1 333 5034 127

**Australia**  
+61 2 8844 9500

**Austria**  
+43 1 333 50340

**Belgium**  
+32 2 482 30 30

**Canada**  
+1 800 530 8447

**China**  
+86 10 8419 3588

**Denmark**  
+45 70 23 62 60

**Europe-Other**  
+43 1 333 5034 127

**France**  
+33 1 60 92 48 00

**Germany**  
+49 6103 408 1014

**India**  
+91 22 6742 9434

**Italy**  
+39 02 950 591

**Japan**  
+81 45 453 9100

**Latin America**  
+1 608 276 5659

**Middle East**  
+43 1 333 5034 127

**Netherlands**  
+31 76 579 55 55

**South Africa**  
+27 11 570 1840

**Spain**  
+34 914 845 965

**Sweden/Norway/  
Finland**  
+46 8 556 468 00

**Switzerland**  
+41 61 48784 00

**UK**  
+44 1442 233555

**USA**  
+1 800 532 4752

[www.thermo.com](http://www.thermo.com)

### Legal Notices

©2004, 2008 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.



Thermo Fisher Scientific  
(Bremen) GmbH is certified  
DIN EN ISO 9001:2000

AN30048\_E 01/08C