

# Normalization methods and selection strategies for reference materials in stable isotope analyses – review



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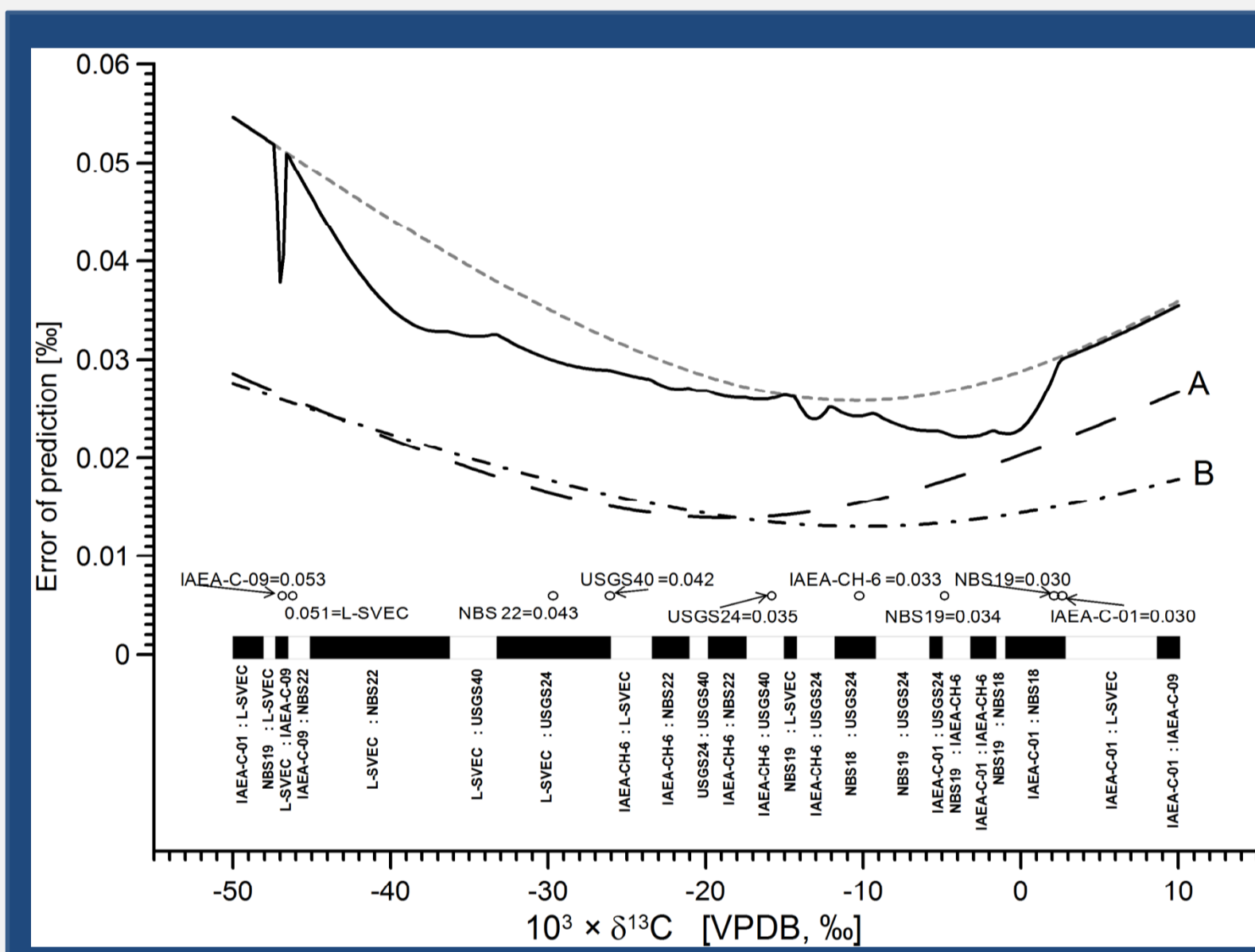


## Conclusions:

- The selection of reference materials, numbers of replicates,  $\delta$ -values of reference materials and normalization technique have been identified as crucial in determining the uncertainty associated with the final results.
- The range of observed differences justifies the need to employ world-wide the same sets of standards for each element and each stable isotope analytical technique.
- A 50% reduction of normalization error can be achieved either by analyzing two standards four times each or four standards two times each.
- For stable carbon isotope analyses using the EA technique, the optimal set (IAEA-C-09, L-SVEC, NBS19 and IAEA-CO-1) or suboptimal (L-SVEC, NBS22, USGS24 and NBS19) set of standards may be used.
- For stable oxygen isotope analyses using the TC/EA technique, the optimal pairs to minimise normalisation errors are: USGS35 and USGS34 for nitrates; IAEA-SO-6 and IAEA-SO-5 for sulphates; and IAEA-601 and IAEA-602 for organic materials. The overall optimal selection among is the IAEA-602 and IAEA-SO-6 pair.

## Introduction

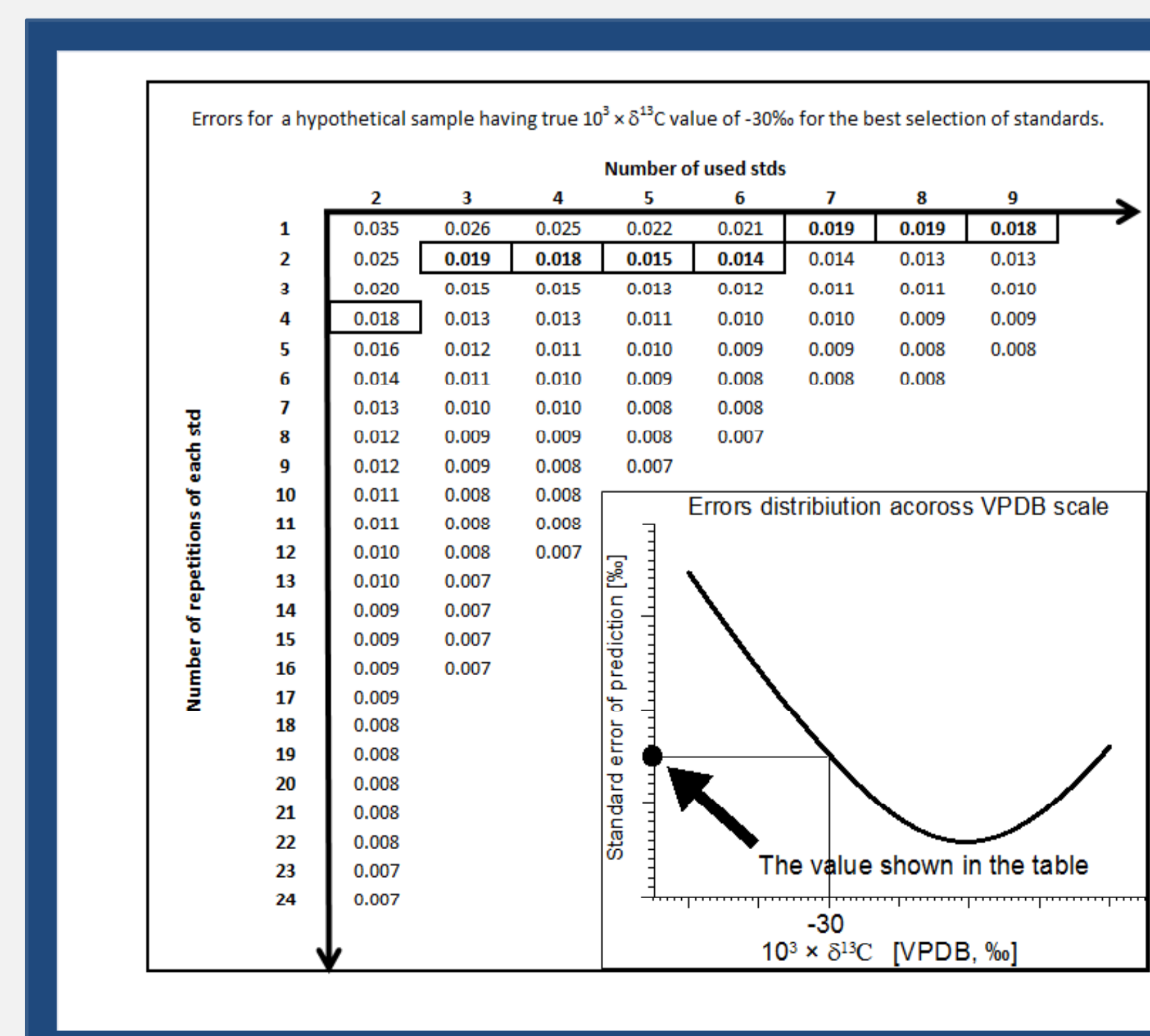
A stable isotope analyst has to make a number of important decisions regarding how to best determine the "true" stable isotope composition of analysed samples in reference to an international scale. It has to be decided which reference materials should be used, the number of reference materials and how many repetitions of each standard is most appropriate for a desired level of precision, and what normalization procedure should be selected. In this paper we summarise what is known about propagation of uncertainties associated with normalization procedures and propagation of uncertainties associated with reference materials used as anchors for the determination of "true" values for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ .



**Fig. 1.** The normalization error in predicting true  $10^3 \times \delta^{13}\text{C}$  values for the optimal pair-wise selection of standards at each  $\delta^{13}\text{C}$  value (solid line), compared to the error (dashed line) resulting from normalization using two point anchor (L-SVEC and NBS 19). The alternating black and white rectangles show the range of  $10^3 \times \delta^{13}\text{C}$  values for which each labelled pair of standards are locally optimal. The L-SVEC and NBS 19 pair is locally optimal, or close to optimal, for only ~20% of the range of  $\delta^{13}\text{C}$  values considered here. Line A is the normalization error for the set of four standards (LSVEC, NBS19, USGS24 and NBS22) the maximum error located at  $-50\text{‰} = 0.029\text{‰}$ . Line B is the normalization error for the globally optimal four standards (IAEA-C-09, L-SVEC, NBS19, IAEA-CO-1), replicated twice; the maximum error at  $-50\text{‰} = 0.028\text{‰}$ .

## Normalization methods

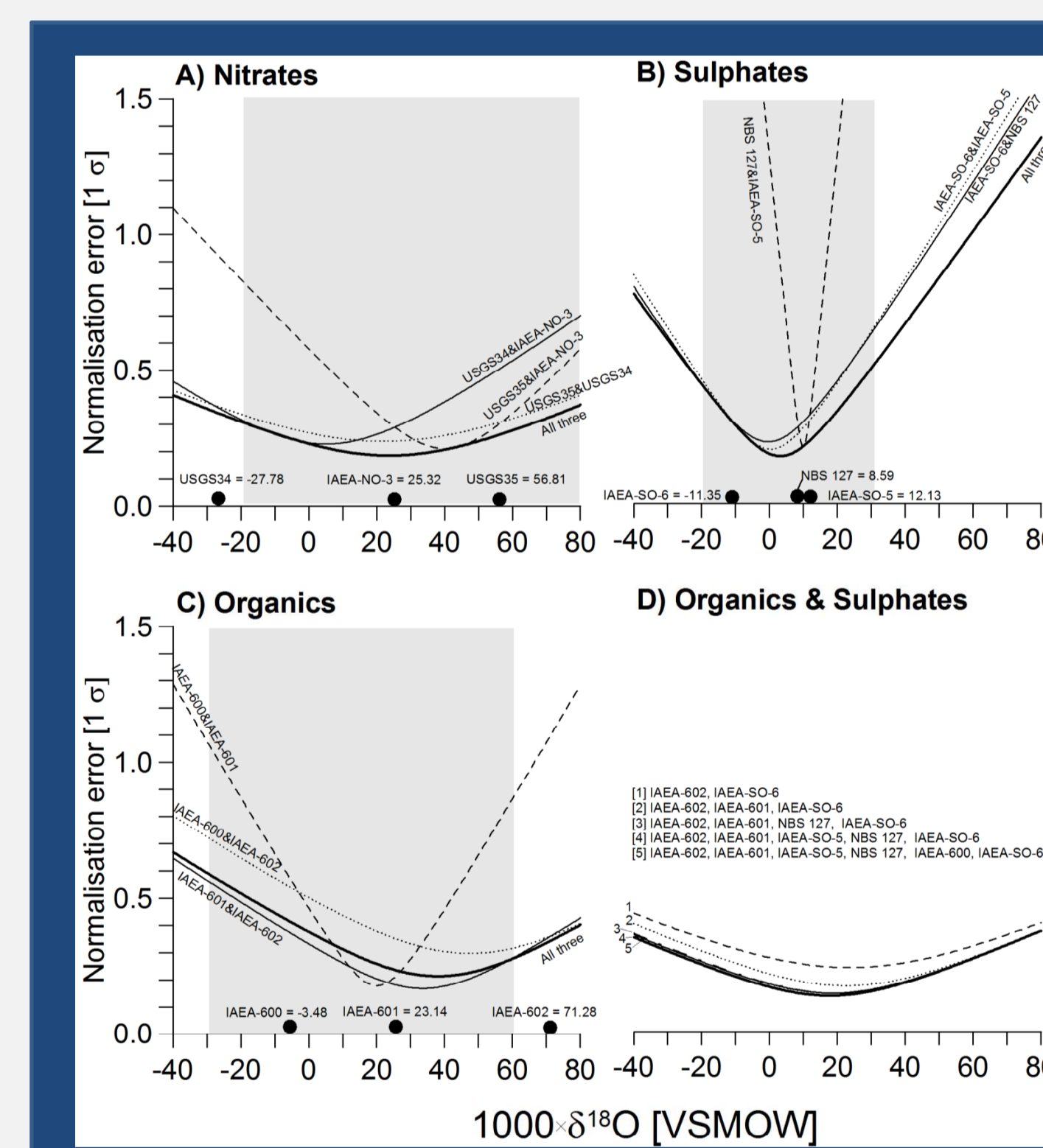
Several normalization methods transforming the "raw" value obtained from mass spectrometers to one of the internationally recognized scales has been developed. However, as summarised by Paul et al. [2007,] different normalization transforms alone may lead to inconsistencies between laboratories. The most common normalization procedures are: single-point anchoring (versus working gas and certified reference standard), modified single-point normalization, linear shift between the measured and the true isotopic composition of two certified reference standards, two-point and multi-point linear normalization methods. The accuracy of these various normalization methods has been compared by using analytical laboratory data by Paul et al. [2007], with the single-point and normalization versus tank calibrations resulting in the largest normalization errors, and that also exceed the analytical uncertainty recommended for  $\delta^{13}\text{C}$ . The normalization error depends greatly on the relative differences between the stable isotope composition of the reference material and the sample. On the other hand, the normalization methods using two or more certified reference standards produces a smaller normalization error, if the reference materials are bracketing the whole range of isotopic composition of unknown samples. These conclusions are in agreement with the approach proposed by Coplen et al. [2006] for the stable carbon isotope referencing. These studies strongly support the use of two-point or multi-point normalisation methods based on regression line as producing the most reliable results Paul et al. [2007].



**Fig. 2.** Monte Carlo derived normalization errors taking into account 1s uncertainties associated with the 9 international standards (Coplen et al., 2006). The computed normalization errors are for the error minimizing combination of standards for a hypothetical sample having true  $10^3 \times \delta^{13}\text{C}$  of  $-30\text{‰}$ . These normalization errors are given for number of different standards and different numbers of replicates. All values of normalization error are given as standard error of prediction in per mil (‰). For example, standard error of prediction for  $10^3 \times \delta^{13}\text{C} = -30\text{‰}$  equals 0.018‰ when the normalization was based on two different standards, each replicated four times.

## Selection of reference materials for $\delta^{13}\text{C}$

Even when a multi-point normalization method is used, the final normalization error still depends highly on the selection of reference materials, their  $\delta$ -values and the number of their replicates [Fig. 1,2]. Furthermore, the uncertainties associated with the calibration of each of the reference materials influences the overall normalization error. Skrzypek et al. [2010] performed both Monte Carlo simulations and laboratory experiments to investigate aspects of error propagation during the normalization of stable carbon isotope data. The normalization error can be reduced by approximately 50% for  $\delta^{13}\text{C}$  (compare to the two-point normalization) if either two standards are analysed four times each, or four standards two times each. It has been concluded that increasing both the number of different reference standards and the number of repetitions of each of these standards reduces the normalization error [Fig.2]. For instance, for stable carbon isotope analyses using the EA technique, the optimal set of reference materials is IAEA-C-09, L-SVEC, NBS19 and IAEA-CO-1, with the next best option being L-SVEC, NBS22, USGS24 and NBS19 [Skrzypek et al., 2010]. Despite the availability of several other reference materials, these two sets should be used to best minimize the errors.



$$\delta_{Spl}^T = \frac{\delta_{Std1}^T - \delta_{Std2}^T}{\delta_{Std1}^M - \delta_{Std2}^M} \times (\delta_{Spl}^M - \delta_{Std2}^M) + \delta_{Std2}^T$$

Two-point normalization method, symbols:  
 $\delta^M$  - measured and  $\delta^T$  - true,  
 Spl - sample, Std - standard.

**Fig. 4.** Normalisation error across the VSMOW scale between  $-40$  and  $+80\text{‰}$ . Each reference material was considered as analysed once (no replicates). The gray shadow indicated the approximate typical range of  $\delta^{18}\text{O}$  variability in natural samples from various environments.

## Selection of reference materials for $\delta^{18}\text{O}$ analyses

The level of reduction in errors is especially visible when the reference materials employed have relatively high uncertainty, due to the precision of the analytical technique (e.g., High Temperature Conversion) or its chemical composition (e.g.,  $\delta^{18}\text{O}$  yield as CO from nitrate materials) [Brand et al., 2009; Skrzypek and Sadler, 2011]. Similarly, for stable carbon isotope composition, a much lower uncertainty in stable oxygen isotope analyses can be easily achieved through an optimal selection of reference materials. Skrzypek and Sadler [2011], calculated that the best performing pairs minimizing the errors for nitrates are USGS35 and USGS34; for sulphates IAEA-SO-6 and IAEA-SO-5; and for organic materials IAEA-601 and IAEA-602 [Fig.3]. For both carbon and oxygen analyses, the observed errors were lowest when reference materials were located nearest to the extremes of the considered range, and highest for reference materials covering only a narrow range of  $\delta$ -values. However, the difference is especially distinct for  $\delta^{18}\text{O}$  solid reference materials due to their higher uncertainties. A possible memory effect should also be taken into account during the selection of reference materials.

## ACKNOWLEDGEMENTS

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