Three keratin lab standards for daily laboratory use in isotopic studies

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Introduction

❑Reference materials provided by the International Atomic Energy Agency (IAEA) are expensive and distributed in small quantities, so that they are not intended for daily use, but only for calibration of in-house lab standards

❑No suitable certified organic reference material for δ^2H and $\delta^{18}O$ determinations in keratinous tissues are yet available from the IAEA

❑ In order to measure δ^2H in keratin material is necessary to have a keratin reference accounting for H that is exchanged with ambient laboratory air moisture

Objectives

❑ To prepare three different keratin reference standards for routine daily use in the stable isotope laboratory

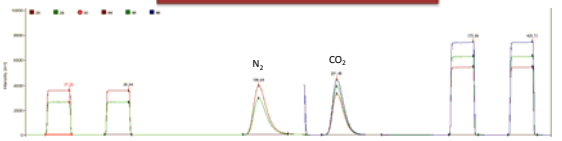
Keratin Standard Origins

1- Fin whale (*Balaenoptera physalus*) baleen (**BB**): beached whale found in Malaga in 2012
2- Cow (*Bos taurus*) horn (**CV**): Obtained from a endemic cow from the Natural Area of Doñana
3- Razorbill (*Alca torda*) feathers (**PA**): Beached razorbill found in Huelva

Preparation

1) Cleaned keratin samples were cut into <1 cm pieces
2) Samples were solvent cleaned to remove fats and oils
3) Ball Milling to < 120 um particle size
4) Shaker sieving with < 120 um mesh, followed by blending
5) Random sampling for isotopic variance testing

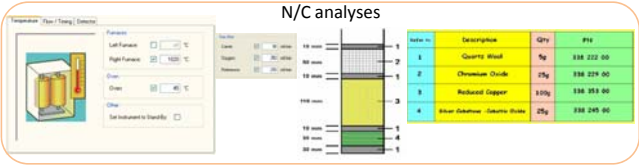
Analyses



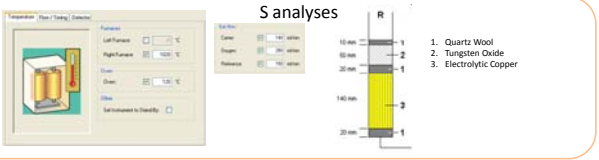
❑ Isotopic analyses in the "Laboratorio de Isótopos Estables" of the Estación Biológica de Doñana (LIE-EBD) were carried by means of a Thermo-Scientific Flash HT Plus elemental analyzer coupled to a Delta-V advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific)

❑ Analyses of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ were performed in Dynamic Flash Combustion mode, and δ^2H and $\delta^{18}O$ by High Temperature Conversion, with the following configurations:

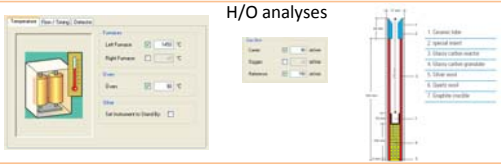
N/C analyses



S analyses



H/O analyses



Result

BB $\delta^{15}N$= +9.97 +/- 0.07 air (n=6) $\delta^{13}C$= -18.6 +/- 0.08 V-PDB (n=6) $\delta^{34}S$= +19.17 +/- 0.2 VCDT (n=2)* δ^2H = -46 +/-1.8 ‰VSMOW (n=11) **	**CV** $\delta^{15}N$= +10.27 +/- 0.08 air (n=5) $\delta^{13}C$= -22.24 +/- 0.07 V-PDB (n=5) $\delta^{34}S$= +10.89 +/- 0.2 VCDT (n=2)* δ^2H = -82.1 +/-1.8 ‰VSMOW (n=3) ** $\delta^{18}O$ = +16.69 +/-0.3 ‰ VSMOW (n=3) **
PA $\delta^{15}N$= +16.47 +/- 0.09 air (n=8) $\delta^{13}C$= -15.69 +/- 0.08 V-PDB (n=8) δ^2H = +24 +/-1.2 ‰VSMOW (n=9) **	**Spectrum Keratin Fine Powder Lot # 2AJ3011***** $\delta^{15}N$ = 6.02 +/-0.04 ‰ air (n=5) $\delta^{13}C$ = -15.02 +/-0.03 ‰ V-PDB (n=5)

*Pending for validation at other stable isotope laboratories.
**Isotopic values were normalized with Caribou Hoof Standard (CBS) and Kudu Horn Standard (KHS) supplied by Environment Canada, which are pending for international validation.
***Powdered keratin from Spectrum Chemicals.

BB and CV were simultaneously analyzed at LIE-EBD, the Reston Stable Isotope Laboratory (RSIL, U.S. Geological Survey, USA), and the Stable Isotope Hydrology and Ecology Research Laboratory (SIHERL, Environment Canada, Canada), whereas PA has been exclusively analyzed at LIE-EBD and SIHERL.

To the best of our knowledge, no positive standards for δ^2H are available to apply a suitable correction to PA. This material was normalized with CBS and KHS with value of δ^2H of -197 and -54.1 respectively. Therefore, this value is provisional, pending of further calibrations. At the present, PA is the only available keratin standard with positive value for δ^2H analyses.

Acknowledgements. We thank to Carlos Gutiérrez Expósito, Renaud de Stephanis and the Scientific Collections of the EBD for providing pictures and samples. We also thank RSIL and SIHERL for the complementary analyses