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Direct Analysis of δ^2H and δ^{18}O in Natural and Enriched Human Urine Using Laser-Based, Off-Axis Integrated Cavity Output Spectroscopy

Elena S. F. Berman,*[,†] Susan L. Fortson,[†] Steven P. Snaith,[†] Manish Gupta,[†] Isabelle Chery,[‡] Stephane Blanc,[‡] Edward L. Melanson,[§] Peter J Thomson,[⊥] and John R. Speakman[⊥,∥]

[†]Los Gatos Research, 67 East Evelyn Ave, Suite 3, Mountain View California 94043, United States

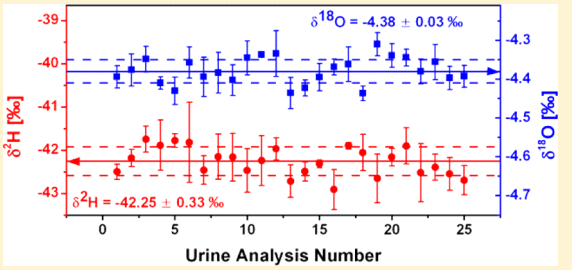
[‡]Department of Ecology, Physiology and Ethology, Hubert Curien Multidisciplinary Institute, 23 rue Becquerel, 67087 Strasbourg cedex 2, France

[§]Division of Endocrinology, Metabolism, and Diabetes/Division of Geriatrics, University of Colorado Anschutz Medical Campus, 12801 East 17th Ave, Aurora, Colorado 80045, United States

[⊥]Institute of Biological and Environmental Sciences, University of Aberdeen, Tillydrone Ave Aberdeen, AB24 2TZ, Scotland, U.K.

[∥]State Key laboratory of Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

ABSTRACT: The stable isotopes of hydrogen (δ^2H) and oxygen (δ^{18}O) in human urine are measured during studies of total energy expenditure by the doubly labeled water method, measurement of total body water, and measurement of insulin resistance by glucose disposal among other applications. An ultrasensitive laser absorption spectrometer based on off-axis integrated cavity output spectroscopy was demonstrated for simple and inexpensive measurement of stable isotopes in natural isotopic abundance and isotopically enriched human urine. Preparation of urine for analysis was simple and rapid (approximately 25 samples per hour), requiring no decolorizing or distillation steps. Analysis schemes were demonstrated to address sample-to-sample memory while still allowing analysis of 45 natural or 30 enriched urine samples per day. The instrument was linear over a wide range of water isotopes (δ^2H = −454 to +1702 ‰ and δ^{18}O = −58.3 to +265 ‰). Measurements of human urine were precise to better than 0.65 ‰ 1σ for δ^2H and 0.09 ‰ 1σ for δ^{18}O for natural urines, 1.1 ‰ 1σ for δ^2H and 0.13 ‰ 1σ for δ^{18}O for low enriched urines, and 1.0 ‰ 1σ for δ^2H and 0.08 ‰ 1σ for δ^{18}O for high enriched urines. Furthermore, the accuracy of the isotope measurements of human urines was verified to better than ±0.81 ‰ in δ^2H and ±0.13 ‰ in δ^{18}O (average deviation) against three independent isotope-ratio mass spectrometry laboratories. The ability to immediately and inexpensively measure the stable isotopes of water in human urine is expected to increase the number and variety of experiments which can be undertaken.



Analysis of the stable isotopes of hydrogen (δ^2H) and oxygen (δ^{18}O) in human body water is used in a variety of biomedical applications including measurement of total energy expenditure (TEE) by the doubly labeled water (DLW) method,[1−3] measurement of total body water,[4] and measurement of insulin resistance by glucose disposal[5,6] among other applications. Currently, the vast majority of studies use isotope-ratio mass spectrometry (IRMS) for analysis of δ^2H and δ^{18}O in body waters. For IRMS analysis, bodily fluids (e.g., urine) require either extensive purification, such as cryogenic distillation followed by decolorization,[7] or analysis by CO_2 equilibration for ^{18}O measurements and zinc or chromium reduction for ^{2}H measurements.[8,9] These preparation methods and IRMS analyses are labor-intensive, costly, and limited to only a few measurement laboratories worldwide. However, in order for the aforementioned biomedical applications to become widely available, measurements of a large number of samples must be completed quickly, accurately, and inexpensively, preferably at a location near the site of sample generation.

Ultrasensitive laser absorption spectroscopy, such as off-axis integrated cavity output spectroscopy (OA-ICOS) and cavity ring down spectroscopy (CRDS), provides the opportunity to measure δ^2H and δ^{18}O rapidly, accurately, and inexpensively.[10−12] Furthermore, laser-based instrumentation does not require highly trained operators and has a small footprint, allowing measurements to be made by researchers generating the samples. While studies have shown that laser-based instruments require corrections for organic contamination of samples,[11,13,14] two laboratories have recently shown that the organic component of urine does not adversely affect laser-based isotope measurements.[7,15] O'Grady et al. utilized CRDS to measure natural isotopic abundance human urines that had been either cryogenically distilled or decolorized with carbon black.[7] Thorsen et al. used CRDS to measure natural and

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