FOOD ANALYSIS

APPLICATION OF THE AGILENT 4500 SERIES FTIR TO THE STABLE ISOTOPE TECHNIQUE FOR ASSESSING INTAKE OF HUMAN MILK IN BREASTFED INFANTS

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SOLUTION NOTE

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Abstract

The World Health Organization recommends that infants should be breastfed for the first six months of their lives, prior to introduction of other foods, which is in agreement with UN Millennium Development Goals.

Introduction

The method for assessing the actual amount of milk consumed by infants has been problematic, since the conventional approach is to weigh infants before and after feeding, which is time consuming and can affect feeding patterns.

In the 1980's, a method was developed [1] for measuring the amount of milk consumed that uses nonradioactive deuterium oxide as a traceable analyte. In this method, a mother consumes a bolus of deuterium oxide (^2H_2O), and the elimination of the ^2H_2O isotope in the mother and the appearance of the isotope in the baby is measured as a function of time from samples taken of their saliva. Deuterium oxide is passed to the child during breast feeding and thus the amount of breast milk that has been consumed by the child is determined.

Two analytical methods can be employed for measuring the amount of deuterium oxide in saliva: isotope ratio mass spectrometry (IRMS) and Fourier transform infrared spectroscopy (FTIR). The former technique is more sensitive, but the instrumentation is more costly and requires more expertise to use. The FTIR method requires the consumption of larger amounts of deuterium oxide, but tracer costs are low and it uses less costly instrumentation that is easier to maintain. Therefore, FTIR is preferred in regions where funding is limited.

Part of the mandate of the International Atomic Energy Agency (IAEA) is to provide information on the use of nuclear techniques for promoting human health, and as such, the IAEA publishes information on a number of practical uses in medicine, including stable (nonradioactive) isotope tracer techniques. In the IAEA Human Health Series No. 7 [2], the procedure for assessing the amount of breast milk that is consumed by an infant through the deuterium oxide method is described in detail. Chapter 4 of that publication specifically discusses the method for determining the amount of deuterium oxide present in saliva using FTIR spectroscopy.

The method has been applied successfully in a number of locations worldwide. As described, it uses a bench top, lab FTIR and a traditional liquid transmission cell for carrying out the measurement. This application note addresses the numerous advantages of using the Agilent 4500 Series FTIR, a battery powered, fully portable system, equipped with Agilent exclusive TumblIR sample technology, for measuring the amount of deuterium oxide in saliva (Figure 1).

Instrumentation

When the seminal work on measuring the levels of deuterium oxide in saliva was originally developed, the cost effective FTIR technology available at that time consisted of a routine, bench top FTIR that used an internally mounted CaF_2 liquid transmission cell. This older technology necessitates certain use and environmental requirements to ensure analytical accuracy and precision. For example, the FTIR instrument needs to be placed in a temperature controlled room on a bench top that is free of vibration. Since this instrumentation has an internal sample compartment, it is necessary to clean up any liquid spills and to purge the sample compartment to eliminate atmospheric carbon dioxide and water vapor interferences.

Figure 1. Agilent 4500 Series FTIR, a battery powered, portable FTIR, equipped with 100-µm pathlength TumblIR sample interface for the measurement of ^2H_2O.

The CaF_2 sealed cells must be carefully filled to ensure that bubbles are not present, which would likely affect the results. Also, the cells tend to easily scratch, and leakage is an issue with classical liquid transmission cells.

The Agilent 4500 Series FTIR equipped with TumblIR Sample Interface represents a completely new approach to measuring deuterium oxide in body fluids through FTIR. The Agilent system is designed and engineered to remove many of the constraints imposed by using a conventional bench top FTIR, as described in the original method. These advantages include:

- Rugged opto-mechanics and electronics—The 4500 Series FTIR is intended for field use, that is, it is portable. It does not need to be placed on a vibration-free bench top.
- The system can operate at peak efficiency without the necessity for an air-conditioned, humidity controlled environment
- TumblIR Technology is a far more convenient transmission IR sample interface than the traditional CaF_2 sealed liquid cell described earlier. This Agilent exclusive technology eliminates all of the issues associated with using classical sealed liquid cells. Since the new sample technology is far easier to use, the likelihood of operator error is reduced.

- There is no internal sample compartment—All sample measurements are carried out on the outside top surface of the instrument. Samples cannot be spilled inside the instrument, as is the case with traditional FTIR with an internal sample compartment.
- Since power is provided by an on-board battery, local fluctuations in power supply do not have any influence on the instrument or on the accuracy of the measurement results.
- The sample technology is never removed from the instrument for sample loading or post analysis cleanup, as is required for the CaF_2 sealed cell. Analytical accuracy and reproducibility is optimized and no re-alignment,which can affect results, is ever necessary.
- The TumblIR technology requires less sample size than conventional CaF_2 liquid transmission cells. Only 50 µL of sample is required for accurate results using the TumblIR compared to 1 mL required for traditional liquid cells. This is very helpful since it is difficult to obtain large saliva samples from infants.
- The ZnSe window material used in the TumblIR technology is suitable for analysis of saliva, water, blood, serum, and urine.
- The instrument is controlled through a handheld computer and software is highly visual, intuitive, and straightforward to use.

TumblIR and DialPath Technology:
A better sample interface for measuring deuterium oxide in human body fluids

The Agilent TumblIR and DialPath sample interfaces are proprietary, innovative methodologies for measuring liquids, and offer many advantages over traditional sealed transmission cells. The TumblIR technology provides a single, 100-µm pathlength transmission capability for the measurement of deuterium oxide in saliva.

The DialPath sample interface features an optical head that can be rotated to select one of three factory-calibrated, fixed pathlengths varying from 30 to 200 µm. To analyze a sample, a drop of the liquid sample is placed on a stationary infrared-transparent window mounted on the top surface of the analyzer, and then a second window is rotated into position, thus sandwiching the sample between the two windows (Figure 2).

This creates a highly reproducible, fixed sample pathlength between the two windows. After analysis, simple wiping cleans the two windows and prepares the device for the next sample. This methodology has many advantages over the older transmission cell technology:

Figure 2. TumblIR operation: Place sample on window; rotate second window into position; analyze sample; clean windows by wiping.

- No spacers are required—cell leakage and fringing are eliminated.
- No syringes are needed to introduce the sample.
- Liquids of varying viscosity can be handled with equal effectiveness.
- Volatile solutes and solvents are accurately measured.
- Sample cleanup is very quick.
- Samples can be analyzed rapidly.
- No supplies or consumables (cell windows, spacers, cleaning solvents and so forth) are required.
- DialPath technology permits nearly instantaneous selection of three different pathlengths as needed.
- No disassembly is required to change pathlengths.

Results and Discussion

The experimental procedure to measure ^2H_2O in aqueous media using the Agilent 4500 FTIR and 100-µm TumblIR sample interface uses drinking water as background for the deuterated water calibrants. Predose or basal saliva is used as matrix background for each post-dose saliva sample. In this manner, the broad O-H water absorbance at approximately 3,300 cm^{-1} is minimized and the O-D absorbance band at 2,500 cm^{-1} can be readily observed (Figure 3).

A series of standards were prepared ranging from 0–3,000 mg/kg ^2H_2O and measured by the 4500 Series FTIR and by isotope ratio mass spectroscopy. The two data sets show excellent agreement with a R2 value of 1.000 (Figure 4).

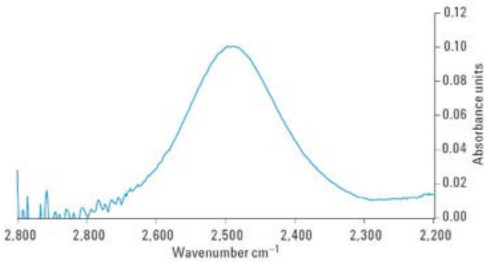

Figure 3. O-D absorbance band in spectrum measured with Agilent 4500 Series FTIR with 100-µm pathlength TumblIR sample interface.

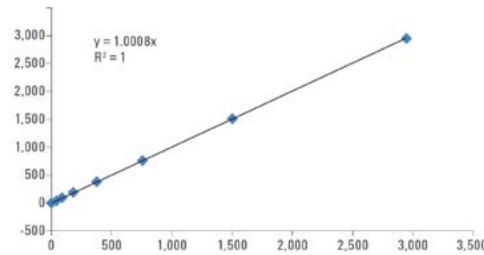

Figure 4. Plot of ^2H_2O concentration as measured by IRMS and an Agilent 4500 Series FTIR shows excellent correlation.

Measurements recorded of 50 µL saliva samples from an adult male after consumption of a 30 g ^2H_2O bolus demonstrate the expected performance, following the IAEA Dose-to-Mother protocol (Figure 5).

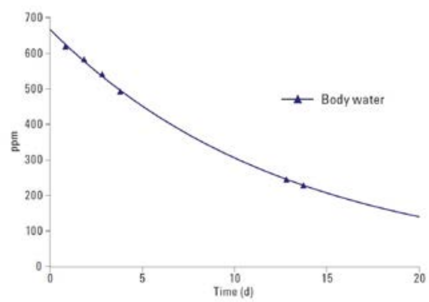

Figure 5. Loss of ^2H_2O in saliva as a function of time as measured by Agilent 4500 Series FTIR. Expected behavior following IAEA Dose-to-Mother protocol is observed. In this example, body water was calculated to have a mean residence time of 13 days.

The measurement of ^2H_2O concentrations by the 4500 FTIR with TumblIR has been extended to include levels in serum and in urine. The results for measurement in blood mirror that for the analysis of ^2H_2O in saliva, that is, excellent correlation with IRMS measurements.

For urine, the results are acceptable, but some mathematical refinement of the spectra is required due to the presence of urea, which interferes with the O-D stretching vibration. Further work will explore improved mathematical treatment of the spectra to minimize the effect of the organics present in urine on the analytical accuracy of the ^2H_2O measurement. The intent is that each method will have an associated function that automatically removes spectral interference.

To date, the following limit of detection (3 × SD) values have been established for the measurement of ^2H_2O in body fluids by the 4500 Series FTIR equipped with DialPath:

- Water 12 mg/kg
- Serum 18 mg/kg
- Saliva 20 mg/kg
- Urine 50 mg/kg

Conclusion

This application note outlines the benefits derived from using the Agilent 4500 Series FTIR and DialPath Sample Interface for the measurement of ^2H_2O in saliva in support of breastfed milk uptake studies. The major advantages of this new technology for the application are as follows:

- The Agilent 4500 FTIR is a highly compact, battery powered, portable FTIR designed for field use. It is far more robust than traditional laboratory based FTIR systems and can be used in remote locations without concern for local power fluctuations or other variable ambient conditions. This enables the analysis to be carried out directly on-site and minimizes the need to transport samples or require a subject to travel to a distant lab.
- The overall performance of the Agilent 4500 FTIR with TumblIR or DialPath technology provides data that is equivalent to or better than that obtained from the conventional lab FTIR and sealed liquid cell approach.
- The TumblIR sample interface eliminates the all the difficulties associated with using classic sealed transmission cells and the effect of these inherent problems on measurement accuracy.
- In summary, the Agilent 4500 FTIR system and supporting analysis methodology is far easier to use, implement, and maintain. This increases the likelihood of accurate analytical results as well as increases the number of samples that can be processed per time unit.

The availability of this new technology offers expanded capability for the measurement of ^2H_2O in body fluids as well as new possibilities for deployment of FTIR technology. Moreover, other applications where the measurement of ^2H_2O in aqueous media is important, such as in body composition analysis, will also be positively influenced by this new capability.

References

This application note outlines the benefits derived from using the Agilent 4500 Series FTIR and DialPath Sample Interface for the measurement of ^2H_2O in saliva in support of breastfed milk uptake studies. The major advantages of this new technology for the application are as follows:

1 - W.A. Coward, et al. "Breast-Milk Intake Measurement in Mixed-Fed Infants by Administration of Deuterium Oxide to Their Mothers" Hum. Nutr. Clin. Nutr. 36C (1982) 141-148.

2- IAEA Human Health Series No. 7, Stable Isotope Technique to Assess Intake of Human Milk in Breastfed Infants, International Atomic Energy Agency, Vienna (2010).

Aknowledgment

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