Precision Isotope Measurements using FT-IR
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Energy from infrared (IR) radiation is generally insufficient to cause the electronic transitions commonly seen in UV-VIS spectroscopy, however, IR photons are energetic enough to cause transitions between vibrational and/or rotational states. If the vibrational or rotational frequency of a molecular dipole exactly matches that of the incident IR radiation, absorption can occur causing a net increase in the vibrational amplitude or rotational frequency of that dipole. Only those molecules containing a permanent dipole moment can absorb infrared radiation and are said to be IR active. Rotational transitions are of relatively low energy and are limited primarily to the far IR region (0.50 – 1,000 μm or 200 – 10 cm^{-1}), whereas vibrational transitions are more energetic and found predominantly in the mid IR region (2.5 - 50 μm or 4,000 - 200 cm^{-1}) [1].

Vibrations within a molecular dipole can be categorized as either bending or stretching: A bend is a change in the bond angle of the dipole, whereas a stretch is a change in the distance between the two atoms of the dipole. A molecule with a permanent dipole can exhibit many types of bends or stretches, only a few of which may be IR active. For example, the symmetric stretch of a triatomic molecule such as CO_2 results in no net change in dipole moment and is therefore IR inactive while the asymmetric bend produces a change in dipole moment and is IR active.

Until the 1980’s, examination of the mid IR was primarily through dispersive instruments commonly used for UV_VIS inspection. The advent of Fourier Transform IR (FT-IR) instrumentation made this region of the spectrum much more accessible.

The heart of most commercially available FT-IR instruments today is the Michelson interferometer. This spectrometer consists a “hot” source that produces light of many closely spaced frequencies. The incident light impinges on a beam splitter that directs half of the beam to a fixed mirror and the other half to a movable mirror, which continually moves back and forth. These two beams are reflected, recombine, pass through the sample, which may absorb light at several frequencies and are measured by the detector. Constructive or destructive interference of these two beams is mitigated by the position of the movable mirror. The resulting interferogram is a plot of output power of the detector vs. mirror position. The frequency of this interferogram is directly proportional to the frequency of the absorbed light. The interferogram obtained is then converted by Fourier Transform to a plot of intensity vs. frequency or wavenumber.

Kindness and Marr [2] describe a novel “simple IR” method to measure the $^{13}C/^{12}C$ isotopic ratio in CO_2 utilizing a technique termed “pressure broadening”. In this methodology, a known quantity of analyte gas is added to the IR cell followed by a high-pressure non-IR absorbing gas. This effectively broadens the very fine rotational lines, reducing distortion due to finite slit width.

A Nicolet Model 7199 FT-IR was used with 1 cm^{-1} resolution and a path length of 8.04 cm. For both background and sample, 100 scans were signal averaged. Separate pressure gauges were used to monitor both the sample gas (CO_2 at 9 Torr) and the broadening gas (N2 at 10 bar). A mixed isotope calibration curve ranging from 0.1 to 5 atom % ^{13}C was prepared using known amounts of ^{13}C and ^{12}C $BaCO_3$ boiled in phosphoric acid. Organic samples were placed in a combustion vessel that was subsequently evacuated to remove natural CO_2. The vessel was then filled with oxygen and the sample combusted using two quartz-iodine lamps. In both the standard and sample preparation, the evolved CO_2 was trapped with liquid nitrogen. Calibration curve data was plotted as 0.5*(area of P branch of $^{13}CO_2$)/ (total P + R area of $^{13}CO_2 + ^{12}CO_2$) and although no calibration curve data was provided, the “typical” calibration curve plotted appeared to be linear from 0.100 to 5.005 atom percent ^{13}C.

An example of this pressure broadening technique can be seen on the following page:

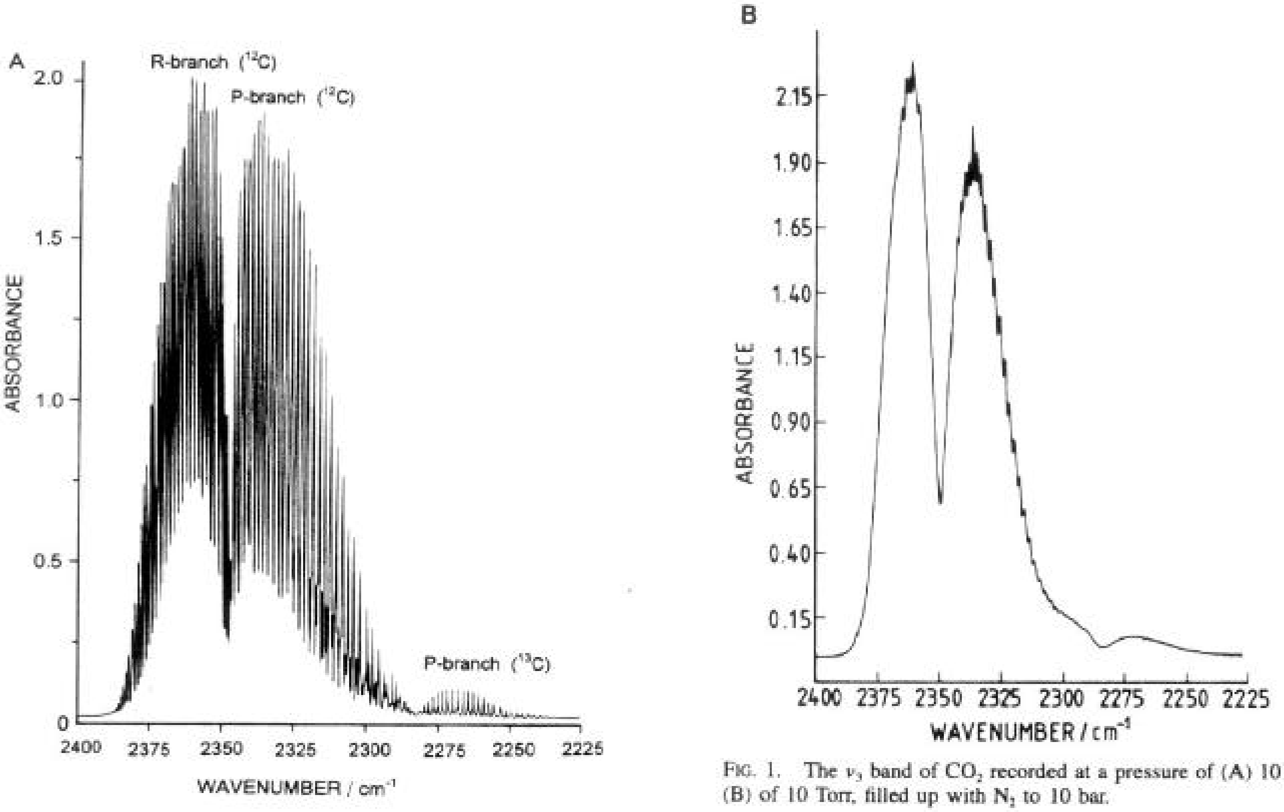

FIG. 1. The ν_3 band of CO_2 recorded at a pressure of (A) 10 Torr and (B) of 10 Torr, filled up with N_2 to 10 bar.

Pressure broadening in this example resulted in a forty fold increase in area of the broadened band relative to the non-broadened band. Relative standard deviation of repeated analyses at the natural abundance level of $^{13}CO_2$ (1.110 atom percent ^{13}C) was reported as 0.78 % with a minimum level of detection of 0.017 atom percent ^{13}C above natural abundance. The primary reason for this increase in precision relative to earlier work was the great signal to noise enhancement achieved by “deresolving” the finer rotational lines observed in the non-broadened band.

Esler et al [3,4] have developed a novel approach to precisely determine the $^{13}C/^{12}C$ isotopic ratio of CO2 in breath and air calibrating with synthetically calculated absorbance spectra. A Bomem MB100 FT-IR spectrometer, with a 1 cm^{-1} maximum resolution, globar source and a variable path length cell were encased in a thermostated box purged with dried nitrogen to minimize instrumental drift. Sample temperature and pressure were precisely monitored at the IR cell. Sample averaging over 64 scans was used to minimize high frequency noise.

Analysis of absorbance spectra was accomplished by a three-step process. Initially, a set of absorbance spectra, which closely approximated that obtained on the actual instrument, was generated by the multiple atmospheric layer transmission program (MALT). This program utilizes a database (HITRAN) containing the line frequency, integrated line strength and the pressure and temperature dependent half width data of each absorbance line for each molecule in the data set. A classical least squares algorithm was then applied to the generated spectra to produce a calibration matrix, which relates concentration to absorbance strength for the spectral region and the compounds or isotopomers included in the calibration data set. This calibration matrix was subsequently used to fit the generated spectrum to the spectrum obtained experimentally. In effect, this process treated each isotopomer of CO_2 as an independent species. This calibration procedure was also utilized to determine the optimum spectral window for each species within the data set (see figure above).

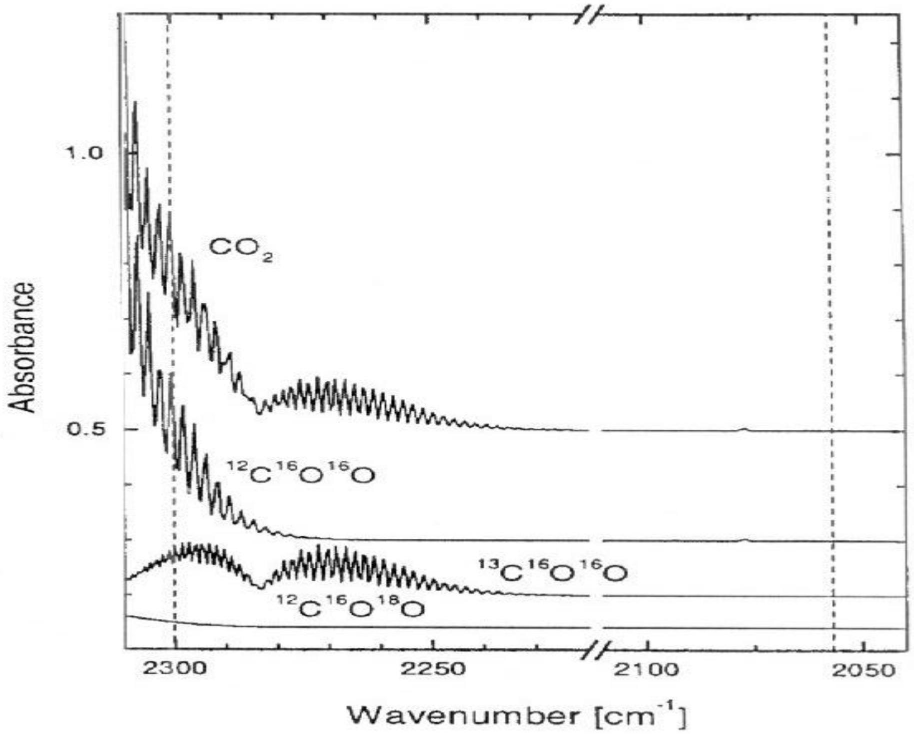

Figure 2. Optimal spectral window (bounded by dashed lines) for $^{12}CO_2$, $^{13}CO_2$, and hence $\delta^{13}CO_2$ analysis, 2300−2060 cm^{-1}.

This modeling method has also been proposed for the resolution and analysis of deuterated water (see below), with a predicted precision of 1 part per thousand.

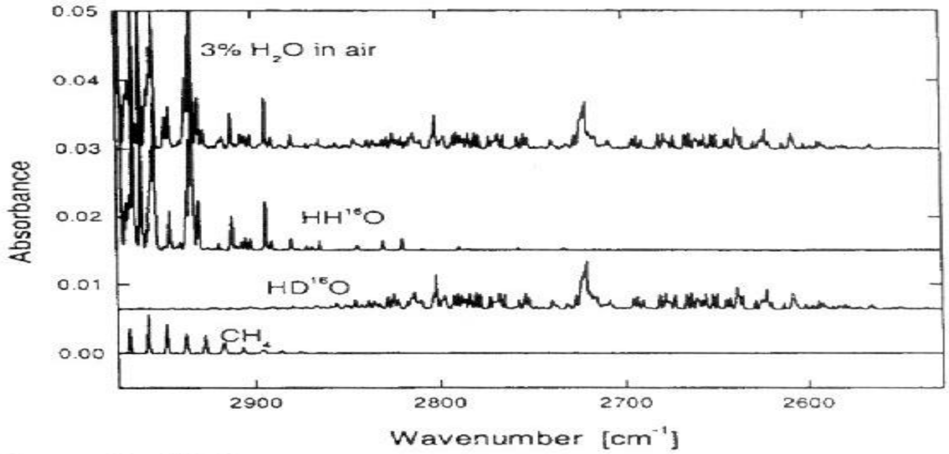

Figure 7. FT-IR spectrum of moist air showing that the deuterated water isotopomer $HD^{16}O$ is resolved from the parent species $HH^{16}O$ at low (1-cm^{-1}) spectral resolution.

This method reports precision for the $^{13}C/^{12}C$ isotopic ratio of CO2 in breath on the order of 0.1 parts per thousand, which is only slightly higher than that obtainable by dual inlet Isotope Ratio Mass Spectrometry. This high level of precision can be attributed to several factors; namely, the correct choice of the optimal spectral window, minimization of detector noise by cooling the detector in liquid nitrogen, limiting high frequency noise by signal averaging and, finally, by minimizing low frequency noise (instrument or baseline drift) by the modeling process, precise temperature and pressure monitoring at the IR cell and by enclosing the instrument in a thermostated box.

In the references cited above, isotope dilution studies have primarily focused on gas analysis of the enrichment of $^{13}CO_2$ relative to natural CO_2 in both atmospheric samples and human breath. In another application of this technique, Diehl et al [5] employed precision isotope measurements by GC\FT-IR for the accurate and precise quantitation of aromatic hydrocarbons (benzene, toluene, ethylbenzene, o-,m- and p-xylene, collectively referred to as BTEX) in reformulated gasoline. Calibration curves were prepared using the perdeuterated analogue of each BTEX component as an internal quantitation standard. Standard components were separated by gas chromatography (see chromatogram below),

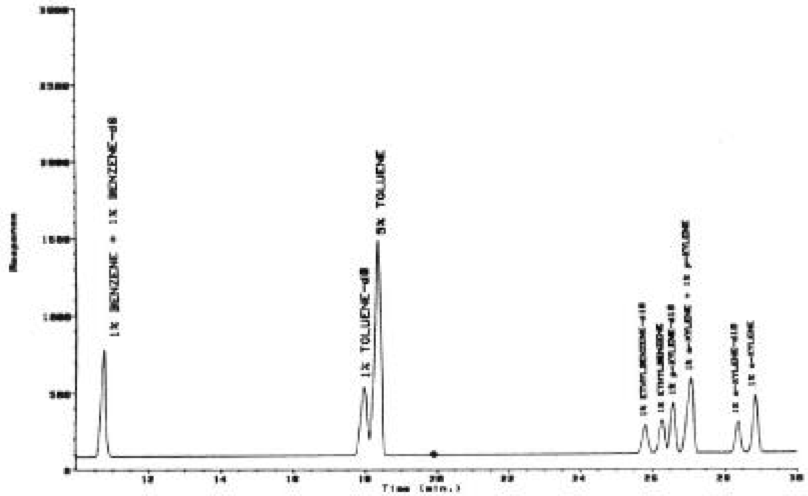

Figure 4. Gram–Schmidt chromatogram of the analytes and their respective internal standards. Some pairs such as ethylbenzene and ethylbenzene-d_{10} are completely resolved while others such as benzene and benzene-d_6 are not separated at all. Coelution had no adverse effect on quantitation.

and then analyzed by FT-IR with resolution of 8 cm^{-1}. For each spectrum, six interferograms were signal averaged. Second difference reconstruction was used to increase signal to noise. Spectra collected over the first 0.5 minutes of each chromatogram were averaged to provide a reference spectrum for this reconstruction. The frequencies monitored result from aryl C-H out-of-plane bending for the unlabelled standards and samples, and from aryl C-D stretching for the perdeuterated internal standards (see table below).

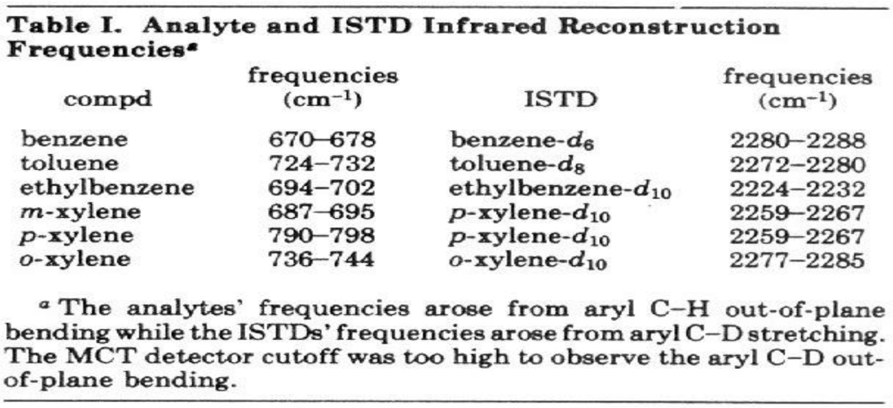

Table I. Analyte and ISTD Infrared Reconstruction Frequencies[a]

compd	frequencies (cm^{-1})	ISTD	frequencies (cm^{-1})
benzene	670–678	benzene-d_6	2280–2288
toluene	724–732	toluene-d_8	2272–2280
ethylbenzene	694–702	ethylbenzene-d_{10}	2224–2232
m-xylene	687–695	*p*-xylene-d_{10}	2259–2267
p-xylene	790–798	*p*-xylene-d_{10}	2259–2267
o-xylene	736–744	*o*-xylene-d_{10}	2277–2285

[a] The analytes' frequencies arose from aryl C–H out-of-plane bending while the ISTDs' frequencies arose from aryl C–D stretching. The MCT detector cutoff was too high to observe the aryl C–D out-of-plane bending.

Although calibration curve data was not provided, response for each BTEX component relative to its deuterated analogue was reported as linear. Repeated BTEX analyses had an average relative standard deviation of 0.8% and an average percent accuracy of 0.5% when compared to alternative analytical techniques. Improvements in precision compared to earlier works of this type result from second difference reconstruction as well as selection of the proper reconstruction frequencies.

In the references cited here, FT-IR may, in some cases, provide a “lower” cost alternative to mass spectrometric techniques for isotope dilution analysis. Although the signal to noise enhancements discussed for each of the preceding examples can dramatically increase the measurement precision and lower the detection limits relative to earlier works, both are still at least an order of magnitude (and often more) away from those attainable by IRMS and/or GCMS. In situations where absolute precision is not required, FT-IR may be a feasible methodology for isotope precision measurements.

References

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