Simple Sampling Method and Routine Gas Chromatography-Mass Spectrometry Analysis in $^{13}$C Breath Tests, Ágnes Keszler, László Kötaí, and Klára Szentmihályi (Institute of Chemistry, Chemical Research Center, Hungarian Academy of Sciences, P.O. Box 17, H-1525 Budapest, Hungary; * author for correspondence: fax 36-1-325-7554, e-mail agi@cric.chemres.hu)

The $^{13}$C-labeled CO$_2$ breath tests are convenient methods for various uses. They can be used to measure gastric emptying rate of solids (1), detect fat malabsorption (2), and evaluate pancreatic lipase activity (3), exocrine pancreatic insufficiency (4), glucose adsorption and utilization (5), liver function control (6), and lactase deficiency (7) with appropriate $^{13}$C isotope-labeled compounds. In recent years, the $^{13}$C-urea breath test has been used to detect Helicobacter pylori (8).

Although several infrared or mass spectrometric methods (9) are available, the transport of large volumes of gaseous samples to analytical centers as well as storage before measurement involves great problems. Therefore, we have developed a simple method that allows storage of these samples in minimal liquid volume. By this technique, the liberation of isotope-labeled CO$_2$-containing gas occurs immediately before measurements. Because most medical centers are not equipped with expensive and complex isotope-ratio mass spectrometers but generally have a routine low-mass-resolution gas chromatography-mass spectrometry (GC-MS) apparatus, we have also used a simple detection method for $^{13}$CO$_2$ by standard GC-MS techniques. Analysis requires only a few minutes, and the method is suitable for selection of $^{13}$CO$_2$-containing samples.

In our test experiments, we used an air stream containing $^{13}$CO$_2$ obtained from $^{13}$C-labeled carbamide tablets or from $^{13}$C-labeled benzoic acid. Their $^{13}$C content was known. The CO$_2$ content of the air was absorbed in a 2 mol/L ethanolic solution of 3-methoxypropylamine (total from 13C-labeled benzoic acid. Their 13C content was solution by the addition of 0.1 mL of sulfuric acid. The

**MAT GCQ type GC-MS apparatus. A medium polarity CP WAX 52CB (ChromPack) column (30 m × 0.25 mm i.d.; 0.25-μm film thickness) was used. The column temperature setting was programmed from 40 °C (hold for 5 min) with a 4 °C/min increase up to 140 °C (hold for 10 min), followed by a 20 °C/min increase up to 210 °C. The injection temperature was kept at 220 °C. The linear velocity of the helium carrier gas was 35 cm/s. We used the electron impact (EI$^+$) mode at 70 eV electron energy in a mass range of 10–650 atomic mass units (amu).

To facilitate handling and transportation of the gaseous samples, we absorbed the CO$_2$ content of the gas samples in a small volume of storage liquid. Although CO$_2$ is absorbed in water or in alkaline solutions (10), rapid and quantitative absorption can hardly be achieved in these solutions. As has been described (11, 12), some organic amine-containing nonaqueous solutions are more suitable for CO$_2$ absorption. These solutions contain the carbamate anions formed and the protonated ammonium cations of the starting amine (11, 12). Therefore, the use of 2-phe nylylamine, ethanolamine, or 3-methoxypropylamine (12) in pure form or dissolved in alcohol-type solvents is preferable. Because the absorption of CO$_2$ is most efficient in 3-methoxypropylamine with a high gas bubbling rate (12), we used the ethanolic solution of this amine in our experiments.

The reaction can be described as follows:

$$
\text{MeO-CH}_2\text{CH}_2\text{CH}_2\text{NH}_2 + \text{CO}_2 =
\text{MeO-CH}_2\text{CH}_2\text{CH}_2\text{NH-C(=O)}^- + \text{H}_3\text{N-CH}_2\text{CH}_2\text{CH}_2\text{OMe}
$$

The salt formed is stable in ethanolic solution, and the volume of the sample container is small; it is easy to handle and greatly facilitates storage and transportation. The samples are acidified at the site of measurement when the free carbamic acid formed, which is unstable in acidic solution, immediately decomposes to amine and CO$_2$. The amine formed is fixed in salt form in the acidic solution so that equilibrium is shifted completely toward the liberation of CO$_2$. The gas sample is removed from the sealed sampling vessel via a septum and is measured directly by GC-MS. The retention time of the CO$_2$ gas is short (1.4 min); thus, the measurement time is short (10 min). The relative amount of $^{13}$CO$_2$ can be readily distinguished from mass spectra obtained from the extracted ion chromatogram (M = 44 or 45, $^{12}$CO$_2$ or $^{13}$CO$_2$). The chromatographic peak-area ratio of the 44- or 45-amu ions depends on the $^{13}$CO$_2$ content of the air sample. We studied the correlation between the $^{13}$C content of the gas and the $^{12}$CO$_2$/$^{13}$CO$_2$ peak-area ratios (Table 1). This peak-area ratio continuously decreased from the nonlabeled sample to the undiluted, labeled sample.

This method is quick and inexpensive, and the presence or absence of $^{13}$CO$_2$ can be readily observed. Although the analytical method applied typically is qualitative, by calibration of the mass detector it may be extended to quantitative measurements. The relationship between $^{13}$CO$_2$ content and the peak-area ratio of the 44/45 peak
was linear (Table 1). At higher dilutions, the $^{13}$C content is very low (close to the natural abundance of $^{13}$C), and the accuracy decreases because of the error of sample handling and GC-MS measurement.

The $^{13}$CO$_2$ content of expired air was measured by both infrared and GC-MS methods (9), and the results were in good agreement ($r^2 = 0.992$). Comparison of these data with histological results showed that the clinical sensitivity of both methods (infrared and GC-MS) was ~95%, and the specificity was 70–80%. The concentration of $^{13}$CO$_2$ in the expired air was high and, after 15 min, quasi-constant (9). The recovery of CO$_2$ from the solution was nearly quantitative (Table 1). Because we used exact selective-ion monitoring chromatography, the accuracy of this method is currently in progress.

To simplify sample treatment, we have also studied the possibility of direct measurements of other fragments of identical mass from $^{13}$C-containing materials in liquid phase. The isotope-labeled carbon derives from the carboxyl group of carbamate salt. The signals of the molecular ions (M) and of its $^{13}$C-containing fragments (F), chosen for M/M+1 or F/F+1 peak selective monitoring, show low intensity, and some other fragments of identical mass may produce interfering signals.

Because the molecular ions of N-alkylated carbamic acids are stable during GC-MS analysis, the M/M+1 and F/F+1 peak ratios can be easily measured by this technique. The study of the absorption and release of CO$_2$ by the use of secondary amines (with N-alkylcarbamate formation) is currently in progress.

We thank Dr. J. Szammer for synthesizing the $^{13}$C-labeled benzoic acid.

**Table 1. Correlation between the $^{12}$CO$_2$/$^{13}$CO$_2$ peak-area ratios in the chromatogram and the $^{13}$CO$_2$ content of the expired air.**

<table>
<thead>
<tr>
<th>No.</th>
<th>$^{12}$CO$_2$/$^{13}$CO$_2$ peak-area ratio</th>
<th>$^{13}$CO$_2$ content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.19</td>
<td>50.0</td>
</tr>
<tr>
<td>2</td>
<td>0.85</td>
<td>66.7</td>
</tr>
<tr>
<td>3</td>
<td>0.25</td>
<td>100.0</td>
</tr>
</tbody>
</table>

$^a$ Normalized to the maximal value.

$^b$ Recovery of $^{13}$CO$_2$ in the expired gas.

**References**


**Effect of Oral Creatine Supplementation on Random Urine Creatinine, pH, and Specific Gravity Measurements, Jeri D. Ropero-Miller,1 Helen Page-Wilkes,2 Paul L. Doering,3 and Bruce A. Goldberger4 (1 Office of the Chief Medical Examiner, Campus Box 7580, Chapel Hill, NC 27599-7580; 2 Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, FL 32610-0275; 3 Department of Pharmacy Practice, University of Florida College of Pharmacy, Gainesville, FL 32610-0486; * address correspondence to this author at: Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, P.O. Box 100275, Gainesville, FL 32610-0275; fax 352-846-1586, e-mail bruce-goldberger@ufl.edu)**

Forensic urine drug-testing laboratories routinely evaluate the suitability of urine specimens to assess potential adulteration, substitution, or dilution. For example, the measurement of urinary creatinine and specific gravity is performed by these laboratories to determine whether a specimen is abnormally dilute (1).

Creatine is synthesized endogenously and is stored in skeletal muscle in a high-energy phosphorylated form. During muscle contraction, creatine and creatine phosphate are spontaneously converted to creatinine. Creatinine is eliminated from the body by renal excretion at a relatively constant rate, making it a clinically chosen measurement to indicate renal function and a forensically chosen measurement to detect dilute urine and potentially adulterated specimens (2).

Creatine has been available since the early 1960s as a dietary supplement purported to ergogenically enhance short-term, high-intensity exercise (3, 4). However, it was not until the 1990s, when oral creatine supplements became widely available over the counter, that creatine