Measuring Water Content of Feces by the Karl Fischer Method

R. Jensen,1 D. Buffangeix,2 and G. Cov1

We describe a technique for measuring the water content of stools by the Karl Fischer method. The analysis is based on removal of water into a mixture of methanol/chloroform (1/2), after dispersion of the stool by sonication in presence of solvent. An aliquot of the solution thus obtained is placed in the measuring cell of a Karl Fischer apparatus and then analyzed in the classic way. We further describe the advantages of this method (odorless, precise, reproducible) in contrast to other current methods. In addition the same organic solution can also be used in determining the lipid content of stools.

Additional Keyphrases: lipid measurement in stool • water content in diagnosis and as basis for expressing results of other analyses

The measure of the water in feces is an important element in the biological diagnosis of digestive ailments (1–11). Modigliani says (2), “The daily weighing of the feces is the best index of water loss.” The water content of feces depends not on dietary intake, but on digestive function, of which it is an accurate reflection (1–2). Intestinal peristalsis and the degree of water absorption are related, as well as water absorption and intestinal blood circulation (5, 6). In every case, “Fecal hyperhydration is one of the first signs of irritation of the colonic mucosa” (3). Normally stool contains 75% water. When measured directly from the transverse colon, water content is 82–83%; from the left colon, 84–85%; and from the ileocecum, 90–95% (3).

Many clinicians consider that measuring the relative water content of feces, together with microscopic and chemical analysis, permits an excellent evaluation of digestive syndromes such as diarrhea, constipation, and malabsorption (3, 7–9).

This is confirmed by our work (Table 2), where the diagnostic conclusions suggested by comparative study of fecal consistency, pH, color, and degree of hydration have been upheld by the analysis of Goiffon’s 28 parameters (3) as well as by a complete bioclinical investigation (12). This method permits us to detect even subtle syndromes such as “false diarrhea” (Table 2, sample 2) which is defined as: “constipated feces, secondarily eliminated broken up, in the flood of a reactional hypersecretion, resulting from the irritation by stasis of the pelvic and sigmoid colons” (11).

Water content of stool is thus considered by physicians as a preliminary test, permitting a better orientation toward complementary examinations such as radiography and endoscopy. Also, from a purely analytical point of view, the quantitative measurement of fecal water is indispensable whenever results of other determinations are given in terms of fecal dry-weight; in particular lipid measurement on a normal diet (9, 11) or various other fecal variables such as protein, nitrogen, and electrolytes.

Fecal water can be measured by simple evaporation in an oven at 105 °C (11) or, more rapidly, by drying under infrared rays.1 Both are simply measures of the weight loss of the sample.4 Measurement of fecal lipids, another fundamental measurement, is then done by ether extraction of previously dehydrated feces, but this technique has been shown to be inaccurate, and we present a new method, which permits the separation of water and a solution of lipids into an organic solvent (12). Azeotropic distillation also accomplishes this double operation, but even though it greatly diminishes odor it is not accurate, as the water is measured volumetrically. The technique we propose is based on measurement of water by the Karl Fischer method. The water is desorbed into Folch solvent—a methanol/chloroform (1/2 by vol) mixture (13)—the standard mixture for extracting lipids from biological materials. We chose this solvent because it removes both fecal water and fecal lipids.

The resulting solution allows one to measure the various lipid fractions—free fatty acids, triglycerides, phospholipids, and free and esterified cholesterol—by thin-layer chromatography, by a technique described by one of us (15).

Advantages of our method include rapidity, reproducibility, and precision, by virtue of automation; and convenience, because of use of a closed vessel and because of obtaining simultaneously a solution of fecal lipids.

1 Laboratoire de Biochimie Analytique, U.E.R. 1, Université de Bordeaux II, Place de la Victoire, 33076 Bordeaux, France.
2 Laboratoire Central de Biochimie, Hôpital Saint-André, 1 rue Jean Burguet, 33000 Bordeaux, France.

Received Mar. 2, 1976; accepted May 24, 1976.

1 Merland, R., personal communication.
Material and Methods

Principle

An aliquot of feces is treated with a mixture of Folch solvent. The fecal suspension thus obtained is placed in the measuring cell of the Karl Fischer apparatus, and the reagent is added to the end-point of the reaction.

Apparatus

For these determinations, we used the Karl Fischer E 547/1 semi-automatic titrimeter (Methrohm AG, CH-100 Herisau, Switzerland). It is equipped with a manual burette for the introduction of the Karl Fischer reagent into the measuring cell, and a mechanical burette that assures automatic addition of hydrated methanol, stopped electronically when the reaction is over. (This termination is detected by means of a double platinum electrode immersed in the solution.) The different volumes of reagents added are measured by a volumetric counter graduated in 0.01 ml.

We used a special reaction vessel that has an orifice large enough to permit the introduction of solid substances with a spatula. This recipient fits under a stationary unit to which are attached the measuring electrode, the vessel with openings that permit the introduction of the reagents, and a column containing a drying agent. Mixing of the different reagents is assured by a magnetic stirrer.

Choice of Solvent

Use of the Karl Fischer reagent. A series of experiments was done to determine the most appropriate solvent mixture for this determination. In the first, we placed a fraction of feces directly into the reaction vessel, in contact with the Karl Fischer reagent, of which we measured the excess by difference. This method is preferred by certain authors for the measure of water in various solids (2). Three aliquots weighing between 15 and 20 mg were obtained successively from each stool sample.

Our results are shown in Table 1, which demonstrates that in this type of measurement, the classic method of Karl Fischer, results are not reproducible because certain of them deviate more than 10% from the mean. This method, then, is not suitable for biological measures because, as has been previously stated, variations of more than 2% can lead to erroneous diagnostic interpretations.

These variations could have several origins: a non-negligible fraction of Karl Fischer reagent is consumed for a chemical reaction different from that with water; errors in weighing, especially when very small samples are taken; and (or) the stool specimens are not homogeneous.

Considering average values, the results closest to those given by the evaporation method were obtained for pasty stools; pasty stools are in principle the most homogeneous and the water is more easily accessible to the reagents.

The largest differences (always inferior) were obtained for liquid stools: these differences seem essentially attributable to evaporation during the time necessary to obtain and weigh the sample. For hard stools, the same errors were observed, apparently because all the water was not removed, owing to the nature of the material.

It would seem possible to diminish the experimental errors by analyzing a larger sample. However, given the abundance of water in the feces (between 70 and 90%), that would necessitate too great an increase in the volume of the Karl Fischer reagent, which would be incompatible with the size of the reaction vessel and the simplicity of the method.

Use of the Folch reagent (3). The impossibility of realizing a direct reproducible measurement led us to work with a dilution of stool in the Folch solvent. An aliquot of this dispersion is placed in a measuring cell and then measured. This mode of operation presents the following advantages:

- increase in the sample size: 100 mg instead of 15 to 20 mg gives a more precise weighing; 10% of the volume of the dispersion is sufficient to obtain a precise measurement by the Karl Fischer reagent without excessive consumption of reagent, even for very liquid stools;
- the fecal water is extracted into the solvent, as well as the fecal lipids, with an optimal recovery. Thus, another aliquot of the stool dispersion will permit the measurement of the diverse lipid fractions (cf. Table 1).

The aluminum-foil weighing paper and stool are placed together in a ground-glass-stoppered 100-ml Erlenmeyer flask, and 25 ml of Folch solvent, measured exactly with a graduated flask, is poured into the flask. Extraction efficiency depends on the consistency of the feces and their tendency to fragment. To increase fragmentation we sonicate the feces, to increase surface area and to remove all particles that adhere to the aluminum weighing paper. We determined that the contact time between sample and solvent as well as the length of sonication were optimum by measuring aliquots of the extracted water every hour until the maximum value

| Table 1. Results Obtained by the Classical Method of Karl Fischer for 11 Specimens |
|---------------------------------------------|------------------|------------------|
| H2O measured by Karl Fischer method, %    | Sample no.       |
| Stool consistency | 1   | 2   | 3   | Av  | H2O by evap, % |
| Pasty           | 68.9 | 60.1 | 101.6 | 76.3 | 76           |
| Liquid          | 77.6 | 77.1 | 66.5  | 73.7 | 88           |
| Pasty           | 89.2 | 71.3 | 75.7  | 78.7 | 77           |
| Hard            | 62.1 | 59.7 | 67.4  | 63.1 | 71           |
| Liquid          | 92.6 | 78.9 | 77.6  | 83.0 | 90           |
| Pasty           | 65.8 | 71.3 | 66.1  | 67.7 | 75           |
| Foamy           | 93.5 | 78.1 | 71.9  | 81.1 | 87           |
| Pasty           | 77.5 | 76.2 | 83.6  | 79.1 | 79           |
| Pasty           | 78.2 | 77.3 | 68.7  | 74.7 | 75           |
| Foamy           | 96.3 | 84.8 | 94.2  | 91.7 | 87           |
was reached. The most practical combination consisted of a 30-min sonication followed by a 24-h contact and then a second sonication for 30 min.

For water measurement, the fecal suspension thus obtained is placed in the measuring cell without further treatment; for the measurement of fecal lipids the solvent is separated from particles in suspension by filtration, then evaporated at a low temperature and the residue resuspended in an appropriate solvent (12).

Measurement Procedure

The measurement consists of extraction, measurement, and calculation.

Extraction. The stool specimen is homogenized as completely as possible, and a 100-mg sample of the homogenate is added to a ground-glass-stoppered, 100-ml round flask containing 25 ml of Folch solvent (13). This preparation is subjected to sonication for 30 min (the apparatus we used was the cleaner ultrasonic Aerograph; Varian Aerograph; Walnut Creek, Calif.). After 24 h of contact, the sample is again sonicated for 30 min. The resulting suspension is used as such for the water measurement by the Karl Fischer electrochemical method. However, it should be noted that in the case of very dense or mucus-containing stools, which are difficult to homogenize, reproducible results require analysis of three different samples, averaging the results.

Measurement. (1) Adjustment of the Karl Fischer apparatus: The apparatus is adjusted in the classic way by determining the equivalence between hydrated methanol and the Karl Fischer reagent and between Karl Fischer reagent and sodium tartrate-2H₂O, containing 15.66% water (14).

(2) Assay for the water content of stools: Usually 10 ml of the suspension obtained after sonication is placed into the measuring cell (S₂ ml). While being agitated on a magnetic stirrer, the Karl Fischer reagent (K₃ ml) is added to the suspension. Excess Karl Fischer reagent is measured with hydrated methanol; m₃ ml represents the volume of hydrated methanol used for this measurement.

Calculation. The percentage of water contained in the stool specimen is calculated as follows:

\[
\left( \frac{a \times 15.66}{p} \right) \left( \frac{S_1}{S_2} \right) \left( \frac{m_1K_2 - m_2K_1}{m_1K_1 - m_2K_1} \right)
\]

= percentage of water in stool

where: a = weight, in mg, of sodium tartrate (15.66% water) used for the titration of the Karl Fischer reagent. p = weight, in mg, of stool used in the assay. S₁ = volume, in ml, of solvent added to liquefy the stool. S₂ = volume, in ml, of the stool suspension placed in the measuring cell. K₁ = volume, in ml, of Karl Fischer reagent used to titrate the water contained in the hydrated methanol. m₁ = volume, in ml, of hydrated methanol that corresponds to the volume K₁ of Karl Fischer reagent. K₂ = volume, in ml, of Karl Fischer reagent equivalent to the water contained in the sodium tartrate. m₂ = volume, in ml, of hydrated methanol corresponding to the excess of K₂ (volume of Karl Fischer reagent un-reacted with sodium tartrate. K₃ = volume, in ml, of Karl Fischer reagent used to react with the stool suspension. m₃ = volume, in ml, of hydrated methanol used to neutralize the excess of volume K₃ which had not reacted in the stool suspension.

Results and Discussion

Table 2 shows some of our results for stools of different consistencies. Determination by the classical method of the coefficient of regression for the method that we propose results in a straight line of regression of slope 0.88 and a coefficient of regression of 0.98.

Thus the correlation between results by the two techniques is good; moreover, the electrochemical

---

Table 2. Examples of Diagnostic Results in Relation to Consistency, Color, pH, and Percentage of Water in the Stools (10 Patients)

<table>
<thead>
<tr>
<th>Consistency</th>
<th>Color</th>
<th>pH</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Av</th>
<th>Diagno tic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frothy</td>
<td>Ochre</td>
<td>6.0</td>
<td>84.95</td>
<td>85.10</td>
<td>83.50</td>
<td>84.51</td>
<td>Diarrhea; fermentation</td>
</tr>
<tr>
<td>Grumous</td>
<td>Brown</td>
<td>6.0</td>
<td>88.20</td>
<td>88.07</td>
<td>88.27</td>
<td>88.18</td>
<td>&quot;False diarrhea&quot;</td>
</tr>
<tr>
<td>Hard, formed</td>
<td>White</td>
<td>5.5</td>
<td>63.13</td>
<td>63.00</td>
<td>64.26</td>
<td>63.46</td>
<td>Constipation, severe biliary insufficiency</td>
</tr>
<tr>
<td>Hard, formed</td>
<td>Ochre</td>
<td>6.5</td>
<td>65.31</td>
<td>66.29</td>
<td>65.12</td>
<td>65.57</td>
<td>Constipation, slight biliary insufficiency</td>
</tr>
<tr>
<td>Hard, formed</td>
<td>Brown</td>
<td>7.5</td>
<td>73.00</td>
<td>74.21</td>
<td>74.10</td>
<td>73.77</td>
<td>Constipation, intestinal atony</td>
</tr>
<tr>
<td>Pasty</td>
<td>Ochre</td>
<td>6.0</td>
<td>85.18</td>
<td>83.90</td>
<td>84.00</td>
<td>84.36</td>
<td>Diarrhea, caecal irritation</td>
</tr>
<tr>
<td>Well formed</td>
<td>Brown</td>
<td>6.5</td>
<td>78.23</td>
<td>78.15</td>
<td>79.37</td>
<td>78.58</td>
<td>Normal stool</td>
</tr>
<tr>
<td>Mucus</td>
<td>Ochre</td>
<td>7.5</td>
<td>87.00</td>
<td>89.40</td>
<td>89.27</td>
<td>88.55</td>
<td>Diarrhea, small bowel irritation</td>
</tr>
<tr>
<td>Pasty</td>
<td>Ochre</td>
<td>6.5</td>
<td>77.29</td>
<td>75.60</td>
<td>75.10</td>
<td>75.99</td>
<td>Post-gastrectomy malabsorption</td>
</tr>
<tr>
<td>Hard, formed</td>
<td>Ochre</td>
<td>6.5</td>
<td>71.82</td>
<td>71.23</td>
<td>72.10</td>
<td>71.71</td>
<td>Constipation, slight biliary insufficiency</td>
</tr>
</tbody>
</table>
method gives a higher value for water content than does the evaporation method for a given stool sample. However, as we said, to obtain the best results it is necessary to analyze each sample in triplicate. The reproducibility of this method increases with the water content of the stool. The greatest deviations in the results were for samples of mucus-containing stool.

This raises the problem of the homogenization of fecal matter, which also is shown to be accomplished better with our method than with the classic method.

From a physiopathological viewpoint, fecal water content is an essential variable to measure in functional coprology. The concentration of lipids, proteins, and nitrogen (e.g.) are usually given in terms of dry weight of feces, although for reasons of simplicity, it is usual to do the reactions without drying the feces and then to correct the results for water content of the feces.

Otherwise, used as a rapid diagnostic approach, one can reach correct conclusions based on the comparative study of the consistency, pH, color, and water content of stool, as is shown in Table 2, where the diagnostic conclusions were confirmed in every case by complete functional coprology based according to Goiffon and Goiffon's recommendation (3) on the comparative study of 28 variables, of which water is one.

In current biological practice, the conclusions suggested by the study of our four variables are with one or two exceptions confirmed by the functional analysis of Goiffon and Goiffon and by a complete bioclinical examination. In this same table the diagnostic interpretation that one can make from water measurements are similar for the two methods, except for stool No. 6. In this case functional coprology and bioclinical examination confirmed the presence of a caecal irritation, which corresponds well with the measurement by the Karl Fischer method, for this sample more revealing than our method.

We conclude that measurement of fecal water by the electrochemical method of Karl Fischer gives results comparable to those obtained by evaporation. However, the Karl Fischer method has the advantage of being faster, even though it is necessary to measure three aliquots for each stool sample, this by reason of the small sample used and the heterogeneity of the stool. The method has the further advantage of being odorless. Its simplicity makes it suitable for wide use in all laboratories and allows one to obtain, by relatively simple techniques, a very good solution of fecal lipids suitable for chromatographic analysis.

The advantages of this method include not only on its great precision, which is in fact superior to current clinical needs, but also on the fecal lipid solution simultaneously obtained, and the fact that from very simple manipulations one can obtain different results crucial to the diagnosis of a digestive pathology.

References