Gas Chromatographic Determination of Water in Natural Products by Reaction with 2,2-Dimethoxypropane

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Abstract ☐ The reaction between 2,2-dimethoxypropane and water to form acetone and methanol was applied to the determination of water in natural products by GLC. Water was extracted from the natural products with methanol and analyzed with a column of 30% tetrahydroxyethylethylenediamine on diatomite, using cyclohexane as an internal standard. Peak area ratios were used to calculate the water content. A linear relationship exists between the ratios of the observed peak areas of acetone to the internal standard and the actual weight ratios of water to the internal standard. The water content of 24 natural products representing a variety of plant parts was analyzed by GLC utilizing the above reaction. The results compare well with those obtained by gravimetric and azeotropic methods. The new method is simple, rapid, and has a very good precision and accuracy. A major advantage of the method is that water estimation can be carried out using a flame-ionization detector.

Keyphrases ☐ Water determination—natural products ☐ 2,2-Dimethoxypropane-water reaction—analysis method ☐ GLC—analysis

A wide variety of analytical methods are used in many fields for the determination of water. Ewing (1) has found more than 25 distinct methods for this purpose. Among the procedures which have been the subject of recent investigation, GLC has proved to be particularly well-suited for the analysis of water in different organic systems. The technique has been used by a number of investigators as a means of determining water in gases (2–4), mixed solvents (3, 5–8), pharmaceutical preparations (9,10), foods (11–13), and cosmetics (14). Recently, this laboratory has described a gas-liquid chromatographic procedure for the determination of moisture in crude drugs and related natural products (15). In all of these methods, the water is determined directly after separation and elution from the chromatographic column. Because of the insensitivity of the flame-ionization detector to water, these direct methods have relied on the thermal conductivity detector.

Indirect gas-liquid chromatographic methods have also been employed in the analysis of water in many systems. These methods are based on the quantitative conversion of water to some other substance which can be separated by gas chromatography and detected by the more sensitive flame-ionization detector. One such method involves the conversion of water to acetylene by reaction with calcium carbide and the subsequent separation and determination of the acetylene (16–18). Another approach is based upon the rapid and quantitative acid-catalyzed reaction between 2,2-dimethoxypropane (DMP) and water to form acetone and methanol; the acetone formed in the reaction is then determined by GLC.

The DMP reaction was first reported by Erley (19) in 1957 as a means of removing water from nonvolatile samples intended for IR analysis. Critchfield and Bishop (20) applied the reaction to determine the water content of various solvents and inorganic hydrates by using IR absorption to measure the acetone formed. The use of the reaction for the determination of water by GLC was first suggested by Hager and Baker (21). Badinand *et al.* (22) and Martin and Knevel (23) studied the reaction and effected a quantitative gas-liquid chromatographic method for water determination in some organic solvents. No other report relating to the use of the DMP reaction in the gas-liquid chromatographic analysis of water in various systems has appeared in the literature. In a preliminary study (24), the author explored the applicability of the DMP reaction to the analysis of water in natural products. Although the results obtained proved promising, certain difficulties were encountered in the extraction procedure, sampling technique, and choice of a suitable internal standard. The calculations which were involved were also tedious and time-consuming. The present paper outlines the details of a simple, specific, and rapid gas-liquid chromatographic procedure, based upon the use of the DMP reaction. The method does not suffer from the previously encountered problems and it has been found to be well suited for the determination of water in a variety of crude drugs and similar natural products.

EXPERIMENTAL

The natural products used in this work were obtained from a commercial source.[1]

Gravimetric Method for Water Determination—The water content of all the products, except caraway, cinnamon, clove, ginger, and peppermint, was determined by the NF XII procedure (25) for vegetable drugs containing no constituents volatile at 105°. The water content of caraway, cinnamon, clove, ginger, and peppermint was determined by the NF XII procedure (25) for vegetable drugs containing ether-soluble constituents volatile at 105°.

Azeotropic Method for Water Determination—The toluene distillation method given in the USP XVII (26) was used in determining the water content of all the natural products.

Gas-Liquid Chromatographic Method for Water Determination

Instrumentation—A gas chromatograph[2] equipped with a hydrogen flame-ionization detector and a 0-1 mv. recorder[3] with a chart speed of 2 in./min. and a 1-sec. full-scale response, was used throughout the experimental work. All samples were injected with a 10-μl. syringe.[4]

Column—The column used in this study was a stainless steel coil, 1.89 m. (6 ft.) long and 0.32 cm. (0.125 in.) o.d. packed with 30% tetrahydroxyethyl ethylenediamine on diatomaceous earth,[5] 80–100 mesh.[6] The packed column was conditioned at 100° for 48 hr. before use.

[1] S.B. Penick and Co., New York, N. Y.
[2] Perkin-Elmer model 811.
[3] Speedomax G.
[4] Hamilton No. 701.
[5] Chromosorb W, Johns-Manville, New York, N. Y.
[6] Purchased from Perkin-Elmer, Norwalk, Conn.

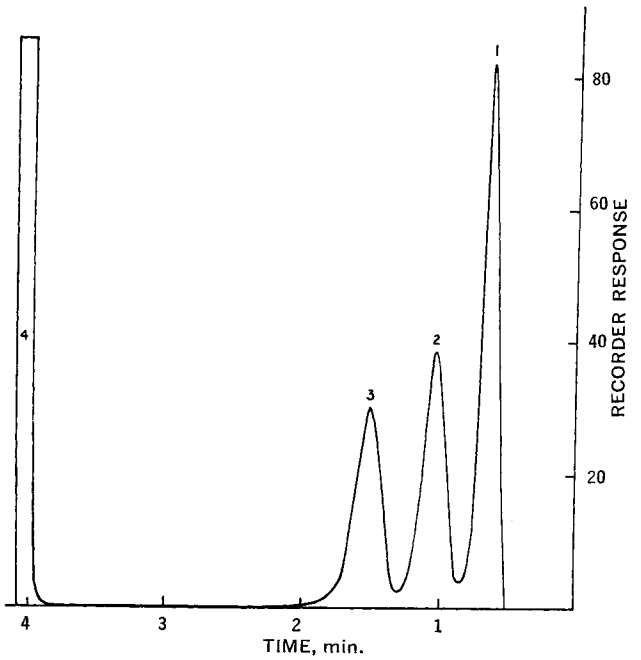

Figure 1—*Typical gas chromatogram obtained from the extract of the natural product (agar) after reaction with 2,2-dimethoxypropane. Key: 1, cyclohexane (internal standard); 2, 2,2-dimethoxypropane; 3, acetone; 4, methanol.*

Operating Conditions—The column temperature was 100°. The sample injection port was maintained at 140° and the detector block at 160°. Helium was used as the carrier gas, at a rate of 25–30 ml./min. with an inlet pressure of 20 psig. Air and hydrogen inlet pressures were 56 and 46 psig., respectively. Three-microliter injections of all samples were used throughout. All determinations were made with an attenuation setting X2K.

Reagents—The reagents used were methanol,[7] methanesulfonic acid,[8] 2,2-dimethoxypropane,[9] and cyclohexane.[10]

Standard Curve—A standard curve was prepared from a stock solution made by adding exactly 1.0 ml. of water to a 100-ml. volumetric flask and diluting to volume with methanol. Aliquots of exactly 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 ml. of the stock solution were transferred to 25-ml. volumetric flasks and to each were added exactly 2.0 ml. of DMP, 5.0 ml. of freshly prepared 0.1 *N* methanolic methanesulfonic acid, and 0.50 ml. of the internal standard, cyclohexane. A blank was prepared at the same time with each set of analytical determinations. The flasks were shaken for a few minutes to insure completeness of the DMP reaction, and the contents diluted to volume with methanol. Sample solutions were chromatographed and the peak areas of acetone (formed in the reaction) and cyclohexane were measured by multiplying their peak height times their width at half height. A standard curve was then established by plotting the ratio of peak areas of acetone to cyclohexane against the weight[11] ratios of water to cyclohexane. A new stock solution and set of standards were prepared every day that extracts of natural products of unknown water quantity were analyzed.

Extraction Procedure and Sample Preparation—*Method A—Blender Extraction*—A 10-g. sample of the natural product to be analyzed was transferred to a stainless steel blender[12] fitted with air-tight screw cap and threaded sampling plug. One hundred milliliters of methanol was added and the product was disintegrated for 5 min. After blending, the mixture was allowed to settle for 1 min., and duplicate 10.0-ml. aliquots of the supernatant were transferred with a pipet to each of two 25-ml. volumetric flasks and treated in exactly the same manner as described in the above procedure for the standards. After diluting the sample solutions to volume with methanol, 3 μl. was injected into the gas chromatograph under conditions identical to those used for the standards.

This procedure was used in the analysis of the water content of all the natural products included in this study.

Method B—Reflux Extraction—A 10-g. sample of the plant material was heated for 1 hr. under reflux with 100.0 ml. of methanol. After cooling the mixture, duplicate 10.0-ml. aliquots of the clear supernatant were transferred quantitatively into 25-ml. volumetric flasks and analyzed in exactly the same manner as indicated above.

This procedure was used in the analysis of the water content of pectin, belladonna, cinchona, and rauwolfia serpentina.

Calculations—The amount of water in the natural product was determined by computing the acetone-cyclohexane peak area ratio from the chromatogram, obtaining the corresponding weight ratio of water-cyclohexane from the standard curve, and multiplying by the weight of cyclohexane. The value so obtained was then converted to percent moisture in the original natural product sample.

Recovery Studies—To determine the recovery of water added to a natural product, five drugs containing different amounts of water were chosen for the recovery experiment. A 10-g. sample of the powdered product was placed into the blender, the product was extracted with 100.0 ml. methanol to which an accurately known amount of water was added, and a 10.0-ml. aliquot of the extract was assayed as above to estimate the recovery of the added water.

RESULTS

Resolution of Products of the DMP Reaction—Figure 1 is a chromatogram of the reaction mixture given by the agar extract; it is typical of the type of chromatogram obtained from the DMP reaction mixtures. As seen in Fig. 1, acetone was well separated

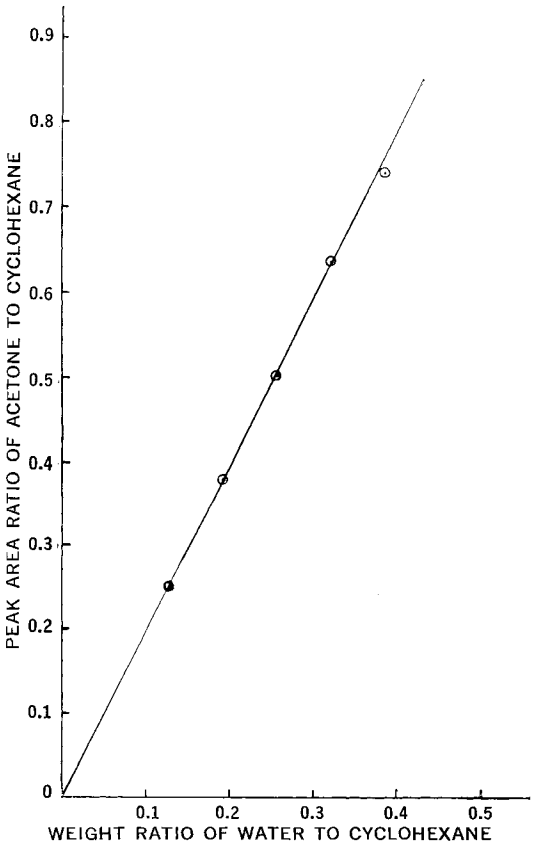

Figure 2—*Peak area ratio of acetone to cyclohexane as a function of weight ratio of water to cyclohexane.*

[7] Spectro grade, Distillation Products Industries, Eastman Organic Chemicals Department, Rochester, N. Y.
[8] Practical grade, Distillation Products Industries, Eastman Organic Chemicals Department, Rochester, N. Y.
[9] Purissimum, Aldrich Chemical Co., Inc., Milwaukee, Wis.
[10] Chromatoquality, Matheson, Coleman & Bell, East Rutherford, N. J.
[11] Water and cyclohexane were measured by volume and converted to weight using specific gravity calculations.
[12] Waring Products Co., Winsted, Conn.

from both the excess DMP and the methanol as well as from the internal standard, cyclohexane.

Relationship between Water Concentration and Acetone Area—For accurate quantitative analysis, the internal standardization technique was used. The relationship between the weight ratios of water to the internal standard and the area ratios of the acetone produced in the DMP reaction to the internal standard was found to be linear for weights of water from 25.0 to 150.0 mg. (Fig. 2).

Estimation of Water by Gravimetric, Azeotropic, and Gas-Liquid Chromatographic Methods—Comparison of assay results obtained from 24 natural products using the gravimetric, azeotropic, and gas-liquid chromatographic methods is given in Table I. The water content of the different products analyzed by the three methods was found to vary from as low as 4% for digitalis to as high as 15% for agar. Table I also compares results of gas-liquid chromatographic analysis of extracts from five natural products prepared by blender and reflux extraction. The comparison shows that, for pectin, water is more completely removed from the product when reflux extraction is used.

Quantitative Recovery of Added Water—The results obtained when accurately known amounts of water were added to five of the natural products chosen for this experiment, are presented in Table II. Recoveries on duplicate analyses are within ±0.5% for the five plant products analyzed.

Reproducibility of Gas-Liquid Chromatographic Procedure—The reproducibility of the gas-liquid chromatographic method was established by examining a series of repeated analyses of the water content of seven natural products selected from the list in Table I. The results of these analyses are shown in Table III. The figures indicate that the reproducibility of the chromatographic analysis is satisfactory.

Table I—Comparative Results of Percent Water in Natural Products as Determined by Gravimetric, Azeotropic, and Gas-Liquid Chromatographic Methods

	Method of Analysis			
			GLC[b]	
Natural Product	Gravimetric[a]	Azeotropic[a]	Blender Extraction	Reflux Extraction
Roots and Rhizomes				
Gentian	6.07	6.00	6.81	
Ginger	6.84	6.50	7.02	
Ipecac	8.50	8.08	8.66	
Licorice	7.50	7.28	8.53	
Podophyllum	8.76	8.50	8.60	
Rauwolfia serpentina	7.78	7.45	7.83	7.96
Barks				
Cascara sagrada	7.24	7.75	8.41	
Cinchona	7.32	7.15	7.86	7.50
Cinnamon	6.56	7.00	8.34	
Wild cherry	6.24	6.30	7.35	
Leaves				
Belladonna	7.17	6.70	6.88	6.82
Digitalis	4.21	4.06	4.48	
Peppermint	7.12	7.18	8.21	
Senna	7.08	6.71	7.29	
Flowers and Fruits				
Clove	5.42	5.44	5.88	
Caraway	5.34	6.00	5.44	
Seeds				
Areca	7.94	7.17	8.22	
Strophanthus	5.57	5.00	6.45	
Miscellaneous				
Agar	15.13	14.00	14.73	
Aloe (cape)	5.00	4.16	6.74	
Ergot	5.64	5.03	6.37	
Starch (corn)	8.57	9.43	9.45	
Pectin (lot No. 1)	12.33	13.97	7.35	13.17
Pectin (lot No. 2)	9.61	9.54	4.00	9.20

[a] Each value is the average of two or more determinations. [b] Each value is the average of results obtained from six chromatographic injections, representing two or more extractions of the natural product.

Table II—Recovery Data[a]

Natural Product	Water, % Added	Water, % Recovered
Caraway	6.00	5.50
Digitalis	10.00	10.26
Rauwolfia serpentina	7.50	7.28
Starch	3.00	3.12
Tragacanth	5.00	5.25

[a] Mean results of two estimations.

DISCUSSION

The flame-ionization detector is an extremely sensitive detector which responds to virtually all compounds with the exception of permanent inorganic gases, notably air, water, ammonia, and carbon disulfide (27). The great avantages of the flame-ionization detector have caused investigators to explore means to extend the applicability of this device to substances which normally respond very poorly. One of the most successful approaches has been to convert a poorly detectable substance to one which is readily detectable prior to the chromatographic process. The reaction of water with 2,2-dimethoxypropane is one such reaction. It is practically instantaneous to form one mole of acetone and two moles of methanol (20). The equilibrium constant indicates that approximately 96% of the water reacts with the reagent at 30° (19). Mild heating (19) or acid catalysis (20–23) may be necessary for the quantitative conversion of water in a reasonable length of time. Methanesulfonic acid was selected to catalyze the DMP reaction because it is a strong acid that is soluble in organic solvents and is commercially available as a high purity compound suitable for the purpose of this investigation.

Water was extracted from the natural products by comminution of the drug with methanol in a blender. This procedure has been utilized, with good quantitative results, in the removal of water from a variety of food products and crude drugs (12–14). The water was measured by the proportional increase of the acetone formed in the DMP reaction. Water could have also been measured by the proportional decrease in the DMP area if the analytical procedure were designed toward that direction. The proportional increase in the area of the methanol peak could also provide the basis for water measurement, but in this study, the poor shape of the methanol peak and the use of this solvent in the extraction of water from the natural products precluded such a consideration.

The completeness of the DMP reaction was checked periodically by injecting into the gas chromatograph aliquots of both standard and unknown solutions immediately and after 24 hr. of standing. No increase in the amount of acetone formed could be found after this lapse of time.

The column used was that employed by Martin and Knevel (23) in their analysis of water in organic solvents. This column was evaluated in preliminary studies in this laboratory (24) and was found to be satisfactory for the separation of the DMP reaction products as well as the internal standard, cyclohexane. It afforded symmetrical peaks of the desired compounds, low retention time, and good performance during several months of continued use. The analysis time for each sample was relatively short (5 min.).

Table III—Reproducibility of the Gas-Liquid Chromatographic Analysis[a]

Natural Product	No. of Runs	Water, %
Agar	4	14.65, 14.70, 14.76, 14.80
Caraway	3	5.14, 5.38, 5.84
Cascara sagrada	4	8.10, 8.42, 8.56, 8.57
Cinchona	4	7.66, 7.71, 7.92, 8.15
Licorice	3	8.32, 8.60, 8.68
Rauwolfia serpentina	3	7.69, 7.85, 7.94
Senna	2	7.17, 7.40

[a] Each value recorded represents the mean of six chromatographic injections from two aliquots of an extract of the natural product.

Because of its accuracy, the internal standard technique was used. After a number of trials with other materials, cyclohexane was chosen as the internal standard because of its commercial availability as a chromatographically pure reagent and its adequate separation from the other compounds. It was found that, under the experimental conditions cited, a linear relationship exists between the ratios of the observed peak areas of acetone to the internal standard and the actual weight ratios of water to the internal standard. The standard curves so established were checked and redetermined with every set of analytical estimations. The use of an internal standard greatly simplified the analytical procedure and calculations.

The results of the water analyses of the natural products performed by the indirect gas-liquid chromatographic method are quantitatively in accord with those obtained by the gravimetric and azeotropic methods. The results are also in good agreement with those obtained previously from a direct gas-liquid chromatographic procedure developed in this laboratory (15, 24). The precision of the chromatographic analysis is satisfactory; the maximum standard deviation found was 0.540. To determine the accuracy of the gas-liquid chromatographic procedure, known amounts of water were added to five different natural products of known water content. In all cases quantitative recoveries of the added water were obtained. The accuracy of the procedure can also be estimated by comparing the results with the values obtained by gravimetric and azeotropic analyses.

In the course of analysis by GLC of the extracts prepared by blender extraction, it was noted that one of the products, pectin, gave values for water which were markedly lower than those given by the other two methods. When this product was refluxed with methanol for 1 hr. and the extract subsequently analyzed by GLC, results were obtained which were more in accord with those obtained with the other methods. Analysis of extracts prepared by refluxing other products with methanol in a manner similar to pectin, did not change the values obtained when such products were extracted with methanol in a blender. It appears, therefore, that some of the water in pectin exists in a bound form which is not available for extraction with methanol by disintegration in a blender but can be readily removed by a more rigorous extraction procedure in which the product is refluxed with the solvent for 1 hr. Some natural products in which the carbohydrate content is characteristically high, are known to retain part of their water in a bound form (28).

The results obtained in this study clearly demonstrate that the indirect gas-liquid chromatographic method is an effective method for the quantitative analysis of water in natural products. It is quite simple, rapid, sensitive, and has a very good precision and accuracy. A major advantage of the method is that it can make use of the extremely high sensitivity of the flame-ionization detector. The new method can be easily adapted to the analysis of water in other crude drugs, foods, biological materials, and similar products.

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