drug. Possibly this is the explanation for the good *in vivo* antibacterial activity of the 2-propionate, 2-pivalate, and 2-(3,3-dimethyl)-butyrate monoesters of lincomycin, even though they are enzymatically hydrolyzed *in vitro* relatively slowly.

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Free and Bound Water in Crude Drugs: Effect of Extraction Method on Subsequent Analysis by GLC

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Abstract □ The effect of the extraction procedure on the recovery of water from crude drugs was related with its quantitative analysis by GLC. Some crude drugs seem to retain part of their water in a bound form, which is not available for extraction with methanol by disintegration in a blender but can be readily removed by boiling the product with the solvent under reflux for 1 hr. A number of crude drugs, representing a variety of plant parts and products, were extracted with methanol by the two procedures; the extracts were subsequently analyzed for their total water by GLC. Reflux extraction is the method of choice for the preparation of extracts of crude drugs that contain bound water prior to their analysis by this technique.

Keyphrases □ Water, recovery from crude drugs—effect and comparison of extraction procedures □ Reflux extraction—preparation of crude drug extracts □ Crude drugs—extraction procedures, effect on GLC analysis □ GLC—analysis of crude drugs, effect of extraction procedures

A previously published paper from this laboratory (1) described a GLC method for the quantitative determination of water in natural products by reaction with 2,2-dimethoxypropane. In the course of analysis by this method, it was observed that the extraction procedure normally used to remove water from these products (disintegration with methanol in a blender) gave, in one instance (pectin), results that markedly deviated from the values given by the official methods. Pectin seems to retain part of its water in a bound form which does not lend itself readily to extraction by this procedure.

Further studies on this problem showed that some degree of water binding also exists in other crude drugs and natural products besides pectin. For accurate quantitative analysis, water in this form must be completely extracted from such products prior to determination by GLC. The purposes of this paper are to record the effect of extraction on water recovery from crude drugs and to relate this effect with quantitative analysis by this technique.

EXPERIMENTAL

The plant materials used in this investigation were obtained, in powdered form, from a commercial source[1].

Water Determination by Direct GLC

Extraction and Sample Preparation—*Procedure A: Blender Extraction*—In a typical analysis, 10.00 g. of the crude drug was placed in a blender jar[2] containing 100.00 ml. of anhydrous methanol[3] and 3.00 ml. of *n*-propanol[4] as the internal standard. After blending for 5 min., the mixture was allowed to settle; then a sample of the clear supernatant was drawn into a vial, and 4.00 μl. was injected with a microliter syringe[5] into a gas chromatograph[6] equipped with thermistor detector.

Procedure B: Reflux Extraction—A 10.00-g. sample of the plant material was heated for 1 hr. under reflux with 100.00 ml. of anhydrous methanol and 3.00 ml. of *n*-propanol as the internal standard. After cooling the mixture, a sample of the clear supernatant was transferred into a vial, and 4.00 μl. was injected into a gas chromatograph equipped with thermistor detector.

Calculations—The percent water in the original sample of the crude drug was determined by computing the ratio of peak height of water to *n*-propanol from the chromatogram, obtaining the corresponding weight ratio of water to *n*-propanol from a standard curve prepared by chromatographing samples containing various amounts of water in mixtures of 100.00 ml. anhydrous methanol and 3.00 ml. *n*-propanol, and multiplying by the weight of *n*-propanol[7].

[1] S. B. Penick and Co., New York, N. Y.
[2] Waring Products Co., Winsted, Conn.
[3] Reagent grade, Merck and Co., Inc., Rahway, N. J.
[4] Matheson, Coleman and Bell, East Rutherford, N. J.
[5] Hamilton No. 701, Hamilton Co., Whittier, Calif.
[6] Perkin-Elmer model 154 vapor fractometer, Norwalk, Conn.
[7] Water and *n*-propanol were measured by volume and converted to weight using specific gravity calculations.

Table I—Percent Water in Crude Drugs That Do Not Retain Bound Water as Determined by GLC, Gravimetric Analysis, and Azeotropic Distillation

Crude Drug	GLC[a] Direct Analysis: Blender Extraction	GLC[a] Direct Analysis: Reflux Extraction	GLC[a] Indirect Analysis: Blender Extraction	GLC[a] Indirect Analysis: Reflux Extraction	Gravimetric Analysis[b]	Azeotropic Distillation[b]
Areca	8.22	8.34	8.22	8.61	7.94	7.17
Belladonna leaf	6.35	6.40	6.88	6.82	7.17	6.70
Cascara sagrada	7.11	7.35	8.41	8.00	7.24	7.75
Ipecac	8.10	8.22	8.66	8.96	8.50	8.08
Podophyllum	8.08	8.00	8.60	9.25	8.76	8.50
Tragacanth	8.26	9.31	8.37	9.20	8.03	8.38

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

Table II—Percent Water in Crude Drugs That Retain Part of Their Water in a Bound Form as Determined by GLC, Gravimetric Analysis, and Azeotropic Distillation

Crude Drug	GLC[a] Direct Analysis: Blender Extraction	GLC[a] Direct Analysis: Reflux Extraction	GLC[a] Indirect Analysis: Blender Extraction	GLC[a] Indirect Analysis: Reflux Extraction	Gravimetric Analysis[b]	Azeotropic Distillation[b]
Acacia	7.33	12.37	8.10	12.70	12.95	11.90
Colchicum corm	5.38	8.40	5.76	8.88	8.75	8.50
Pectin (Lot 1)	—	—	7.35	13.17	12.33	13.97
Pectin (Lot 2)	4.65	9.53	4.00	9.20	9.61	9.54
Nux vomica	6.44	8.82	6.22	8.65	8.02	8.16

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

The column, instrumentation, and chromatography conditions used in this study were described previously (2).

Water Determination by Indirect GLC

Extraction—*Procedure A: Blender Extraction*—A 10.00-g. sample of the crude drug was disintegrated with 100.00 ml. methanol[8] for 5 min. in a stainless steel blender. After allowing the mixture to settle, a 10.00-ml. aliquot of the clear supernatant was transferred with a pipet to a 25-ml. volumetric flask.

Procedure B: Reflux Extraction—A 10.00-g. sample of the plant material was refluxed for 1 hr. with 100.00 ml. methanol. After cooling the mixture, a 10.00-ml. aliquot of the clear supernatant was transferred to a 25-ml. volumetric flask.

Sample Preparation and Calculations—The extract of the crude drug prepared by each of the two procedures was analyzed for its water content by the GLC method previously reported (1). The analytical method is based upon the quantitative acid-catalyzed conversion of water to acetone by 2,2-dimethoxypropane; the acetone formed in the reaction is determined by GLC and related to the amount of water present in the original sample.

Determination of Accuracy of GLC Analysis

The water content of three of the crude drugs was assayed by direct and indirect GLC. Known quantities of water were then added to the plant materials prior to extraction by the reflux procedure, and the extracts were reassayed to estimate the recovery of the added water.

Water Determination by Gravimetric Method

The water content of the plant products was determined by the NF XIII procedure (3) for vegetable drugs containing no constituents volatile at 105°.

Water Determination by Azeotropic Distillation

The toluene distillation method given in NF XIII (3) was followed in determining the water content of the plant materials.

[8] Spectrograde, Distillation Products Ind., Eastman Organic Chemicals Department, Rochester, N. Y.

RESULTS AND DISCUSSION

Water is believed to exist in several forms in organic matter. As a constituent of materials of plant origin, it may be present free or partially bound to macromolecular components of the cells. A reliable estimate of the moisture content in crude drugs by GLC can be made when the extraction procedure assures the total removal of water from these products. When present in the free form, water can be extracted readily by comminution of the crude drug with methanol in a suitable blender for few minutes. Bound water, on the other hand, is tightly linked into the complex structure of the larger molecules and is mechanically, although not chemically, a part of the structure (4); in this form, it is difficult to remove or extract by the usual blending procedure. When a more rigorous extraction procedure (refluxing with methanol) is used, however, the removal of bound water from the crude drug is found to be more efficient and complete.

In the case of crude drugs that do not retain bound water (Table I), the moisture values obtained by GLC, either by blender or reflux extraction, are quantitatively in accord with those obtained by gravimetric analysis and azeotropic distillation. Water in these products exists entirely in a free form, which can be quantitatively extracted by either procedure prior to analysis by GLC.

On the other hand, some crude drugs and similar natural products retain part of their water in a bound form (Table II). Water in this form is not amenable to blender extraction but is readily and efficiently removed by reflux extraction. When blender extraction is used in the GLC analysis of moisture in such products, low and erratic results are obtained if bound water represents a significant portion of the total water content. This is clearly evident from an examination of the results shown in Table II, where blender extrac-

Table III—Effect of Reflux Time on Water Extraction[a]

Crude Drug	Water, %: Direct GLC, 1 hr. Reflux	Direct GLC, 3 hr. Reflux	Indirect GLC, 1 hr. Reflux	Indirect GLC, 3 hr. Reflux
Acacia	12.37	12.06	12.70	12.74
Colchicum corm	8.40	8.61	8.88	8.70
Ipecac	8.22	8.18	8.96	8.37
Tragacanth	9.31	9.11	9.20	9.48

[a] Mean results of two or more estimations.

Table IV—Recovery Studies of GLC Determination (Reflux Extraction), Gravimetric Analysis, and Azeotropic Distillation[a,b]

Crude Drug	Water, Percent in Crude Drug: Originally Present	Added	Total Calculated	Total Found	Recovery Difference, %
Direct GLC					
Belladonna leaf	6.40	5.00	11.40	10.50	−7.90
Pectin	9.53	3.00	12.53	12.33	−1.60
Nux vomica	8.82	7.00	15.82	16.68	+5.44
Indirect GLC					
Belladonna leaf	6.82	5.00	11.82	12.30	+4.06
Pectin	9.20	3.00	12.20	12.81	+5.00
Nux vomica	8.65	7.00	15.65	15.00	−4.16
Gravimetric analysis					
Belladonna leaf	8.07	5.00	13.07	12.55	−3.98
Pectin	12.41	3.00	15.41	15.29	−0.78
Nux vomica	9.45	7.00	16.45	16.04	−2.50
Azeotropic distillation					
Belladonna leaf	8.00	5.00	13.00	11.50	−11.54
Pectin	12.50	3.00	15.50	15.00	−3.23
Nux vomica	9.00	7.00	16.00	15.00	−6.25

[a] Each recovery value is the mean of two determinations. [b] Gravimetric and azeotropic analyses were made on lots of belladonna leaf, pectin, and nux vomica other than those used in Tables I and II.

tion, in each case, gave a discernible difference in the GLC values as compared to gravimetric analysis and azeotropic distillation. Reflux extraction, however, gave values for water content by GLC that are in good agreement with those given by the other two methods. The results in Table III show that an increase in the reflux time from 1 to 3 hr. did not substantially increase the values for water content.

Water binding is known to exist in natural products in which the carbohydrate content is characteristically high (4). In this study, a number of such products appeared to contain bound water in amounts ranging from 30 to 55%.

The good agreement obtained between the GLC technique and official procedures for water determination in crude drugs suggests that the results represent an accurate assessment of the actual water content of these materials. The accuracy of the GLC procedure was further established by determining the recovery of added water to three selected crude drugs. Table IV shows a comparison of the recovery data obtained by GLC with those given by gravimetric analysis and azeotropic distillation. The results indicate that the accuracy of analysis by direct and indirect GLC (5 and 4.40%, respectively) is within the accuracy range obtained by the official methods (2.42% for gravimetric analysis and 7% for azeotropic distillation). The reproducibility of the GLC analysis is satisfactory. This can be readily seen from the results shown in Table V; the maximum standard deviation obtained for direct and indirect procedures after reflux extraction was 0.20 and 0.31, respectively.

Table V—Reproducibility of GLC Analysis (Reflux Procedure)[a]

Crude Drug	Water, %: Direct GLC	Indirect GLC
Acacia	11.93, 12.81	12.62, 12.78
Nux vomica	8.73, 8.80, 8.92	8.31, 8.68, 8.97
Pectin	9.10, 9.45, 10.04	9.01, 9.15, 9.44
Tragacanth	9.30, 9.33	9.02, 9.14, 9.45

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

SUMMARY

The data obtained in this study underline the fact that crude drugs bind water to different degrees within their cellular composition. Water in this form cannot be completely removed by blender extraction for subsequent analysis by GLC. To assess accurately the moisture content of such products by GLC, the reflux procedure described herein should be used. For crude drugs that do not retain part of their water in a bound form, the blending and reflux procedures appear to be equally accurate.

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