

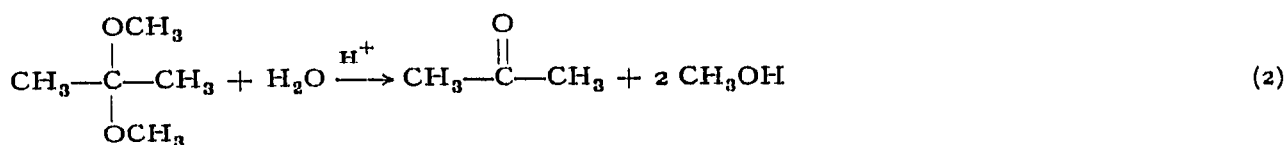
### Comparative study of a direct and a new indirect gas-liquid chromatographic procedure for the estimation of water in natural products

The water content of natural products is very significant for the potency of many of these materials insofar as it affects the stability of the active principle(s). Such significance is attested by the definition of methods for moisture determination in the official compendia, the specification of permissible limits of moisture in the monographs of certain crude drugs, and the specification of methods of storage designed to limit moisture accumulation.

There are a great variety of analytical methods for determining the water content of natural products. The classical methods often used include gravimetric, azeotropic and titrimetric procedures. Many official compendia, including the United States and British Pharmacopeias, recognize one or more of these procedures. In each case, there exist certain disadvantages and limitations. The gravimetric methods are time-consuming and volatile substances other than water may effect the determination<sup>1</sup>. Water analysis by azeotropic methods requires considerable care and attention during operation, needs large samples of the material and, in many cases, may fail to obtain the release of all the water from the natural product<sup>1</sup>. Titrimetric procedures, on the other hand, suffer from interferences of compounds that readily undergo oxidation-reduction side reactions with the Karl Fischer reagent, and the difficulty in getting a clearly defined end-point; in addition, they require special precautions in the preparation, storage and use of the Karl Fischer reagent.

The use of gas-liquid chromatography in the study of water analysis has increased in recent years. Using this technique, assays have been developed by a number of investigators for the determination of water in a variety of natural products<sup>1-4</sup>. These assays combine direct separation and quantitation of water and, therefore, must utilize the less sensitive thermal conductivity detector because of the unsuitability of flame ionization detectors for the analysis of water. Despite this limitation, these methods still possess considerable advantages over other methods of analysis.

Indirect gas-liquid chromatographic procedures, in which water is converted to more readily analyzed substances, also have appeared recently in the literature. In these methods, the analysis of water is based upon its conversion to acetylene with calcium carbide (eqn. 1) or to acetone with 2,2-dimethoxypropane (DMP) (eqn. 2); the acetylene or acetone formed in these reactions is subsequently determined by gas-liquid chromatography, using either thermal conductivity detectors or the more highly sensitive flame ionization detectors.



Although gas-liquid chromatographic procedures based on the calcium carbide reaction have been applied in the quantitative determination of water in various materials<sup>5-9</sup>, such procedures have not resulted in a good general method for water determination. The stoichiometry of the reaction is not simple and the optimum conditions for carrying out the conversion of water to acetylene have not been adequately established. Furthermore, the reaction is inapplicable in the presence of alcohols which interfere to give acetylene and a wide range of other products<sup>10, 11</sup>. In this study methanol is used to extract water from the natural products, therefore the application of the calcium carbide reaction was precluded.

The reaction of DMP with water has been studied by a number of investigators<sup>12-16</sup>. It is practically instantaneous to form the theoretical amount of acetone<sup>12</sup>. The equilibrium constant indicates that approximately 96% of the water reacts at 30° if mixed with DMP in a 1 to 1 mole ratio<sup>13</sup>. Mild heating<sup>13</sup> or acid catalysis<sup>12, 14-16</sup> may be necessary for the quantitative conversion of water. Although use of the DMP reaction has been investigated in the gas-liquid chromatographic analysis of water in a number of organic systems with good results<sup>14-16</sup>, its applicability for the determination of water in natural products has not yet been described. This investigation was undertaken to demonstrate that the reaction of water with DMP can be utilized for its determination in natural products by gas-liquid chromatography, using a flame ionization detector. In addition, it compares the results obtained by this method to those previously obtained in this laboratory from a direct gas-liquid chromatographic procedure.

### *Experimental*

A Perkin Elmer Model 811 gas chromatograph, equipped with a hydrogen flame ionization detector and a 0-1 mV Speedomax G recorder with a chart speed of 2 in./min and a 1 sec full scale response, was used. The chromatographic column was a stainless steel coil, 1/8 in. in O.D., 6 ft. in length and packed with 30% tetrahydroxyethylethylenediamine on Chromosorb W, 80/100 mesh. The column was equilibrated for 72 h at the operating conditions before use. The operating conditions were: hydrogen pressure, 46 p.s.i.g.; air pressure, 56 p.s.i.g.; helium flow rate, 30 ml/min; injection block temperature, 140°; detector block temperature, 160°; oven temperature, 100°.

In a typical analysis 10 g of the natural product was placed in a Waring blender (500 ml, screw-on-lid) containing 100 ml of spectro-grade methanol. The material was comminuted for 5 min, allowed to settle, then a 10.0 ml aliquot of the clear supernatant was drawn into a pipette and transferred quantitatively to a 25 ml volumetric flask. Two milliliters of DMP and 5.0 ml of 0.1 *N* methanesulfonic acid (to catalyze the reaction<sup>14, 16</sup>) were added, the flask was shaken for a few minutes and the reaction mixture was diluted to volume with methanol. Three microliters were then injected with a Hamilton microliter syringe into the gas chromatograph.

The concentration of water in the sample analyzed was obtained by absolute calibration. The peak area of the acetone formed in the reaction was related to the peak areas of acetone formed in standard solutions containing 25.0-150.0 mg of water prepared in exactly the same manner as described above for the plant extract. Under the experimental conditions cited, each unknown was bracketed with two standard solutions before proceeding to the next unknown. A blank was run with each set of analytical determinations.

*Results and discussion*

Fig. 1 shows a typical chromatogram of an extract obtained from ergot. The water content of the natural products analyzed by this procedure are given in Table I. The results are compared with those previously obtained in this laboratory from a direct gas-liquid chromatographic method<sup>1</sup>. The correlation between the values obtained in each case is satisfactory. Analysis by the indirect method gave results slightly higher than those obtained by the direct method. This may be due to the greater sensitivity of the flame ionization detector used in the indirect gas-liquid chromatographic method. Direct thermal conductivity detection lacks adequate sensitivity and indirect methods have been employed to increase sensitivity in the analysis of water in various materials<sup>7-9</sup>.

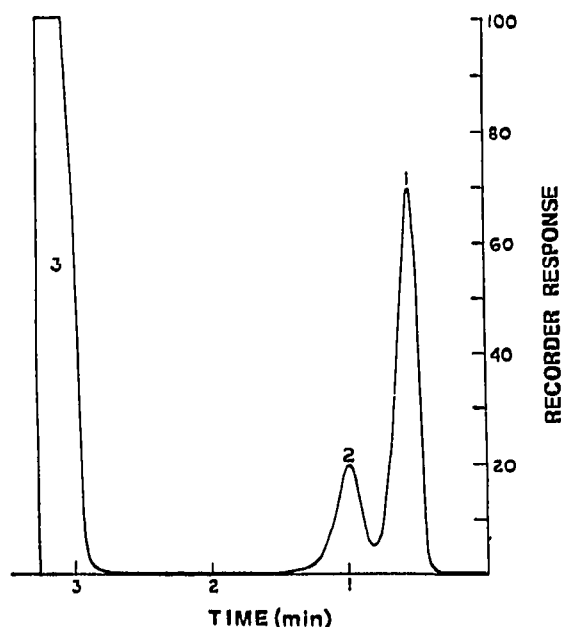


Fig. 1. Gas chromatogram of the methanol extract from ergot. 1 = 2,2-Dimethoxypropane; 2 = acetone; 3 = methanol.

MARTIN AND KNEVEL<sup>10</sup> described a gas chromatographic method for water determination in organic solvents which utilized the DMP reaction and which took into account both the peak heights of the acetone produced and the DMP unreacted for the calculation of the water content. In the light of this report, we calculated water content in the natural products utilizing our results (based instead on area calculations) and the formula derived by MARTIN AND KNEVEL. The values obtained are shown in the third column of Table I; they are quantitatively in accord with the values obtained by the much less painstaking absolute calibration procedure based on the acetone peak, alone.

It appears, therefore, that water analysis of natural products by the indirect method is both feasible and reliable. A most noteworthy characteristic of the new method is the ability to use the flame ionization detector for the detection and determination of water not only in natural products but in other organic systems as well. The new procedure is simple, rapid and, owing to its greater sensitivity, requires

TABLE I

COMPARISON OF WATER CONTENTS AS DETERMINED BY DIRECT AND INDIRECT PROCEDURES

Natural product	Water content (%)		
	Direct <sup>a</sup>	Indirect <sup>b</sup>	
		Absolute calibration	Martin and Knevel's formula
Agar	14.72	14.90	14.62
Belladonna	6.43	7.07	6.76
Cascara Sagrada	7.11	8.50	8.42
Clove	4.60	5.85	5.75
Digitalis	3.89	4.16	4.22
Ergot	5.55	6.30	6.15
Gentian	6.08	6.80	6.77
Ginger	5.95	6.91	6.72
Peppermint	6.91	8.21	7.87
Starch	9.00	9.18	9.25

<sup>a</sup> The values are obtained from ref. 1.

<sup>b</sup> Each value is the mean of six chromatographic injections representing two or more extractions of the natural product.

a smaller amount of the plant material than methods employing direct analysis. It should be indicated, however, that water analysis by the new method would be improved considerably and calculations would be simplified greatly if a suitable internal standard is added to the DMP reaction mixture before injection into the gas chromatograph. Experiments are currently in progress with this objective in mind.

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