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Keyphrases

α-5,9-dimethyl-6,7-benzomorphans, *N*-substituted—synthesis
Pharmacological screening—5,9-dimethyl-6,7-benzomorphans
Analgesic activity
Depressant activity
Hypothermic activity

Titration of Residual Moisture in Lyophilized Vials

By EDGAR E. THEIMER and JOSEPH J. PAVELEK

Karl Fischer titration of residual water in lyophilized vials can be performed very simply, without specialized equipment, and gives results which are more reliable than loss-on-drying methods.

IN THE determination of moisture, the Karl Fischer (K.F.) titrimetric method presents many advantages over the weight-loss-on-drying method. The literature abounds with comparative studies, most of which favor the K.F. method. Levy, Murtaugh, and Rosenblatt (1) titrated water in penicillin vials, assuming a zero blank. Wiberley (2) stressed the use of micro-equipment and introduced some improvements intended to broaden the use of the K.F. reagent. Burger and Polderman (3) used similar technique in some precise work on effect of lyophilizing conditions on residual water, using mannitol, *etc.* For lyophilized proteinaceous preparations, Sobel (4) suggested a colorimetric procedure, and Alajos (5) devised a special two-chambered vial for extraction of water and subsequent K.F. titration, to prevent direct contact of K.F. reagent with the protein.

Figure 1 depicts a vial containing a lyophilized mixture of 100 mg. of mannitol and 20 mg. of acetylcholine chloride.[1] It was desired to set up a specification limiting the water content of these vials in production batches. The primary aim of this study was to devise a simple quantitative test for water, of reasonable precision, without the use of specialized apparatus, and as free as possible from sources of error.

The first test method used involved drying in a vacuum oven to constant weight. Since it seemed likely that the rubber center seal might lose some weight during this treatment, similar blank vials were simultaneously treated and a correction applied. After further trials, it became evident that this correction value was variable, sometimes becoming as large as 0.6 mg. Also, it was found difficult to prevent weight gain between drying and weighing. Another difficulty was the need for microweighing technique in handling relatively large vials. In addition, there was some evidence that slight differences in drying temperature gave different results. In order to deal with these difficulties, various rather awkward dodges were devised, including weighing the vials while still hot, withdrawing and discarding the center seal, and pairing of samples with individual blank vials, but eventually a different water determination method was sought.

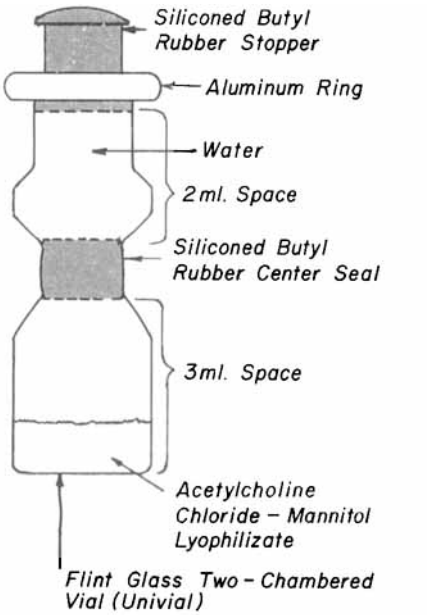

Fig. 1—Vial containing lyophilized mixture of 100 mg. mannitol and 20 mg. acetylcholine chloride.

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[1] Marketed as Miochol by Smith, Miller & Patch, Inc., New Brunswick, N. J.

The technique in the following method is simple and probably free from important sources of error. It does not involve the use of a magnetic stirrer, titrating vessel with special cover, or humidity-controlled work chamber, nor special drying of the diluent, methanol. In the case dealt with here, with the white background afforded by the methanol-insoluble mannitol, the end point can be detected visually when strong K.F. reagent is used and no electrometric equipment is needed. By using the vial as titrating vessel, it is possible to avoid sample transfer and exposure to the atmosphere. Also, the vial being designed to allow penetration of the stopper with a hypodermic needle for withdrawing the contents without exposure to the atmosphere, a hypodermic needle can be similarly used for addition of K.F. reagent. These advantages are important because the amount of water determined is less than 1 mg. and the sample is hygroscopic.

The actual amount of water in the methanol and the "handling blank" involved in the manipulations need not be determined directly, since they cancel out automatically.

TITRIMETRIC DETERMINATION OF WATER IN FREEZE-DRIED ACETYLCHOLINE CHLORIDE-MANNITOL

Reagents—*Methanol*—Rinse adequately an automatic buret equipped with a silica-gel drying tube, with absolute or anhydrous grade methanol; then fill it with methanol. Various brands were used in this work. A.C.S. anhydrous methanol "low in acetone" (Matheson, Coleman, and Bell) was found to give the lowest blank.

Water in Methanol—Rinse adequately a 100-ml. volumetric flask with a needle-penetrable stopper, using methanol from the same container. Add exactly 0.1 ml. water. Add methanol to volume.

Karl Fischer Reagent—Use the stabilized reagent available commercially with each ml. equivalent to approximately 5 mg. water. K.F. Reagent, single solution, stabilized (Fisher Scientific Co.) was used.

Protect all liquids from atmospheric moisture as much as possible.

Procedure—Preparation of vials and stoppers for blanks and standards. Obtain 10–20 vials similar to the sample vials, with siliconed stoppers and center-seals. Remove metal rings. Discard water in the top chambers. Push center seals through, using a metal rod or similar tool. Clean vials and stoppers by rinsing, as necessary, with water, acetone, and an air stream. Place them in a 105–110° oven for 1 hr. Remove the vials and stoppers from the oven, and stopper the vials at once. Store in a desiccator until used.

Preparation of Samples—Remove stoppers and metal rings. Pour water out from top chamber. Dry stoppers and top chambers with acetone and a stream of air. Place vials and stoppers in 105–110° oven for 1 hr. Remove, and at once insert oven-dried stoppers. Store in a desiccator until used.

Titration of Blanks—Remove stopper from vial, quickly add 2.0 ml. of methanol from buret and replace stopper. Release pressure by puncturing stopper with breather needle. Remove needle. Shake well. Titrate with K.F. reagent from a 1-ml. tuberculin syringe and needle. Repeat until precision confidence is established.

Titration of Standards—With a calibrated syringe and needle, withdraw water in methanol through the stopper, first rinsing the syringe several times, then measuring 2.0 ml. Remove stopper from Univial and quickly add the 2.0 ml. water in methanol and replace stopper. Insert breather needle. Shake well. Titrate with K.F. reagent from 1-ml. tuberculin syringe and needle. Repeat until precision confidence is established.

Titration of Samples—Remove stopper, quickly push center seal through, using a suitable instrument, add 2.0 ml. methanol from buret and replace stopper. Release pressure by puncturing stopper with breather needle. Remove needle. Shake well. Titrate with K.F. reagent from 1-ml. tuberculin syringe and needle. Fading of amber color is due to slow extraction of the last bit of water from the crystals by K.F. reagent. The end point should be stable for 15 sec.

Calculation—Sample titration = V_{sam}, blank = V_B, standard = V_{stan}. Then the weight of water in the lower chamber of the sample is:

$$\frac{V_{sam} - V_B}{V_{stan} - V_B} \times 2 \text{ mg.}$$

DISCUSSION AND RESULTS

The blank titer combines K.F. reagent comsumption due to: (*a*) water in the methanol reagent. (*b*) moisture unavoidably introduced in the handling.

Although some attempts were made to use as blanks vials containing oven-dried samples, the values showed unsatisfactory reproducibility. It was felt that any difference in titer between such blanks and the empty-vial blanks eventually adopted was due to the handling errors caused by the extreme hygroscopicity of acetylcholine chloride.

In order to justify the use of empty-vial blanks it was necessary to determine whether completely dry acetylcholine chloride or mannitol consume K.F. reagent. Two-gram samples of each were oven-dried to constant weight in flasks, closed with rubber-stoppers fitted with a short needle-penetrable rubber core, and cooled in a desiccator. Twenty-five milliliters of methanol was added quickly by pipet and the flasks were titrated with K.F. reagent. An empty flask was similarly treated, as a blank.

It was found that mannitol was easily dried at 105° to give zero net titer, and that acetylcholine chloride could be dried to a negligibly small or zero net titer by heating at 110° for 16 hr., although 105° was not hot enough to drive off the last 0.1% moisture in some cases even after 4 days heating.

In order to study the effect of the drying temperature used in preparing blanks, the following experiment was run. Two groups of samples, all from the same batch, were prepared:

(*A*) Top chamber dried with acetone, an air-stream, and 10 min. warming close under an infrared lamp. (Infra-red Economy Heater, Fisher Scientific Co.).

(*B*) Top chamber dried with acetone, an air stream, and heating entire vial at 110° for 3 hr.

The vials were then stoppered with oven-dried stoppers, and titrated in the usual way. The titration values are shown in Table I. The following conclusions may be drawn from this experiment.

(*a*) The brief warming of the top chamber of the Group A vials is sufficient to dry them, even though the center seal has been in contact with water for a

Table I—Reagent Needed to Titrate Samples Subjected to Varying Pretreatment, ml.

A	B
0.23	0.24
0.24	0.25
0.28	0.30
0.25	0.29
0.23	0.22
0.28	0.24
0.25	0.23
0.27	0.24
0.24	0.30

authors found that a lyophilized B complex containing 10 mg. riboflavin could be titrated to an easily identified end point despite the color in the sample. Other types of sample have not been tested but it would seem that the method is applicable to any parenteral-type vial containing lyophilized or powdered sample low in water content, unless it is deeply colored or contains a substance which interferes with the titration, such as ascorbic acid.

Simple vials do not require the preliminary preparation described for the double-chambered vial, but can be titrated directly.

Table II—Determination of Water in Freeze-dried Acetylcholine Chloride–Mannitol Mixture

Blanks	Standards	Samples			
		Lot X		Lot Y	
Titer, ml.	Titer, ml.	Titer, ml.	H_2O, mg.	Titer, ml.	H_2O, mg.
0.07	0.45	0.22	0.77	0.23	0.83
0.07	0.44	0.28	1.09	0.18	0.56
0.08	0.45	0.21	0.72	0.17	0.50
0.07	0.45	0.38	1.63	0.19	0.61
0.09	0.45	0.22	0.77	0.25	0.93
Av. = 0.076	Av. = 0.448	0.20	0.66	0.17	0.50
		0.23	0.83	0.21	0.72

long time and might be expected to have absorbed some water not easily driven off.

(*b*) There is no significant change in K.F. titer even at 110° for 3 hr., either through volatilization of water through the center seal or through chemical reaction.

For simplicity, oven heating was adopted for the procedure.

Table II shows some results obtained by the proposed method, the values for water in samples of Lot X and Y being calculated using the average blank and standard titers.

A recalibrated pipet was used in preparing the water in methanol standard. A weighed quantity of standard hydrated sodium acetate might be considered as an alternate.

Applicability to Other Types of Sample—The

CONCLUSION

It is believed that the proposed titrimetric procedure is the method of choice for determining water in certain applicable cases.

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Keyphrases

Lyophilized substances—moisture in vials
Vials—moisture determination
Karl Fischer titration—analysis
Methanol—reagent

Preliminary Phytochemical Investigation of Wild Rice Ergot

By GREGORY T. SINNER* and LEE C. SCHRAMM†

Ergot parasitizing wild rice was analyzed for moisture, fat, and alkaloid content. Results indicated 4.6 percent moisture, 32 percent fat, and a total alkaloid content of approximately 0.1 mg. percent. The fungus was cultured in a modified *Claviceps* culture medium, but alkaloids could not be detected in culture filtrates.

Species of Claviceps have been found as parasites of nearly all members of the grass family (1).

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The occurrence of this fungus on wild rice (*Zizania aquatica*) was first reported by Dennison in 1900 (2). In 1915, Fyles (3) published a preliminary morphological description of wild rice ergot and noted its host specificity. Although she was able to cause infestation of healthy wild rice by inoculation with conidial suspensions and honey dew from diseased wild rice, she was repeatedly unable to infect other healthy grains (normally susceptible to *Claviceps* infestations) with this organism. Presumably because of this, Fyles later referred to the ergot of wild rice as a distinct species, *Spermoedia zizaniae*, a