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(TENTATIVE. NOT FOR PUBLICATION)

DETERMINATION OF SECONDARY, TERTIARY AND TOTAL ALKALOIDS IN TOBACCO (1)

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REAGENTS, SOLUTIONS, AND APPARATUS

Acetic acid, A.C.S. grade

Acetic anhydride, A.C.S. grade

Filter-Cell Filter aid, Johns-Manville Corp.

Perchloric acid, 70-72%, A.C.S. grade

Potassium acid phthalate, primary standard grade

Benzene-chloroform solution. Mix equal parts by volume of benzene

and chloroform. Saturate the solution with 50% sodium hydroxide.

Sodium hydroxide solution, 50%. Dissolve 500 g. sodium hydroxide in water and dilute to 1 liter.

Acetic acid solution, 5%. Dilute 50 ml. of glacial acetic acid to 1 liter with water.

Crystal violet indicator solution. Dissolve 0.5 g. crystal violet in 100 ml. of glacial acetic acid.

Perchloric acid solution, 0.025 N. Add 4.7 ml. of 72% perchloric acid to a freshly opened 5-lb. bottle of glacial acetic acid and mix.

Standardize as follows: Accurately weigh 0.1 g. potassium acid phthalate into a 125-ml. Erlenmeyer flask. Add 50 ml. glacial acetic acid and heat to effect solution. Cool, add 2 drops crystal violet indicator solution and titrate to a blue-green and

- (1) Modification of procedure by the same authors published in Anal. Chem. 27, 1650 (1955).

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point. Perform a blank titration on 50 ml. acetic acid and 2 drops indicator solution and correct the volume of titrant accordingly (See Note 1).

$$N = \frac{\text{Sample Wt.} \times 4.89668}{\text{Volume of Titrant}}$$

Wrist-action shaker, Model BB, Burrell Corp., Pittsburgh, Pa.

PROCEDURE

Accurately weigh 2.5 grams of finely ground tobacco into a 250-ml. standard tapered Erlenmeyer flask. Add 15 ml. 5% acetic acid solution, and swirl the flask until the tobacco is thoroughly wetted. Pipet 100 ml. of benzene-chloroform solution (1:1 v/v) into the flask, then 10 ml. of 50% sodium hydroxide solution. Stopper the flask tightly and shake for 20 minutes using the shaker. Add 4.5-5 g. (two teaspoonsful) of Filter-Cell, filter aid, mix, and filter the majority of the hydrocarbon layer through Whatman No. 2 filter paper into a second flask. If the filtrate has any turbidity add 2-2.5 g. (1 teaspoonful) additional Filter-Cell and refilter through Whatman No. 2 paper (See Note 2). Pipet 25 ml. aliquots of the filtrate into each of two 125-ml. Erlenmeyer flasks. Pass a stream of air over the surface of the solution in the first flask for five minutes, add 2 drops of crystal violet indicator and titrate to a green end point with 0.025 N perchloric acid. Add 1.0 ml. of acetic anhydride to the second flask and allow to stand at least 15 minutes, add 25 ml. acetic acid and 2 drops of crystal violet indicator solution (See Note 3) and titrate to a blue-green end point with 0.025 N perchloric acid. For each series of analyses perform blank titrations and correct the respective volumes of titrant (See Note 1).

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COMPUTATIONS

$$\% \text{ total alkaloids, as nicotine} = \frac{V_1 \times N \times 32.43}{\text{Sample Wt.}}$$

$$\% \text{ tertiary alkaloids, as nicotine} = \frac{(2V_2 - V_1) \times N \times 32.43}{\text{Sample Wt.}}$$

$$\% \text{ secondary alkaloids, as nor nicotine} = \frac{2(V_1 - V_2) \times N \times 29.64}{\text{Sample Wt.}}$$

V_1 = volume of titrant for non-acetylated aliquot

V_2 = volume of titrant for acetylated aliquot

N = normality of perchloric acid

Note 1 - The reagent blank values, both in standardization of the titrant and in analysis of tobacco samples, are quite small; however, they should be applied for the most accurate results. If not applied in standardization, they should not be applied in analysis of tobacco samples.

Note 2 - It is most critical that a clear filtrate be realized at this point, otherwise high results will be obtained.

Note 3 - The addition of the acetic acid to the acetylated aliquot precludes the necessity of a potentiometric titration in analysis of tobacco extracts. Potentiometric titrations should be employed, however, in titration of acetylated nicotine-nornicotine solutions obtained from the picates.

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