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In connection with some work on the physiological effects of ethyl alcohol on rats being done in this laboratory, it was essential to have a rapid and accurate method for the determination of ethyl alcohol in small samples of blood.

A large number of methods has been reported in the literature, most of them being based upon the oxidation of ethyl alcohol by potassium dichromate in strong sulfuric acid solution as devised by Widmark (1) and Nicloux (2). Heise (3) and Abels (4) compare the color produced by the oxidation products with that produced by known amounts of ethyl alcohol using the same amount of oxidizing agent (potassium dichromate and sulfuric acid) in the same total volume. A disadvantage to these methods is the fact that the color standards are unstable. Heise distills the alcohol from the sample previously treated with picric acid-tartaric acid solution. Miller and Getchell (5) use steam distillation from picric acid solution. They determine the excess potassium dichromate by titration of the iodine liberated from excess potassium iodide with standard sodium thiosulfate. Beeman (6) obtains the alcohol from the sample by steam distillation. The alcohol is oxidized by excess dichromate in sulfuric acid. The acetic acid produced is distilled under diminished pressure and titrated with standard sodium hydroxide. A large amount of sample is necessary.

Abels' method removes the alcohol from the sample by absorbing the latter on a roll of filter paper and suspending it in a stoppered flask above a measured amount of potassium dichromate-sulfuric acid mixture and heating. The color is compared with a series of standards. Sheftel (7) employs the same method of obtaining the alcohol from the sample but determines the excess dichromate by the colorimeter. Cavett (8) places the sample in a glass cup suspended in a glass stoppered flask containing standard potassium dichromate and sulfuric acid and after heating determines the excess potassium dichromate by titration as described by Harger (9). Harger prepares blood filtrate from which the alcohol is removed by distillation. After the oxidation of a suitable aliquot of the distillate by standard potassium dichromate in strong sulfuric acid the excess dichromate is deter-

mined by titration with a solution of methyl orange and ferrous sulfate. Fleming and Stotz (10) distill the alcohol from blood filtrate. The alcohol is determined by oxidation with excess potassium dichromate. The excess of the latter is determined by the addition of a measured amount of standard ferrous sulfate. The excess ferrous sulfate is determined by titration with standard potassium permanganate.

Newman (11) removes the alcohol from the sample by the application of a vacuum. The alcohol is oxidized by potassium dichromate and sulfuric acid. The excess dichromate is determined by titrating the iodine liberated from potassium iodide with standard sodium thiosulfate. Gibson and Blotner (12) determine the excess potassium dichromate by use of a photoelectric colorimeter equipped with a filter to correct for the absorption of light by the $Cr_2(SO_4)_3$ formed in the reaction. Friedemann and Klaas (13) distill the alcohol from the diluted sample after treatment with sodium tungstate and mercuric sulfate-sulfuric acid mixture. The alcohol is determined by oxidation with standard alkaline potassium permanganate. After the oxidation is completed the excess permanganate is determined iodimetrically. This method is recommended by Hinsberg (14) for its specificity. Levine and Bodansky (15) propose a method very similar to the method of Cavett (8) except that the sample is absorbed on a filter paper. The titration is based upon the reducing fluid first proposed by Harger (9).

In most of these methods the amount of sample necessary rendered them unsuitable for our purposes. Our method is designed to combine specificity with speed using a minimum of sample.

Experimental

The sample, 0.1 ml. of blood, is diluted with water and then sodium tungstate solution and mercuric sulfate-sulfuric acid mixture are added to precipitate the blood proteins. The alcohol is distilled from this mixture in a manner similar to that of Friedemann and Klaas (13). The distillate is treated with potassium dichromate and sulfuric acid and the excess exidizing agent determined as in the method of Harger (9).

Apparatus. An all glass distillation apparatus is used consisting of a 50 ml. distillation flask fitted to a vertical condenser by a ground glass joint.

The distillate is received in a hard glass reaction tube, 22 mm. x 175 mm., graduated to contain 5 ml.

To facilitate titration it is convenient to use an air stirring device as described by Harger (9).

Reagents. Standard potassium dichromate, 0.0434 N containing 2.129 grams of pure potassium dichromate per liter, 1 ml. of this solution is equivalent to 0.50 mg. of ethyl alcohol.

Concentrated sulfuric acid C. P.

Sulfuric acid, 60 per cent by weight. A 1:1 mixture of water and concentrated sulfuric acid.

Methyl orange, a 0.1 per cent solution in 0.025 N sodium hydroxide.

Ferrous sulfate (20 per cent) containing 120 ml. of concentrated sulfuric acid per liter. This solution will keep about one year.

Sodium tungstate, a 10 per cent solution in water.

Mercuric sulfate-sulfuric acid solution containing 100 grams of mercuric sulphate and 56 ml. of concentrated sulfuric acid per liter.

Red reducing fluid is prepared by adding 15 ml. of the methyl orange solution to 30 ml. of 60 per cent sulfuric acid solution and then 1 ml. of the ferrous sulfate solution is added. Mix and cool. This solution keeps about 2-3 days but should be standardized each day.

Procedure. With an accurate pipette measure 0.1 ml. of freely flowing or oxalated blood and rinse into the distilling flask with 10 ml. of distilled water. Add 1 ml. of 10 per cent sodium tungstate and 1 ml. of mercuric sulfate-sulfuric acid solution. Mix. Distill, collecting 5 ml. of distillate. This distillation should be at such a rate that the 5 ml. are distilled in about 15 minutes. The distillate is collected in the reaction tube. Add 1 ml. of standard potassium dichromate from an accurate micro burette. Mix. Add 5 ml. of concentrated sulfuric acid and mix carefully so as to avoid boiling. Allow to stand 10 minutes then cool to room temperature and titrate with the red reducing fluid using an accurate burette graduated to 0.02 ml. The first permanent pink tinge is the end point.

Treat 5 ml. of distilled water exactly the same as the distillate. This is the water blank which serves to correct for the reducing substances in the sulfuric acid.

After titrating a sample to the end point add 1 ml. of standard potassium dichromate, mix, and titrate to the same end point in order to standardize the red reducing fluid against the standard potassium dichromate.

Calculations.

$$\frac{\text{(ml. water blank)} - \text{(ml. titration of sample)}}{\text{ml. equivalent to 1 ml. } K_2Cr_2O_7} \times 500 = \text{mg. percent ethyl alcohol.}$$

To check the accuracy of the method a series of blood samples containing known amounts of ethyl alcohol were analyzed by the micro method and by the method of Friedemann and Klaas (13). The results are shown in table 1.

Table 1

	Concentration of alcohol in mg. per cent		
Sample	Actual	Micro	Friedemann and Klaas
1	47.5	48.5	44.5
2	129.0	128.5	130.4
3	171.7	172.3	166.5
4	258.0	256.5	240.0
5	309.0	307.0	307.8

The samples were preserved by using as an anticoagulant 0.15 per cent of a mixture of equal parts of sodium fluoride and potassium oxalate.

Another series of blood samples was prepared and preserved in the same way. These samples were stored in a refrigerator at 1°C. Determinations were made at intervals of one week for three weeks, using the micro method. The results are shown in table 2.

Table 2

	Concentration of alcohol in mg. per cent		
Sample	Actual	Found	Found after three weeks
1	Normal	6.0	5.3
2	35.2	35.3	36.8
3	69.0	69.0	68.5
4	119.0	119.5	116.0
5	161.0	156.0	145.0
6	228.5	228.0	218.0
7	299.0	300.0	292.0
8	338.0	336.0	333.0
9	375.0	376.0	363.0
10	404.0	403.0	396.0

The samples were warmed to 20°C. before measuring the sample and then returned to the refrigerator at once. This experiment serves to illustrate the preservative action of the mixture of sodium fluoride and potassium oxalate as well as to check the accuracy of the method.

Discussion

Our method differs from that of Harger in the way that the alcohol is removed from the blood. We find that at least 1 ml. of blood is necessary to prepare sufficient blood filtrate for use in Harger's method. The method used to obtain the alcohol from the sample is similar to that used by Friedemann and Klaas except that the amount of sodium tungstate and mercuric sulfate-sulfuric acid solutions is reduced to 1 ml. instead of 5 ml. and the amount of sample, 0.1 ml., is satisfactory for all alcoholic concentrations up to 450 mg. per cent.

Our method presents certain advantages not heretofore combined in any one method. The small sample makes it possible to use finger tip blood or to obtain twelve or more samples from a small experimental animal at short intervals of time.

The time necessary for analysis is short. As many as fifteen analyses may be run easily in a day. In addition, one set of reagents involving two standard solutions is adequate for all samples usually encountered.

The method is accurate and quite specific for alcohol under usual conditions. At least most of the volatile non-alcoholic reducing constituents are separated from the alcohol by the distillation. As little as 0.05 ml. of blood is satisfactory for determination of concentrations of alcohol above 30 mg. per cent.

Summary

1. A new micro method has been given for the determination of ethyl alcohol in blood.

2. The micro method is rapid and is of the same order of accuracy as that of Friedemann and Klaas which is highly recommended for its accuracy.

3. The apparatus is simple and trouble free.

4. The reagents necessary are easily prepared and, with the exception of the red reducing fluid, are stable for at least one year.

5. The addition of 0.15 per cent of a mixture of 1:1 sodium fluoride and potassium oxalate to blood serves to prevent coagulation and preserves the blood for two to three weeks. The alcoholic content of the blood will remain quite constant for that time.

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