

A SIMPLE METHOD FOR THE DETERMINATION OF ACETONE IN BLOOD AND URINE

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With the increasing use of volatile solvents in industry, simple and rapid methods for their detection in body fluids are becoming more important both from a medicolegal and a clinical point of view. Recently we described such a technique for the determination of ethyl alcohol in blood (1) and saliva (2), and the present paper is an extension of the same method for estimating acetone and other volatile ketones.

The reaction of acetone with Nessler's solution to form a cream-white precipitate is a familiar observation, and we have made use of this reaction for the quantitative detection of as little as 0.002 mg. of acetone in a 0.5 ml. sample of blood or urine. The acetone is first absorbed into a solution of sodium bisulfite which is then treated with the Nessler's solution; precipitates form, varying in intensity from a faint haze to definite turbidities, depending upon the amount of acetone present. These turbidities are finally compared against a set of standards made from known amounts of acetone. The ketone can be determined in the blood or urine without any preliminary protein precipitation, dilution, or distillation of acetone. The whole determination takes 20 minutes, and the actual manipulation less than 5 minutes.

Method

Reagents—

1. Sodium bisulfite, 5 per cent solution in water.
2. Nessler's solution (Koch and McMeekin's reagent (3)).
3. Sulfuric acid, 1:1 dilution by volume.
4. Standard acetone solution. A stock solution containing 0.1

mg. per ml. of acetone is prepared and checked gravimetrically by the method of Van Slyke (4). This solution retains its titer for about 2 weeks. The standard solutions used are made by a 1:10 dilution of this, so that 1 ml. contains 0.01 mg. This should be prepared daily.

Technique—The apparatus consists of a 50 ml. Erlenmeyer flask and a tightly fitting cork. Suspended from the under surface of the cork by means of a pin is a cm. length of cotton pencil. This pencil is a compressed roll of absorbent cotton, and is commonly supplied by many firms (Johnson and Johnson) to dentists under the name of dental rolls. It is best to use a pin about 6 cm. long, and with a large, beaded head. The pin is stuck first through the cotton plug and then into the cork.

The urine is made acid to Congo red with 1:1 sulfuric acid, and 0.5 ml. is pipetted onto the cotton roll. In the case of the blood, no previous acidification is necessary, and the 0.5 ml. sample may be used directly. 0.5 ml. of the 5 per cent sodium bisulfite is spread over the bottom of the flask, and, the cork is carefully and firmly inserted so as to allow the blood or urine sample to suspend about 1 cm. over the level of the bisulfite. The flask is heated in a boiling water bath for 15 minutes, and when cool, the cork and cotton roll are removed. 1 ml. of water is added, then 1 ml. of Nessler's solution, bringing the total volume to 2.5 ml. The solution is poured into a test-tube (6 inches \times 0.5 inch) and the amount of turbidity compared with the standard set.

The standards are prepared at the same time in similar test-tubes from 0, 0.002, 0.004 . . . 0.010 mg. of acetone, water to 1 ml., 0.5 ml. of 5 per cent sodium bisulfite, and 1 ml. of Nessler's solution. The full development of the turbidity takes 15 minutes, during which time the tubes should be shaken occasionally. If exposed as little as possible to the light, they keep for about 24 hours. When abnormal amounts of acetone are suspected in the blood or urine, a stronger set of standards containing 0, 0.005, 0.010 . . . 0.050 mg. of acetone should be prepared.

Results

Ten blank determinations with water alone on the cotton roll failed to reveal any acetone value. We then applied the method

to the estimation of acetone and acetoacetic acid in the blood and urine of normal individuals, and obtained the following values.¹

	No. of observations	Acetone mg. per cent
Blood.....	30	0.3-2.0
Urine.....	50	0.2-2.5

Recovery experiments were also carried out on samples of human blood and urine with the results shown in Table I.

TABLE I
Recovery of Acetone from Blood and Urine

Ten experiments were performed in each case.

	Acetone added to 100 ml.	Acetone found in 100 ml. (blank corrected)	Recovery
	mg.	mg.*	per cent
Blood	0.50	0.45 \pm 0.04	90
	1.00	1.10 \pm 0.05	110
	2.00	2.20 \pm 0.10	110
	4.00	3.80 \pm 0.20	95
	8.00	8.50 \pm 0.50	106
Urine	0.50	0.46 \pm 0.04	94
	1.00	0.95 \pm 0.05	95
	2.00	2.20 \pm 0.08	110
	4.00	4.20 \pm 0.25	105
	8.00	7.60 \pm 0.45	95

* \pm average deviation.

Table II gives the values obtained by this method as compared with those obtained by that of Van Slyke (4) and of Ravin's modification of the Behre and Benedict technique (5).

DISCUSSION

It is to be noted that this method is by no means specific for acetone, for other ketones volatile in sufficient quantity under the

¹ These, and many of the following experiments, were carried out by using filter paper rolls instead of the cotton plugs. The preparation of these filter papers is described in a previous paper (1); twenty-five blank determinations with these papers similarly failed to show any acetone values, so the papers are quite safe to use, although less convenient.

conditions of the experiment are absorbed by the bisulfite and form similar precipitates with the Nessler's solution. We have found this to be so with butanone, and this may be quantitatively recovered from blood or urine in amounts as little as 0.002 mg. from 0.5 ml. samples.

There are, however, interfering substances which must be considered, principally ammonia and aldehydes. Only in the case of the urine is the former important, and unless the specimen is sufficiently acidified, the volatile ammonia dissolves in the bisulfite solution, and a heavy orange precipitate results upon the addition of the Nessler's solution. Fabre (6) and Supniewski (7) have found acetaldehyde to be the chief aldehyde of the blood,

TABLE II
Determination of Acetone and Acetoacetic Acid

Urine No.	This method	Van Slyke	Behre-Benedict
	<i>mg. per cent</i>	<i>mg. per cent</i>	<i>mg. per cent</i> *
1	1.60	1.45	
2	2.00	1.80	
3	1.80	1.70	
4	1.00		1.20
5	0.80		0.90
6	1.50		1.60
7	1.10	1.20	1.05
8	5.90	6.05	6.00
9	5.40	5.30	5.25
10	5.00	5.15	5.10

and to occur in normal concentrations of from 0.02 to 0.06 mg. per cent. Formaldehyde may also exist in larger amounts in the urine after certain medication (urotropine). Ordinarily the aldehydes are differentiated by this reaction from the ketones by the character of the precipitate formed: the ketones cause cream-white turbidities, while equal amounts of aldehyde form dark, metallic gray precipitates, so that when they occur in the urine or blood in excess their presence is readily manifested. However, in their normal concentrations, in the presence of a large excess of ketone, they must figure in the error of the determination, and may thus occasionally form as much as 15 per cent of the "found ketone" before lending a grayish color to the precipitate. This last is

obtained when the acetone is at the lower limit and the acetaldehyde at the upper limit of their normal ranges; usually the aldehyde will form only about 5 per cent of the estimated ketone.

Methyl and ethyl alcohols, and ethyl and butyl acetates, do not react with the Nessler's solution except in relatively large concentrations (about 1 per cent).

For the complete absorption of the ketones we have found it necessary to use a large excess of the bisulfite reagent. The dissociation constant of the acetone-bisulfite complex is relatively large, and it is only by means of the excess that an adequate absorption is obtained (8).

In these determinations we have found it necessary to use fresh samples of blood and urine, for as much as 20 per cent of the acetone is destroyed within 1 day. This is probably in a large measure due to bacterial decomposition, for when toluene is used to preserve the specimen, only about 5 to 10 per cent of the ketone is found to have disappeared. This, again, may be due to the acetone passing into solution in the toluene in which it is quite soluble (the partition coefficient of acetone between water and toluene is 2:1). Thus it is desirable to avoid using an excess of toluene as a preservative.

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SUMMARY

A rapid and simplified method for the determination of ketones in blood and urine is presented.

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