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Rapid Qualitative and Quantitative Microanalysis of Organic Substances by Means of a Glass-ring Oven Technique. III. Determination of a Trace Amount of Brucine in Strychnine Nitrate

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Synopsis. A rapid and highly selective method for the microdetermination of brucine in strychnine nitrate by the ring oven technique is presented. Nitric acid was used as a developing solution and color reagent. The detection limit was 0.2 μg, and the time needed for the determination about 5 min.

The spot test and the ring oven technique, among others, have been proposed in the literature for the simple determination of an alkaloid. The trace of brucine has been adulterated by strychnine production. It was determined in various alkaloids from 10 to 15 μg by use of the ring oven technique.[1] This work deals with a new simplified procedure, in which the detection limit and the determination range have been improved.

Experimental

Apparatus. A glass ring oven[2] was used. As the bath liquid 100 ml of water was used, the analytical procedure being carried out at 90 °C.

Brucine Standard Stock Solution. Brucine (Tokyo Chemical Industry Co., Ltd.) was recrystallized from water and ethanol. After it had been dried for 48 h in a sulfuric acid desiccator, a 100-mg portion of brucine was dissolved in 100 ml of 10 vol% ethanol. This standard stock solution was diluted as required.

Color Reagent. (1.0 M) Nitric acid was used both as color reagent and as developing solution.

Filter Paper. Toyo No. 54 filter paper, diam. 55 mm, was used. The other reagents were of analytical reagent grade.

Procedure. A filter paper was set upon a ring oven maintained at 90 °C. The sample solution was dropped in the center of the filter paper. After the spot had dried, the developing solution was dropped on the same place. Brucine was removed to the ring zone with 15 μl of 1.0 M nitric acid. After the developing solution had dried, brucine was removed with 20 μl of 1.0 M nitric acid. After the developing solution had dried, the amount of brucine was determined by comparing the color intensities of all the standard rings. We used 20, 30, and 50 μl for the sample solutions. The procedure was repeated three times. The amount of brucine was obtained by averaging the values of the three measurements.

Standard Rings. A filter paper was set upon the ring oven at 90 °C. Ten microliters of the brucine standard solution containing 0.2—1.0 μg per ml was dropped in the center of the paper. After the standard solution had dried out, 15 μl of a 1.0 M nitric acid solution was dropped in the center of the paper. After this solution had dried, 20 μl of a 1.0 M nitric acid solution was then dropped. Standard ring scales containing 0.2—2.0 μg of brucine were prepared at an interval of 0.02 or 0.05 μg.

Results and Discussion

Clear rings were not formed below 90 °C. The paper sometimes burned when heating was carried out above 100 °C. Well defined rings were obtained at 90 °C. Filter paper No. 54 was found to be the best of six kinds of chromatography paper.

NO_2 gas, which gives immediate coloration, was used. Brucine was concentrated by the use of water, ammonia and hydrochloric acid. When there was not enough to concentrate in the ring zone, the concentration ring was wide and spreading.

Nitric acid was used since it can be concentrated and thus conveniently serves both as developing solution and color reagent. The ring was wide and spreading when 0.1—0.2 M nitric acid was used, but clear ring formed when 0.5—8 M nitric acid was used. Strychnine showed identical results. The ring zone often burned when nitric acid concentration exceeded 2.0 M. Thus 1.0 M solution was used. No thin and clear ring could be obtained by a single developing procedure, the error being large. A good ring could, however, be obtained by repeating the procedure twice. When the amounts of the developing solution were 15 and 20 μl, the results were better than when 10 and 10 μl, or 10 and 15 μl were used. Fifteen and 20 μl of 1.0 M

TABLE 1. EFFECT OF STRYCHNINE NITRATE ON THE DETERMINATION OF BRUCINE

Amount of brucine (μg)	Amount of strychnine nitrate(μg)	Determination error(%)
0.2	10	0
	20	0
	50	+10
	100	+20
1.0	5	0
	10	0
	20	0
	50	0
	100	+5
	200	+10
5.0	25	0
	50	0
	100	0
	250	0
	500	+5
10.0	50	0
	100	0
	200	0
	500	+5

Table 2. Determination of brucine in strychnine nitrate

Sample	Brucine detected[a)]	Concentration of brucine (μg/l g-strychnine nitrate)
A	1.5	75
B	2.0	100
C	1.0	50

a) In 100 μl.

nitric acid were used. Brucine was concentrated in the ring zone perfectly. Concentration was determined according to color intensity and width of the ring as observed under a microscope.

The ring zone was 20 mm in diameter and 0.2 mm wide, the detection limit 0.2 μg, and the determination range 0.2—20 μg. The average relative error for this technique is 5% over the whole range of concentration of brucine, 0.2—20 μg. When the developing solution dried, the concentration ring turned blood-red, then to yellow-red with the elapse of time. The coloration remained unchanged for some years.

The results are given in Table 1. When the amount of brucine is small, strychnine nitrate has little or no effect on the results. When the standard solution is added to the sample, the error becomes a little greater.

One gram of strychnine nitrate was recrystallized from water and ethanol, and mother liquor was concentrated to 5.0 ml. Brucine was determined with the use of 20, 30, and 50 μl of the mother liquor. The results are given in Table 2. Commercial strychnine nitrate contains a trace of brucine. The analytical procedure took about five minutes, the accuracy being unsatisfactory.

The sample was concentrated with 10 μl of water and its quality was determined by NO_2 gas. The method can also be applied to the analysis of medicine.

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References

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