Journal of Criminal Law and Criminology

Volume 43 | Issue 2 Article 12

1952

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IDENTIFICATION AND DETERMINATION OF STRYCHNINE BY ULTRAVIOLET SPECTROPHOTOMETRY

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It is fairly well established that many of the alkaloids have characteristic absorption bands in the ultraviolet region of the spectrum. Thus, ultraviolet spectrophotometry lends itself very well to toxicologic analysis of strychnine from body fluids, and many long tedious hours can be saved and the present less reliable methods superseded.

Absorption bands were plotted with a DU Universal Beckman spectrophotometer with an ultraviolet light source using strychnine standards. More than twenty-five analyses were plotted, and each conformed to the same characteristic absorption band with a maximum density at 255 m μ and a minimum density at 230 m μ (Fig. 1).

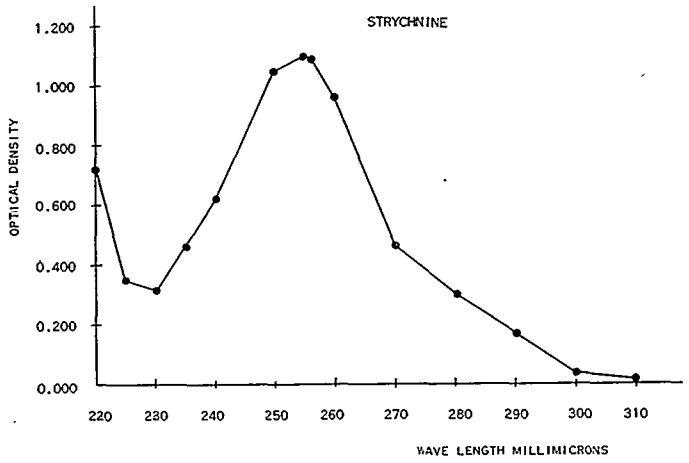

Figure 1

Strychnine standards were prepared, representing 0, 5, 10, 15, 20, 25, and 30 micrograms of strychnine per ml in an 0.5 N sulfuric acid solution. Each standard was transferred to a silica cuvette, and its optical density was determined at 255 m μ (maximum density). Plotting optical density against concentration results in a linear graph following the Beer-Lampert Law of concentration (Fig. 2).

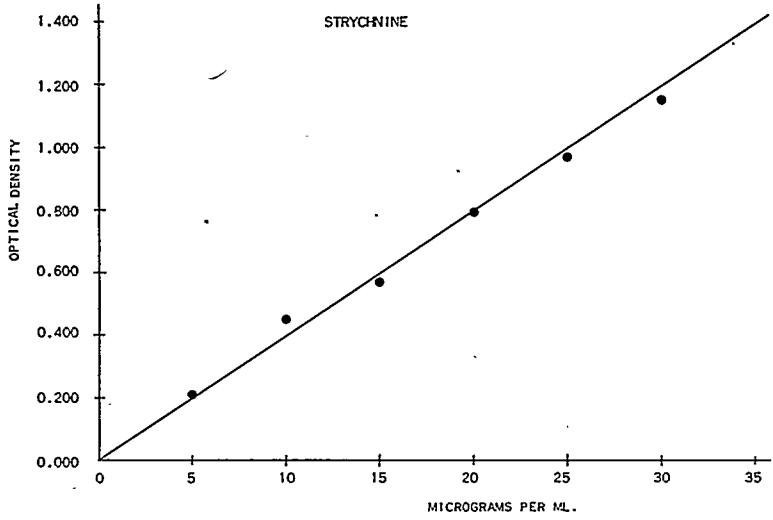

Figure 2

PROCEDURE

Varying concentrations of standard strychnine solutions were diluted to approximately 5 ml with water and then made slightly alkaline with one drop of concentrated ammonia. Twenty-five ml of chloroform were placed in a separatory funnel and the alkalinized strychnine added, and then gently shaken for about 5 minutes and extracted. The chloroform layer was allowed to settle and was then separated. This chloroform extract was filtered through filter paper to remove excess water droplets, and exactly 20 ml of filtrate collected and then extracted with 5 ml of 0.5 N sulfuric acid. The acid aqueous layer was allowed to stand and finally separated into a centrifuge cone and excess chloroform dropets removed by centrifugation.

Three ml of acid extract were transferred to a Beckman-Silica cuvette and read with the spectrophotometer using a 0.5 N sulfuric acid blank that has been saturated with one drop of chloroform. The absorption spectra were plotted, wave length against optical density. For qualitative identification the resultant curve must always be identical with that plotted in Figure 1.

For quantitative determinations, the optical density was recorded at its maximum 255 m μ, and then computed from standard graph of Figure 2. Correction factors must be applied to compensate for the aliquots used in the various steps. Percentage error was found to be within -6% recovery from pure solutions. Sensitivity was found to be

approximately 5 micrograms per 5 ml solution. This method is applicable to blood.

Determination of Strychnine (Alkaloid) in Blood. Fifteen samples of fresh normal human blood of 5 ml each were inocculated with 10, 25, 50, or 100 micrograms of strychnine alkaloid, extracted with chloroform, and the strychnine removed from the chloroform with 0.5 N sulfuric acid and then determined with the spectrophotometer, in a similar manner described above. (See Table I.)

TABLE I

Sample Number	Micrograms of Strychnine Added	Micrograms of Strychnine Recovered	Percent Error
1.	10	9	— 10
2.	10	9	— 10
3.	10	10	0
4.	25	23	— 8
5.	25	24	— 4
6.	25	23	— 8
7.	25	25	0
8.	50	46	— 8
9.	50	45	— 10
10.	50	47	— 6
11.	100	94	— 6
12.	100	88	— 12
13.	100	97	— 3
14.	100	92	— 8
15.	100	89	— 11

DISCUSSION

Recent papers (1, 2, 3, 4, 5, 6) have described ultraviolet spectrophotometric methods for determining various organic compounds such as barbiturate derivatives, quinine, chlorophyl, and others. Each of these compounds is characterized by intense ultraviolet absorption with specific maximum and minimum absorption peaks. In the present study, the isolation and quantitative determination of strychnine was carried out in slightly alkaline medium (approximately pH 10) and was found to give characteristic absorption bands with a maximum at 255 m μ and a minimum at 230 m μ and satisfactory recovery yields. Significant optical density readings were obtained with strychnine concentrations as low as 5 micrograms per 3 ml of blood. The sensitivity of the method is sufficient to detect and determine strychnine in small quantities of blood in lethal or even sub-lethal cases of strychnine poisoning.

Using fairly fresh blood, this method is adequate. However, when

decomposed or hemolyzed blood is tested, interfering absorption curves may occasionally be encountered. These were found to have a consistent maximum density at 260 m μ and minimum at 240 m μ. This imposes a serious limitation in the application of this method. Caution must be exercised before making positive identification, since absorption curve patterns of the many other available drugs and poisons are not yet worked out. In medicolegal cases, the method is of value in conjunction with history and other tests. The following case illustrates a practical application.

Case Report of Strychnine Poisoning. A 35 year old white male was found dead in bed in the early morning. There was no evidence of foul play. Autopsy and microscopic studies did not reveal the cause of death, and toxicological analyses were indicated. An organized systematic scheme (modified Stas-Otto) for the isolation of all of the possible common poisons was instituted. Simultaneously blood samples were also specially analyzed for alcohol, carbon monoxide, cyanide, barbiturates, etc., and also by the procedure described in this paper, for strychnine. The absorption bands were positive and characteristic for strychnine (identical with those illustrated in Fig. 1) and on computation, the strychnine concentration was determined to be 1.3 milligrams per 100 ml of blood.

Interim analysis with 500 gm specimens of liver, kidney, brain, and stomach contents utilizing multiple extractions and purifications, ruled out other common possible toxic agents and confirmed the strychnine identification. The following tests were positive for strychnine: (1) Wagner's test (typical crystals), (2) Mayer's test (typical crystals), (3) Picric acid test (typical crystals), (4) Dichromate-sulfuric acid (fading purple test), (5) Physiologic tests with frogs.

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