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SPECTROPHOTOMETRIC DETERMINATION OF STRYCHNINE IN THE PRESENCE OF BRUCINE

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A spectrophotometric method for the determination of strychinine in the presence of brucine has been developed. The determination of strychnine has also been carried out from tincture Nux vomica. The above mentioned compounds when treated with *N*-bromosuccinimide in the presence of 40-65% sulphuric acid give a pink colour which has an absorption maximum between 510-520 nm. A separation procedure for strychnine and brucine has also been described. The method is convenient, accurate, and precise. The maximum error was 4% when 500 μg strychnine was determined in the presence of same amount of brucine.

INTRODUCTION

The determination and detection of strychnes alkaloids, especially of strychnine and brucine, is very important for forensic scientists.

Strychnine has been determined in tablets containing starch sugar mixture [1]. The absorption was measured at 254 nm in a sulphuric acid medium. Beer's law was obeyed from 0.001 to 0.005%. Codeine and atropine have been investigated along with strychnine but interference due to brucine has not been described. In another semi-quantitative method strychnine gives red colour when it is made to react with zinc, $HgCl_2$ and sodium nitrite [2]. This is quite a sensitive test but interference due to brucine has not been studied. A paper chromatographic method for the determination of strychnine in Nux vomica has also been described [3]. In this method the Nux vomica extract was dissolved in $CHCl_3$ followed by the addition of pilocarpine nitrate as internal standard. After the application of the sample to filter paper the ascending solvent technique was applied in butanol - citric acid water medium. Iodoplatinate reagent was used as spray for the detection of strychnine. Calibration was effected using 0.2 to 0.4% solutions of strychnine. This method is again semi-quantitative and no reference is made to the spot of brucine which is one among the major alkaloids in Nux vomica. An amperometric method has been tried [4] in which 0.25 to 2.5 mM codeine, morphine, moscapine, papaverine, thebaine, strychnine or brucine was treated with 1M-KCl and 0.001% triton X. This mixture was then titrated with the use of dropping mercury electrode against a 0.25 to 2.5 mM solution of potassium iodotri-iodothallate at –0.7V vs the SCE. The method is prone to considerable interferences and has only theoretical value. In another method spot tests for codeine, morphine, thebaine, narcotine, papaverine, strychnine and brucine have been investigated [5]. In these spot tests niobic, telluric, bismuthic, titanic and stannic acids have been used together with 34% sulphuric acid. These spot tests are not specific and therefore have only limited ultility. Potassium iodotri-iodothallate (I) has been used by Horak and Zyka [6] as a precipitant for a number of alkaloids and ultimately for their indirect photometric determination by estimating thallium with crystal violet. The drawback of this method is that it is applicable to only individual alkaloid. Phosphotungstic and silicotungstic acids [7–9] have also been used for the determination of these alkaloids but again these are employed for individual alkaloids. Nitranilic and flavianic acid [11-12] have also been used with limited application in the amperometric determination of opium and strychnes alkaloids. There is another spectrophotometric method for the determination of strychnine and brucine. The pH of the acid or alkaline Nux vomica mixture is adjusted to 5 with NaOH or HCl. Methyl orange is added and the complexes formed with alkaloids are extracted into $CHCl_3$. The dye is removed by washing $CHCl_3$ with 0.1M NaOH and then with 0.05M H_2SO_4. The absorption of strychnine is measured at 262 nm and of brucine at 300 nm. The absorption measurement is in the uv region which is prone to a number of errors. Paper chromatographic methods are also tried for the separation of opium and strychnes alkaloids using zirconium antimonate paper

and metal salt impregnated silica gel and alumina layers [13-14]. These are semi-quantitative methods and can only be used for identification. Extractive spectrophotometric determination of brucine has also been developed by the same authors but again the method is not specific and strychnine interferes in this method [15]. Solochrome green dye has been used as complexing agent; chromium (III) forms coloured aducts with brucine and other alkaloids [16]. The study is almost of only theoretical value. There are some good methods for the determination of strychnine but only from pure solutions [17]. A polarographic indication method for the determination of strychnine has been described [20] in which strychnine nitrate is titrated with CdI_3 in solution of some neutral salt (0.1M KNO_3 or NaCl), but the method is only applicable to individual alkaloids. Strychnine can be separated from extraneous interfering material but not from brucine by the use of oxidized cellulose as a cation exchanger and then can be determined photometrically [21]. Chromatographic methods to determine brucine and strychnine in powdered Nux vomica have also been tried [22-23]. These are good methods for the identification of these alkaloids but quantitative determination is rather difficult. In the present investigation *N*-bromosuccimide in the presence of sulphuric acid has been used for the spectrophotometric determination of strychnine and brucine in the presence of each other. The method is convenient, rapid, and specific.

EXPERIMENTAL

Reagents. All reagents used were of analytical grade or of comparable purity.

(1) Strychnine hydrochloride: 100 mg of strychnine hydrochloride (E. Merck BRD) were dissolved in distilled water and volume was made to 100 ml in a measuring flask.

Brucine: 100 mg of brucine (E. Merck BRD) were dissolved in 20 ml of 1 N H_2SO_4 and diluted to 100 ml with distilled water.

N-Bromosuccinimide (NBS): 100 mg of the compound were dissolved in 100 ml of distilled water.

Sulphuric acid: 40% and 65% of solutions were prepared in distilled water.

Equipment. All absorbence measurements were made with Double Beam Beckman spectrophotometer using 1 cm cells.

PROCEDURE

Spot Test for Strychnine. To 0.01 ml of the test solution containing 10 μg of strychine 0.01 ml of 65% sulphuric acid was added in a microtest tube. The contents were cooled under tap water and 0.01 ml of *N*-bromosuccinimide containing 10 μg of the compound was added and the resulting solution was heated on a water bath for 10 min. The appearance of pink colour indicates strychnine. The visual limit of identification is 3 μg/0.01 ml.

Spot Test for Brucine. To a 0.01 ml of brucine solution containing 10 μg of the compound, 0.01 ml of 40% sulphuric acid was added in a microtest tube. The contents were cooled under tap water and 0.01 ml of *N*-bromosuccinimide containing 10 μg of the compound was added. The tube was then heated in a water bath for about 10 min. A pink colour indicates the presence of brucine. The visual limit of identification is 1 μg/0.01 ml.

Differentiation of Brucine from Strychnine. 1 ml of the test solution containing about 100 μg of brucine was taken in a test tube. To this were added 2 ml of 40% sulphuric acid and the contents were cooled. Then 1 ml of NBS containing 1 mg of the compound was added carefully touching the sides of the microtest tube. A pink coloured ring is formed at the junction of the two solutions. This ring does not appear in case of strychnine.

Colour with brucine can be produced even in the cold and without the addition of sulphuric acid.

PROCEDURE FOR THE DETERMINATION OF BRUCINE

1 ml of test solution containing 1 mg of brucine was taken in a test tube. To this was added 2 ml of 40% sulphuric acid and the contents were cooled under tap water. After this 1 ml of NBS solution containing 1 mg of the substance was added and the tube was shaken and heated on the water bath for 10 min. The contents were then diluted to 5 ml and the pink colour was measured at 520 nm, using 1-cm cells.

PROCEDURE FOR THE DETERMINATION OF STRYCHNINE

1 ml of test solution containing 1 mg of strychnine was taken in a test tube. To this was added 2 ml of 65% H_2SO_4 and the contents were cooled under tap water. 1.5 ml of NBS containing 1.5 mg of the compound was added and the tube was shaken and heated on the water bath for 10 min. The contents were then diluted to 5 ml

and the pink colour was measured at 520 nm using 1-cm cells.

DETERMINATION OF STRYCHNINE IN THE PRESENCE OF BRUCINE

A synthetic mixture solution of strychnine and brucine containing 5 mg of each was prepared. Then 5 ml of NBS containing 5 mg of the substance was added to this mixture. The resulting solution was then heated on water bath for about 10 min. The contents were then cooled and 10 ml of 10% NaOH solution was added and after shaking it was cooled in Ice bath. The ice cooled mixture was then transferred to a separatory funnel and 5 ml of carbon tetrachloride were added. After shaking, the contents were allowed to separate into two layers. Carbon tetrachloride layer was then drawn out in a China dish from the separatory funnel and this extraction was repeated twice. The carbon tetrachloride so obtained was then evaporated putting the China dish on a sand bath. The residue was then dissolved in 40% sulphuric acid and the procedure for the determination of strychnine was performed.

DETERMINATION OF STRYCHNINE FROM TINCTURE NUX VOMICA

10 ml of tincture Nux vomica was taken and to this was added 5 ml of NBS (equivalent to 5 mg) and the mixture was heated on a water bath for about 10 min. 10 ml of 10% NaOH was added to the mixture and the contents were cooled to about 1°. The mixture was then transferred to a separatory funnel containing 5 ml of chloroform. After shaking carefully the chloroform layer was separated and collected in China dish. Two more such extractions were performed, collected and heated to dryness. The residue was then dissolved in 40% sulphuric acid and the volume was made up to 25 ml in a volumetric flask. A definite aliquote was then taken and procedure for the determination of strychnine was then performed as described earlier.

RESULTS AND DISCUSSION

The results for the determination of brucine and strychnine are shown in Tables 1–4.

The results shown in the tables were calculated from the calibration graphs (Figs. 1 and 2) for brucine and strychnine.

Table 1. Determination of brucine

Amount taken (μg)	Amount found (μg)	% error
250	245	–2.00
300	297	–1.00
350	351	+0.57
400	400	0.0
450	452	+0.44
500	498	–0.44

Every value determined is an average of three determinations.

Table 2. Determination of strychnine

Amount taken (μg)	Amount found (μg)	% error
200	201	+0.50
250	250	0.00
300	295	–0.66
350	345	–0.28
400	402	+0.50
450	447	–0.66

Every value determined is an average of three determinations.

Table 3. Determination of strychnine in presence of brucine

Strychnine taken (μg)	Brucine added (μg)	Strychnine found	% error
500	500	520	+4
600	500	590	–1.6
700	500	710	+1.4
800	500	810	+1.2
900	500	920	+2.2

Every value found is an average of three determinations.

SPECTRAL CHARACTERISTICS OF BRUCINE AND STRYCHNINE

Colours. The pink colour which is produced as result of the reaction between brucine, sulphuric acid and *N*-bromosuccinimide and later of strychnine and sulphuric acid and *N*-bromosuccinimide show maximum absorption approximately at the same wave length, i.e., between 510-520 nm (Fig. 1). It is clear from this figure that the absorption characteristics are similar in both cases.

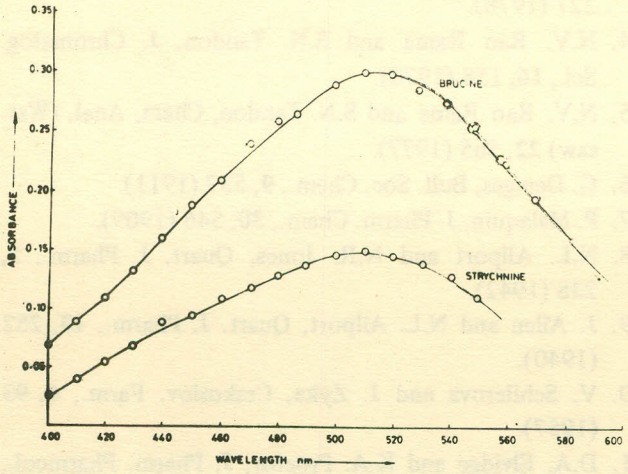

Fig. 1. Absorption spectrum of brucine and strychnine

Order of mixing the reagents. The order of mixing the reagents does not have any significant effect on the production of colour.

Effect of sulphuric acid concentration on the production of colour. The acid concentration has significant effect on the production of colour. In the case of brucine 40% sulphuric acid was used for the production of colour but in the case of strychnine 65% sulphuric acid was used.

Stability of the colour. The pink colour which is produced with brucine is stable for at least 2 hr. but with strychnine it is only stable for 30 min.

Effect of temperature. The colour reaction was carried out at 80° in a water bath and the contents were heated for about 10 min.

Effect of reagent concentration. It has been found that for the complete development of colour that 1.5 mg of *N*-bromosuccinimide were required for 1 mg of strychnine in a volume of 5 ml. Even a double amount of NBS could be used. When more than 2 mg were used it had adverse effect on the production of colour. For 1 mg of brucine, 1 mg of *N*-bromosuccinimide was used for complete development of colour. Higher amounts of NBS had adverse effect on the production of colour with this compound.

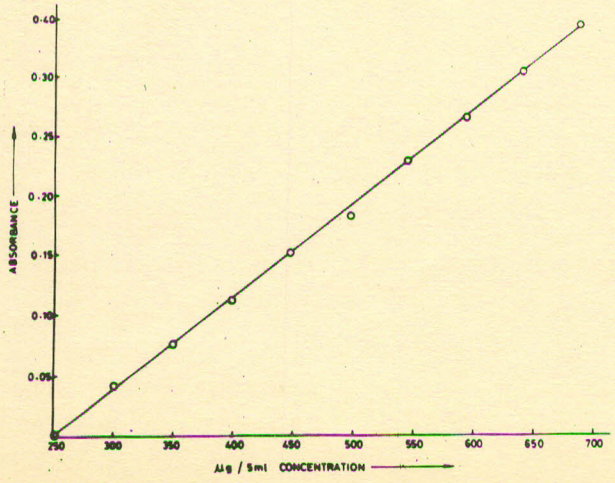

Fig. 2. Calibration graph for brucine.

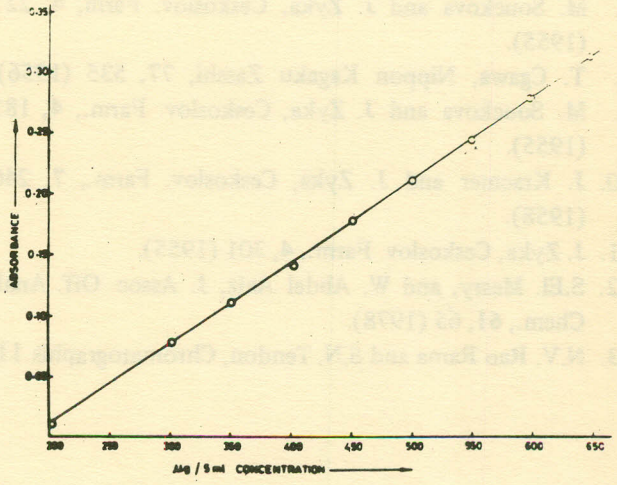

Fig. 3. Calibration graph of strychnine.

Table 4. Determination of strychnine from tincture Nux vomica

Amount of tincture taken (ml)	Amount of strychnine declared (mg)	Amount of strychnine found (mg)	% error
10	12.5	12.8	+ 2.6
15	18.7	19.0	+ 1.6
20	25.0	24.8	+ 0.8
30	37.5	37.5	0.0
40	50.0	50.5	+ 1.0

Every value determined is agaverage of 5 determinations.

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