

# THE ULTRA-VIOLET SPECTROPHOTOMETRIC ASSAY OF ALKALOIDS

## PART I. STRYCHNINE IN THE PRESENCE OF BRUCINE

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Received April 28, 1952

SALTS of strychnine are often used parenterally in a very low concentration. The injection prepared from the hydrochloride, is now included in the British Pharmacopœia, 1948. Apart from its use as a single salt, strychnine in combination with other alkaloids and/or other metallic salts is often prescribed and methods of assay for such preparations are required. Following the method of Brownlee<sup>1</sup> work was being carried out in this Institute on the estimation of strychnine in various mixtures. In the course of these studies it was considered advantageous to estimate the alkaloid by ultra-violet spectrophotometry. This work is in progress; but in the meantime a paper by Ridi and Khalifa<sup>2</sup> on the assay of strychnine in galenicals and other preparations came to our notice and we consider it desirable to note down the observations on the same problem so far recorded in our laboratory.

Strychnine or brucine can be estimated when present alone, or after solvent extraction when present together, by titration with standard perchloric acid in acetic acid medium using crystal violet as indicator.<sup>3</sup> Titration methods require somewhat large quantities of bases for accurate determination, and when more than one is present they need to be separated. The accuracy of the results obtained depends mainly on the efficiency of separation and extraction.

### EXPERIMENTAL

Pure strychnine and brucine obtained by repeated recrystallisation were used. Dilute standard solutions of the bases were prepared in 0.001 N sulphuric acid. Percentage transmission and optical density of the solutions and their mixtures were determined against 0.001 N sulphuric acid. All dilutions of the stock solutions were made in 0.001 N sulphuric acid: A Beckman spectrophotometer, Model DU, calibrated against hydrogen lines was used for the measurements. Fused silica cells of light path 1.004 cm. were used in all cases.

### RESULTS AND DISCUSSION

Figure 1 gives typical transmission-wavelength curves for pure strychnine and pure brucine in dilute sulphuric acid. Strychnine shows maximum absorption in the curves at 252 to 254  $m\mu$  while brucine shows maximum absorption at 262  $m\mu$  and another transmission minimum at 300  $m\mu$ . The wavelengths correspond to those obtained by previous workers.<sup>2,4</sup> Ridi and Khalifa<sup>2</sup> record the wavelengths as 254  $m\mu$ ,

264  $m\mu$  and 301  $m\mu$ , although their experiments were made in a different solvent, ethanol. A comparison of the curves obtained by them with those in Figure 1, shows no significant shift with change of solvent from dilute sulphuric acid to ethanol. It is to be observed from Figure 1 that at 300  $m\mu$ , there exists a significant absorption by strychnine. A correction is required, or compensation must be made in the method for this absorption by strychnine in solution, when estimating brucine from absorption measurements at 300  $m\mu$ . Also when strychnine and brucine

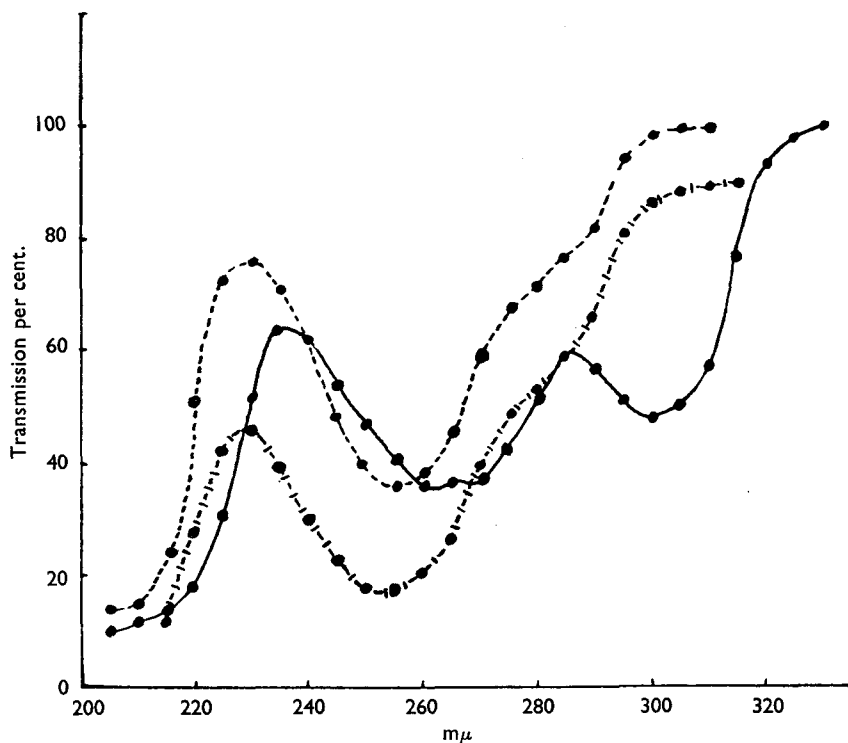


FIG. 1. Transmission-wavelength curves.

- Brucine 0.0016 per cent. in dilute sulphuric acid.
- |-|-|-|- Strychnine 0.00218 per cent. in dilute sulphuric acid.
- Strychnine 0.00126 per cent. in dilute sulphuric acid.

are present together in estimable quantities, they influence absorption by each of the bases to some extent (*vide infra*).

In Figures 2A and 2B are given straight line graphs obtained with pure strychnine and pure brucine in dilute sulphuric acid, by plotting the optical densities against their respective concentrations at wavelengths of 300  $m\mu$ , 262  $m\mu$  and 252  $m\mu$ . The ratios of optical density per cm. and concentration in g. per cent. are tabulated as  $E_{1\text{ cm.}}^{1\text{ per cent.}}$  in Table I.

Progressive deviation from Beer's law is to be noticed in Figures 2A and 2B in the concentration range above 5 mg. per cent. in the case of strychnine and above 8 mg. per cent. in the case of brucine.

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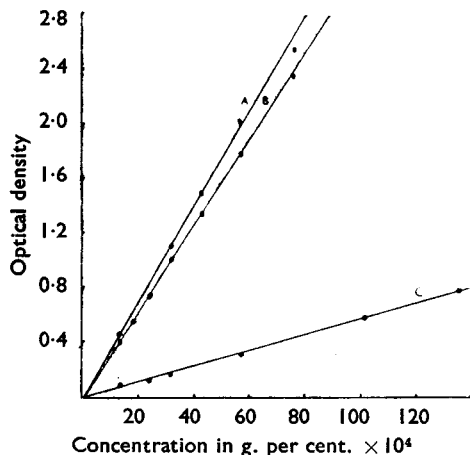


FIG. 2A. Concentration-optical density graphs for strychnine.  
 A at 252  $m\mu$   
 B at 262  $m\mu$   
 C at 300  $m\mu$  (optical density magnified 10 times).

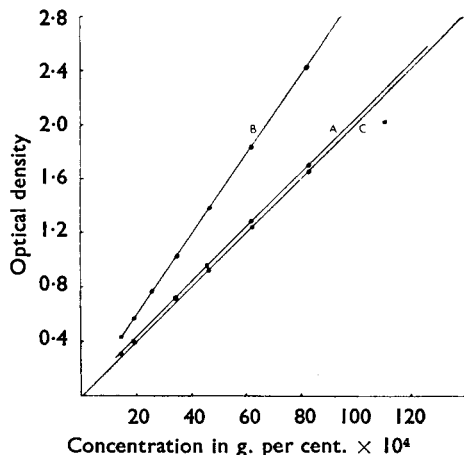


FIG. 2B. Concentration-optical density graphs for brucine.  
 A at 252  $m\mu$   
 B at 262  $m\mu$   
 C at 300  $m\mu$

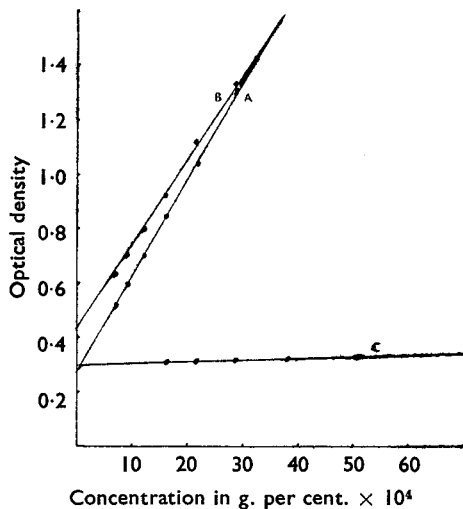


FIG. 3A. Concentration-optical density graphs for strychnine in the presence of a fixed concentration (0.00147 g. per cent.) of brucine.  
 A at 252  $m\mu$ .  
 B at 262  $m\mu$ .  
 C at 300  $m\mu$ .

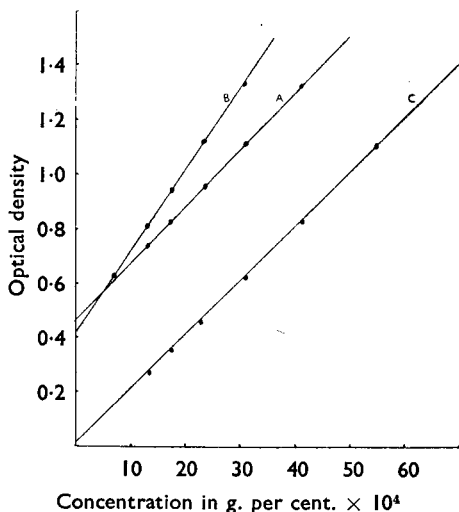


FIG. 3B. Concentration-optical density graphs for brucine in the presence of a fixed concentration (0.00135 g. per cent.) of strychnine.  
 A at 252  $m\mu$ .  
 B at 262  $m\mu$ .  
 C at 300  $m\mu$ .

TABLE I

$E_{1\text{ cm.}}^{1\text{ per cent.}}$  VALUES FOR PURE STRYCHNINE AND PURE BRUCINE

|                  | 300 m $\mu$ | 262 m $\mu$ | 252 m $\mu$ |
|------------------|-------------|-------------|-------------|
| Strychnine .. .. | 5.6         | 310         | 330         |
| Brucine .. ..    | 199.2       | 291.4       | 202.6       |

The graphs in Figures 3A and 3B were obtained by varying the concentration of strychnine in a solution of "fixed" brucine concentration and *vice versa*. In Table II  $E_{1\text{ cm.}}^{1\text{ per cent.}}$  values for the systems at different wavelengths are given. In Table III are recorded for comparison the values, of optical densities for the "fixed" concentrations of brucine and strychnine at different wavelengths (i) as read from the intercept of the straight lines on the optical density axis and (ii) those obtained experimentally with the particular concentrations of pure brucine and pure strychnine in the absence of the other.

TABLE II

$E_{1\text{ cm.}}^{1\text{ per cent.}}$  VALUES FOR STRYCHNINE IN FIXED CONCENTRATION OF BRUCINE (0.00147 g. per cent.)

$E_{1\text{ cm.}}^{1\text{ per cent.}}$  VALUES FOR BRUCINE IN FIXED CONCENTRATION OF STRYCHNINE (0.00135 g. per cent.)

|                  | 300 m $\mu$ | 262 m $\mu$ | 252 m $\mu$ |
|------------------|-------------|-------------|-------------|
| Strychnine .. .. | 5.3         | 309         | 347         |
| Brucine .. ..    | 195.6       | 298         | 207         |

TABLE III

OPTICAL DENSITIES OF BRUCINE AND STRYCHNINE IN CONCENTRATIONS OF 0.00147 G. PER CENT. AND 0.00135 G. PER CENT. RESPECTIVELY (1) as read from Figure 3 and (2) as obtained directly by measurements in such concentrations

|                  | 300 m $\mu$ |       | 262 m $\mu$ |       | 252 m $\mu$ |       |
|------------------|-------------|-------|-------------|-------|-------------|-------|
|                  | (1)         | (2)   | (1)         | (2)   | (1)         | (2)   |
| Strychnine .. .. | 0.009       | 0.009 | 0.415       | 0.415 | 0.460       | 0.457 |
| Brucine .. ..    | 0.300       | 0.302 | 0.425       | 0.430 | 0.290       | 0.290 |

From Tables I and II it should be clear that strychnine and brucine when present together produce changes in the slopes of the curves indicating interference. The strict validity of Beer's law in the concentration range of the mixtures and the agreement in the values tabulated in Table III suggest that the interference is not dependent on the concentration ratio of the two bases (in the concentration range used) and that the optical density is additive, i.e., the total optical density is the sum of the densities at zero concentration of the variable constituent and the optical density due to the variable constituent in the mixture. The validity of Beer's law and the agreement in the values of intercepts with the experimentals were checked at other wavelengths

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(not cited in the figures) such as at 228  $m\mu$ , 236  $m\mu$  and 286  $m\mu$  and also at concentration ranges of 6 mg. per cent. of strychnine and 8 mg. per cent. of brucine.

In Figure 4 the straight lines were obtained—(i) by plotting concentrations of brucine in mixtures containing brucine and strychnine in a fixed ratio of 0.788, against optical densities of the mixtures determined at 252  $m\mu$ , 262  $m\mu$  and at 300  $m\mu$ . It is important to note that the slope of curve C at 300  $m\mu$  (viz. 201) is not the same as that obtained with brucine in fixed concentration of strychnine (viz. 195.6). The curve D is obtained by calculating the absorption due to brucine alone at 300  $m\mu$  in the mixtures using the  $1.004 \times E_{1\text{ cm.}}^{1\text{ per cent.}}$  value (196.4) of the base in presence of strychnine. The two straight lines are significantly different, and a straight line practically coinciding with curve C is obtained by plotting the sums of the absorptions by the two bases. It may also be noticed that curve C is also reproduced if the  $1.004 \times E_{1\text{ cm.}}^{1\text{ per cent.}}$  value (200) for pure brucine solutions at 300  $m\mu$  is taken to calculate the absorption and no allowance is made for absorption by strychnine (cf. ii) present in them. The latter procedure is at least not theoretically justified when there exists significant absorption by strychnine at 300  $m\mu$ .

(ii) Another straight line (A) coinciding with the 252  $m\mu$  line is obtained by plotting the sum of the individual optical densities of the bases at respective concentrations, read from Figure 3 or by calculating the sums of the absorptions by the bases using their  $1.004 \times E_{1\text{ cm.}}^{1\text{ per cent.}}$  values at 252  $m\mu$  given in Table II. If the  $1.004 \times E_{1\text{ cm.}}^{1\text{ per cent.}}$  values at 252  $m\mu$  for pure solutions be taken for such calculations, a curve widely different from the curve A is obtained, hence as a general procedure the slopes of the curves for the individual bases in presence of one another should be accepted when working with their mixtures. The curve B is obtained by plotting the experimental values of absorption by the bases at 262  $m\mu$  and also by plotting the sums of individual absorptions calculated by

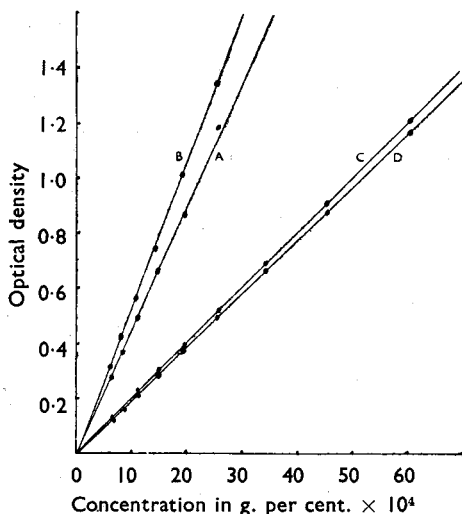


FIG. 4. Brucine concentration-optical density graphs for brucine-strychnine mixture in the weight ratio of 1:0.788.

- A at 252  $m\mu$ .
- B at 262  $m\mu$ .
- C at 300  $m\mu$ .
- D at 300  $m\mu$ , for brucine only in the presence of strychnine.

Experimental points on curves A, B and C also correspond to results obtained by calculation from Fig. 3.

the method envisaged. The very close coincidence of the lines obtained at different wavelengths renders obvious the feasibility of estimating strychnine and brucine accurately in presence of one another.

For the determination of strychnine and brucine in an unknown sample, a solution of suitable concentration is prepared. Two determinations of optical densities are required, say one at 252  $m\mu$  and the other at 262  $m\mu$ . The concentrations of the bases are found by solving the simultaneous equations:—

$$E_{252}^S \cdot S + E_{252}^B \cdot B = e_{252}^M$$

$$E_{262}^S \cdot S + E_{262}^B \cdot B = e_{262}^M$$

Where  $E_{252}^S$ ,  $E_{262}^S$ , and  $E_{252}^B$ ,  $E_{262}^B$  are  $E_{1 \text{ cm.}}^{1 \text{ per cent.}}$  values for strychnine and brucine present together at 252  $m\mu$  and 262  $m\mu$  respectively, S (strychnine) and B (brucine) are the concentrations of bases in g./100 ml. of solution and  $e_{252}^M$  and  $e_{262}^M$  are the optical densities per cm. of the solution at the two wavelengths. The error involved in this method is usually less than 1 per cent. for both the constituents.

#### SUMMARY

1. A simple and rapid ultra-violet spectrophotometric method of assay of mixed solutions of strychnine and brucine has been developed with an accuracy within 1 per cent.

2. Change of solvent from water to ethanol does not produce a shift in the maximum and minimum of the ultra-violet absorption curves.

3. The slopes of the straight line graphs—optical density against concentration of the bases are altered when the two are present together.

The authors wish to express their sincere thanks to Dr. U. P. Basu, D.Sc., F.N.I., for suggesting the problem and taking a keen interest in this work.

#### REFERENCES

1. Brownlee, *Quart. J. Pharm. Pharmacol.*, 1945, **18**, 163.
2. Ridi and Khalifa, *J. Pharm. Pharmacol.*, 1952, **4**, 190.
3. Herd, *J. Amer. pharm. Ass., Sci. Ed.*, 1942, **31**, 9.
4. Brewstier and Blanc, *Bull. Soc. Chem.*, 1934, **1**, 712.