THE ULTRA-VIOLET SPECTROPHOTOMETRIC ASSAY OF ALKALOIDS

PART II. QUININE AND STRYCHNINE

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IN Part I[1] a spectrophotometric method for the estimation of strychnine and brucine in presence of one another was described. In the present paper a method for the assay of strychnine in presence of quinine is described. Salts of strychnine are often incorporated with those of quinine in pharmaceutical preparations wherein the concentration ratios of the two vary from 1:30 to much lower values. Correct estimation of strychnine concentration in such proportions presents difficulties. The fluorimetric method for the estimation of quinine is accurate at low concentrations but it demands the absence of other substances in solution. Carol[2] estimated quinine spectrophotometrically in dilute hydrochloric acid solution from absorptions at 250·5, 318 and 347 mμ (cf. also Grant and Jones[3]). Other methods for the estimation of quinine in alcoholic solutions are also known[4,5].

EXPERIMENTAL

Materials used. Pure strychnine and quinine bases, obtained by repeated crystallisation, were taken and their standard solutions prepared in 0·1N hydrochloric acid. Percentage transmissions of the solutions and their mixtures were determined against the 0·1N acid (except in a few cases mentioned in the text). Dilutions of the stock solutions were made in the 0·1N acid. A Beckman spectrophotometer, model DU, calibrated against hydrogen lines, was used for measurements. Measuring solutions were placed in fused silica cells of light path length 1·004 cm.

RESULTS

Pure bases. Figure 1 gives typical specific extinction coefficient–wavelength curves of the individual bases. A point of particular interest in the curves is that a quinine solution is comparatively transparent at the transmission maxima at 270 mμ, whereas a solution of strychnine has considerable absorption at this point. Straight line graphs were obtained with pure quinine and pure strychnine solutions respectively by plotting the optical densities against concentrations at selected wavelengths. Specific extinction coefficients ($E^{1\,\text{per cent.}}_{1\,\text{cm.}}$) are tabulated in Table I.

It is apparent that for the determination of quinine, absorption measurement at 231 mμ can also be adopted, besides those at the three wavelengths used by Carol[2].

Mixtures of bases. Straight line graphs were obtained by varying the concentration of quinine in a solution of "fixed" strychnine concentration and also by varying the concentration of strychnine in a solution

of "fixed" quinine concentration. In Table II, $E_{1\,cm.}^{1\ per\ cent.}$ values of the variable constituents in such mixtures are given.

In Table III are recorded the values of optical densities for the "fixed" concentrations of strychnine and quinine at different wavelengths obtained experimentally with the particular concentrations of pure bases.

TABLE I

$E_{1\,cm.}^{1\ per\ cent.}$ VALUES FOR PURE QUININE AND PURE STRYCHNINE SOLUTIONS

	231 mμ	252 mμ	270 mμ	317 mμ	347 mμ
Quinine	319·4	825·2	21·12	131·3	164·1
Strychnine	116·3	355·2	178·3	0·00	0·00

TABLE II

$E_{1\,cm.}^{1\ per\ cent.}$ VALUES FOR QUININE IN "FIXED" STRYCHNINE CONCENTRATION (0·00125 PER CENT.)

$E_{1\,cm.}^{1\ per\ cent.}$ VALUES FOR STRYCHNINE IN "FIXED" QUININE CONCENTRATION (0·003 PER CENT.)

	231 mμ	270 mμ	317 mμ	347 mμ
Quinine	319·7	21·3	131·5	164·0
Strychnine	112·0	178·0	0·00	0·00

TABLE III

CONTRIBUTION TO THE OPTICAL DENSITIES BY QUININE AND STRYCHNINE IN CONCENTRATIONS OF 0·003 AND 0·00125 g. PER CENT. RESPECTIVELY

As obtained directly

	231 mμ	270 mμ	317 mμ	347 mμ
Quinine	0·967	0·0641	0·393	0·492
Strychnine	0·146	0·224	0·00	0·00

From the agreement in the extinction values given in Tables I and II and in the optical density values tabulated in Table III it is clear that quinine and strychnine in presence of one another do not appreciably influence their individual absorptions (cf. Part I). Absorption measurements at 317 mμ or at 347 mμ offer the advantage of ascertaining the quinine concentration by a single measurement.

1:40 *Mixture of the bases.* In a mixture of strychnine and quinine in the proportions 1:40 (1 per cent. of quinine + 0·025 per cent. of strychnine) the $E_{1\,cm.}$ values at 231, 252 and 270 mμ would be 322·3, 834 and 25·7 respectively, of which 319·4, 825·2 and 21·1 respectively would be due to quinine alone. The contribution of strychnine to the specific extinction coefficient values in the mixture falls nearly within the limits of experimental accuracy in the cases of absorption at 231 and 252 mμ. For still lower ratios of the mixture the extinction values would not differ significantly from those for pure quinine, and so an accurate estimation of strychnine cannot be made by measurements of absorption

at 347 (or 317) mμ and at 231 (or 252) mμ. For higher proportions of strychnine (say 1:30 or 1:40) measurements at 347 and 270 mμ give fairly accurate results.

For mixtures of lower ratio than 1:40. In estimating a very low concentration of strychnine in presence of, say, a hundredfold excess of quinine; the absorption due to strychnine calculated from measurements at 347 and at 270 mμ will give inaccurate results due to the facts: (1) an error of 1 per cent. in the estimation of quinine is reflected as a 10 per cent. error in the optical density value for strychnine; (2) the specific extinction coefficient of quinine at 270 mμ suffers significant changes at different concentrations. This practical difficulty in the assay of strychnine when present in lower proportions may be overcome by the following procedure.

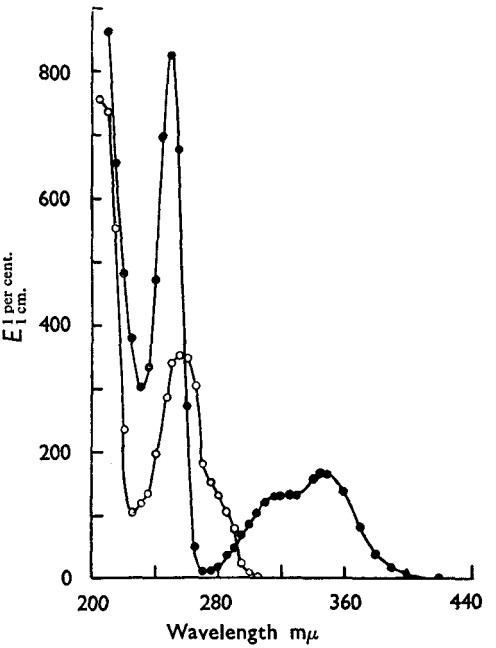

FIG. 1. Specific extinction coefficient—wavelength curves.

–●– Quinine (0·00116 per cent. in dilute hydrochloric acid).

–○– Strychnine (0·00113 per cent. in dilute hydrochloric acid).

From Figure 1 it is clear that the absorptions by quinine at 270 and at 385 mμ are similar. But at the latter the curve is very steep at the concentrations of quinine that must be used in the method described below. This similarity in absorption at the two wavelengths is used in the method developed. A proportion of the unknown solution of strychnine and quinine in ratios below 1:40 is suitably diluted and quinine estimated from the absorption at 347 mμ. From this knowledge of the quinine content of the solution another portion of the same is so diluted that the optical density at 270 mμ due to quinine alone would be of the order of unity (solution A). Next from a standard stock solution of pure quinine a dilute solution whose absorption at 270 mμ would also be of the order of unity (solution B) is prepared. The procedure adopted for measurement is, first to determine the optical density of the pure quinine solution (B) at 270 mμ and then to set the wavelength dial of the instrument (about 385 mμ) exactly at the point where the absorption equals that at 270 mμ. Absorption by the diluted test solution (A) is then read at the last setting of the wavelength dial. The dial setting is now altered and the absorption measured at 270 mμ. The difference in optical densities

gives an accurate estimate of the absorption due to strychnine in the mixture at 270 mμ.

Results obtained thus are incorporated in Table V. 0·5N hydrochloric acid was used in these experiments.

TABLE IV

OPTICAL DENSITY VALUES OF STRYCHNINE AT 270 mμ FOR DIFFERENT RATIOS OF STRYCHNINE TO QUININE AS OBTAINED (*a*) EXPERIMENTALLY, (*b*) BY CALCULATION

Strychnine concentration per cent.	Strychnine quinine ratio (approximately)	Optical density at approximately 385 mμ (1)	Optical density at 270 mμ (2)	Optical density due to strychnine (2)–(1) (*a*)	Optical density calculated (*b*)	Error per cent.
0·000981	1:45	0·889	1·067	0·178	0·176	+1·1
0·000817	1:55	0·821	0·966	0·145	0·146	−0·7
0·000577	1:85	0·989	1·093	0·104	0·103	+1·0
0·00044	1:110	0·972	1·050	0·078	0·0788	−1·0
0·00022	1:250	0·976	1·015	0·039	0·0394	−1·0

The agreement in the experimental and calculated values is thus within 2 per cent.

SUMMARY AND CONCLUSIONS

1. A simple ultra-violet spectrophotometric method for assay of mixed solutions of strychnine and quinine has been developed.

2. Strychnine in solutions containing strychnine and quinine down to the ratio of 1:250 could be estimated within 1 per cent. of the actual quantity present.

3. For ultra-violet spectrophotometric assay of strychnine and quinine it is imperative that the mixture should have little or no fluorescence under an ordinary ultra-violet lamp. For assay of the alkaloids in pharmaceutical preparations containing other ingredients, the bases are extracted and estimated as hydrochlorides. The accuracy of the method is largely dependent on the efficiency of such extractions.

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