THE CHROMATOGRAPHIC PURIFICATION AND ULTRA-VIOLET SPECTROPHOTOMETRIC ASSAY OF STRYCHNINE IN GALENICALS

By M. S. El Ridi and K. Khalifa

From the Biochemistry Department, Kasr El Aini Faculty of Medicine, Fouad I University, Cairo, Egypt

Received November 12, 1951

The British Pharmacopœia method for the assay of strychnine in galenicals comprises: (1) Purification of the galenical by extracting with chloroform in an acid medium to remove the chloroform-soluble substances; liberation of the alkaloid by alkali and extraction with chloroform; elimination of brucine, without destruction of strychnine, by oxidation with nitric acid in presence of 3 per cent. sulphuric acid; extraction of the relatively pure strychnine with chloroform, after rendering alkaline with sodium hydroxide. (2) Estimation of the strychnine by acid titration.

The methods of assay of different national Pharmacopœias are in principle similar but differ in detail.

The Expert Committee on the Unification of Pharmacopœia, World Health Organisation, recently recommended in connection with the proposed aim of an acceptable International Pharmacopœia that more simple methods of assay of vegetable drugs should be sought. The Committee further recommended that if such methods are found to be satisfactory they should be included in the International Pharmacopœia.

The following procedure differs in principle from the B.P. method. The chromatographic adsorption technique as described by Brownlee,[1] modified to suit the use of smaller quantities, is substituted for the B.P. method of purification of the galenical containing strychnine (see also Reimers, Gottlieb and Christensen[2]). The strychnine is estimated in the purified product directly by ultra-violet spectrophotometry. This technique has the advantage over the titration method of being directly applicable without the further solvent extractions made necessary by the presence of titratable impurities.

Spectrophotometric Examination of Strychnine and Brucine

In galenical preparations the undesired alkaloid brucine always accompanies the strychnine. A chromatographic separation of brucine and strychnine does not appear to have been effected. Brucine will interfere with the direct spectrophotometric estimation of strychnine.

Strychnine absorbs in the ultra-violet only with maximum absorption at 255μ (Brustier and Blanc,[3] Elvidge[4]).

We have examined the ultra-violet absorption spectra of strychnine and brucine using absolute ethanol as solvent by the Unicam spectrophotometer. Strychnine shows a maximum absorption at 254 mμ; brucine shows a maximum absorption at 264 mμ and another less distinct band at 301 mμ.

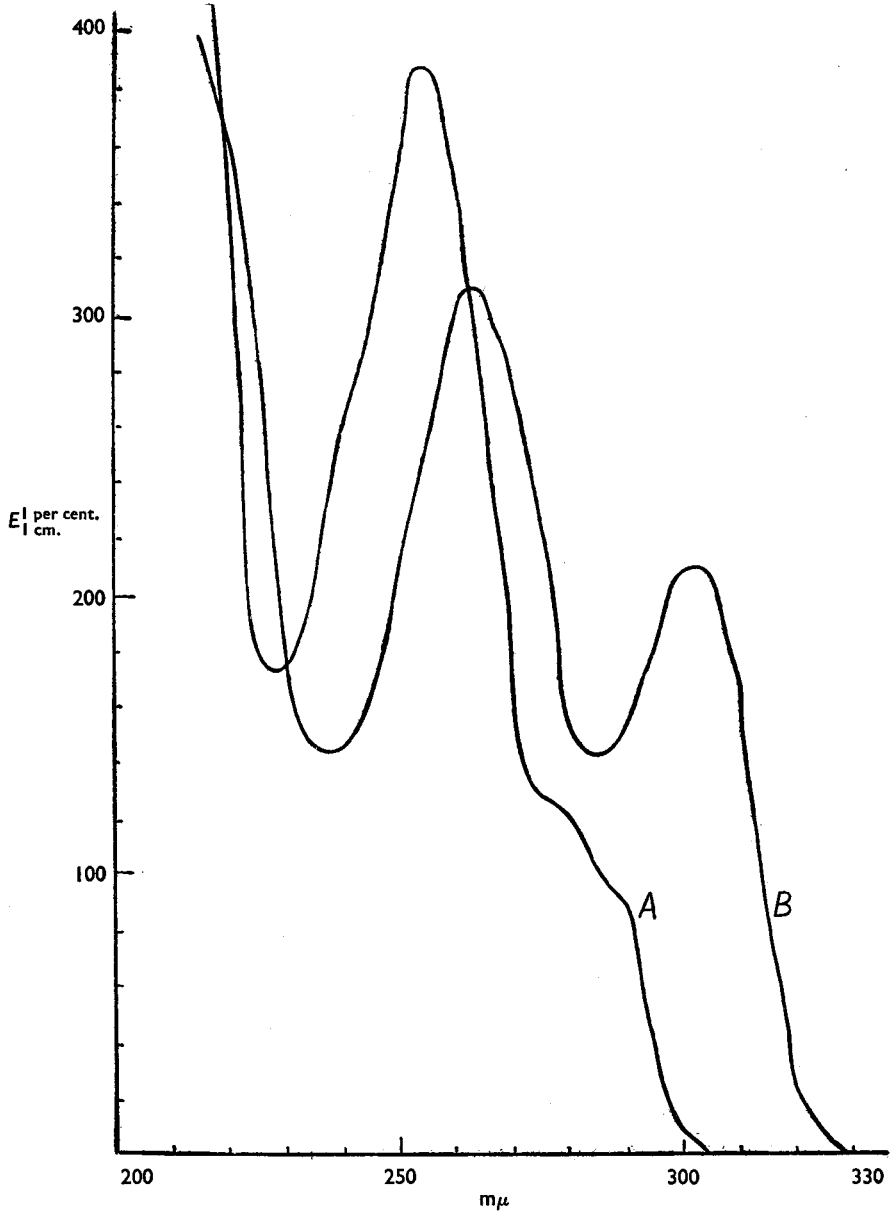

FIG. 1. Showing absorption curves and $E^{1\%}_{1\,cm.}$ of (A) strychnine (m.pt. 270° to 280° C.) in absolute ethanol, and (B) anhydrous brucine (m.pt. 178° C.) in absolute ethanol.

EXPERIMENTAL

Using pure strychnine (m.pt. 270° to 280° C.) and pure anhydrous brucine (m.pt. 178° C.) in absolute ethanol, the absorption curves were determined from the spectra taken with the Unicam instrument. Figure 1 shows the $E_{1\,cm.}^{1\,per\,cent.}$ of each alkaloid is plotted against wavelength. It is clear that the absorption at 301 mμ is due only to brucine. Therefore, in a pure mixture of strychnine and brucine, only the amount of brucine can be calculated from the extinction measured at 301 mμ and the strychnine obtained by the extinction difference at 254 mμ. In galenical preparations other unknown ultra-violet-absorbing impurities interfere with these direct calculations. However, the effect of the other impurities may be estimated by eliminating the spectrum of brucine which is readily oxidised to a non-absorbing product without destruction of strychnine.

OXIDATION OF BRUCINE

Since the nitrate ion absorbs at 302 mμ, oxidation with nitric acid cannot be used in the spectrographic assay. It was found that when pure brucine is treated with potassium persulphate at 60° to 70° C. in presence of 3 per cent. sulphuric acid for 1 hour, oxidation takes place with complete disappearance of the brucine absorption bands at 264 mμ and at 301 mμ. This was confirmed by making the oxidised solution alkaline and extracting with chloroform; no brucine was obtained.

When strychnine is treated by the prescribed potassium persulphate procedure it remains unchanged. To confirm this, different weights of each of strychnine and of brucine in different proportions were dissolved in 10 ml. of 3 per cent. sulphuric acid, 0·5 g. of potassium persulphate was added and dissolved by shaking and the solution was kept at 60° to 70° C. for 1 hour. The solution was then completed to 100 ml. with distilled water and the amount of strychnine determined by measuring the E 254 mμ value. Another portion was rendered alkaline with sodium hydroxide and the unchanged strychnine was extracted with chloroform. The residue left after distillation of the chloroform was dissolved in absolute ethanol, made up to a convenient volume with the same solvent, and the amount of strychnine determined in the same way. The same weight of the mixture of strychnine and brucine was analysed for its strychnine content by the B.P. procedure for comparison. Results of a series of determinations are shown in Table I.

DETERMINATION OF STRYCHNINE IN GALENICALS

Liquid Extract of Nux Vomica

Assay procedure. Into a glass tube 25 to 30 cm. long, 1·3 cm. in diameter, with a constricted end, 15 g. of active alumina was packed dry in portions forming an adsorption column 14 cm. long. The column was connected to a suction apparatus, 2 ml. of the liquid extract was poured on and gentle suction was applied. Before the liquid began to disappear from above the adsorption column, 86 per cent. ethanol was added little by little to wash down the alkaloids on the sides of the tube.

TABLE I

COMPARISON OF RECOVERY OF PURE STRYCHNINE FROM STRYCHNINE–BRUCINE MIXTURES BY THE B.P. METHOD AND SPECTROPHOTOMETRIC ANALYSIS

Prepared amounts of alkaloid mixture		Pure strychnine recovered from the mixture					
				Spectrophotometric method after potassium persulphate treatment			
Brucine g.	Strychnine g.	B.P. method g.	Error per cent.	Direct measurement in 0·3 per cent. sulphuric acid g.	Error per cent.	After chloroform extraction measured in absolute ethanol g.	Error per cent.
0·1942	0·2512	0·2420	−3·0	0·2562	+1·9	0·2390	−4·8
0·1001	0·1230	0·1180	−4·0	0·12314	+0·2	0·1225	−0·4
0·1001	0·1845	0·1790	−2·0	0·1790	−2·0	0·1760	−4·5
0·0515	0·0610	0·0542	−1·1	0·0624	+2·0	0·0605	−0·8
0·0734	0·0861	0·0829	−3·0	0·0869	+0·9	0·0880	+2·2
0·0212	0·0245	0·0234	−5·0	0·0250	+1·0	0·0236	−3·5
0·0432	0·0492	0·0460	−6·0	0·0500	+1·0	0·0480	−2·4
0·0683	0·0608	0·0576	−5·0	0·0576	−5·0	0·0600	−1·3
0·0121	0·0608	0·0559	−8·0	0·0605	−0·4	0·0576	−5·2
Average error per cent.			−4·7		−0·04		−2·3

The adsorption column was then washed with greater amounts of 86 per cent. ethanol until the percolate was alkaloid free.

Usually about 50 ml. of 86 per cent. ethanol was sufficient for complete washing. The clear percolate was transferred quantitatively to a 100-ml. flask and completed to volume with 96 per cent. ethanol. An aliquot portion, usually 20 to 30 ml., of this was distilled, the residue was dissolved in 10 ml. of 3 per cent. sulphuric acid, 0·5 g. of potassium persulphate was dissolved in the solution by shaking and the solution was kept in a water-bath at 60° to 70° C. for 1 hour. After cooling, the solution was transferred quantitatively to a 100-ml. flask with distilled water. The solution was mixed by shaking, filtered through a dry filter-paper and dry funnel into a dry flask. The strychnine was then determined by measuring the E 254 mμ value of the solution in a Unicam spectrophotometer. Strychnine could be calculated according to the well-known relationship

$$C = E/E^{1\ \text{per cent}}_{1\ \text{cm.}}$$

where C = concentration in g. per cent., $E^{1\ \text{per cent.}}_{1\ \text{cm.}} = 390$ at 254 mμ, E = measured extinction at 254 mμ.

Tincture of Nux Vomica

10 ml. of the tincture were allowed to flow through an adsorption column of 10 g. of active alumina packed dry in the glass tube already described, and the assay completed as for the liquid extract.

To measure the sensitivity of the described procedure it was carried out on the galenicals with and without addition of known volumes of standard solution of pure strychnine in 86 per cent. ethanol. At the same time the galenical was assayed by the B.P. procedure with and without the addition of known quantities of strychnine for comparison. The results are shown in Tables II and III.

TABLE II

COMPARISON OF SENSITIVITY OF THE B.P. METHOD AND THE SPECTROPHOTOMETRIC

Individual extract	Added g.	Recovered	Error per cent.				
1·40	0·0608	0·0580	−3	1·51	0·02430	0·02320	−4
1·43	0·1210	0·1160	−4	1·47	0·00488	0·00466	−4
1·41	0·0912	0·0850	−6	1·49	0·00365	0·00357	−2
1·46	0·0860	0·0820	−4	1·47	0·00681	0·00710	
1·40	0·0950	0·0900	−5	1·52	0·00852	0·00834	
		0·1190	−3	1·45	0·00511	0·00513	
		0·1250	−4	1·46	0·00340	0·00328	
		0·0930	−5	1·42	0·00272	0·00256	

				Spectrophotometric method after potassium persulphate treatment			
				Strychnine	Strychnine		
0·119	0·060	0·054	−10	0·130		0·00251	+3
0·122	0·085	0·075	−11	0·125		0·00275	−5
0·119	0·098	0·092	−6	0·121		0·00334	−1
0·126	0·101	0·093	−8	0·125		0·00609	+4
0·121	0·076	0·073	−4	0·120	0·00438	0·00440	+0·5
0·119	0·082	0·078	−5	0·122	0·00365	0·00359	−1
0·122	0·086	0·082	−4	0·127	0·00486	0·00490	+0·8
0·119	0·112	0·107	−4	0·130	0·00365	0·00359	−1
0·122	0·094	0·088	−6	0·126	0·00242	0·00236	−1
Average: 0·121			−6	0·125			−0·07

DISCUSSION

The accuracy of estimation of strychnine spectrophotometrically depends largely upon the success of elimination of the interfering ultra-violet-absorbing impurities from the galenical preparation.

This in turn depends upon the method of purification of the galenical. Using chromatographic purification the kind and nature of the adsorbing agent was important in achieving an approximate degree of purification. Pure active alumina was tried and on spectrophotometric examination of the purified galenical after potassium persulphate treatment the spectrum of pure strychnine was obtained. This indicates that the absorbing impurities were completely eliminated. Using activated recovered alumina the absorbing impurities were not completely removed. The lack of absorption of the impurities at 254 mμ is the critical factor in determining the accuracy of the method.

The absorption spectrum of the interfering impurities may be estimated

by subtracting the absorption of pure strychnine from the total absorption of strychnine and impurities in the galenical (see Fig. 2).

The curves A and B were obtained using the average of the experimental data in Table II. The 8 times repeated average with the B.P. method gave 1·42 per cent. of strychnine (the manufacturers order single determination method has 1·50 per cent. of strychnine $\pm$ 0·05). That average with spectrophotometric procedure was 1·47 per cent. of

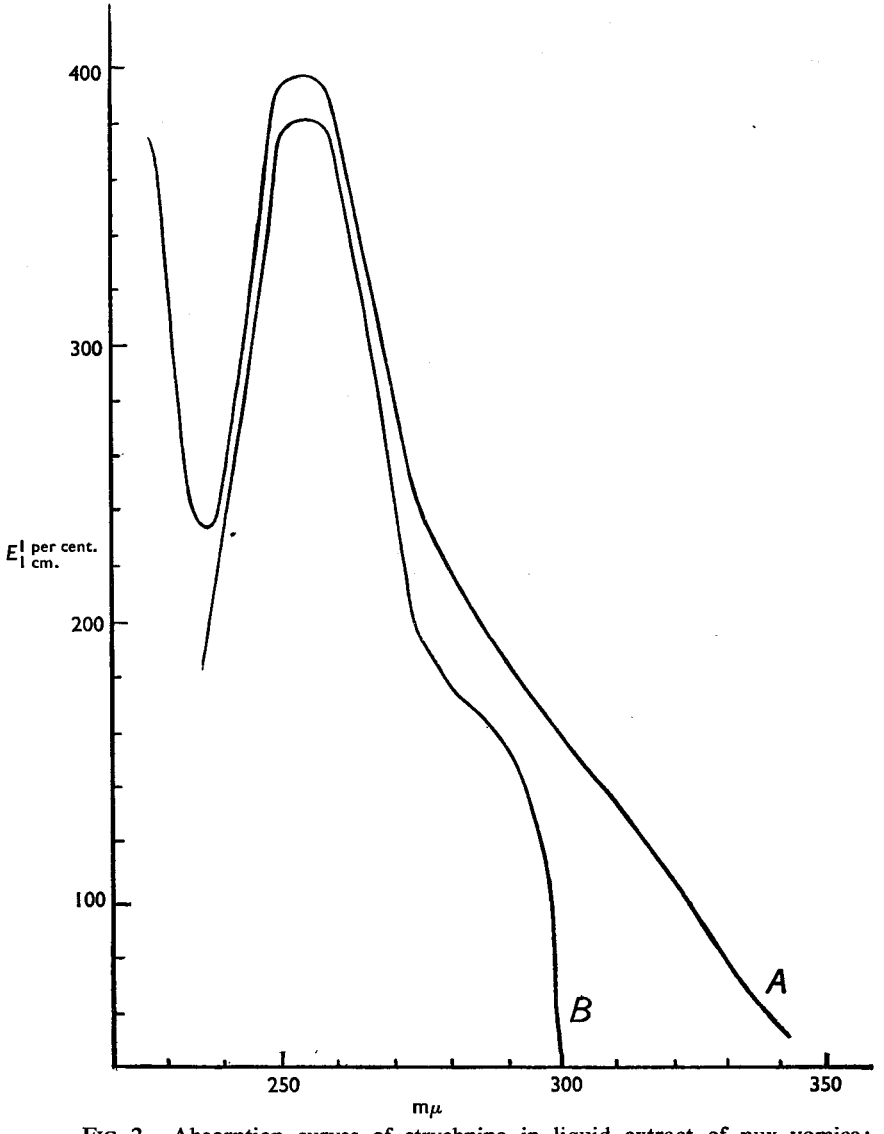

FIG. 2. Absorption curves of strychnine in liquid extract of nux vomica: (A) after chromatographic purification and potassium persulphate treatment; (B) calculated from the B.P. procedure.

strychnine. Taking the average of the B.P. procedure as the minimum value for strychnine, the spectrophotometric method gives 0·05 per cent. higher and will not exceed 2 per cent. error as shown in Table II.

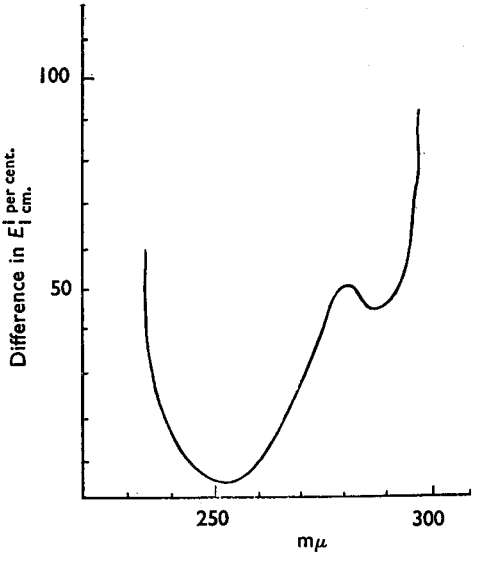

FIG. 3. Absorption spectra of impurities in liquid extract of nux vomica obtained by difference between the average spectrophotometric values and the minimum value by the B.P. procedure.

The average extinction at each wavelength for the 8 samples as found by the spectroscopic process after potassium persulphate treatment is plotted in curve A, Figure 2. For curve B the B.P. method was employed to obtain a standard minimum value of the strychnine concentration. The difference of absorption between curves A and B (due to ultraviolet absorbing impurities) is plotted against wavelengths as in Figure 3. From the curve it is seen that the amount of absorption due to these impurities is at a minimum at 250 to 260 mμ at which the absorption is maximal.

This means that the ultraviolet-absorbing impurities have a negligible amount of absorption in the region of maximal absorption of strychnine and do not interfere in spectrophotometric determinations of strychnine in galenical preparations.

SUMMARY

1. A modified chromatographic purification of nux vomica galenicals from pigments and resinous substances is described.

2. A new oxidising agent for brucine is recommended.

3. A spectrophotometric procedure for the estimation of strychnine in galenicals is described with an accuracy of not less than 98 per cent.

4. The method is comparatively rapid, and avoids difficulties, such as emulsification, which occur during extraction procedures.

Our thanks are due to Prof. I. R. Fahmy for suggestions and advice, to Prof. R. A. Morton, F.R.S., of Liverpool University, for editorial assistance, and to H. Shaker for his assistance in reading and checking the absorption curves and data.

REFERENCES

1. Brownlee, *Quart. J. Pharm. Pharmacol.*, 1945, **18**, 163.
2. Reimers, Gottlieb and Christensen, *ibid.*,, 1947, **20**, 99.
3. Brustier and Blanc, *Bull. Soc. Chem.*, 1934, **1**, 712.
4. Elvidge, *Quart. J. Pharm. Pharmacol.*, 1940, **13**, 224; 1942, **15**, 209.