# METHOD FOR THE QUANTITATIVE ESTIMATION OF STRYCHNINE AND BRUCINE IN NUX VOMICA BY PAPER ELECTROPHORESIS

By D. K. Bardhan and Diptish Chakravarty

*From the Research and Analytical Division, Smith Stanistreet and Co. Ltd., Calcutta-14, India*

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A method for the quantitative separation and estimation of strychnine and brucine in nux vomica seeds has been described. The method involves extraction of total alkaloids, electrophoretic separation in a buffer medium and subsequent estimation in spectrophotometer after elution in suitable solvent. The method is precise and accurate.

Numerous methods have been published for the assay of strychnine and brucine. Waligora and Bylo (1953) used potentiometric chromatography with an antimony microelectrode. The pure alkaloids in admixture have been estimated from optical density values at their ultra-violet absorption maxima (Bhattacharya and Ganguly, 1952; Biggs, 1952). Chromatographic separation on a column of activated alumina (Fischer and Buchegger, 1950; Ridi and Khalifa, 1952) and kieselguhr (Jensen and Svendsen, 1950) have been reported, and separation and estimation have also been achieved by paper partition chromatography (Gore and Adshed, 1952; Briner, 1958).

We have applied horizontal zone electrophoresis on paper to separate and estimate the alkaloids from a crude extract of the drug. Since electrophoresis itself purifies the materials, the presence of impurities or other alkaloids of dissimilar mobilities does not interfere. On the basis of our observations we describe a method in which a simultaneous estimation of both the alkaloids is possible with an accuracy of $\pm 5$ per cent.

## Experimental

*Standard strychnine solution.* 98·9 mg. of strychnine sulphate N.F. was accurately weighed after drying and was dissolved in water to a volume of 50 ml.

*Standard brucine solution.* 98·8 mg. of brucine sulphate N.F. was similarly treated.

*Standard buffer solution.* 100 ml. of 0·1 N solution of citric acid (B.P.) neutralised with 20 ml. of 0·1 N solution of sodium hydroxide (B.P.) at room temperature (25°). The solution had a pH of 3·10 when measured with the glass electrode.

*Sodium nitrite solution.* 5 g. of sodium nitrite (pure) dissolved in water to make 500 ml. was used as electrolyte.

*Modified Dragendorff's reagent. Solution A.* Bismuth subnitrate 1·062 g. was dissolved in 50 ml. water and 12·5 ml. glacial acetic acid.

*Solution B.* Potassium iodide 25 g. was dissolved in 62·5 ml. water. The spray reagent was prepared from 1 ml. of solution A, 1 ml. of solution B with 2 ml. of glacial acetic acid and 10 ml. of water.

*Extract of Nux Vomica*

Powdered seeds of nux vomica (40 mesh) (10 g.) were shaken with 100 ml. of a mixture of ether and chloroform (2:1) and 5 ml. of dilute ammonia for about 2 hr. The extract was transferred completely into a percolator and extracted with the same solvent mixture till the percolate became free from alkaloid (about 6 hr.). The percolate was filtered through paper and the filtrate was extracted with successive volumes of 20 ml., 10 ml., 10 ml. and 10 ml. of N sulphuric acid which was also used to dilute the extract to 100 ml.

*Apparatus.* Electrophoresis was conducted in an apparatus, locally made from Perspex sheet with an arrangement for adjusting the length of the paper strip according to needs.

Whatman No. 1 paper was used after washing with 1 per cent hydrochloric acid and a flush of water and subsequent drying in air.

Power was supplied from a stabilised constant voltage/current power pack (locally made) operating from 220V A.C. line with a maximum output of 1000V D.C. at 50 mA.

A Hilger "Univispek" spectrophotometer with a matched pair of quartz cells of path length 1·00 cm. was used for measuring optical density values.

*Method*

An aliquot of 0·05–0·1 ml. of a mixture of equal volumes of the standard solutions of strychnine sulphate and brucine sulphate containing 50–100 $\mu$g. of each of the alkaloids was spotted with an Alga syringe on a paper strip of 6 in. $\times$ 14 in. at two places along a straight line, about 4 in. from one end, one to locate the position of the substances after spraying, the other for the quantitative elution and estimation. The paper was then moistened with a fine spray of the buffer solution.

The whole arrangement was placed in an air-tight compartment and was allowed to saturate for 15 min. and then 250 V at 5–10 mA was applied. Electrophoresis was continued for 6 hr. The paper was then air dried and separated into two strips, one of which was developed by spraying with modified Dragendorff's reagent. Both strychnine and brucine were found to migrate to the negative pole with different mobilities, the former about 16 cm. the latter 13 cm. from the base line. The respective positions were then located on the second strip and the alkaloids were cut out and eluted separately in 10 ml. of 0·01 N sulphuric acid solution at 36° for 6 hr. A blank was run by cutting a similar portion from the same paper and eluting in the same quantity of the solvent. After elution, the solutions were centrifuged and the supernatants were assayed in the spectrophotometer against the respective blanks. The $\lambda_{max.}$ of strychnine and brucine being at 252 m$\mu$ and 262 m$\mu$ respectively, their $E$ (1 per cent, 1 cm.) values were calculated from the concentration density values of pure samples and were found to be as follows: strychnine, $E$ (1 per cent, 1 cm.) 252 m$\mu$ = 299·5; brucine, $E$ (1 per cent, 1 cm.) 262 m$\mu$ = 232·5 (in 0·01 N sulphuric acid).

Results obtained from the known quantities of the mixtures of standard strychnine and brucine solutions have been listed in Table I (A).

For the quantitative estimation of the alkaloids in nux vomica seeds, an aliquot of 0·05–0·1 ml. of the extract of nux vomica was similarly treated on the paper. Results have been shown in Table I (B).

A comparative study was conducted by estimating strychnine and brucine content of the samples according to the method as described

TABLE I

COMPARISON OF RESULTS OBTAINED BY ELECTROPHORESIS WITH THOSE OBTAINED BY CHEMICAL ASSAY

| | Strychnine | | | Brucine | | |
|---|---|---|---|---|---|---|
| Group | Present μg./ml. | Found by electrophoretic method μg./ml. | Found by chemical method μg./ml. | Present μg./ml. | Found by electrophoretic method μg./ml. | Found by chemical method μg./ml. |
| (A) | 9·88 | 9·40 | 9·56 | 9·45 | 10·05 | 9·41 |
| | 10·00 | 10·16 | 10·08 | 9·80 | 9·30 | 9·78 |
| | 4·92 | 4·85 | 4·90 | 5·00 | 5·15 | 5·06 |
| | 25·00 | 26·30 | 25·10 | 25·00 | 26·60 | 25·18 |
| (B) | — | 6·01 | 6·30 | — | 10·50 | 10·04 |
| | — | 12·10 | 12·32 | — | 15·90 | 15·00 |
| | — | 13·70 | 13·02 | — | 20·00 | 19·50 |
| | — | 7·93 | 7·50 | — | 13·15 | 13·30 |

in the B.P. (1958). The total alkaloid was first determined gravimetrically followed by strychnine assay by volumetric titration. The difference between these two values was considered to be the brucine content of the sample.

The results of chemical assays are also recorded in Table I.

## DISCUSSION

We have observed that both strychnine and brucine obey Beer's law at their respective absorption maxima within concentrations of 5 to 40 μg./ml. of solution in 0·01 N sulphuric acid. The alkaloids are stable in acidic buffer medium. The absorption curve after electrophoresis showed no change in their physical properties.

The positions of the alkaloids on the paper can also be located by contact ultra-violet photography using a chlorine gas filter, when the same strip of paper can be eluted.

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