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# The assay of nux vomica and its preparations

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A method is proposed for the extraction of strychnine and brucine, from nux vomica and some of its preparations. An ammoniacal suspension of the drug is extracted with chloroform in a downward displacement liquid-liquid extractor. The strychnine and brucine are then extracted from the chloroform with normal sulphuric acid and the strychnine determined spectrophotometrically. In general the results are in good agreement with those obtained by official methods and the method effects a considerable saving of time.

THE assay of nux vomica and its preparations essentially involves the separation of the strychnine, together with brucine, followed by the isolation and estimation of the strychnine. Solvent extraction, followed by separation and nitration (B.P. 1963), column chromatography (B.P.C. 1959; 1963), or ion-exchange resins (Elvidge & Proctor 1957) are the usual methods involved in the isolation step and the use of any of these is both difficult and time consuming. We have overcome this by the simple extraction of an ammoniacal aqueous suspension of the sample with chloroform in a downward displacement liquid-liquid extractor. In the preparations examined, no substances which interfere with the spectrophotometric assay at 262 and 300 m$\mu$ (Elvidge & Proctor, 1957; B.P.C. 1959; 1963) are present in the final solution.

## Experimental

*Apparatus.* A downward displacement liquid-liquid extractor (Quickfit and Quartz Type EX 10/23, nominal capacity 60 ml) and disc baffle (Type EX 10/20). The apparatus was completed by a condenser and a 100-ml flat bottomed flask. 3 Conical separating funnels 250 ml capacity.

*Reagents.* Ammonia solution: 10% v/v solution of AR ammonia in water. AR chloroform. 70% v/v ethanol in water. N Sulphuric acid.

### GENERAL METHOD

Using distilled water (25 ml) and ammonia solution (5 ml) transfer the sample (as prepared below) to a liquid-liquid extractor containing chloroform (80 ml). Reflux for 4 hr, cool, and transfer the chloroform in the flask to a separating funnel. Successively extract the chloroform with N sulphuric acid (4 × 20 ml) combine the acid solutions, and wash once with chloroform (10 ml). Transfer the acid solution to a flat dish and warm gently with stirring to remove the dissolved chloroform. Cool, transfer to a 100 ml graduated flask (filtering if necessary), and make up to volume with N sulphuric acid. This is Solution S.

From a knowledge of the approximate strychnine content of the original sample, dilute solution S with N sulphuric acid so that it contains approximately 1 mg strychnine per 100 ml of solution. (Optimum absorbance

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between 0·6 and 0·9). Measure the absorbance at 262 mμ and 300 mμ in a 1 cm cell against N sulphuric acid as a blank.

If the formula of Elvidge & Proctor (1957) is applied to the present case,

$$\text{the percentage strychnine} = \frac{0{\cdot}318\ a - 0{\cdot}460\ b}{\%\ \text{concentration}}$$

Where a = absorbance at 262 mμ

b = absorbance at 300 mμ

$$\%\ \text{concentration} = \frac{\text{ml or g sample} \times c}{100}$$

c = ml of solution S used in final dilution.

SPECIFIC METHODS

*Liquid extract of nux vomica B.P.* Extract 2·0 ml of the sample and dilute 5·0 ml of solution S to 100 ml before measurement. Results are given in Table 1.

TABLE 1. STRYCHNINE % W/V IN LIQUID EXTRACT OF NUX VOMICA AND TINCTURE OF NUX VOMICA B.P.

| Sample No. | Proposed method | | B.P. 1963 | |
|---|---|---|---|---|
| | No. of tests | Range of results | No. of tests | Range of results |
| *Liquid extract* | | | | |
| 1 | 6 | 1·45–1·52 | 2 | 1·44–1·54 |
| 2 | 3 | 1·43–1·48 | 3 | 1·43–1·44 |
| 3 | 3 | 1·53–1·54 | 2 | 1·51–1·61 |
| 4 | 3 | 1·63 | 2 | 1·60–1·67 |
| 5 | 3 | 1·67 | 4 | 1·63–1·67 |
| 6 | 4 | 1·55–1·57 | 3 | 1·47–1·54 |
| *Tincture* | | | | |
| 1 | 4 | 0·116–0·118 | 3 | 0·109–0·125 |
| 2 | 2 | 0·136 | 2 | 0·125 |
| 3 | 3 | 0·123–0·125 | 2 | 0·123–0·128 |
| 4 | 3 | 0·128–0·130 | 2 | 0·128–0·133 |

*Tincture of nux vomica B.P.* Extract 5·0 ml of the sample and dilute 20·0 ml of solution S to 100 ml before measurement. Results are given in Table 1.

*Nux vomica beans B.P. and prepared nux vomica B.P.* 1953. Two methods were tried on these materials and both are given below.

*Method* 1. Accurately weigh about 400 mg of the finely powdered sample into a centrifuge tube and mix well with ethanol solution (25 ml). Centrifuge, and transfer the supernatant liquid to an evaporating dish. Repeat the above extraction three times. Evaporate the combined

extracts to about 5 ml and using the ammonia solution, water and chloroform (20 ml) transfer to a liquid-liquid extractor containing chloroform (60 ml). Extract and dilute 25 ml of solution S to 100 ml before measurement.

*Method* 2. Accurately weigh about 400 mg of the finely powdered sample into a beaker and mix with ethanol solution (2 ml). Add ammonia solution (5 ml), mix, and using water (25 ml) and chloroform (20 ml) transfer to a liquid-liquid extractor containing chloroform (60 ml). Extract and dilute 25 ml of solution S to 100 ml before measurement.

With Method 2, lumps of sample must be broken down with a glass rod. When transferring to the extractor no sample should be left adhering to the top of the disc baffle before the rest of the chloroform is added. With solid samples it is advisable to swirl the contents of the extractor gently from time to time during the extraction period. Failure to observe these conditions usually leads to incomplete extraction. Results are given in Table 2.

TABLE 2. STRYCHINE % W/W IN NUX VOMICA BEANS, PREPARED NUX VOMICA (B.P. 1953) AND NUX VOMICA DRIED EXTRACT B.P.C.

| Sample No. | Proposed method | | B.P. 1963 B.P. 1953 | |
|---|---|---|---|---|
| | No. of tests | Range of results | No. of tests | Range of results |
| *Nux vomica beans and prepared nux vomica* | | | | |
| 1 | 3 | 1·21–1·26 | 2 | 1·14–1·16 |
| 2 | 2 | 1·20–1·22 | 2 | 1·22–1·26 |
| 3 | 2 | 1·07–1·11 | 2 | 1·09–1·10 |
| 4 | 4 | 1·11–1·19 | 4 | 1·06–1·12 |
| 5 | 3 | 1·93–1·96 | 3 | 1·84–1·98 |
| 6 | 4 | 1·83–1·91 | 2 | 1·84–1·88 |
| *Dried extract* | | | | |
| 1 | 10 | 6·18–6·74 | 5 | 5·64–6·76 |
| 2 | 3 | 5·25–5·30 | 3 | 5·17–5·66 |
| 3 | 3 | 5·13–5·17 | 2 | 4·90–5·12 |
| 4 | 4 | 5·83–5·95 | 2 | 5·65–5·95 |
| 5 | 4 | 7·44–7·92 | 5 | 7·39–8·10 |
| 6 | 2 | 5·32–5·36 | 2 | 5·40 |

*Dry extract of nux vomica B.P.C.* As for nux vomica B.P. and prepared nux vomica B.P. 1953, diluting 5 ml of Solution S to 100 ml before measurement. Results are given in Table 2.

## Results and discussion

Tables 1 and 2 show the results obtained. The standard deviation was calculated for the first sample of dried extract only. By the proposed method a mean of 6·38% (s.d. $\pm$ 0·20) was obtained whilst by official methods the mean was 6·19 (s.d. $\pm$ 0·49).

The proposed method gives results as reproducible as those obtained by existing methods. For liquid extracts and tinctures it is more readily and quickly carried out with no loss of accuracy.

The assay of solid products particularly dry extract of nux vomica B.P.C. is subject to wider variation irrespective of the method used. By Method 2 the results were more reproducible and the assay technique was trouble free provided the conditions of operation were strictly observed. For the assay of nux vomica beans and prepared nux vomica (B.P. 1953) Method 2 gave comparable results in less than half the time taken by the B.P. methods.

## References

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