Table 2. The enthalpies (ΔH), entropies (ΔS) and free energies (ΔG) of transfer of 1a and 1c from water to 1-octanol[a].

	ΔH (KJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔG (KJ mol^{-1})[b]
1a	+2·66 (0·16)	+13·1 (0·5)	−1·42 (0·02)
1c	−3·29 (0·30)	−3·4 (0·9)	−2·24 (0·10)

[a] The values are the means from 5 experiments; the standard deviations are given in parentheses.
[b] At 37 °C.

The partition coefficient of 1a is slightly less than that of 1c over the whole temperature range (288–308 K). Interestingly, however, the ΔH and ΔS of transfer are substantially *greater* for 1a than 1c. This strongly suggests that 1a *does* form an intramolecular hydrogen bond in 1-octanol, because similar partition studies on 2 and 4 substituted phenols (Dearden & Bresnen 1982) have shown that the ΔH and ΔS of transfer are *less negative where intramolecular hydrogen bonding occurs*, presumably due to a reduction in the hydrogen bonding interactions between the solute and 1-octanol.

Evidently, the solvating properties of 1-octanol attenuate the effects of intramolecular hydrogen bonding on ΔG. Therefore, reliable conclusions regarding the intramolecular hydrogen bonding properties of a solute in the 1-octanol-water solvent systems can only be drawn from measurements of ΔH and ΔS.

Finally, these results suggest that compounds related to 1a, such as the histamine H_2-receptor antagonists metiamide (1b) and cimetidine (1d), probably adopt intramolecularly hydrogen bonded conformations in much more polar environments than previously considered.

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The rapid estimation of strychnine in tincture of nux vomica BP by high-performance liquid chromatography

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Strychnine is separated from other alkaloids in nux vomica tincture in less than 6 min on a 12·5 cm Hypersil column using a mobile phase of methanol, 2 M ammonium hydroxide, M ammonium nitrate (27 : 2 : 1), uv detection at 254 nm. Quantitative estimation may be obtained by comparing peak area or height against an external standard (0·150% w/v strychnine base in 45% ethanol). The results obtained were comparable to those obtained by the BP method which takes 5 h.

The estimation of strychnine in tincture of nux vomica by the British Pharmacopoeia (BP) method is a spectrophotometric determination after a 4 h liquid–liquid extraction. Absorption methods of this type are lacking in specificity when alkaloids of similar structure occur together, as they do in this example. The rapid quantitative estimation of strychnine in tincture of nux vomica by a modification of the high-performance liquid chromatography (hplc) system used by Jane (1975) is now reported. Other hplc separations involving strychnine alkaloids have been described (Wu & Siggia 1972; Murgia & Walton 1975; Verpoorte & Baarheim Svendsen 1975; Twitchett 1975).

Materials and methods

All chemicals and reagents were reagent grade (BDH) with the exception of methanol (Analar). Standard strychnine and brucine solutions were 0·15% w/v in 45% ethanol.

Samples of nux vomica tincture obtained from different sources were assayed for strychnine by the BP method and then by hplc.

The chromatographic column (125 × 4·6 mm) was packed by upward displacement (Bristow et al 1977) with 5 μm Hypersil (Shandon Southern, Runcorn). A variable wavelength uv photometer (Pye Unicam, Cambridge) operating at 254 nm was used as a detector and a Minigrator (Spectra-Physics, St Albans) was used to measure peak areas and retention times. Samples (10 μl) were introduced onto the column using a Rheodyne injection valve (model 7125) fitted with a 10 μl loop. The eluent, methanol–2 M ammonium hydroxide–M ammonium nitrate (27 : 2 : 1) degassed under vacuum at ambient temperature and used at a flow rate of 2·0 ml min^{-1} was delivered from an Altex 110 pump (Altex Scientific Inc., Berkeley, California). The chart recorder was a Leeds and Northrup XL flat bed recorder. Each result (Table 1) is the mean of three replicate determinations. Calibration plots were similarly determined using standard solutions of strychnine and brucine. Concentrations were calculated using either peak areas or heights.

Results and discussion

The method, adapted from Jane (1975), used a shorter column, changed the stationary phase to spherical particles (Hypersil) and increased the flow rate; the net effect being to decrease the retention time of strychnine from 14 to 3·6 min. Under these conditions, 6 min sufficed to separate strychnine, brucine and the minor alkaloids. Sensitivity was enhanced by changing the detector wavelength from 278 to 254 nm.

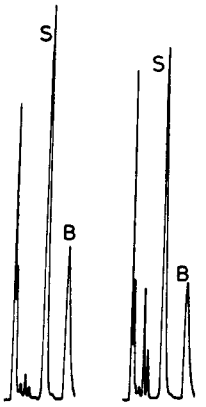

FIG. 1. Chromatogram of nux vomica tincture showing the strychnine to brucine variation and the content of minor alkaloids: S = strychnine, B = brucine.

A comparison of the results between the official method and the hplc technique is shown for eight samples in Table 1. The strychnine content of two of these failed to comply with the BP limits of 0·119–0·131% w/v strychnine but in most instances the methods gave comparable values.

Table 1. Estimation of strychnine and brucine in nux vomica tincture.

Sample no.	Official assay Strychnine (% w/v)	Hplc assay Strychnine (% w/v)	Hplc assay Brucine (% w/v)	Ratio (S/B)
1	0·125	0·124	0·078	1:1·6
2	0·128	0·119	0·091	1:1·3
3	0·131	0·136	0·095	1:1·4
4	0·134*	0·137	0·058	1:2·4
5	0·125	0·125	0·110	1:1·1
6	0·130	0·128	0·093	1:1·4
7	0·143*	0·146	0·120	1:1·2
8	0·121	0·129	0·086	1:1·5

* Sample does not comply with the BP limits of 0·119–0·131% w/v strychnine.

The BP method is a lengthy and non-specific procedure involving a 4 h liquid–liquid extraction, solvent transfer and determination of absorbance at 262 and 300 nm. The expression used to calculate the percentage of strychnine is designed essentially to take into account the presence of brucine but does not allow adequately for the presence of minor alkaloids which may contribute to the final absorbance. In the samples examined, the content of minor alkaloids varied and this is the possible cause of variation between the hplc and official results. Thus Sample 2 had a significantly lower strychnine content by the hplc method compared with the BP assay figure and the chromatogram for this tincture showed a higher concentration of minor alkaloids compared with other samples. The possibility that the major peak is due to strychnine plus one or more minor alkaloids, is contra-indicated by the fact that the absorbance ratio calculated from the peak area measured at 254 nm and 280 nm is the same as that for the strychnine standard.

Whereas Garratt (1955) reported the ratio of strychnine to brucine as 1 : 1 or 1 : 1·2, Table 1 shows the ratio varying from 1 : 1·1 to 1 : 2·4 when determined by the hplc method. Although brucine has only 1/8–1/10 the activity of strychnine, variations of this proportion would indicate tinctures having different overall potencies even though the strychnine content may be similar.

The hplc method is specific in that strychnine is separated from minor alkaloids and from brucine. If required, the amount of brucine can be separately reported. Six replicate determinations made on one of the tinctures gave coefficients of variance of 0·491 (peak area) and 0·403 (peak height) for the strychnine peak. Although the mobile phase is alkaline (pH 9) the high methanol concentration protects the silica from degradation and no significant loss of performance was noticed over several months.

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