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Short Communication

Separation of *Strychnos nux-vomica* alkaloids by high-performance liquid chromatography

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ABSTRACT

The common alkaloids present in *Strychnos nux-vomica* L. were separated using normal-phase high-performance liquid chromatography.

INTRODUCTION

Various methods of analysis of *Strychnos nux-vomica* L. alkaloids involving strychnine and brucine by high-performance liquid chromatography (HPLC) have been reported. Wu and Siggia [1], Twitchett [2] and Murgia and Walton [3] separated a number of alkaloids including strychnine. Verpoorte and Baerheim Svendsen [4], Dennis [5] and Hayakawa *et al.* [6] separated *S. nux-vomica* alkaloids including strychnine and brucine. However, none of these studies dealt with the separation of the two major alkaloids of *S. nux-vomica* (strychnine and brucine) and of the more important minor bases (icajine, novacine, vomicine, pseudostrychnine, pseudobrucine, α-colubrine and β-colubrine). This paper describes the separation of all these alkaloids and the determination of strychnine and brucine.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Millipore-Waters (Milford, MA, USA) Model 510 pumping system, a Waters UCK 029970 injector, a Waters automated gradient controller and a Waters Model 440 absorbance detector. The detector output was recorded with an SE 120 BBC Goerz Metrawatt chart recorder.

Chemicals

Chloroform for HPLC (Spectrochem, Bombay, India), cyclohexane for HPLC (S.D. Fine Chem, Boisar, India) and redistilled and filtered diethylamine of AnalaR grade (BDH, Poole, UK) were used. Strychnine and brucine (Smith, Stanistreet, Calcutta, India) were used without further purification.

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Chromatographic procedure

A Waters μPorasil column (30 cm × 3.9 mm I.D.) (particle size 10 μm) was used. Separations were performed at 20°C. The mobile phase was chloroform–cyclohexane–diethylamine (60:40:1) and was mixed in batches of 300 ml. The flow-rate was 0.5 ml/min. The eluate was monitored at 280 nm. For assays the chart speed was set at 0.1 cm/min.

Calibration

A stock standard solution of strychnine or brucine was prepared by dissolving 5 mg of the alkaloid in 10 ml of chloroform. A 1-ml of the stock solution was diluted to 5 ml with chloroform and volumes of 10, 15, 20, 25 and 30 μl were injected. Calibration graphs were obtained by plotting peak height (y) against concentration (x) (strychnine: regression equation $y = 35.2x - 1.6$, correlation coefficient 0.9926; brucine: regression equation $y = 20.4x + 3.2$, correlation coefficient 0.9983).

Method of extraction and analysis of nux-vomica tincture

A 5-ml volume of mother tincture of nux-vomica was evaporated to dryness and the dry tincture was transferred to a separating funnel with 5 × 10 ml of 10% glacial acetic acid. The acidic solution was made alkaline with 25% ammonia solution and was extracted with 5 × 30 ml of chloroform. The combined chloroform extracts were evaporated to dryness and the dry extract was transferred to a 10-ml volumetric flask with chloroform for HPLC and diluted to volume with chloroform. A 0.5-ml volume of this solution was transferred to a 5-ml volumetric flask and diluted to volume with chloroform. Volumes of 30 μl were injected for assay.

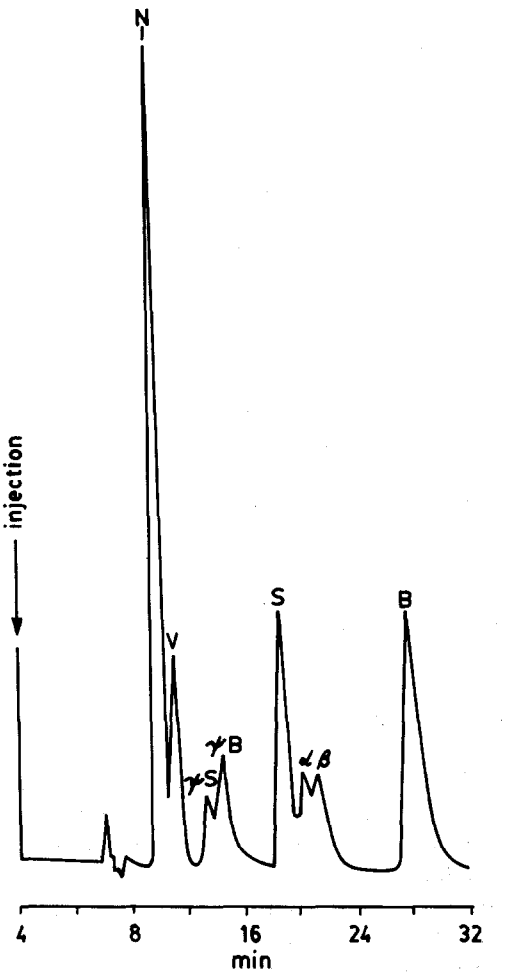

Fig. 1. Chromatogram of *Strychnos nux-vomica* alkaloids. N = Novacine; I = icajine; V = vomicine; ψS = pseudostrychnine; ψB = pseudobrucine; S = strychnine; α = α-colubrine; β = β-colubrine; B = brucine.

TABLE I

CAPACITY FACTORS OF ALKALOIDS

Alkaloid	k'	Alkaloid	k'
Icajine	0.67	Strychnine	2.13
Novacine	0.67	α-Colubrine	2.4
Vomicine	0.86	β-Colubrine	2.6
Pseudostrychnine	1.2	Brucine	3.66
Pseudobrucine	1.4		

TABLE II

RECOVERIES OF STRYCHNINE AND BRUCINE

Parameter	Strychnine	Brucine
Amount taken (mg)	5.00	5.00
Mean recovery (mg) ($n = 3$)	4.83	4.93
Standard deviation (mg)	0.125	0.094
Standard error of mean	0.07	0.05
Recovery (%)	96.6	98.6
Coefficient of variation (%)	2.59	1.91

TABLE III

DETERMINATION OF STRYCHNINE AND BRUCINE IN NUX-VOMICA TINCTURE

Alkaloid	Sample	Mean weight in 10 ml of tincture ± standard deviation (mg) (n = 3)	Concentration (%, w/v)	Coefficient of variation (%)
Strychnine	A	4.13 ± 0.125	41.3	3.03
	B	4.30 ± 0.16	43.0	3.72
Brucine	A	6.30 ± 0.29	63.0	4.6
	B	6.10 ± 0.14	61.0	2.3

For recovery tests, solutions of strychnine (20 mg) and brucine (20 mg) in 10 ml of chloroform were prepared and 2.5 ml of the solutions were added to 5 ml of nux-vomica tincture, dried and then extracted following the above procedure. Volumes of 30 μl were injected for assay.

Recoveries were calculated from the difference between the amount of strychnine or brucine present in the mother tincture and that in the same sample with known amounts of the two alkaloids added.

RESULTS AND DISCUSSION

Using the experience acquired during the thin-layer chromatographic analysis of tertiary nux-vomica alkaloids [7], different proportions of chloroform–cyclohexane–diethylamine were tried as mobile phases. Chloroform–cyclohexane–diethylamine (60:40:1) gave the best results, strychnine and brucine being separated with no interfering peaks. The most important alkaloids of nux-vomica extract were separated, except icajine and novacine, which showed the same retention time. This separation was found to be reproducible. Elution with baseline separation of a synthetic mixture of the authentic alkaloids was completed within 30 min (Fig. 1). The capacity factors (k') for different alkaloids are given in Table I. A higher proportion of chloroform in the mobile phase did not separate all the alkaloids completely and a reduction in the chloroform concentration resulted in an increase in pea width.

Detection was carried out at 280 nm despite th fact that the UV absorbance maximum of strych nine is 254 nm, because the diethylamine in the mo bile phase had a UV cut-off point (λ_{max}) higher tha 254 nm and thus interfered with the detection of th alkaloids.

The recoveries of strychnine and brucine we 96.6% and 98.6%, respectively. The results of r covery tests are given in Table II.

Strychnine and brucine were determined in tw samples of nux-vomica tincture. The results are gi en in Table III.

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