GLC Determination of Strychnine and Brucine in Pharmaceutical Preparations

DAVID L. SONDACK▲ and WILLIAM L. KOCH

Abstract □ The quantitative analysis of strychnine and brucine in pharmaceutical preparations is described. Samples are made alkaline and extracted with chloroform. The organic layer is collected and evaporated. The residue is dissolved in an aliquot of the internal standard solution in chloroform. Favorable quantitation has been achieved using papaverine as the internal standard for samples containing both strychnine and brucine. For samples containing strychnine only, brucine may be used. The rapidity and precision of this method represent an improvement over previous assays.

Keyphrases □ Strychnine and brucine—GLC analysis in liquid and tablet formulations □ Brucine and strychnine—GLC analysis in liquid and tablet formulations □ GLC—analysis, strychnine and brucine formulations

Strychnine and brucine are the most common tertiary *Strychnos* alkaloids (1). Many methods for their estimation have been described, including gravimetric and titrimetric analyses (2), paper chromatography (2–4), TLC (5), ion-exchange chromatography (6), spectrophotometry (7), and NMR spectroscopy (8). The two alkaloids have been separated by GLC (9, 10). This article describes the application of GLC to the estimation of strychnine and brucine in some liquid and tablet formulations.

EXPERIMENTAL[1]

Materials—Helium was used as a carrier gas, and electrolytic hydrogen and oxygen were used in the detector. The stationary phase was 3.0% OV-1 on Gas Chrom Q[2], 80–100 mesh, and was packed in borosilicate glass columns, 0.91 m. × 0.64 cm. o.d. All chemicals used were reagent grade or the best quality available.

Operating Conditions—The column was operated isothermally at 280° with the detector block and injection port at 295°. The helium flow rate was 55 ml./min. with an inlet pressure of 40 p.s.i. The electrometer range was 10 with an attenuation of 128. Sample injections of between 1 and 5 μl. were made.

Internal Standard—Papaverine hydrochloride, 1 mg./ml. in chloroform, was used as an internal standard for the formulation containing both strychnine and brucine. The quinine-containing elixirs displayed a large peak in a position to interfere with the use of papaverine. Since brucine was absent from the formulations, it was used. Either brucine or papaverine could be used for samples containing strychnine alone.

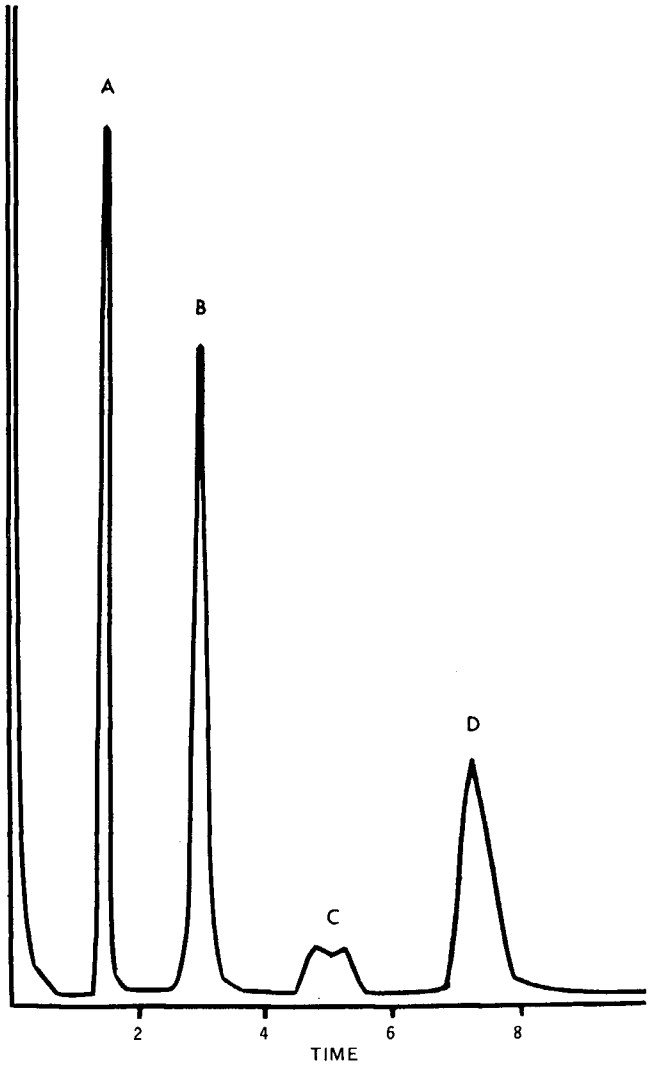

Figure 1—*GLC of nux vomica tincture with internal standard. Key: A, papaverine (internal standard); B, strychnine; C, probably α- and β-colubrine and icajine (10); and D, brucine.*

Standard Reference Solutions—*For Nux Vomica Tincture*—Accurately weigh 75 mg. of strychnine sulfate and 35 mg. of brucine. Quantitatively transfer the material to a 50-ml. volumetric flask. Dilute to the mark with deionized water. Pipet 10 ml. of the solution into a 60-ml. separator. Add 2 ml. of 10% sodium hydroxide solution and extract three times with 10-ml. portions of chloroform. Filter the organic phase through anhydrous sodium sulfate. Collect the filtrate in a small beaker and evaporate to dryness without heat. Redissolve the residue in 10 ml. of the papaverine hydro-

[1] A gas chromatograph (Hewlett-Packard model 402) equipped with a flame-ionization detector was used. The detector signal was fed to an IBM 1800 computer for peak integration and to a 1-mv. recorder (Honeywell Electronik 16) with a chart speed of 38.1 cm. (15 in.)/hr. and a 1-sec. full-scale response. Samples were injected with a 10-μl. syringe (Hamilton No. 701).

[2] Applied Science Laboratories, State College, Pa.

Table I—Precision and Accuracy

Formulation	RSD		RE	
	Strychnine	Brucine	Strychnine	Brucine
Nux vomica tincture	+2.0%	±3%	+3%	+1%
Iron quinine and strychnine tonic	±1.0%	—	−1%	—
Iron quinine and strychnine phosphates	±0.5%	—	+2%	—
Strychnine sulfate tablets	±2%	—	+2%	—

chloride internal standard solution. Inject 1-μl. quantities for chromatography.

For Elixirs and Tablets—Accurately weigh 50 mg. of strychnine sulfate and quantitatively transfer the material to a 250-ml. volumetric flask. Dilute to the mark with deionized water. Pipet 10 ml. into a 60-ml. separator. Add 2 ml. of 10% sodium hydroxide solution and extract as above. Redissolve the residue in 1 ml. of the brucine internal standard solution. Inject 1-μl. quantities for chromatography.

Formulations—*Nux Vomica Tincture*—Pipet 10 ml. of the tincture into a 60-ml. separator. Direct a stream of filtered dry nitrogen at the surface for 1 hr. to evaporate most of the alcohol. Add 5 ml. of 10% sodium hydroxide and treat as described for the reference standard.

Elixirs: Iron Quinine and Strychnine Tonic and Iron Quinine and Strychnine Phosphates—Pipet 10 ml. of the elixir into a 60-ml. separator. Direct a stream of filtered dry nitrogen at the surface for 1 hr. to drive off most of the alcohol. Add 5 ml. of 10% sodium hydroxide (the pH should be greater than 11) and proceed as described for the reference standard.

Tablets: Strychnine Sulfate—Obtain an average tablet weight using 10 tablets. Grind them to a fine powder. Accurately weigh and quantitatively transfer the equivalent of 1 mg. of strychnine sulfate to a 60-ml. separator. Add 10 ml. of deionized water and 2 ml. of 10% sodium hydroxide. Proceed as described for the reference standard.

Calculations—For each chromatogram, determine the ratio of the area of the strychnine peak to that of the internal standard. For the nux vomica tincture, determine the ratio of the areas for brucine and the internal standard also. The concentration of strychnine in liquid formulations is then given by:

$$\frac{R\ \text{sample}}{R\ \text{standard}} \times \frac{\text{mg. standard}}{\text{volume standard stock solution}} \times \text{standard purity} \times \frac{10\ \text{ml. standard}}{10\ \text{ml. sample}} \times 0.7804^{3} = \text{mg. strychnine (free base)/ml.} \quad \text{(Eq. 1)}$$

The concentration of brucine is obtained by using the appropriate ratios and standard weights in a similar equation. The strychnine content in tablets is obtained from:

$$\frac{R\ \text{sample}}{R\ \text{standard}} \times \frac{\text{mg. standard}}{\text{volume standard stock solution}} \times \text{standard purity} \times \frac{\text{average tablet weight}}{\text{weight of sample}} \times 10\ \text{ml.} \times 0.8949^{4} = \text{mg. strychnine sulfate/tablet} \quad \text{(Eq. 2)}$$

RESULTS AND DISCUSSION

The linearity of response of the chromatographic system to 1-μl. injections made from solutions containing 0.2–4 mg./ml. was ascertained. Five replicate samples of each formulation were analyzed to determine the precision of the method. Known amounts of reference standard were added to samples prior to extraction to obtain accuracy data. The results are shown in Table I. A typical chromatogram is shown in Fig. 1.

Samples of nux vomica tincture were assayed by this method and by the method described in NF XI (11). The results are compared in Table II. The NF method requires oxidative destruction of brucine and other interfering alkaloids prior to titration of the excess acid used to neutralize extracted strychnine. Typically, a standard deviation of ±5% is obtained, and as much as 2 days may be used in analyzing many samples.

Table II also lists the results obtained for samples of the elixirs and tablets. NF XI does not describe a quantitative assay for strychnine in such formulations. Thus, the present assay compares favorably in its rapidity, precision, and accuracy. No sacrifice in these parameters is experienced even with such a mixture as the nux vomica tincture.

Table II—Assay of Some *Strychnos* Alkaloid Formulations

Sample	NF Method, mg. Strychnine/ml.	GLC	
		mg. Strychnine/ml.	mg. Brucine/ml.
Nux vomica			
1	1.32	1.10	0.46
2	1.22	1.20	0.45
3	1.22	1.26	0.55
4	1.22	1.16	0.48
Iron quinine and strychnine tonic	—	0.144	—
Iron quinine and strychnine phosphates	—	0.171	—
Tablets	—	0.819[a]	—

[a] Milligrams strychnine sulfate (anhydrous) per tablet.

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▲ To whom inquiries should be directed.

[3] 0.7804 is the fraction of strychnine free base in the pentahydrated sulfate salt.

[4] 0.8949 is the fraction of strychnine sulfate in the pentahydrate salt.