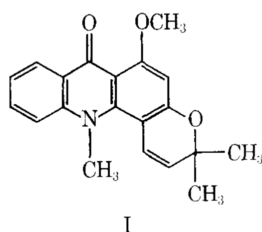
Quantitative Determination of Acronine, an Antitumor Alkaloid, by Gas Chromatography

FRANK E. GAINER and WILLIAM A. ARNETT

Abstract □ A method for the direct quantitative determination of the alkaloid, acronine, by gas chromatography is described and evaluated. Results are presented which show that the method is reliable after separation of the acronine from various pharmaceutical excipients. Methodology concerning the use of fluorescence and UV spectroscopy is also presented.

Keyphrases □ Acronine dosage forms—analysis □ GLC—separation, identification □ Cholesterol—GLC internal standard

Acronine[1] (I) is a naturally occurring alkaloid which



was obtained by extraction from the bark of the Australian plant *Acronychia Bower:Schott* (1, 2). More recently Beck *et al.* (3) reported the synthesis of the alkaloid. Acronine shows significant antitumor activity in experimental animals (1), and it is being considered for evaluation as an antitumor agent in humans. A study of the UV absorption spectrum of acronine is reported in work done by Brown and Lahey (4) but no mention is made of the measurement of the UV absorbance to quantitatively determine the alkaloid. A quantitative assay procedure is necessary in order to evaluate clinical trial material and formulations.

An extensive review of general and specific assay methods for alkaloids has been written by Theivagt *et al.* (5). Brochmann-Hanssen and Svendsen (6) have demonstrated the adaptation of gas chromatography to the analysis of alkaloids and alkaloid extracts. They used GLC in developing a technique for direct analysis of alkaloidal salts with temperature programming and for phenolic alkaloids as the TMS ethers. More recently Takagi *et al.* (7, 8) reported the quantitative assay of two alkaloids as their TMS ethers using a polyester stationary phase column.[2]

The method described here for acronine is direct and does not require derivative formation. In this study, gas chromatography was selected as the analytical method of choice because it was more readily adaptable to a stability assay method for acronine than either UV or fluorescence spectroscopy.

Preliminary investigations indicated that acronine had an a, 1% of 1045 at 297 mμ (Fig. 1) and that de-

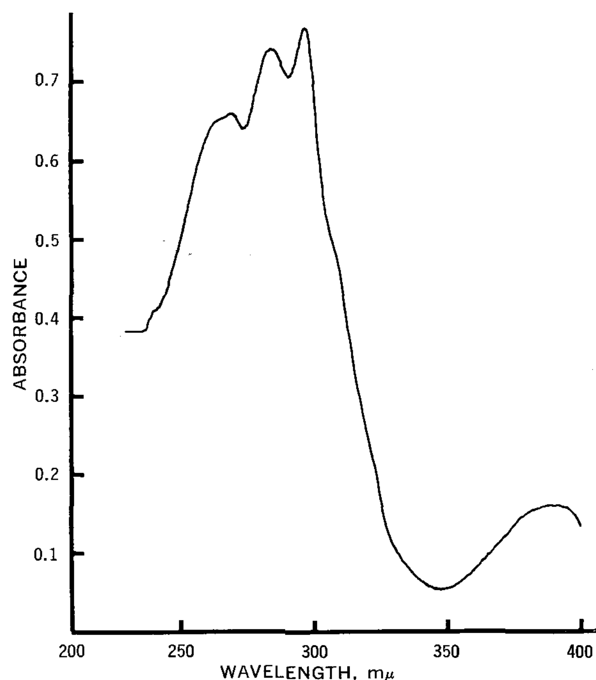


Figure 1—*UV absorption spectrum of acronine in chloroform.*

graded materials absorb in the same region. The UV absorbance in some cases was independent of the stability of the molecule. Dilute solutions of acronine in chloroform are yellow with a bright green fluorescence. The fluorescence spectra of acronine in this solvent show excitation peaks at 305 and 394 mμ and one emission peak at 455 mμ (Fig. 2). When the molecule was degraded artificially, an apparent increase in fluorescent intensity was observed. This phenomenon precluded the use of fluorescence spectroscopy. Chloroform solutions of acronine are apparently sensitive to light and should be stored in the dark or in amber glassware.

EXPERIMENTAL

Apparatus—F and M model 402 equipped with dual hydrogen flame detectors.

Column—A U-shaped glass column, 0.61 m. (2 ft.) × 3 mm. i.d. was used. The column was cleaned with chromic acid cleaning solution and silanized before packing.

Liquid Phase—A silicone gum rubber.[3]

Inert Support—A diatomaceous earth (80/100 mesh) specially treated and silanized.[4] The glass wool used to plug the ends of the column was also silanized.

Preparation of Column Packing—A 0.5% solution of the gum rubber in toluene was prepared by dissolving 1.0 g. of the rubber in 200 ml. of toluene with constant stirring on a steam bath. Forty grams of 80/100 mesh diatomaceous earth was added to the rubber solution in a 1.0-l. filter flask. Vacuum was applied to degas the solution and it was allowed to stand for 5 min. The support was

[1] Approved nonproprietary name (USAN); however, this compound is called acronycine in earlier publications.
[2] HI-EFF 8B, Applied Science Laboratories, State College, Pa.

[3] OV-17, Applied Science Laboratories, Cat. No. 08240.
[4] Diatoport S, F and M Scientific Co.

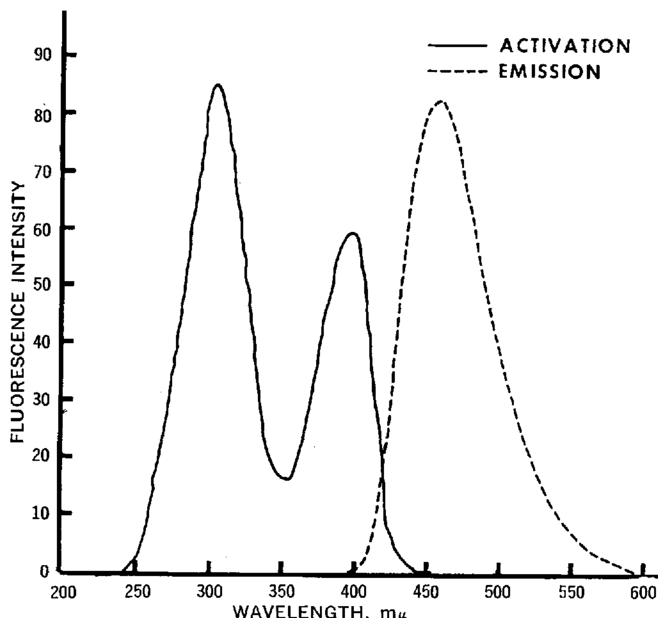


Figure 2—*Activation and emission spectra of acronine.*

then separated from the solution by vacuum filtration. The support was spread and air dried for 24 hr. To remove the last traces of toluene, the support was heated at 100° for 1–2 hr.

Column Conditioning—The column was "no flow" conditioned for 1.0 hr. at 310°. After cooling to room temperature, the carrier gas flow was started and the column was conditioned at 275° for 24 hr.

Instrumental Parameters—The instrument was operated isothermally at a column temperature of 250° and the detector and flash heater temperatures at 290°. Helium was used as the carrier gas with the flow rate at 60–70 ml./min. Oxygen was used for operating the hydrogen flame detector. The range setting was at 10, and the attenuation at 16×.

Preparation of Acronine Standard Solution—An accurately weighed and diluted solution of acronine reference standard in chloroform containing 0.25 mg./ml. was used as the standard solution.

Internal Standard—A cholesterol (Mann Research Laboratories) solution of 0.125 mg./ml. in chloroform was used as the internal standard solution.

Preparation of Standard Curve—Internal standard solution in the amount of 4.0 ml. was added to each of five 10-ml. flasks. A 4.0-ml. aliquot from each of five different standard dilutions was then added to the flasks to give a series of standard concentrations. Approximately 5λ of each resulting solution was injected into the gas chromatography. The peak heights of cholesterol and acronine were measured from the top of the peak to the corrected baseline. The standard ratio of the acronine peak height to the cholesterol peak height was calculated and plotted graphically against the concentrations.

General Extraction Procedure—In order to carry out preformulation studies it was necessary to extract the acronine from many of the commonly used pharmaceutical excipients. A sample containing approximately 25 mg. of acronine was weighed and transferred to a 125-ml. separator containing 25 ml. of water. The mixture was then extracted with four 20-ml. portions of chloroform. The chloroform extracts were passed through anhydrous sodium sulfate and the combined extracts collected in a 100-ml. volumetric flask. The flask was diluted to volume and after mixing an aliquot of the sample with an appropriate amount of internal standard, the sample was submitted for analysis by gas chromatography.

Table I—Composite Assays of Hand-Mixed Samples of Acronine and Talc

Capsule Content Wt., mg.	mg./capsule	n	$\bar{x}$	*RSD*, %	Accuracy, %
300	2.50	4	2.50	±4.33	0.00
380	5.00	5	4.90	±4.21	−2.00
380	10.00	5	9.71	±1.40	−2.90
380	25.00	5	25.00	±2.62	−0.02

Table II—Recovery of Acronine from Mixes with Various Excipients

Excipient	Acronine Recovered, mg./g.	Recovery, %
	1004.0	100.4
Talc	508.3	101.7
Stearic acid	500.6	100.1
Magnesium stearate	521.0	104.2
Silica gel	499.8	100.0
Tartaric acid	503.8	100.8
Ascorbic acid	495.5	99.1
Dipotassium phosphate	426.1	85.2

RESULTS AND DISCUSSION

The selection of cholesterol for use as an internal standard was important to the success of the proposed GLC method for acronine. The peaks are well shaped with very little tailing for both acronine and cholesterol (Fig. 3).

The quantitative determination of acronine is made by comparing the sample ratios of acronine to internal standard with the standard ratio of acronine to internal standard. Data for the calibration curve were obtained by assaying five concentrations of acronine standard solutions, including the normal working standard concentration. Solution concentrations extended over a range of ±50% of the normal working standard concentration. The method proved to be linear in response throughout this concentration range. The acronine concentration was varied but the internal standard peak height was held constant for each sample.

The reproducibility of measurement by this GLC technique was demonstrated by making six replicate injections of the same stan-

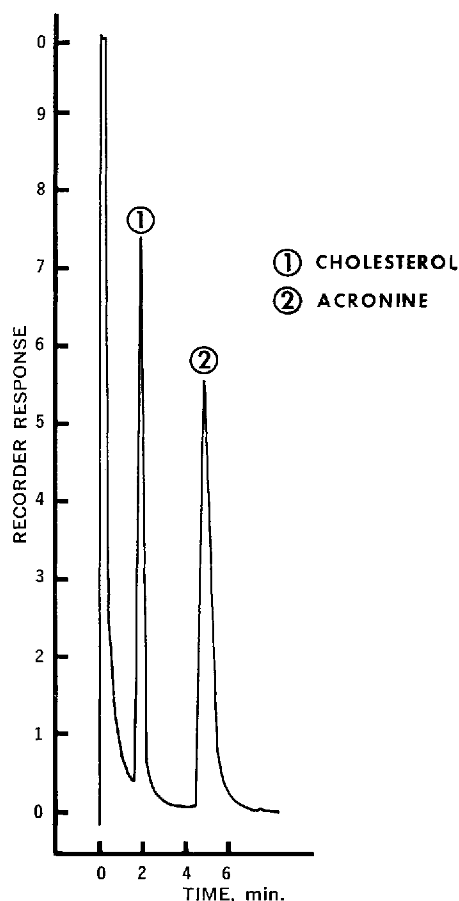


Figure 3—*Chromatogram of cholesterol/acronine mixture.*

Table III—Assay of Finished Capsules

Content Wt., mg.	Excipient	Acronine, mg./Capsule	N	Precision (RSD), %
170	Microcrystalline cellulose	24.9	5	±1.63
290	Silica gel	23.0	5	±1.17
300	Mg. stearate and starch	23.7	4	±2.77
300	Stearic acid and starch	24.8	5	±1.16
300	Starch	25.3	5	±1.74
380	Talc	25.5	5	±1.59

dard solution. The peak height ratios were calculated and used to determine the precision of the method. These calculations gave a relative standard deviation of ±0.99%.

Accurately weighed amounts of acronine and talc were mixed in four different proportions and the simulated products were assayed for acronine by the described GLC method. The results of these assays are presented in Table I. The applicability of the method was further tested by assaying admixtures of acronine and seven different potential excipients. All samples were hand-mixed in a 1:1 ratio, and the results of these assays are shown in Table II, along with a comparative lot of acronine raw material. The recovery of acronine from all excipients except dipotassium phosphate was excellent. The use of dipotassium phosphate as an excipient resulted in consistently low assay results, indicating an apparent binding effect with the acronine.

The final evaluation of the method was made on six lots of acronine capsules prepared in our pharmaceutical pilot plant. Each lot of capsules contained the same amount of acronine (25 mg.) with varying amounts of different excipients. The results are reported in Table III and are based upon composite assays using 10 capsules.

Acronine is an alkaloidal compound with newly discovered antitumor activity. A simple, rapid, accurate, and precise method of determining acronine has been described. The method is suitable for the determination of acronine either as the raw material or in admixtures of pharmaceutical excipients. In general, extractions are clean and simple, and the assay can be performed with a minimum of familiarization with the method. It is believed that this method will find use in the assay of final pharmaceutical formulations and in further studies involving acronine.

REFERENCES

(1) G. H. Svoboda, G. A. Poore, P. J. Simpson, and G. B. Boder, *J. Pharm. Sci.*, **55**, 758(1966).

(2) F. N. Lahey and W. C. Thomas, *Australian J. Sci. Res.*, **2A,** 423(1949).

(3) J. R. Beck, R. N. Booher, A. C. Brown, R. Kwok, and A. Pohland, *J. Am. Chem. Soc.*, **89,** 3934(1967).

(4) R. D. Brown and F. N. Lahey, *Australian J. Sci. Res.*, **3A,** 593(1950).

(5) J. G. Theivagt, V. E. Papendick, and D. C. Wimer, *Anal. Chem.*, **39,** 191R(1967).

(6) E. Brochmann-Hanssen and A. B. Svendsen, *J. Pharm. Sci.*, **51,** 1095(1962).

(7) S. Takagi, T. Katagi, and K. Takebayashi, *Chem. Pharm. Bull. (Tokyo)*, **16,** 1116(1968).

(8) *Ibid.*, **16,** 1121(1968).

ACKNOWLEDGMENT AND ADDRESSES

Received June 30, 1969 from the *Analytical Development Laboratories, Eli Lilly and Company, Indianapolis, IN 46206*

Accepted for publication August 6, 1969.

The authors wish to thank Mr. S. H. Cox for his preliminary investigations involving the UV and fluorometric assays and Mr. H. F. Hugar for his handling of the routine assays.

Studies with Ion-Exchange Resins on Cinchona Alkaloids III: Exchange Rates

S. S. KANHERE, A. H. VYAS, C. V. BHAT, B. R. KAMATH, R. S. SHAH, and S. L. BAFNA

Abstract ☐ The exchange rates of four cinchona alkaloid sulfates with styrene divinylbenzene copolymer-based sulfonic acid cation-exchange resins of different degree of crosslinking and particle size have been studied and the results are discussed.

Keyphrases ☐ Cinchona alkaloids, exchange rates—sulfonic acid cation exchange resins ☐ Sulfonic acid cation exchange resins—degree of crosslinking and particle size.

Earlier (1) the effect of the ionic form of the sulfonic acid cation-exchange resins of different degree of crosslinking and of the added sulfuric acid on the equilibrium exchange of four cinchona alkaloid (quinine, quinidine, cinchonine, and cinchonidine) sulfates as well as the equilibrium uptake of the four cinchona alkaloid bases by the hydrogen form of the same resins from six aliphatic alcohols had been studied. This paper includes the study of the exchange rates of the four cinchona alkaloid sulfates with sulfonic acid cation-exchange resins of different degree of crosslinking and particle size at 35 and 45°.

EXPERIMENTAL

Resins[1] (1–3)—Styrene divinylbenzene copolymer-based sulfonic acid cation-exchange resins of degree of crosslinking (percent nominal divinylbenzene content), X = 1, 2, and 4 are referred to as Resins $X1$, $X2$, and $X4$. Each resin was washed, conditioned, regenerated into hydrogen form, air dried, sieved, and stored. A large number ($\simeq$50 to 60) of air-dry particles of each resin was measured for the particle diameter of each fraction of the air-dry resin using a microscope with a mechanical stage at room temperature ($\simeq$30°) and the values for the average particle diameter, a, thus obtained were; $X1$, 0.215 mm.; $X2$, 0.215 mm.; $X4$, 0.66 mm., 0.43 mm., and 0.215 mm. The moisture content and the capacity of the air-dry resins were estimated (1, 4). The percentage moisture and the

[1] Dowex 50W, Dow Chemical Co., Midland, Mich.