High-Performance Liquid Chromatographic and TLC Determinations of Desacetylvinblastine Amide (Vindesine) and Its Monosulfate Salt

R. L. HUSSEY [x] **and W. M. NEWLON**

Received May 18, 1977, from the *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46206.* Accepted for publication November 16, 1977.

Abstract □ High-performance liquid chromatographic and TLC methods were developed for the determination of desacetylvinblastine amide base (vindesine), a chemically modified *Catharanthus* alkaloid presently in clinical evaluation. Both methods permit the detection of 6,7-dihydrovindesine and vindesine N_b'-oxide, the principal by-products in the chemical conversion of vinblastine to vindesine. The methods are applicable to both the sulfate salts and the free bases of the chemical substances described.

Keyphrases □ Vindesine—high-performance liquid chromatographic and TLC analyses, bulk drug □ High-performance liquid chromatography—analysis, vindesine in bulk drug □ TLC—analysis, vindesine in bulk drug □ Alkaloids, *Catharanthus*—vindesine, high-performance liquid chromatographic and TLC analyses, bulk drug

Chemical modification of vinblastine, a clinically effective alkaloid of the Apocynaceae family (1), genus *Catharanthus,* provided the agent desacetylvinblastine amide (vindesine) sulfate (2, 3), which has been in clinical evaluation for antitumor activity since 1975. High-performance liquid chromatographic (HPLC) and TLC methods were developed to determine vindesine free base (I) and/or its monosulfate salt in the presence of two principal by-products (3) obtained during semisynthesis. The first by-product, 6,7-dihydrovindesine (II), is generated in the Raney nickel hydrogenolysis step of the vindesine preparation; the second, vindesine N_b'-oxide (III), is the product of air oxidation of vindesine (Scheme I).

An HPLC method was developed to provide a quantitative assay of I in the final stages of raw material purification. TLC (4, 5) was used in a supplementary, semiquantitative manner.

EXPERIMENTAL

Equipment—The liquid chromatograph[1] was equipped with a UV spectrometer[2] set at 280 nm and a 3.9 × 30-cm stainless steel column containing chemically bonded octadecylsilane on silica[3]. The detector was set with a sensitivity of 1.00 absorbance unit full scale. A strip recorder allowing variable input[4] was employed. Data acquisition and computation were handled by on-line computation[5].

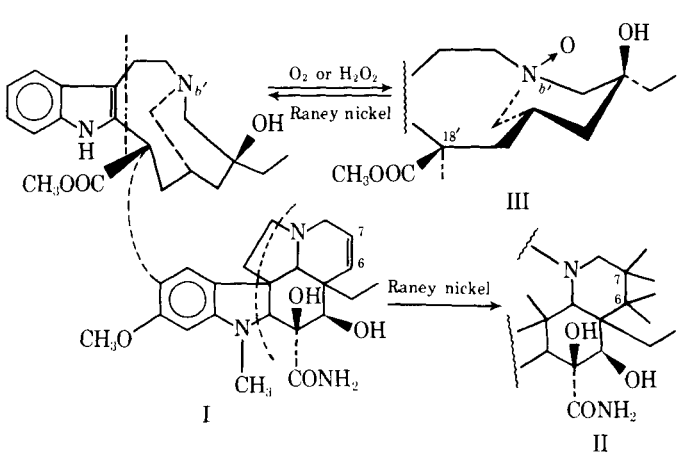
The scanning UV–visible TLC reflectance densitometer[6] was set at 280 nm in the ratio density mode. Commercially available plates[7] of silica gel F-254 and nonfluorescent silica were both employed as alternative detection systems for II and III. The TLC plate was used for making a photographic record, and the nonfluorescent plate was used for semiquantitation in conjunction with the densitometer.

Reagents—Two HPLC solvent systems were employed. The primary system was methanol–water–diethylamine (1000:600:3). The flow rate was adjusted to 1.5 ml/min; the elution volumes from solvent breakthrough were 3.0, 10.9, 13.5, and 20.1 ml for III, I, II, and the internal standard phenanthrene[8], respectively. For the quantitation of I in high levels of II, methanol–water–diethylamine (835:600:3) was employed with a flow rate of 1.5 ml/min. For the latter solvent system, the elution volumes from solvent breakthrough were 3.1, 20.2, 25.6, and 32.8 ml, respectively. In both cases, the column dead volume was 3.2 ml.

Ether–methanol–40% aqueous methylamine (20:4:1) was employed as the TLC solvent system. The average R_f values found for III, I, and II were 0.36, 0.57, and 0.74, respectively.

HPLC Determination of I Raw Material Base and/or Monosulfate—Three replicates of each standard and sample were used for each assay result. The number of replicates chosen for the assay assured that each result would be within ±2% of the true value at the 95% confidence level. Each accurately weighed sample (5 mg) was quantitatively transferred to a 5-ml glass-stoppered flask. Three milliliters of the internal standard was added [45 mg of phenanthrene (IV), reagent grade, prepared in 100 ml of methanol]. To aid the otherwise slow dissolution, 10 sec of sonic vibration was employed.

Aliquots of 20 μl of each sample or standard solution were injected onto the column of the liquid chromatograph. The peak areas (PA) of I and the internal standard were measured by on-line computation. The ratio of each sample (or standard) area to the internal standard was then obtained: $(PA_{sample}/PA_{internal\ standard}) = R$. A five-point calibration was periodically obtained (0–10 mg/3 ml) to verify linearity and passage of concentration through the origin of response. Routinely, however, the



Scheme I

[1] Waters model 6000A.
[2] Varian Vari-Chrom VUV-10.
[3] Waters μBondapak C_{18} No. 27324.
[4] Fisher Recordall series 5000.
[5] Dual Hewlett-Packard 2100 RTE System.
[6] Schoeffel SD3000.
[7] E. Merck.
[8] Aldrich Chemical.

0022-3549/78/0900-1319$01.00/0

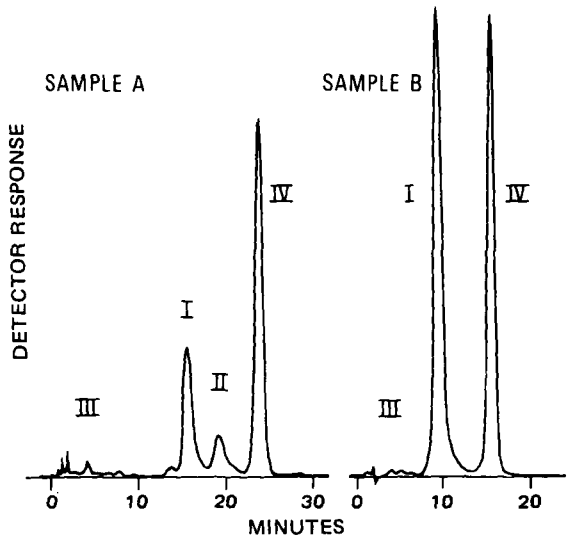


Figure 1—*HPLC separations. Key: Sample A, a sample before prepurification run in the secondary solvent system; and Sample B, a finished I sulfate run in the primary solvent system. The elution times shown are measured from the time of injection.*

purity of each sample was obtained in the following manner:

$$\frac{\text{average sample ratio, } R_{\text{sample}}}{\text{average standard ratio, } R_{\text{standard}}} \frac{\text{mg standard}}{\text{mg sample}} \times \% \text{ purity of standard} = \% \text{ purity of sample} \quad \text{(Eq. 1)}$$

TLC of I Raw Material Base and/or Monosulfate—The I base and/or monosulfate was prepared at a concentration of 20 mg/ml in methanol. This solution was used in two ways. A precoated F-254 plate was spotted with 10 μl of the methanol solution, and a nonfluorescent plate was spotted with 2 μl. Both plates were developed in the aforementioned developing solvent. An equilibration time of just 5 min was employed in a lined tank.

Following development, a photograph was taken of the fluorescent plate, and a TLC reflectance absorbance scan (ratio mode, 280 nm) was taken of the nonfluorescent plate. The linearity of response to concentration was checked periodically from 0 to 20 μg. In most cases, however, the scanned plate was judged on total peak area. In other cases, II was spotted alongside the sample at an equivalent of 5% (1 mg/ml), and a direct semiquantitation was made on a linear response basis.

RESULTS AND DISCUSSION

Both the HPLC and TLC procedures showed the presence of II and III. The HPLC method was linear in response for both the monosulfate salt and the base from 0 to 2.8 mg/ml. For the monosulfate salt, data based on 25 separately weighed samples gave a relative standard deviation of 0.89%. For the free base, the relative standard deviation was 0.99%, also based on 25 separately weighed samples. Methanol solutions of both the sulfate and base proved stable for 18 hr, thus allowing easy automation.

Typical liquid chromatograms are shown in Fig. 1. By-product II was present only in crude vindesine preparations prior to prepurification by preparative high-pressure silica gel chromatography. The secondary solvent system described in *Experimental* was used for the crude vindesine preparations but required a longer assay time. The primary solvent system would have clearly detected II, but the separation would not have been as great. This latter solvent system is of advantage for purified preparations where II is absent since it requires a shorter assay time. The small minor peaks following III were due to compounds of undetermined structure. Phenanthrene (IV), the internal standard for the HPLC analysis, was not used in the TLC semiquantitation.

For very pure finished formulations, 0.5 *M* potassium phosphate buffer can be used instead of diethylamine in the mobile solvent for HPLC. Some additional methanol must be added to the mobile system to reduce the tailing caused by the lowered pH, but the octadecylsilane column packing then has a longer life. With diethylamine, a column life of 3 weeks has been obtained; with the phosphate buffer, a life of several months is obtained.

The TLC procedure was useful principally as a fast, corroboratory check. Thin-layer densitometry still does not have the accuracy or precision of HPLC analysis.

REFERENCES

(1) I. S. Johnson, J. G. Armstrong, M. Gorman, and J. P. Burnett, Jr., *Cancer Res.*, **23**, 1390 (1963).

(2) G. J. Cullinan, K. Gerzon, G. A. Poore, and M. J. Sweeney, XIth International Cancer Congress, Abstract Panel 14, paper 137, Florence, Italy, Oct. 23, 1975.

(3) C. J. Barnett, G. J. Cullinan, K. Gerzon, R. C. Hoying, W. E. Jones, W. M. Newlon, G. A. Poore, R. L. Robison, M. J. Sweeney, G. C. Todd, R. W. Dyke, and R. L. Nelson, *J. Med. Chem.*, **21**, 88 (1978).

(4) I. M. Jakovljevic, L. D. Seay, and R. W. Shaffer, *J. Pharm. Sci.*, **53**, 553 (1964).

(5) N. J. Cone, R. Miller, and N. Neuss, *ibid.*, **52**, 688 (1963).

ACKNOWLEDGMENTS

The authors thank Dr. C. J. Barnett for preparation of purified 6,7-dihydrovindesine and Dr. K. Gerzon for the preparation of vindesine N_b'-oxide. Thanks also are given to Dr. E. C. Rickard and Dr. J. Zynger for the use of their statistical evaluation procedure, to Mr. J. J. Osborne for assistance in performing the assays described, and to Dr. K. Gerzon for review of this article.