Separation and Quantitation of Possible Degradation Products of Procarbazine Hydrochloride in Its Dosage Form

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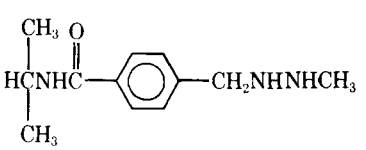
Abstract □ A stability-indicating assay for the degradation products of procarbazine hydrochloride was developed using high-pressure liquid chromatography. The method uses a buffered methanol–water mobile phase on a reversed-phase column. Concentrations of degradation products as low as 0.04 mg/ml, 0.02% degradation, can be quantitated using an internal standard of cinnamyl alcohol. The typical range for degradation products in procarbazine capsules is 0.1–0.5% after as long as 4.5 years.

Keyphrases □ Procarbazine hydrochloride degradation products—high-pressure liquid chromatographic analysis in pharmaceutical formulations □ Degradation products—of procarbazine hydrochloride, high-pressure liquid chromatographic analysis in pharmaceutical formulations □ High-pressure liquid chromatography—analyses, degradation products of procarbazine hydrochloride in pharmaceutical formulations □ Antineoplastic agents—procarbazine hydrochloride, high-pressure liquid chromatographic analysis of degradation products in pharmaceutical formulations

Procarbazine, *N*-(1-methylethyl)-4-[(2-methylhydrazino)methyl]benzamide (I), is an antineoplastic agent used to treat Hodgkin's disease and other diseases involving malignant tumors (1). Procarbazine is capable of undergoing oxidation by atmospheric oxygen or degradation in the presence of moisture to give *N*-(1-methylethyl)-4-[(2-methylazo)methyl]benzamide (II), *N*-(1-methylethyl)-4-[(2-methylamino)hydrazo]benzamide (III), and, to a much lesser extent, *N*-isopropyl-4-formylbenzamide (IV) (2–5).

Procarbazine can be assayed using UV spectroscopy (2, 5), polarography (2, 6), coulometry (2, 7, 8), or direct titration as the hydrochloride salt (2, 5). These methods are applicable to both the pure compound and the dosage forms. TLC on silica gel has been used to separate procarbazine from II and III and to estimate the amount of II and III present (2). However, both oxidation of procarbazine and isomerization between II and III can occur during chromatography, severely lowering the accuracy and precision of this method.

This report presents a method for accurately quantitating small amounts of II–IV in procarbazine hydrochloride capsules using high-pressure liquid chromatography (HPLC).



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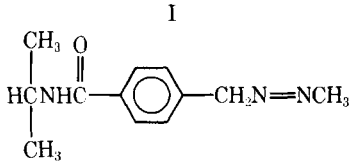

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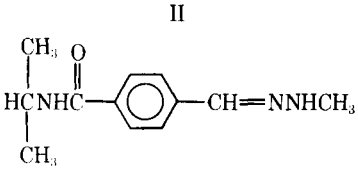

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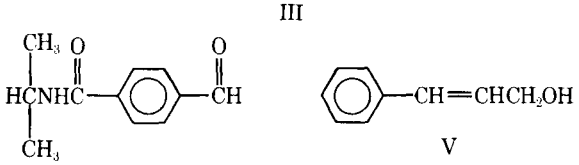

IV V

EXPERIMENTAL

Procarbazine hydrochloride and its degradation products, II–IV, were all reference standard quality[1]. Cinnamyl alcohol[2] (V) and monobasic ammonium phosphate[3] were reagent grade. Certified ACS metol[4] was used to prepare the mobile phase along with distilled, deionized, filtered water. The mobile phase, used for dissolving the drugs in pure form and extracting them from the capsule material as well as for eluting the samples from the column, consisted of 56% water containing the ammonium phosphate and 44% methanol. The final concentration of ammonium phosphate was 0.05 *M* in the mixture, and the pH was 5.5. No pH adjustment was needed after making up the mobile phase.

Instrumentation—The constant-pressure liquid chromatograph[5] was equipped with a 254-nm UV detector and a septum injector. The output signal was measured on a dual-pen recorder[6] with integrator. A 25-cm Partisil PXS 10/25 ODS-2 reversed-phase column[7] with a C_{18}-type bonded phase on silica particles, 10 μm in diameter, was used.

Procedure—The cinnamyl alcohol internal standard was weighed and dissolved in the mobile phase at about 1 mg/ml. This solution, diluted 1:10 with the mobile phase for a final concentration of about 0.1 mg/ml, was used to dissolve the standards and to extract the degradation products from the capsule mixture. Standards were weighed, dissolved in the mobile phase with the internal standard solution, and immediately injected.

The dosage form was assayed by accurately weighing the contents of five capsules[8] of procarbazine hydrochloride (~1.7 g containing approximately 250 mg of procarbazine hydrochloride) into a centrifuge tube. A 10-ml aliquot of the internal standard in the mobile phase was added, and the centrifuge tube was shaken for 15 sec. A small amount of this solution was immediately filtered through a 0.45-μm filter[9] and immediately injected. About 4-μl quantities of standards and samples were injected on the column.

Amber glassware was used throughout this procedure because these compounds are reported to be light sensitive (2). Samples must be chromatographed immediately after dissolution because procarbazine hydrochloride can degrade in these solutions.

RESULTS AND DISCUSSION

Representative chromatograms are shown in Fig. 1. The chromatographic parameters calculated from these chromatograms are listed in

[1] Hoffmann-La Roche, Nutley, NJ 07110.
[2] Eastman Kodak Co., Rochester, NY 14650.
[3] Mallinckrodt Inc., St. Louis, MO 63147.
[4] Fisher Scientific, Fair Lawn, NJ 07410.
[5] Model 848, Instrument Products Division, DuPont Inc., Wilmington, DE 19898.
[6] Model 282, Linear Instruments Corp., Irving, CA 92714.
[7] Whatman Inc., Clifton, NJ 07014.
[8] Matulane, Hoffmann-La Roche Inc., Nutley, NJ 07110.
[9] Millipore Corp., Bedford, MA 01730.

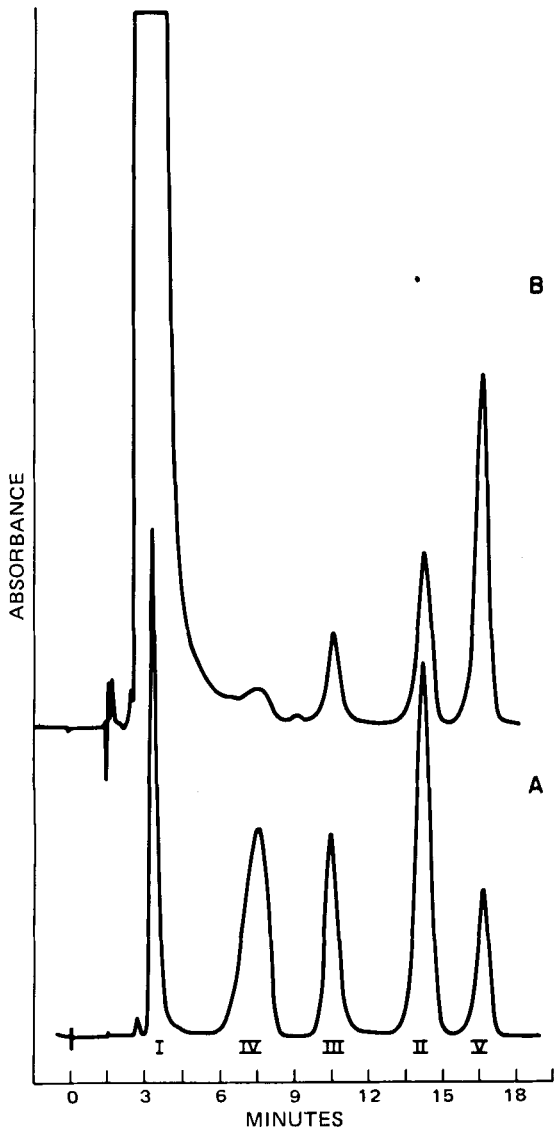

Figure 1—*Chromatograms run with a flow rate of 1.5 ml/min at 1900 psi and 0.04 absorbance full scale. Key: A, set of standards, I = 1.58 mg/ml, II = 0.43 mg/ml, III = 0.81 mg/ml, IV = 1.21 mg/ml, and V = 0.087 mg/ml; and B, typical extraction of procarbazine and degradation products from capsules using the procedure given in the text.*

Table I. All peaks are Gaussian shaped, except for the peak of IV. This peak shows both fronting and excess broadening, resulting in large values of height equivalent to a theoretical plate (HETP).

The method yields baseline separation of procarbazine, its degradation products, and the internal standard. Additionally, since procarbazine is eluted from the column firs., overloading the column with procarbazine should not significantly affect either the retention volumes or resolution of subsequent peaks.

Standard analytical curves were determined for I–IV using their peak

Table I—Chromatographic Characteristics of Procarbazine, Degradation Products, and Cinnamyl Alcohol

Compound	Retention Time, min	Retention Volume, ml[a]	Net Retention Volume, ml[b]	Number of Plates[c]	HETP
I	3.31	4.95	2.70	670	0.37
II	14.21	21.31	19.06	2810	0.089
III	10.61	15.91	13.66	1120	0.22
IV	7.61	11.41	9.16	240	1.04
V	16.85	25.27	23.02	5390	0.046

[a] The flow rate is 1.5 ml/min. [b] The void volume of the column is 2.25 ml. [c] Number of plates was calculated from the equation $N = 5.54$ (retention time/width at half maximum)^2 (9).

Table II—Slopes, Intercepts, and Correlation Coefficients for Procarbazine and the Degradation Products as Pure Compounds and in Capsule Material[a]

Compound	Matrix[b]	Slope, mg/ml[c]	Intercept, mg/ml[c,d]	r
I	None	0.36 (0.01)	0 (0.01)	99.99
II	None	0.43 (0.03)	0 (0.01)	99.88
II	Capsule	0.40 (0.03)	−0.11 (0.05)	99.88
III	None	0.48 (0.02)	0 (0.01)	99.99
III	Capsule	0.52 (0.03)	−0.21 (0.04)	99.98
IV	None	0.24 (0.01)	0 (0.01)	99.97
IV	Capsule	0.22 (0.02)	0.02 (0.02)	99.96

[a] Five data points were used to determine each curve. [b] Analytical curves were determined of either pure compounds dissolved in the mobile phase or compounds added to 1.7 g of capsule material in a standard addition method. These standard addition samples were extracted as described previously. [c] Numbers in parentheses are 95% confidence limits for the slope and intercept. [d] Samples in capsule material have some degradation products in them initially causing the negative intercepts.

heights (PH). The slopes, intercepts, and correlation coefficients are listed in Table II. The values in parentheses listed next to each slope and intercept are the 95% confidence limits. These parameters were calculated using:

$$\text{mg/ml} = \text{slope} \times \frac{\text{PH (sample)}}{\text{PH (internal standard)}} + \text{intercept} \quad \text{(Eq. 1)}$$

The use of an internal standard, cinnamyl alcohol, compensates for any variability in the amount of sample injected. Typically, about 4 μl of sample is injected. Therefore, the amount of sample injected ranges from 1.4 μg to 200 ng. The limit of detection using these chromatographic conditions, as estimated by a signal to noise ratio of 2:1, is approximately 4 μg/ml or 20 ng injected. The limits of quantitation, estimated as 10 times the limit of detection, is 40 μg/ml or 200 mg injected (10). The sensitivity under these conditions is about 0.7 mg/ml for full-scale deflection but can be increased by using less internal standard and raising the sensitivity of the detector from 0.04 to 0.01 absorbance unit. No isomerization between II and III was observed if the standards were chromatographed immediately upon dissolution.

Small amounts of degradation products were added to a sample of procarbazine hydrochloride in capsule material to test whether the extraction was complete and whether the presence of large amounts of procarbazine hydrochloride would interfere with the analysis of the degradation products. The contents of 30 procarbazine hydrochloride capsules were pooled, and 1.7 g of capsule material was added to each centrifuge tube. Small amounts of II–IV were weighed and also added to these tubes. The samples were then extracted as described previously and chromatographed. The analytical curves were determined; the slopes, intercepts, and correlation coefficients, along with their 95% confidence limits, are listed in Table II.

The range of values for the slope of each compound extracted from the capsule matrix corresponds very well with that for the pure compound, confirming that all degradation products are extracted from the capsule matrix into the mobile phase. Complete extraction of these compounds was demonstrated further by extracting the contents of five capsules, filtering off the mobile phase, and reextracting the capsule matrix with the mobile phase. No degradation products were found chromatographically in the second extraction solution. The negative intercepts of the analytical curves for II and III were due to the presence of these

Table III—Typical Values for the Amount of Degradation Products Found in Procarbazine Capsules[a]

Sample[b]	Shelflife, years[c]	Degradation to II[d], %	Degradation to III[d], %	Degradation to IV[d], %
A	4.5	0.29	0.26	0.044
B	4.0	0.31	0.36	0.012
C	4.0	0.31	0.48	0.013
D	2.5	0.29	0.49	0.013
E	1.4	0.25	0.48	0.014
F	0.8	0.27	0.45	0.014

[a] All values are the average of three determinations. [b] A–F represent different lots of material. [c] Number of years since manufacture. All samples were held at 25° over this time. [d] Percent degradation calculated using the equation:

$$\%\ \text{degradation} = \frac{\text{degradation product (mg/ml)}}{\text{25 mg of procarbazine/ml}} \times 100$$

Five capsules, each containing 50 mg of procarbazine (250 mg), were extracted into 10 ml of mobile phase.

degradation products in the capsule mixture itself. Therefore, the extraction with mobile phase removes all degradation products from the capsule matrix.

Representative samples of procarbazine hydrochloride capsules manufactured at various times were assayed for II–IV. A typical chromatogram is shown in Fig. 1B; the values for the percent degradation product found, based on the initial amount of procarbazine being 50 mg/capsule, are given in Table III. These values are all significantly below 0.5% degradation and confirm that II and III are the main degradation products while IV is only seen in a trace amount.

In previous accelerated degradation studies, II and III were found at about the 1% level after 1 month at 55°[10]. Only a very small amount of IV was found at this temperature. Sample A (Table III) had a smaller amount of III and a larger amount of IV than any other sample. This result could, in part, be due to the oxidation of III to IV as described previously (2). Another potential degradation product, *N*-isopropyltoluamide, having a retention volume of 32 ml, was not found in any sample (2–5).

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In Vitro Evidence for Ipecac Inactivation by Activated Charcoal

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Received February 9, 1977, from the *Chemical Engineering Department, Clarkson College of Technology, Potsdam, NY 13676.* Accepted for publication June 15, 1977.

Abstract □ The *in vitro* adsorption of the alkaloid emetine, a primary constituent of ipecac, on activated charcoal was studied. The results support the supposition that syrup of ipecac should not be given to counteract poisonings if activated charcoal is also to be administered.

Keyphrases □ Emetine—*in vitro* adsorption on activated charcoal □ Ipecac—*in vitro* adsorption of constituent emetine on activated charcoal □ Adsorption, *in vitro*—emetine on activated charcoal □ Charcoal, activated—*in vitro* adsorption of emetine □ Alkaloids—emetine, *in vitro* adsorption on activated charcoal

Statements occasionally appear in the literature indicating that syrup of ipecac and activated charcoal should not be given together to counteract poisonings. The reasoning is the hypothesis that the alkaloids emetine and cephaeline, which constitute more than 90% of the alkaloids of ipecac, would adsorb to the charcoal and thereby fail to act as desired, but no proof has yet been presented.

The only reference to ipecac adsorption to activated charcoal appears to be that in an 1846 paper by Garrod, which was quoted by Holt and Holz (1). Studies (2–5) on other alkaloids such as strychnine, nicotine, atropine, morphine, yohimbine, veratrine, and aconitine have clearly established a substantial affinity between alkaloids and activated charcoal. However, the extent of binding varies widely, not only with the alkaloid involved but with pH. Since alkaloids are basic, the extent of adsorption is often much less at pH values corresponding to gastric conditions than it is at a neutral pH. This result is due to the well-recognized fact (2) that ionized forms of solutes usually bind much less strongly than their nonionized forms.

In light of the lack of any data concerning the adsorption of alkaloids of ipecac on charcoal, a study of emetine adsorption was performed. Only emetine was studied since it comprises more than half of the total alkaloid content of ipecac (6). Moreover, cephaeline ($C_{28}H_{38}N_2O_4$) differs from emetine ($C_{29}H_{40}N_2O_4$) only by the lack of one methyl group, so it would be expected to behave similarly to emetine in terms of adsorption.

EXPERIMENTAL

Simulated gastric fluid USP (pepsin omitted), pH 1.2, was used for all solutions. Emetine hydrochloride[1] was dissolved to the extent of either 0.2 or 0.4 g/liter in the gastric fluid. Powdered activated charcoal[2] was washed twice with 6 *N* HCl and six times with distilled water and then was dried at 120° for 24 hr.

Various amounts of the charcoal were mixed with 10-ml aliquots of the emetine solutions in capped glass vials and shaken for 6 hr or longer. After most of the charcoal settled, the supernate was filtered through 0.45-μm pore-size microporous membranes[3]. The clear filtrate was analyzed by UV spectrophotometry at 253.6 nm. Samples of emetine solutions of known concentrations, prepared by dilution with the gastric fluid, were treated in the same manner, except that charcoal was omitted.

RESULTS

Figure 1 shows the adsorption isotherm obtained, as represented by a plot of Q^* (grams of emetine adsorbed per gram of charcoal) *versus* C_f (concentration of emetine in grams per liter in the surrounding fluid at equilibrium). The data could be fit well (by eye) with the Freundlich equation $Q^* = 0.249C_f^{0.182}$ in the range from $0 < C_f < 0.03$ and by another Freundlich expression, $Q^* = 0.177C_f^{0.0793}$, in the range of about $0.06 < C_f < 0.400$.

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Norit A, American Norit Co., Jacksonville, Fla.
[3] Amicon Corp., Lexington, Mass.