Table IV—Effect of Phenylalanine and *N*-Acetylphenylalanine on the Inhibition [a] (Percent) by Trifluoroacetyl-*p*-fluoro-DL-phenylalanine

Reversing Agent [b]	*N*-Trifluoroacetyl-*p*-fluoro-DL-phenylalanine			
	0	1.43 m*M*	3.58 m*M*	7.16 m*M*
None	0	6	21	40
L-Phenylalanine (6.05 m*M*)	+4	9	22	41
N-Acetyl-L-phenylalanine (4.82 m*M*)	8	12	25	47

[a] Maximum growth of controls containing no inhibitor was 141 Klett units. [b] Final concentration of 1 mg/ml. Figures in parentheses are molar equivalents.

of these amino acids and amino acid analogs is independent of the activity of the free parent amino acid. While free *p*-fluoro-DL-phenylalanine and β-3-thienyl-DL-alanine showed considerable activity, their corresponding trifluoroacetyl derivatives did not show as much activity; *o*-fluro-DL-phenylalanine, *m*-fluoro-DL-phenylalanine, and β-2-thienyl-DL-alanine, which showed no activity as free amino acids, showed considerable activity as the trifluoroacetyl derivatives (*cf.*, 3).

It is difficult to propose any mechanism of such inhibition. Interference with phenylalanine metabolism may be involved, since all trifluoroacetyl compounds showing activity are analogs of phenylalanine.

A study was made to note if the inhibition could be overcome or reversed by the addition of suitable quantities of the natural metabolite. In a series of experiments, the active trifluoroacetyl derivative was challenged by various levels of a natural metabolite and of a noninhibitory acyl derivative. No significant degree of reversal was observed with either phenylalanine or acetylphenylalanine (Table IV). This observation, however, does not rule out the involvement of phenylalanine because the area of inhibitory action could be beyond the point of participation of these compounds as free amino acids or as simple acyl derivatives in its metabolic pathway. More sophisticated studies are required to learn the mechanism of inhibition.

In view of the involvement of the trifluoroacetyl derivatives of only phenylalanine-related compounds in this screening system, consideration of these compounds as potential inhibitors of melanomas, wherein the metabolism of phenylalanine is believed to be intimately associated, may be of value. These compounds are now being prepared in larger quantities in preparation for testing in mammalian systems, especially in melanomas.

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Stability-Indicating Assay for Vidarabine

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Received June 12, 1978, from the *Pharmaceutical Research Division, Warner-Lambert/Parke-Davis, Detroit, MI 48232.* Accepted for publication September 29, 1978.

Abstract □ A physicochemical procedure for the analysis of vidarabine in aqueous parenteral formulations was needed to assure potency and to define stability. Concurrent with the development of this method, its decomposition products and route in aqueous solution were determined. A quantitative procedure was developed to determine intact drug in the presence of decomposition products, and the results obtained were validated by microbial assay. Spectral (UV and polarimetric) and TLC evidence indicated that, in aqueous solution, hydrolysis without racemization occurs, yielding adenine and arabinose. The sensitivity of the method to decomposition is improved by ion-exchange separation of adenine and drug before UV measurement. Analysis of partially decomposed solutions of the drug by both ion-exchange and microbiological methods gave comparable results.

Keyphrases □ Vidarabine—stability-indicating assay, analysis in aqueous parenteral formulations, behavior in aqueous solutions, analysis in the presence of decomposition products □ Parenterals—analysis of vidarabine stability □ Stability—vidarabine in aqueous parenteral formulations

Vidarabine (9-β-D-arabinofuranosyladenine, Vira-A or Ara-A) is an antiviral agent with marked activity *in vitro* and *in vivo* against a wide spectra of DNA viruses (1–8). It also inhibits DNA synthesis in bacteria (9), in mouse fibroblasts in cell culture (10), and in experimental tumors in mice (11, 12).

Since the dosage form being developed is essentially aqueous, information pertinent to the drug's chemistry in an aqueous system is of value for predicting characteristics of the finished formulation and designing a stability-indicating assay. This paper presents a physicochemical procedure for the analysis of vidarabine in aqueous parenteral suspension needed to assure potency and define stability.

EXPERIMENTAL

Chemicals and Reagents—All chemicals used in the preparation of 0.2 *M* borate buffer and 0.1 *M* phosphate buffer were reagent grade and were used without further purification. The hydrochloric acid solutions were prepared from a prestandardized volumetric solution[1]. Vidarabine was an experimental batch[2]; adenine[3] was obtained from a commercial source.

Instrumentation—A direct-reading digital pH meter[4] was used. UV spectra were obtained using a recording UV spectrophotometer[5]. The specific rotation was determined using a polarimeter[6].

Degradation of Vidarabine in Acidic Solution—A 1% solution of vidarabine was prepared in 0.1 *N* HCl, and 2-ml aliquots were sealed in glass ampuls. The ampuls were heated at 100°, withdrawn periodically, and cooled to room temperature; then the specific rotation was measured

[1] Acculute, Anachemia Chemicals.
[2] Rx X lot 41020, Warner-Lambert/Parke-Davis, Detroit, MI 48232.
[3] Lot E-1528, Mann Research Laboratories, New York, N.Y.
[4] Sargent, model DR.
[5] Cary model 11 or 14.
[6] Perkin-Elmer model 141.

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Table I—Observed Specific Rotation as a Function of Time

Hours	$[\alpha]^{25°}$ (1%, 0.1 *N* HCl) 589 nm (sodium D)	436 nm	365[a] nm
0	+5.8	+6.6	−2.3
0.5	−49.7	−96.2	−146.3
1	−54.0	−104.9	−159.0
2	−55.2	−107.3	−162.2
4	−55.2	−107.6	−162.2
Expected rotation[b]	−55.1	−106.0	−160.0

[a] It is advantageous to follow the degradation at 365 nm since the specific rotation of arabinose is greater at this wavelength than at 589 nm. [b] Based on rotation of equimolar solution of D-arabinose.

at 589, 436, and 365 nm. After the polarimetric determination, 1-ml aliquots were diluted 250-fold in 0.1 *N* NaOH (50 times and then five times) for UV determination at 278 and 260 nm. The remaining portions of the solutions were stored at 4° until the run was completed.

Aliquots of 20 μl were spotted sequentially on a 20 × 20-cm thin-layer plate coated with silica gel F-254[7]. The plate was developed in ethanol–acetic acid (98:2) and then viewed under short wavelength UV light (254 nm) for the detection of vidarabine and adenine. The percent of vidarabine remaining at any time during hydrolysis was calculated from the polarimetric data as follows:

$$\%\ \text{vidarabine remaining} = \left(1 - \frac{[\alpha]_t^{25} - [\alpha]_{\text{initial}}^{25}}{-\Delta[\alpha]_{\text{total}}^{25}}\right) \times 100 \quad \text{(Eq. 1)}$$

where $[\alpha]_{\text{initial}}^{25}$ and $[\alpha]_t^{25}$ are the specific rotations obtained initially and at time t at a specified wavelength, respectively, and $\Delta[\alpha]_{\text{total}}^{25}$ is the total change in specific rotation at time zero and at time infinity.

From the UV spectrophotometric data:

$$C_{\text{vidarabine}} = \left(\frac{A_{260} - A_{278} \times 1.291}{10.67}\right) \times 10^{-3} \quad \text{(Eq. 2)}$$

$$C_{\text{adenine}} = \left(\frac{A_{278} - A_{260} \times 0.237}{5.13}\right) \times 10^{-3} \quad \text{(Eq. 3)}$$

$$\%\ \text{vidarabine remaining} = \left(\frac{C_{\text{vidarabine}}}{C_{\text{vidarabine}} + C_{\text{adenine}}}\right) \times 100 \quad \text{(Eq. 4)}$$

where $C_{\text{vidarabine}}$ and C_{adenine} are the molar concentrations of vidarabine and adenine at any time during hydrolysis, and A_{260} and A_{278} are the absorbances at 260 and 278 nm, respectively.

Solubility Studies—An excess amount of vidarabine (or adenine), 30–50 mg, was placed in each of several equilibration vials (21 × 70 mm, 16 ml) containing 10 ml of water or 0.2 *M* borate buffer of a suitable pH.

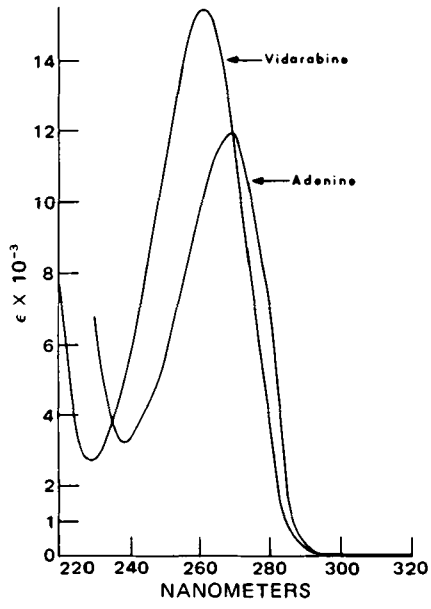


Figure 1—*UV spectra of vidarabine and adenine in 0.1* N *NaOH.*

[7] Merck.

Table II—Recovery Studies

Vidarabine Taken in 20-ml Sample, mg	Adenine Taken in 20-ml Sample, mg	Vidarabine Found, mg	Recovery, %
1.545	0	1.516	98.2
1.545	0	1.524	98.6
1.440	0	1.420	98.6
1.440	0	1.420	98.6
Average			98.5
1.545	0.76	1.499	97.0
1.545	0.76	1.496	96.9
1.413	0.75	1.372	97.2
1.484	1.00	1.473	99.4
Average			97.6

The vials were tumbled end-over-end in a constant-temperature water bath (20°) for at least 24 hr, and then the pH of the mixture was determined. The mixture was then centrifuged, and an aliquot of the clear supernate was diluted 25-fold in 0.1 *M* pH 7 phosphate buffer (further dilution was made if necessary).

The UV spectrum of the diluted solution was recorded using 1-cm silica cells and 0.1 *M* pH 7 phosphate buffer as blank. The solubility of vidarabine (or adenine) was calculated as follows:

$$\text{mg of vidarabine (or adenine)/ml} = \frac{A_{\lambda_{\max}}}{E_1^1} \times 10 \times D \quad \text{(Eq. 5)}$$

where $A_{\lambda_{\max}}$ is the absorbance at $\lambda_{\max}$ (260 nm for vidarabine and 261 nm for adenine), E_1^1 is the absorbance (540 for vidarabine and 981 for adenine) of a 1% solution at $\lambda_{\max}$, and D is the dilution factor.

Recovery Studies—A 250-ml volumetric flask was positioned beneath a glass chromatographic column (1 × 30 cm with polytef stopcock) previously packed with 2 g of 50–100-mesh Dowex 1-X4, chloride form (well soaked in water and washed with 100 ml of water prior to use). A 20-ml aliquot of sample solution in 0.2 *M* pH 10 borate buffer, either a synthetic mixture of vidarabine (75 μg/ml) and adenine (50 μg/ml) or a degraded solution of vidarabine, was charged onto the column. The column was subsequently eluted with 0.2 *M* pH 10 borate buffer until 250 ml was collected.

The UV spectrum of the solution was recorded using 5-cm silica cells

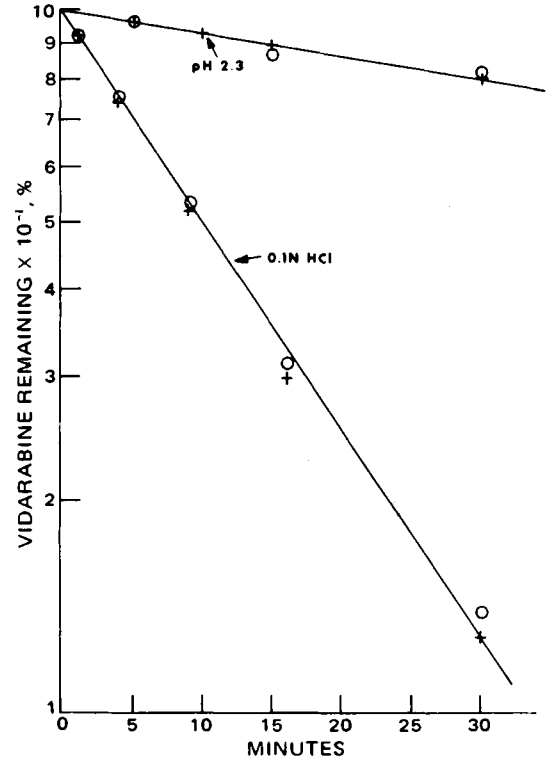


Figure 2—*Hydrolysis of vidarabine at 100°. Key: +, spectrophotometry; and O, polarimetry.*

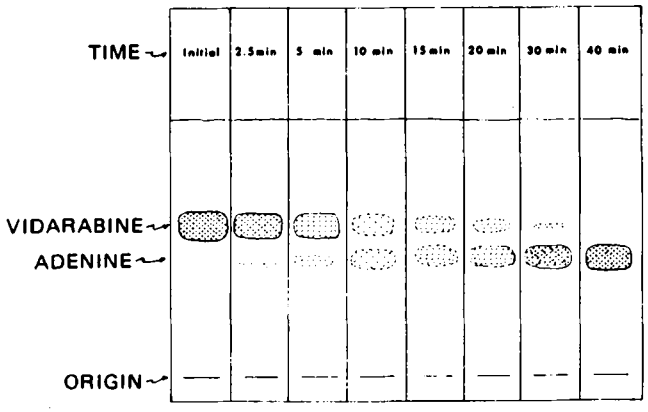


Figure 3—*TLC separation of vidarabine solution degraded in 0.1 N HCl at 100°. The plate was silica gel F-254, the developer was ethanol–acetic acid (98:2), and detection was by shortwave UV light (254 nm).*

and 0.2 *M*, pH 10 borate buffer as the blank. The content of vidarabine was calculated as follows:

$$\text{mg of vidarabine found} = \frac{A_{260}}{540 \times 5} \times 10 \times 250 \qquad \text{(Eq. 6)}$$

Formulation Analysis—*Column Preparation*—A slurry of 0.5 g QAE-Sephadex A-25, chloride form[8], with distilled water was prepared and allowed to hydrate overnight. The hydrated resin slurry was then poured into a chromatographic column[9]. After settling, the resin was converted into borate form by washing the column with 125 ml of 0.1 *M*, pH 10 borate buffer. A layer of solvent was always maintained above the resin bed.

Sample Preparation—Depending on the label claim, 2 ml of the homogeneous suspension was transferred into a volumetric flask with adequate size such that each milliliter contained 0.4 mg of vidarabine. The flask was half-filled with distilled water and heated on a steam bath with occasional shaking until the drug was completely dissolved. The solution was then cooled to room temperature and brought to the mark with distilled water. Twenty milliliters of the resulting solution was further diluted to 25 ml with 0.5 *M*, pH 10 borate buffer and mixed well.

Procedure—A 50-ml volumetric flask was positioned beneath the previously prepared ion-exchange resin column. Three milliliters of the alkaline sample dilution was charged onto the column and allowed to flow through the resin bed at a rate of approximately 1 ml/min. The column was eluted with 0.1 *M*, pH 10 borate buffer until 50 ml was collected.

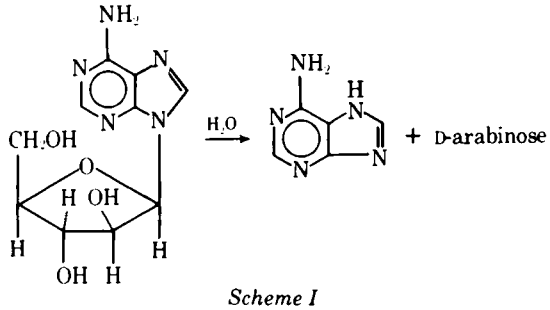
The UV spectrum of the eluate was determined over the 320–220-nm region using 1-cm silica cells and 0.1 *M*, pH 10 borate buffer as the blank. The content of vidarabine in the sample was calculated as follows:

$$\text{mg of vidarabine/ml} = \frac{A_{260}}{540} \times 10 \times \frac{25}{20} \times \frac{50}{3} \times \frac{V}{2} \qquad \text{(Eq. 7)}$$

where V is the volume (milliliters) of the volumetric flask used for the sample preparation.

RESULTS AND DISCUSSION

Past work showed that vidarabine could be hydrolyzed to yield D-arabinose and adenine according to Scheme I. To determine whether this



Scheme I

reaction constituted the only likely route of drug decomposition under these environmental conditions, the hydrolytic reaction was monitored by two independent procedures, one sensitive to possible changes in the optically active pentose moiety and the other sensitive to change in the adenine part of the molecule. Correlation of results by either method provides strong evidence for the occurrence of only one reaction and identifies degradation products that could interfere.

The specific rotation of vidarabine is rather small while that of D-arabinose is substantial. Any process that yields D-arabinose changes the observed specific rotation. If D-arabinose is the only carbohydrate product of the reaction, *e.g.*, if racemization of either drug or D-arabinose does not occur, then the rotation of a completely hydrolyzed drug sample could be predicted based on the known rotation of the sugar. Comparison of observed results from a spent reaction mixture with those expected indicates whether or not racemization occurs.

Polarimetric data obtained on drug solutions subjected to hydrolytic conditions are given in Table I. The observed specific rotation changed with time for up to 2 hr. The rotation after completion of the reaction was that expected for an equimolar solution of D-arabinose.

Change in the adenine portion is conveniently monitored spectrophotometrically. The UV spectra of vidarabine and adenine differ maximally (Fig. 1) when determined in 0.1 *N* NaOH. This spectral difference, although not great, is adequate for the analysis of mixtures containing both where decomposition is in the 20–80% range. The composition of such mixtures can be determined by using observed absorbancy readings at 278 and 260 nm (wavelengths of maximum difference) and application of the conventional mixture formula.

Table III—Comparison of Results by Ion-Exchange and Microbiological Assays

Residence Time of Vidarabine in 0.1 *N* HCl in Steam Bath, min	Vidarabine Found, mg	
	Microbiological	Ion Exchange-UV
0	288[a]	302[a]
4	260	232
7	189	178
10	153	139
13	109	112

[a] 300.9 mg of vidarabine was initially taken.

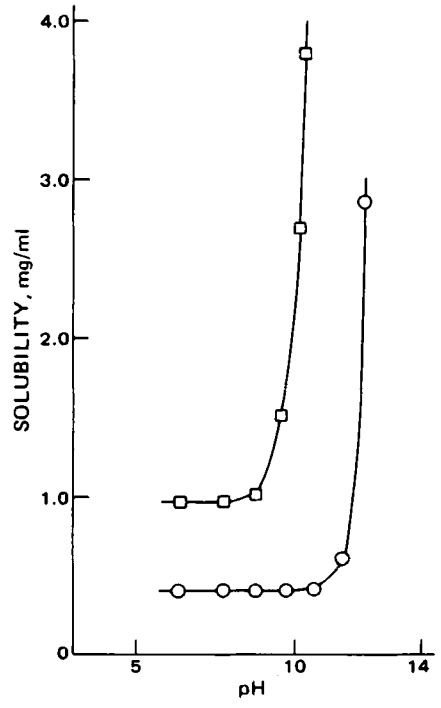


Figure 4—*Solubility profiles of vidarabine (○) and adenine (□) in borate buffer at 20°.*

[8] Pharmacia Fine Chemicals, Piscataway, NJ 08854. This resin was preferable because it produced less extraneous absorption at analytical wavelengths.
[9] Sephadex column, k9/15, Pharmacia Fine Chemicals, Piscataway, NJ 08854.

Table IV—Analysis of Vidarabine Parenteral Suspension Stored at Various Temperatures

Storage Time, weeks	Percent of Label Claim					
	25°[a] or 5°[b]		38°[a] or 25°[b]		45°	
	Upright[c]	Invert	Upright	Invert	Upright	Invert
Batch A						
Initial	100.3	—	—	—	—	—
78	100.8	97.7	—	—	102.2	102.3
156	100.3	99.4	100.9	101.7	—	—
Batch B						
Initial	100.2	—	—	—	—	—
39	—	—	100.9	99.9	98.4	102.9
104	100.8	99.0	100.3	100.4	101.7	102.4

[a] Batch A. [b] Batch B. [c] Vial position.

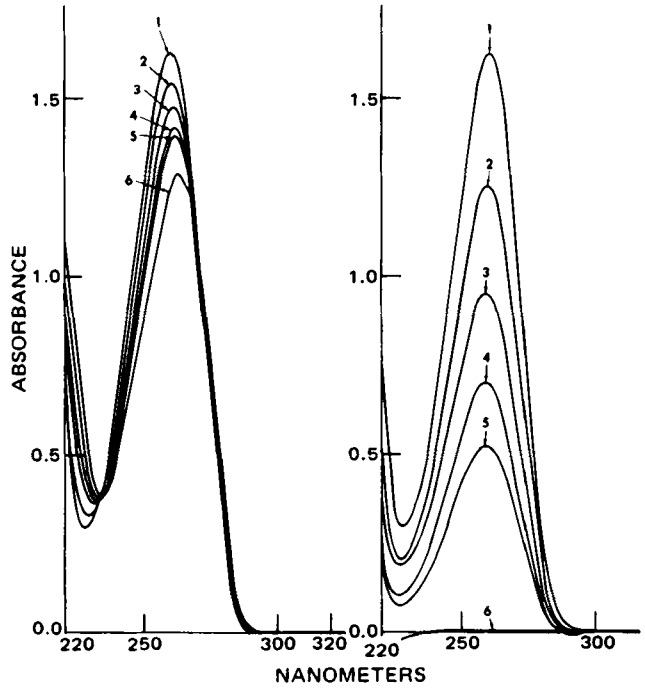


Figure 5—*Sequential spectra of vidarabine maintained at 100° in 0.1 N HCl. Left: Spectra obtained after diluting 12.5-fold into 0.2 M, pH 10 borate buffer. Right: Spectra obtained after passing solution through ion-exchange resin column. Residence times in 0.1 N HCl at 100° were: initial (1), 4 min (2), 7 min (3), 10 min (4), 13 min (5), and 2.5 hr (6).*

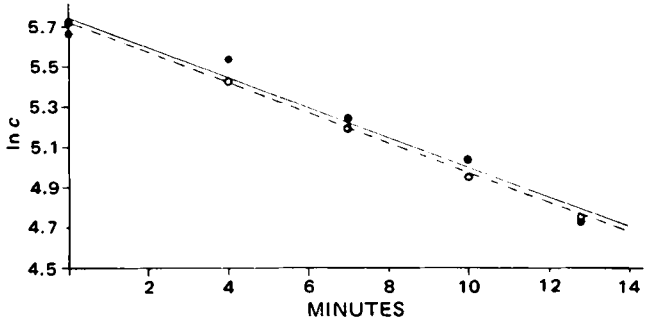


Figure 6—*Least-squares best fits for microbiological (●) and ion-exchange-UV (○) data.*

Internal consistency requires that analysis of partially degraded solutions of vidarabine by polarimetric and UV methods yield the same result. Therefore, 1% solutions of vidarabine were prepared, subdivided in sealed ampuls, and heated at 100°. Samples were withdrawn periodically, diluted in 0.1 *N* NaOH for UV determination, and used directly for optical activity determination.

The results obtained (Fig. 2) demonstrate that the only process occurring is uncomplicated hydrolysis of the *N*-glycoside linkage without racemization or other side reactions. This result was also confirmed by TLC analysis (Fig. 3).

While more complete information is now available concerning the route of decomposition in an aqueous system, work is still necessary to provide analysis of the dosage form. The specific rotation of the drug is too low to have analytical utility, and the UV spectral properties of vidarabine and adenine are such to preclude sensitive analysis. Drug analysis requires that the experimentally determined quantity be a direct measure of drug content and that information regarding identity be obtained simultaneously. A method that separates drug from likely decomposition products would have value.

Results obtained from a study of solubility as a function of pH (Fig. 4) show that vidarabine is a weaker acid than adenine by a difference of approximately 2 pKa units. This difference in acidity should make feasible their separation *via* ion exchange. Adenine at pH 10 is roughly 50% ionized and would be expected to be retained on an anion-exchange resin. Vidarabine would exist as a neutral molecule and should not be retained. Known solutions of vidarabine alone and admixed with adenine were run through an anion-exchange column, giving the results summarized in Table II.

Sequential UV spectra (Fig. 5) were obtained on a hydrolyzed solution of vidarabine after (*a*) direct dilution and (*b*) passage through the ion-exchange column. The spectra obtained by direct dilution change from those produced by vidarabine to those that arise from adenine. The spectra obtained after ion-exchange separation are those from vidarabine only. Results calculated from these spectra plot well as a first-order reaction and compare favorably with microbiological results.

Since this drug is an antiviral agent, any physicochemical method used for assay should reflect potency in the same manner as would microbiological analysis. Therefore, samples were analyzed microbiologically for comparison with results obtained by the ion-exchange procedure (Table III). Intuitively, after allowance for the anticipated variance of the microbiological method, the results appear in good agreement.

Statistically, both sets of data yielded information that, when plotted as a first-order reaction, gave straight lines with the same slope (Fig. 6). The "scatter," as measured by the correlation coefficient, was significantly less for ion-exchange results than microbiological results (0.9988 *versus* 0.9775).

Table IV summarizes the data obtained from two batches of vidarabine parenteral suspensions during stability evaluation. The data are in good agreement with the label claim. The overall average recovery was 100.6%, with a standard deviation of 1.5%.

In summary, a physicochemical stability-indicating procedure was developed for vidarabine parenteral formulations. The procedure is simple and yields results consistent with the microbiological method.

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GLC Determination of Trihexyphenidyl Hydrochloride Dosage Forms

EILEEN BARGO

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Abstract □ A rapid, sensitive, and specific GLC method for the quantitation of trihexyphenidyl hydrochloride in various pharmaceutical dosage forms is described. The procedure involves chloroform extraction of the active ingredient from a weakly acidic solution, followed by GLC determination using a 3% methyl silicone column. The specificity of the system in relation to several compendial drug analogs also is reported.

Keyphrases □ Trihexyphenidyl hydrochloride—GLC analysis in various pharmaceutical dosage forms, analogs □ GLC—analysis, trihexyphenidyl hydrochloride, in various pharmaceutical dosage forms, analogs □ Antiparkinsonism agents—GLC analysis of trihexyphenidyl hydrochloride in various pharmaceutical dosage forms

Several methods for quantitating the antiparkinsonism drug trihexyphenidyl hydrochloride (I) in toxicological samples and dosage forms have been published. These procedures include nonaqueous titration (1–3), colorimetric determination (4–6), polarography (7), and fluorescence (8). The USP procedures for assaying I in elixirs (9) and tablets (10) consist of dye–complex formation with bromcresol purple, chloroform extraction, and colorimetric measurement. All of these analytical procedures are relatively nonspecific and may measure any similarly structured compounds present, leading to erroneous results. Although the more specific technique of GLC has been employed to quantitate I (11–17), little work has been reported on its use in assaying I in dosage forms. In addition, variable results have been encountered in this laboratory with the compendial methods.

The purpose of this work was to develop a rapid, sensitive, reproducible, and discriminating method for the determination of I in pharmaceutical formulations. The procedure presented involves the chloroform extraction of the active ingredient from weakly acidic sample solutions of elixirs, tablets, and sustained-release capsules, followed by GLC determination using a 3% methyl silicone column. The method is an adaption of the GLC procedure developed by Clark[1] for assaying phencyclidine hydrochloride in sample matrixes. This GLC system also permits the differentiation of I from several compendial analogs based on differing retention times.

[1] Charles C. Clark, U.S. Drug Enforcement Administration, Miami, Fla. Method and collaborative study results are currently in press.

EXPERIMENTAL

Reagents and Chemicals—All chemicals and reagents were USP, NF, ACS, or chromatographic grade.

Instrumentation—A pH meter[2] was fitted with a glass–calomel electrode system. The gas chromatograph[3] was equipped with a flame-ionization detector connected to an electronic integrator[4]. The glass column, 4 mm i.d. × 1.8 m long, was packed with 3% OV-1 on 100–120-mesh Chromosorb WHP[5] and conditioned at 260° for 24 hr under nitrogen at a flow rate of 30 ml/min. The instrument parameters were: injected quantity of sample solution, 4–5 μl; injector temperature, 240°; detector temperature, 240°; column temperature, 210° (isothermal); carrier gas (nitrogen) flow rate, 60 ml/min; hydrogen flow rate, 60 ml/min; and air flow rate, 240 ml/min, or according to manufacturer's recommendations.

Column temperature and flow rate were adjusted to elute I in about 7 min and the internal standard in about 8.8 min. (The relative retention time of I *versus* the internal standard is about 0.8.) Electrometer sensitivity was adjusted so that 4–6 μl of the standard solution gave a suitable recorder response, *i.e.*, 40–80% of full-scale deflection.

Preparation of Solutions—The dosage forms[6] studied were an elixir, 2 mg/5 ml; tablets, 2 mg; and sustained-release capsules, 5 mg. The stock solution of 1 mg of *n*-tricosane (II)/ml, the internal standard, was prepared by directly dissolving 100 mg of II in 100 ml of chloroform. The standard solution of I, 0.2 mg/ml, was prepared by treating 10 mg of the standard in the same way as the elixir but with 1% K_2HPO_4 for pH adjustment.

The standard mixture solution of I and its analogs, 1 mg/ml, was prepared by dissolving 10 mg each of cycrimine hydrochloride, biperidin hydrochloride, procyclidine hydrochloride, tridihexethyl chloride, and I in 10 ml of the internal standard solution and mixing.

Sample Preparation—*Elixir*—An amount of sample equivalent to 10 mg of I was pipetted into a 100-ml beaker. Volume was adjusted to about 50 ml with water. The pH was adjusted to 6 ± 0.5 by dropwise addition of 10% K_2HPO_4 (~1 ml) using a pH meter. The sample was then transferred quantitatively with water into a 125-ml separator.

Tablets—Not less than 20 tablets were weighed and finely ground. An accurately weighed portion of the powder, equivalent to about 10 mg of I, was transferred into a 125-ml separator. Twenty-five milliliters of hydrochloric acid (1 in 1000) was added, and the mixture was shaken vigorously mechanically for about 30 min. Then the contents were transferred to a 100-ml beaker, and the pH was adjusted to 6 ± 0.5 with dropwise addition of 1% K_2HPO_4 using a pH meter. The sample was transferred quantitatively with water back into the 125-ml separator.

Capsules (Sustained Release)—The contents of not less than 20 capsules were weighed and finely ground. An accurately weighed portion

[2] Orion model 701A.
[3] Hewlett-Packard model 5830A.
[4] Hewlett-Packard model 18850A.
[5] Applied Science Laboratories, State College, Pa.
[6] Artane, Lederle Laboratories, Pearl River, N.Y.

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