High-Performance Liquid Chromatography of Two Peripheral Vasodilators, Nylidrin Hydrochloride and Isoxsuprine Hydrochloride, in Pharmaceutical Dosage Forms

FRANK VOLPE ˣ, JAMES ZINTEL *, and DONALD SPIEGEL

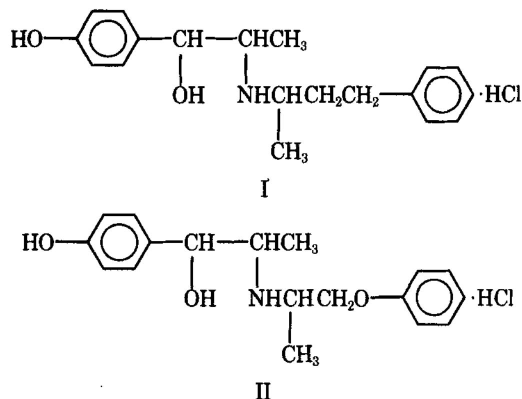
Received November 27, 1978, from the *Quality Services Department, R & D Division, USV Pharmaceutical Corporation, Tuckahoe, NY 10707.* Accepted for publication April 13, 1979. *Formerly of Arlington Laboratories, Montreal, Canada. Present address: Barnes-Hind Pharmaceuticals, Sunnyvale, CA 94086.

Abstract ☐ A reliable and selective high-pressure liquid chromatographic (HPLC) procedure for the quantitative determination of nylidrin hydrochloride or isoxsuprine hydrochloride in pharmaceutical dosage forms is described. The specificity of the stability-indicating HPLC procedure is presented for nylidrin hydrochloride.

Keyphrases ☐ Nylidrin hydrochloride—analysis, high-performance liquid chromatography, tablets, stability indicating ☐ Isoxsuprine hydrochloride—analysis, high-performance liquid chromatography, tablets, stability indicating ☐ Vasodilators, peripheral—nylidrin hydrochloride and isoxsuprine hydrochloride, high-performance liquid chromatographic analysis ☐ High-performance liquid chromatography—analysis, isoxsuprine hydrochloride, nylidrin hydrochloride, tablets, stability indicating

Nylidrin hydrochloride (I, p-hydroxy-α-[1-[(1-methyl-3-phenylpropyl)amino]ethyl]benzyl alcohol hydrochloride) and isoxsuprine hydrochloride (II, p-hydroxy-α-[1-[(1-methyl-2-phenoxyethyl)amino]ethyl]benzyl alcohol hydrochloride) are sympathomimetic agents that presumably act predominantly by β-adrenergic receptor stimulation (1). Analytical methods currently available include UV spectrophotometry (2), nonaqueous titration of the basic amine (2), colorimetry (3, 4), and GLC after derivatization by silylation (5). All of these methods except GLC lack analytical specificity or selectivity for the active drug substances and are, therefore, not suitable stability-indicating assays.

Application of GLC for the derivatization of I was only partially successful because the derivatives formed were sometimes unstable. This result was demonstrated by precipitate formation accompanied by a decrease in peak area. The high-presssure liquid chromatographic (HPLC) method described in this paper is specific and provides direct and accurate determinations of I or II, which could be used to evaluate pharmaceutical product stability.



EXPERIMENTAL

Apparatus—The high-pressure liquid chromatograph[1] was equipped with a microliter loop injector[2], a reversed-phase column[3], and a variable wavelength spectrophotometric detector[4] interfaced to an electronic integrator[5].

Reagents—Nylidrin hydrochloride[6], isoxsuprine hydrochloride[7], UV grade methanol[8], fluorene[9], reagent grade dibasic ammonium phosphate, and phosphoric acid[10] were used.

Chromatographic Conditions—The mobile phase consisted of methanol–aqueous buffer (75:25 v/v). The buffer was prepared by weighing 1.32 g of dibasic ammonium phosphate into a 1000-ml volumetric flask and dissolving with distilled water. The final volume was adjusted to pH 7.5 with 85% phosphoric acid. The methanol and buffer were filtered separately through membrane filters[11] before mixing. The mobile phase flow rate was set at 1.5 ml/min. The column effluent was monitered by UV absorption at 276 nm (maximum for both active ingredients).

Procedure—The internal standard stock solution was prepared by dissolving fluorene in the mobile phase at 0.5 mg/ml. A standard pair

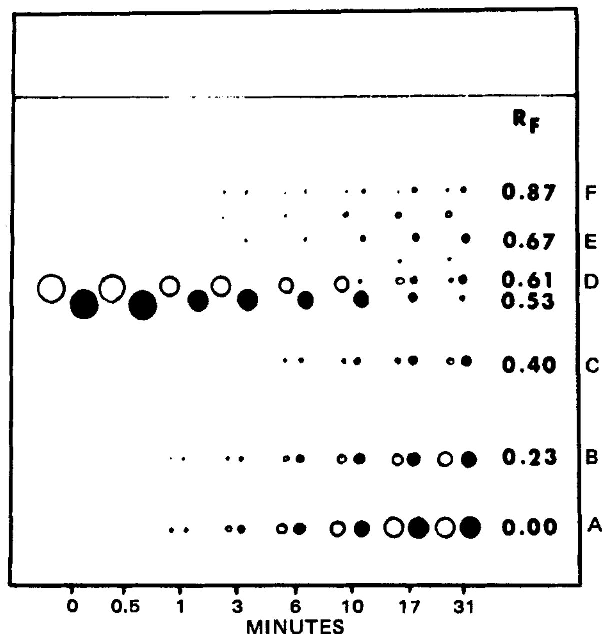


Figure 1—*TLC of partially photothermal-degraded nylidrin and isoxsuprine as a function of exposure time. The intensity of the spots is indicated by size. Key: A–F, degradation products;* ●, *nylidrin; and* ○, *isoxsuprine.*

[1] Model 601, Perkin-Elmer Corp., Norwalk, Conn.
[2] Model 7120, Rheodyne Inc., Berkeley, Calif.
[3] μBondapak C_{18}, 3.9 mm i.d. × 30 cm, Waters Associates, Milford, Mass.
[4] Model LC-55, Perkin-Elmer Corp., Norwalk, Conn.
[5] Model 3385 A, Hewlett-Packard, Paramus, N.J.
[6] USV Pharmaceutical Corp., Tuckahoe, N.Y.
[7] Mead Johnson and Co., Evansville, Ind.
[8] Burdick & Jackson Laboratories, Muskegon, Mich.
[9] Aldrich Chemical Co., Metuchen, N.J.
[10] Fisher Scientific Co., Fair Lawn, N.J.
[11] Type BD, Millipore Corp., Bedford, Mass.

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Table I—Location of the Photothermal Degradation Products (A–F) and Postulated Degradation Products of Nylidrin Hydrochloride as a Function of Retention Time

Compound	Structure	Retention Time, min
A	—	1.6
p-Hydroxybenzoic acid	HO—C₆H₄—COOH	1.6
B	—	2.1
4-Hydroxybenzaldehyde	HO—C₆H₄—C(=O)—H	2.1
4-Hydroxybenzyl alcohol	HO—C₆H₄—CH_2OH	2.2
p-Hydroxyacetophenone	HO—C₆H₄—C(=O)—CH_3	2.2
p-Hydroxyephedrine hydrochloride	HO—C₆H₄—CH(OH)—CH($NHCH_3$)CH_3 · HCl	3.4
α-(1-Aminoethyl)-p-hydroxybenzyl alcohol hydrochloride	HO—C₆H₄—CH(OH)—CH(NH_2)CH_3 · HCl	3.4
C	—	3.6
Isoxsuprine hydrochloride	II	4.0
Nylidrin hydrochloride	I	5.2
1-Methyl-3-phenylpropylamine	$H_2NCH(CH_3)CH_2CH_2$—C₆H₅	6.5[a]
Fluorene (internal standard)	[fluorene structure]	7.0
D	—	9–10
E	—	—[b]
F	—	—[b]

[a] Detected at 254 nm. [b] Not detected.

solution of I (1.2 mg/ml) and fluorene (0.1 mg/ml) was made by accurately weighing ~30 mg of previously dried I and quantitatively transferring it into a 25-ml volumetric flask. Five milliliters of the internal standard stock solution was pipetted into the volumetric flask and diluted to volume with mobile phase. In a similar manner, a standard pair solution of II (1.0 mg/ml) and fluorene (0.1 mg/ml) was prepared.

The respective active ingredients were extracted from tablet preparations by finely powdering 20 tablets and weighing a portion of powder equivalent to ~30 mg of I or ~25 mg of II into 50-ml centrifuge tubes. Five milliliters of the internal standard stock solution was pipetted into each tube, and 20 ml of mobile phase was added. The tubes were sonicated for ~2 min, shaken mechanically for 30 min, and centrifuged. Clear filtrates were obtained by filtering a portion of each supernate through membrane filters[11].

RESULTS AND DISCUSSION

Assay Selectivity—This HPLC assay was intended for stability testing in addition to finished product release testing. To be stability indicating, the method must discriminate between degradation products and the active ingredient. Preferably, a stability-indicating procedure should also quantitatively demonstrate the inverse relationship between the decrease in the active ingredient and the increase in degradation products.

To show that a method is stability indicating, degradation routes and the resulting products may be postulated and tested in the assay system for interference. Alternatively, the active ingredient may be subjected to severe conditions that simulate most modes of expected degradation, and the reaction products, if any, may be tested for interference in the method. Base hydrolysis, acid hydrolysis, and photothermal oxidation were chosen as the most likely modes of degradation. Proof of specificity was obtained only for I. The specificity for II and the structural elucidation of degradation products will be the subjects of a future publication.

Base Hydrolysis—A sample of I was dissolved in 1 *N* NaOH (2 g in 100 ml), and 2-ml aliquots of this solution were placed in 10-ml tubes sealed with polytef-lined screw caps. The tubes were stored at 23 and 70° for 96 hr. The contents were neutralized with acid and evaporated to dryness. HPLC showed there was no significant change in the amount of I since no measurable degradation was detected. A sample was then incubated in 1 *N* NaOH for 36 days at 70°. The chromatograms revealed that the I peak remained unchanged; however, additional unknown peaks appeared with retention times of ~2 min.

Acid Hydrolysis—Two hundred milligrams of I was dissolved in 100

Table II—Linearity of I

Concentration, mg/ml	Peak Area Ratio (I/Fluorene)	Response Factor Ratio[a]
0.90	0.70	0.078
1.01	0.80	0.079
1.10	0.88	0.080
1.23	0.98	0.080
1.33	1.07	0.080
1.42	1.14	0.080

[a] Slope = 0.84, *RSD* = 1.65%, and *y* intercept = 0.05.

Table III—Linearity of II

Concentration, mg/ml	Peak Area Ratio (II/Fluorene)	Response Factor Ratio[a]
0.31	0.36	0.116
0.59	0.69	0.117
0.92	1.06	0.115
1.21	1.44	0.119
1.52	1.80	0.118
1.86	2.20	0.118

[a] Slope = 1.19, *RSD* = 0.84%, and *y* intercept = −0.01.

Table IV—HPLC and NF Analyses of Commercial Tablets

Compound[a]	Sample	Dosage Claimed, mg/tablet	HPLC, mg/tablet	NF, mg/tablet
I	1	6.0	6.16	5.98
I	2	6.0	5.87	5.66
I	3	6.0	6.16	6.06
I	4	12.0	11.9	12.2
I	5	12.0	12.1	11.5
I	6	12.0	12.5	11.9
II	7	10.0	9.81	9.98
II	8	10.0	9.94	9.98
II	9	10.0	9.76	9.99
II	10	20.0	19.6	19.7
II	11	20.0	19.5	19.5
II	12	20.0	19.4	19.7

[a] Compounds I and II were obtained from different manufacturers.

ml of 1 N HCl and refluxed for 2 hr. Aliquots were drawn after 1 and 2 hr, neutralized with sodium hydroxide, evaporated to dryness, dissolved in mobile phase, and chromatographed. There was little difference in the number and size of peaks between the two time intervals; three, possibly four, small peaks had retention times of ~1.5 min. The apparent lack of any dependency on reaction times probably meant that the degradation products did not arise from acid treatment alone but from pyrolysis during the drying step.

Photothermal Decomposition—An alternative approach degraded I in a few minutes. Compounds I (320 μg/spot) and II (160 μg/spot) were applied to a 250-μm silica gel GF plate[12] and irradiated with intense UV light[13] (heat also was emitted from this UV source). The plate was developed using chloroform–methanol–concentrated ammonium hydroxide (90:15:15).

Figure 1 clearly shows the decrease in I and II as well as the concomitant increase in degradation products in both numbers and intensity as a function of exposure time. Since the formation of degradation products (A–F) under the same stress conditions was nearly the same for both compounds, the presence of an ether linkage in II compared to I does not confer additional degradation paths on the former compared to the latter. However, one degradation product (R_f 0.75) originated from II only. The retention times of the degradation products isolated from TLC, known compounds, I, and II are listed in Table I.

To test the selectivity of this method, I was assayed to establish potency (Fig. 2). A portion of this powder was spread thinly on a glass plate and

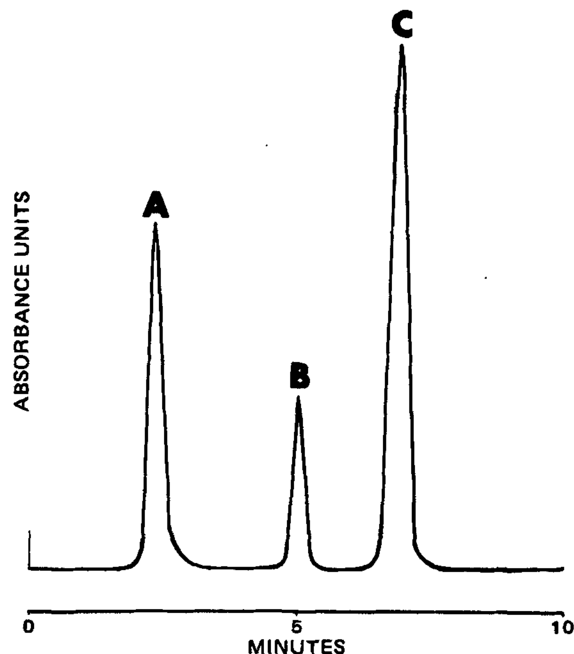

Figure 3—*High-pressure liquid chromatogram of partially degraded nylidrin hydrochloride. Key: A, major degradation product (labeled previously as Product B); B, nylidrin hydrochloride; and C, fluorene (internal standard).*

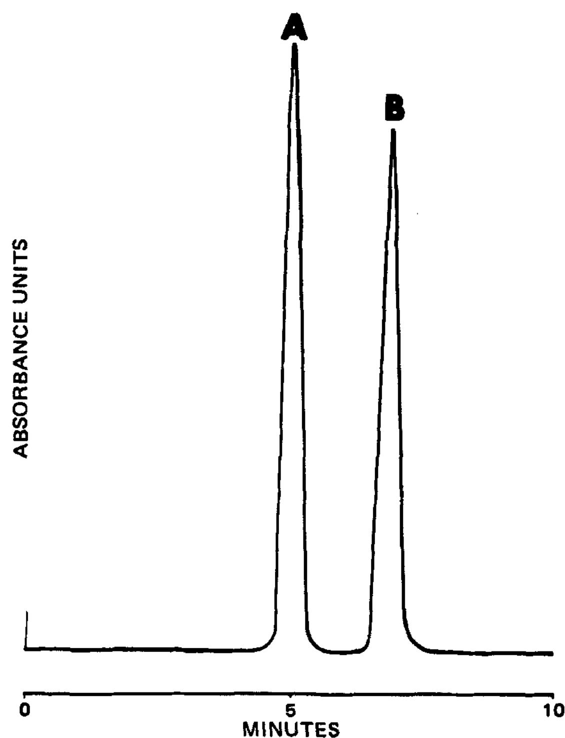

Figure 2—*High-pressure liquid chromatogram of nylidrin hydrochloride. Key: A, nylidrin hydrochloride; and B, fluorene (internal standard).*

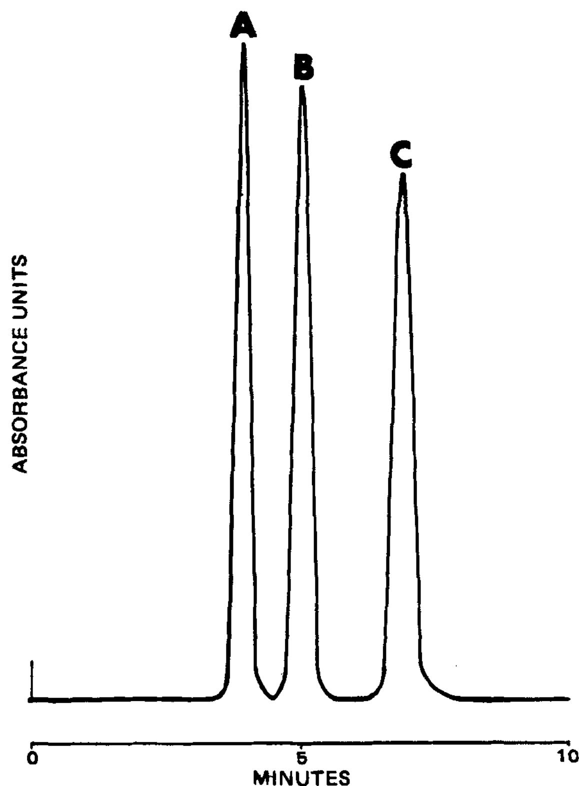

Figure 4—*High-pressure liquid chromatogram. Key: A, isoxsuprine hydrochloride; B, nylidrin hydrochloride; and C, fluorene (internal standard).*

[12] Analtech, Newark, Del.
[13] Model 420-U11, George W. Gates & Co., Long Island, N.Y.

photothermally degraded by irradiating with intense UV light[13] for 30 min. A 100-mg sample of partially degraded I was accurately weighed, quantitatively transferred into a 50-ml volumetric flask, and dissolved in methanol. Then 20.0- and 15.0-ml aliquots were removed, the solvent was evaporated, and the sample was reassayed (Fig. 3). The recovery was 101% by the official NF XIV assay but only 33% by HPLC. The system selectivity is demonstrated further in Fig. 4, where I is resolved from II. These compounds are very similar in structure, size, and polarity.

Statistical Evaluation—The linearity of typical standard curves is summarized in Tables II and III. The response factor ratios, defined as the area ratio (active ingredient/fluorene) times the concentration ratio (fluorene/active ingredient), were quite constant for all concentrations. Precision was demonstrated by a relative standard deviation of 0.4% for nine replicate injections. Analysis of active–placebo mixtures demonstrated the accuracy of the proposed method with an average 99.1% recovery.

The experimental data shown in Table IV were obtained on randomly selected commercial tablet samples. Each sample was analyzed by the HPLC method and by the official NF XIV procedures. The results from both methods are comparable, but since the HPLC method has been shown to be a stability-indicating assay, it is more selective than the NF method and more accurately reflects true tablet potency.

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Solubility Determination of Barely Aqueous-Soluble Organic Solids

TAKERU HIGUCHI, FONG-MEI L. SHIH, TOSHIKIRO KIMURA, and J. HOWARD RYTTING[x]

Received September 15, 1978, from the *Departments of Pharmaceutical Chemistry and Chemistry, University of Kansas, Lawrence, KS 66045.* Accepted for publication April 18, 1979.

Abstract ☐ Solubility determination of organic molecules having very low solubilities is hampered by such problems as slow equilibration during measurement, influence of impurities, and inherent heterogeneity in the energetic content of the crystalline solid. Three approaches to meeting these problems are presented. The first approach involves enhancing the dissolution rate by the addition of a water-immiscible solvent in which the organic solute is more soluble, thereby increasing the surface area available for dissolution. The second method is a combination of experimental data with a group contribution approach that allows the estimation of extremely insoluble solids. This approach involves measurement of the solubility in an organic solvent and calculation of the aqueous solubility from the estimated partition coefficient and the organic solvent data. The third approach is based on using a large excess of the solid and a highly specific analytical determination of the main component. The first two approaches were explored in detail and tested using norethindrone, norethindrone acetate, methyltestosterone, and methyltestosterone acetate.

Keyphrases ☐ Solubility determinations—organic solids, barely aqueous soluble, group contribution, partition coefficient ☐ Organic solids—solubility determinations, barely aqueous soluble, group contribution, partition coefficient ☐ Aqueous solubility—organic solids, barely soluble, solubility determination

The solubilities of solids are very useful parameters in the daily practice of chemistry and pharmacy. Solubility information is particularly vital in formulating products, developing analytical methods, chemical processing, predicting ecological impact, and assessing drug transport and distribution problems.

Solubility determination of solids that are moderately soluble, *i.e.*, $\geq 0.2\%$, normally poses no serious problem. Direct solubility measurement of solids having very low solubilities involves special difficulties, which can lead to large discrepancies in reported values. For example, the reported solubility of cholesterol in water ranges from 0.025 to 2600 μg/ml (1). This paper is concerned with an analysis of these problems and offers several approaches for overcoming them, particularly for determinations carried out in water.

BACKGROUND

Equilibration Kinetics—The low equilibration rate during solubility measurement of slightly soluble species presents a serious problem which has not been adequately addressed from an experimental standpoint. The solubility of a solid in a given solvent is the amount that goes into solution at equilibrium at some selected temperature. Since it is impossible to attain a true equilibrium state in real life, in practice, results are reported for systems that are reasonably close to equilibrium. Such situations are not particularly difficult to attain for moderately soluble substances. An aqueous suspension of benzoic acid crystals, for example, probably reaches a near equilibrium state with moderate stirring at 25° within a few hours. The exact rate depends on the fineness of the crystals, the amount of solid added, and the degree of agitation.

According to various studies on dissolution kinetics, the classical equation (Noyes–Whitney) (2, 3) appears to be an empirically useful relationship. A simplified form of the equation for situations where the various geometric factors are essentially held constant can be written as:

$$\frac{dc}{dt} = k(C_s - C) \quad \text{(Eq. 1)}$$

where dc/dt is the rate of increase in the solute concentration, k is a constant, C_s is the solubility, and C is the solute concentration in solution. Upon integrating, one obtains:

$$\ln \frac{C_s - C}{C_s} = -kt \quad \text{(Eq. 2)}$$

Under these conditions, the kinetics suggest an approximately first-order approach to saturation, with a given degree of saturation being achieved somewhat independently of the solid being studied. Under experimental conditions, however, unless a substantial excess of the solid is used, the effective surface area will decrease significantly during the dissolution process, with a corresponding delay in the attainment of equilibrium.

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