Table III—Antibacterial Activity of Triethylammonium (Acetylcyanoamino)chlorotriphenylstannate and Triethylammonium Dichlorotriphenylstannate

Compound	*B. subtilis*[a]			*E. coli*			*S. aureus*		
	1[b]	10	100	1	10	100	1	10	100
V*a*	2+	2+	2+	—	—	+	2+	2+	2+
VI	2+	2+	2+	—	—	+	+	2+	2+

[a] Bacteria were obtained from the culture collection of the Department of Biological Sciences, St. John's University. [b] Indicates concentration of compounds employed in micrograms per milliliter; — indicates no inhibition of growth, + indicates partial inhibition of growth, and 2+ indicates complete inhibition of growth.

ample, only three of the Series IV compounds completely inhibited *C. globosum* at 100 μg/ml, whereas five of the Series V compounds exhibited this activity. Furthermore, none of the Series IV compounds inhibited *P. notatum* at 100 μg/ml, whereas two of the Series V compounds exhibited this activity. Furthermore, none of the Series IV compounds showed activity against *S. cerevisiae* and *T. viride* at 1 μg/ml, whereas four of the Series V compounds were partially active against *S. cerevisiae* and all of the Series V compounds were partially active against *T. viride* at this concentration. In addition, two of the Series V compounds completely inhibited *S. cerevisiae* at 100 μg/ml. The simple anionic complex, VI, exhibited equal or less activity than the Series V compounds.

The data in Table III show that V*a* and VI behaved in an almost identical manner toward specific test bacteria. Both compounds completely inhibited *Bacillus subtilus* at the lowest level (1 μg/ml) of organotin compound. Both compounds were equal to the previously tested *N*-phenyl-*N'*-cyano-*S*-(triphenylstannyl)isothioureas (II, R = C_6H_5) in this regard (9). On the other hand, the previously tested Series I compounds (8) were completely inactive at this concentration, while the previously tested *N*-phenyl-*N'*-cyano-*O*-(triphenylstannyl)isourea (III, R = C_6H_5) (10) only partially inhibited growth at this concentration. Compound V*a* behaved identically to the Series I compounds and to II (R = C_6H_5) in that it completely inhibited the growth of *Staphylococcus aureus* at 1 μg/ml. Compound III (R = C_6H_5) was inactive at this concentration (10). Compounds V*a* and VI were no more active than some previously studied compounds toward *Escherichia coli* (8–10).

EXPERIMENTAL

The Series IV compounds were individually dissolved in tetrahydrofuran. The Series V compounds and VI were individually dissolved in acetone. The preparation of sterile solutions of the compounds, the fungi employed, the antimicrobial testing procedures, and the determination of growth inhibition were reported previously (10).

Compounds V*a* and VI also were investigated for antibacterial activity according to the procedure reported earlier (10).

REFERENCES

(1) J. S. Thayer, *J. Organomet. Chem.*, **76,** 265 (1974).

(2) "Organotin Compounds: New Chemistry and Applications," J. J. Zuckerman, Ed., American Chemical Society, Washington, D.C., 1976.

(3) K.-D. Freitag and R. Bock, *Pestic. Sci.*, **5,** 731 (1974).

(4) R. D. Barnes, A. T. Bull, and R. C. Poller, *ibid.*, **4,** 305 (1973).

(5) M. E. Getzendaner and H. B. Corbin, *J. Agric. Food Chem.*, **20,** 881 (1972).

(6) R. Bock and K.-D. Freitag, *Naturwissenschaften*, **59,** 165 (1972).

(7) A. J. Chapman and J. W. Price, *Int. Pestic. Control*, 1, 11 (1972).

(8) E. J. Kupchik, M. A. Pisano, H. E. Hanke, and W.-C. R. Tseng, *J. Pharm. Sci.*, **67,** 576 (1978).

(9) E. J. Kupchik, M. A. Pisano, A. V. Raghunath, R. A. Cardona, N. Formaini, and C. Alleguez, *ibid.*, **64,** 1259 (1975).

(10) E. J. Kupchik, M. A. Pisano, D. K. Parikh, and M. A. D'Amico, *ibid.*, **63,** 261 (1974).

(11) E. J. Kupchik and J. A. Feiccabrino, *J. Organomet. Chem.*, **93,** 325 (1975).

(12) J. A. Feiccabrino and E. J. Kupchik, *ibid.*, **73,** 319 (1974).

Determination of Isophenindamine in Phenindamine Tartrate Using an Argentated High-Performance Liquid Chromatographic Mobile Phase

RONALD J. TSCHERNE [x] **and HERMINIA UMAGAT**

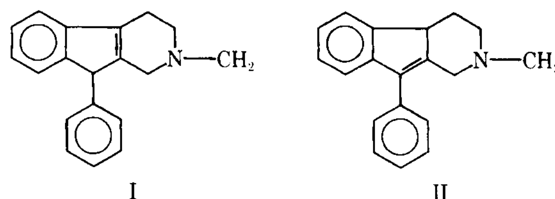
Received May 7, 1979, from the *Quality Control Department, Hoffmann-La Roche Inc., Nutley, NJ 07110.* Accepted for publication September 13, 1979.

Abstract ☐ A high-performance liquid chromatographic procedure was developed for the determination of isophenindamine in phenindamine tartrate bulk powder. The method employs a reversed-phase column and a mobile phase containing methanol, 0.001 *M* HNO_3, and silver nitrate.

Keyphrases ☐ Isophenindamine—analysis, high-performance liquid chromatography, argentated mobile phase, phenindamine tartrate bulk powder ☐ High-performance liquid chromatography—isophenindamine, analysis in phenindamine tartrate bulk powder ☐ Phenindamine tartrate—analysis of isophenindamine in bulk powder, high-performance liquid chromatography, argentated mobile phase ☐ Antihistaminics—phenindamine tartrate, analysis of bulk powder for isophenindamine, high-performance liquid chromatography, argentated mobile phase

The role of histamine in anaphylactic shock and allergic conditions stimulated research for specific histamine antagonists. A series of antihistaminics including phenindamine (I) was synthesized (1). Distinct differences were observed between I and isophenindamine (II) when administered intravenously.

Because of the possible formation of II during the manufacture of phenindamine tartrate, a method for the determination of II was desired. A UV spectrophotometric procedure based on the fact that the wavelength of maximum absorbance for each compound is slightly different



0022-3549/ 80/ 0300-0342$01.00/ 0

© 1980, American Pharmaceutical Association

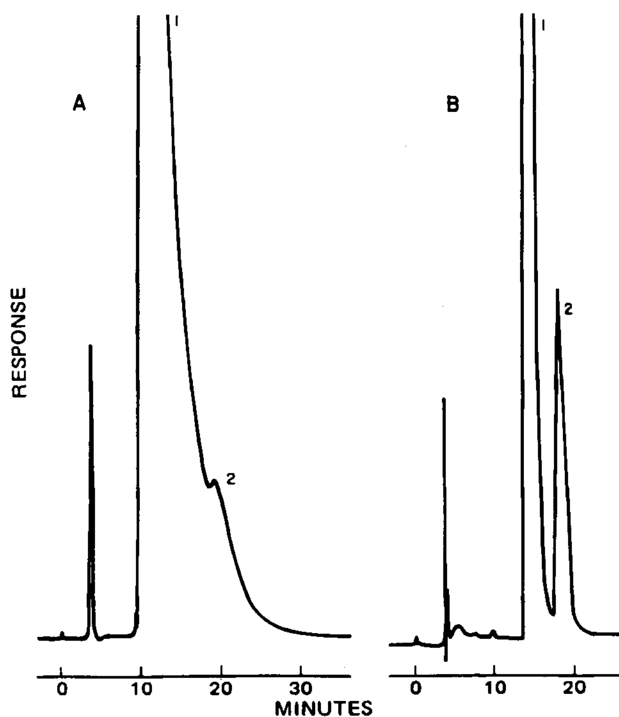


Figure 1—*HPLC separation of isophenindamine from phenindamine using a μBondapak C_{18} column (30 × 3.9 mm i.d.). Key: A, mobile phase of 0.001* M *HNO_3–methanol (1:1) and a flow rate of 0.8 ml/min; B, mobile phase of 0.001* M *HNO_3–methanol (1:1) containing 2% $AgNO_3$ (w/v) and a flow rate of 0.8 ml/min; 1, phenindamine; and 2, isophenindamine.*

(2) would allow quantitation using simultaneous equation treatment. NMR spectroscopy might be used for II detection, but quantitative results have not been reported[1].

A high-performance liquid chromatographic (HPLC) procedure that enables separation and quantitation of II in the presence of phenindamine tartrate bulk powder is described.

EXPERIMENTAL

Reagents and Chemicals—Glass-distilled water, methanol, nitric acid, and silver nitrate[2] were used for preparation of the mobile phase. The mobile phase was filtered[3] prior to use. Samples and standards were prepared using low-actinic glassware. Isophenindamine nitrate[4] was used for standard preparations.

Instrumentation and Apparatus—A constant-volume pump[5] was used in conjunction with a septum-type injector[6] and a variable-wavelength detector[7]. The separation was accomplished using a permanently bonded octadecylsilane partition column[8]. A high-pressure syringe[9] was used for injection of all samples and standards.

Mobile Phase—The mobile phase was prepared by dissolving 20 g of silver nitrate in 500 ml of 0.001 *M* HNO_3 and diluting with 500 ml of methanol. It was prepared fresh daily using low-actinic glassware and filtered through a 0.45-μm filter prior to use.

Standard Preparation—To prepare the standard solution, 13 mg of isophenindamine nitrate reference standard was weighed accurately into a 25-ml low-actinic volumetric flask, and 12.5 ml of 0.001 *M* HNO_3 was added. The flask was shaken until dissolution was nearly or totally complete (~5 min). Then 12.5 ml of methanol was added, and the flask was shaken again until dissolution occurred. A small degree of sonication usually was necessary to solubilize the reference material.

Sample Preparation—To prepare the sample solution, ~100 mg of phenindamine tartrate powder was weighed accurately into a low-actinic 10-ml volumetric flask. The sample was dispersed in 5 ml of 0.001 *M* HNO_3 by gentle shaking of the flask. Then 5 ml of methanol was added, and the solution was shaken until complete dissolution was observed.

Procedure—Five microliters each of the standard and sample solutions were injected alternately into the chromatograph. The peak heights of the isophenindamine standard and the peak that corresponded to it in retention time in the sample chromatogram were measured. The percent isophenindamine (as tartrate) was calculated by:

$$\begin{array}{c}\text{\% isophenindamine}\\ \text{(as tartrate)}\end{array} = \left(\frac{W_{ISTD}}{P_{ISTD}}\right)\left(\frac{P_{ISAM}}{W_{SAM}}\right)\left(\frac{MW_{ITAR}}{MW_{INO3}}\right)\left(\frac{1000}{25}\right) \quad \text{(Eq. 1)}$$

where W_{ISTD} is the weight of isophenindamine nitrate standard in milligrams; W_{SAM} is the weight of the sample in milligrams; P_{ISTD} and P_{ISAM} are the heights, in millimeters, of isophenindamine in the standard and sample runs, respectively; and MW_{ITAR} and MW_{INO3} are the molecular weights of isophenindamine tartrate (411.44) and isophenindamine nitrate (324.37), respectively. The factor 1000/25 is a combination of the dilution factor (10/25) and the conversion to percent (×100).

---

[1] J. H. Johnson and C. Condon, Hoffmann-La Roche Inc., unpublished data.
[2] Analyzed reagent, J. T. Baker Chemical Co.
[3] Millipore Corp.
[4] Hoffmann-La Roche Inc.
[5] Model 396, Laboratory Data Control.
[6] DuPont Instruments.
[7] Model 835, DuPont Instruments.
[8] μBondapak C_{18}, Waters Associates, Milford, Mass.
[9] HP-305, Hamilton Co.

Table I—Comparison of Results Obtained Using UV and Chromatographic Methods

Lot	Percent Isophenindamine: HPLC Determination	Percent Isophenindamine: UV Determination
A	5.6	3.5
B	5.1	3.1
C	5.3	3.9
D	6.3	3.4
E	7.6	4.2

RESULTS AND DISCUSSION

The chromatographic separation of isophenindamine in the presence of phenindamine tartrate was obtained using a μBondapak C_{18} column and an argentated mobile phase. A 2% $AgNO_3$ (w/v) concentration in the mobile phase was necessary for complete compound separation. Lower silver nitrate concentrations reduced the resolution between I and II, while increased silver nitrate concentrations yielded little additional separation. Typical chromatograms using argentated and nonargentated mobile phases are shown in Fig. 1. Ion-pairing systems employing octanesulfonic acid and lauryl sulfate as pairing reagents were tried but did not produce resolution equivalent to the system containing 2% $AgNO_3$.

Several phenindamine tartrate samples were tested for their isophenindamine content using this method, and the results were compared with those obtained using a UV spectrophotometric procedure based on UV absorbance differences (Table I). Since poor correlation of results was found between the two methods, a standard addition study was done using the HPLC procedure. A straight line through the origin (within experimental error) was obtained for a plot of peak height response *versus* amount of isophenindamine added. This experiment showed conclusively that: (*a*) response *versus* concentration was linear between 0 and 10% isophenindamine in the samples and (*b*) discrepancy in results between the two methods must be linked to the inaccuracy of the UV procedure. Intuitively, these conclusions seem logical because the UV procedure suffers inherently from overlapping absorption bands in the measurement region. The relative standard deviation for 10 injections of the same solution into the chromatograph was 2.2%.

When using both the UV and the chromatographic procedures for quantitation studies, isophenindamine nitrate was the reference standard because no isophenindamine tartrate was available. Sample results were corrected for molecular weight differences between the tartrate and the nitrate salts.

Samples of phenindamine free base also were assayed for their isophenindamine content when the sample preparation was modified slightly. Free base samples had to be dissolved in methanol instead of 0.001 *M* HNO_3–methanol (1:1). This alteration did not affect method reliability.

A rechromatographed portion of an eluted phenindamine peak showed that no detectable amount of the iso-compound formed during the analysis. Also, a stability study on prepared solutions indicated that a

small degree of transformation of isophenindamine to phenindamine occurred after standing in the mobile phase for ~1 day.

To take advantage of the properties of silver nitrate, it was necessary to ensure that all materials in contact with the mobile phase were made of 316 stainless steel or other noncorrodible material. The detector balance had to be electronically offset to monitor the effluent at 254 nm because of the relatively high absorbance of the mobile phase. By observing these relatively simple directions and precautions, the system was safe and reliable, producing no column deterioration over ~3 months of use.

REFERENCES

(1) G. Lehmann, *J. Pharmacol. Ther.*, **92**, 249 (1948).

(2) J. Plati and W. Wenner, *J. Org. Chem.*, **20**, 1412 (1955).

ACKNOWLEDGMENTS

The authors thank Dr. J. Plati for the isophenindamine nitrate reference standard, Mr. N. Beratti for spectroscopic data, and Ms. G. Capitano for technical assistance.

Effect of Exercise on Renal Clearance of Atenolol

WILLIAM D. MASON *ˣ, GREGORY KOCHAK *, NATHANIEL WINER *, and IRVING COHEN ‡

Received April 30, 1979, from the **Pharmacokinetics Laboratory, Schools of Pharmacy and Medicine, University of Missouri-Kansas City, Kansas City, MO 64108*, and the *‡Clinical Research Department, ICI Americas, Inc., Wilmington, DE 19897.* Accepted for publication October 1, 1979.

Abstract □ The effect of exercise on plasma atenolol was evaluated using a two-phase complete crossover study in 12 healthy volunteers. In one phase, the volunteers were subjected to physical exertion on a treadmill; in the other phase, they remained in a sitting or standing position. Following a single 100-mg atenolol dose, frequent blood samples and a complete urine collection were obtained over 24 hr. Plasma and urine atenolol levels were assayed by high-pressure liquid chromatography. Plasma atenolol was significantly ($p < 0.05$) higher during the exercise phase of the study, and this result was associated with approximately an 8% decrease in the renal clearance of the drug, probably due to decreased renal blood flow during exercise.

Keyphrases □ Atenolol—effect of exercise on renal clearance □ Pharmacokinetics—atenolol, effect of exercise on renal clearance □ Renal clearance—atenolol, effect of exercise □ β-Adrenergic blocking agents—atenolol, effect of exercise on renal clearance

Recently published reports described the pharmacokinetics (1) and pharmacodynamics (2) of atenolol, 2-[p-[2-hydroxy-3-(isopropylamino)propoxy]phenyl]acetamide, a new cardioselective (3–5) β-adrenergic blocking agent. More than 90% of the bioavailable dose of atenolol is excreted unchanged in the urine within 48 hr of dosing (6). To study the relationship of pharmacokinetics to pharmacodynamics, a two-phase complete crossover design was employed, with the phases differing only in the amount of exercise required of the subjects. A difference in the kinetics of atenolol between the two phases (exercise *versus* nonexercise) is reported.

EXPERIMENTAL

The 12 healthy male volunteers were 20–28 years old and weighed 63.6–85.5 kg (140–188 lb). Each subject was judged healthy based on the absence of any abnormality in his history, physical examination, ECG, hemogram, blood chemistries, and urinalysis.

The study design was a two-phase complete crossover, with the treatments being identical except for the exercise performed by each subject. Following a 10-hr fast, each subject received 100 mg of atenolol as an oral solution in 240 ml of water. The fast continued until after the 4th-hr blood sample was drawn; a standard meal was served at the 5th hr. Following the meal, water was allowed *ad libitum*. Blood samples were collected at 0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, and 24.0 hr. Urine was collected prior to dosing, from 0.0 to 12.0 hr, and from 12.0 to 24.0 hr.

Plasma and urine atenolol levels were determined by a high-pressure liquid chromatographic (HPLC) method described previously (7) and modified as follows. The mobile phase consisted of a 4.00 mM solution of 1-heptanesulfonic acid sodium salt, 1.0% acetic acid, and 60.0% methanol in distilled water. The mobile phase was pumped at 1.5 ml/min and 20° through a stainless steel column packed with a high efficiency bonded-phase packing[1]. Aliquots of 150 μl of the reextraction solution were injected directly on-column through the injector. The atenolol peak was well separated from the procainamide internal standard peak and from artifacts. The retention times of atenolol and procainamide were 5.8 and 12.3 min, respectively.

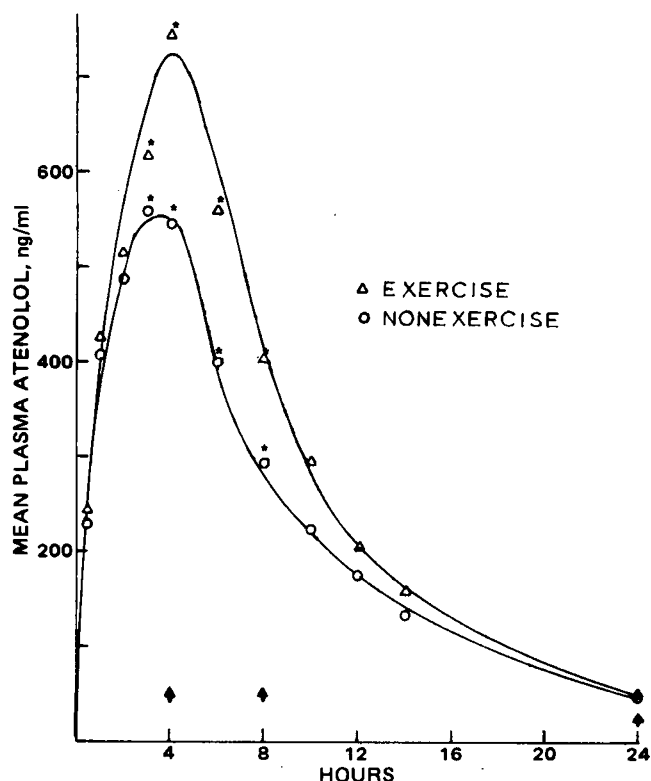


Figure 1—*Plasma atenolol concentration profile for exercised and nonexercised subjects. Each point represents the mean of 12 subjects. Starred (*) points represent significant differences (p < 0.05) between exercised and nonexercised subjects. Arrows indicate times of exercise.*

[1] Spherisorb 5-μm ODS, Laboratory Data Control, Riviera Beach, Fla.

0022-3549/80/0300-0344$01.00/0

© 1980, American Pharmaceutical Association