Ion-Pair High-Performance Liquid Chromatography of Terbutaline and Catecholamines with Aminophylline in Intravenous Solutions

DAVID A. WILLIAMS *x, **ESTHER Y. Y. FUNG** ‡, and **DAVID W. NEWTON** §

Received June 15, 1981, from the *Department of Chemistry, Massachusetts College of Pharmacy and Allied Health Sciences, Boston, MA 02115, the ‡Tiers Drug Store, Uxbridge, Ontario, Canada LOC 1 KO, and the §Department of Pharmaceutics, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68105. Accepted for publication November 12, 1981.

Abstract □ A stability-indicating ion-pair high-performance liquid chromatographic (HPLC) assay that is rapid and specific for epinephrine, isoproterenol, dopamine, norepinephrine, methyldopate, or terbutaline in intravenous solutions with aminophylline has been developed using a spectrofluorometric detector. A HPLC method for the analysis of terbutaline and methyldopate is introduced, and the superiority of fluorometric over UV detection of terbutaline and the catecholamines is illustrated.

Keyphrases □ High-performance liquid chromatography—ion-pair, terbutaline and catecholamines with aminophylline in intravenous solutions □ Fluorescence detector—high-performance liquid chromatographic analysis of catecholamines in presence of aminophylline □ Catecholamines—ion-pair high-performance liquid chromatography with aminophylline in intravenous solutions

Dopamine (I), epinephrine (II), isoproterenol (III), methyldopate (IV), norepinephrine (V), or terbutaline (VI) may be combined with aminophylline (VII) in intravenous admixtures prior to patient administration. Catecholamines and other phenolic sympathomimetic amines are reportedly more unstable in an alkaline media and can undergo rapid autoxidation to biologically inactive quinoid products (1). Thus, the therapeutic efficacy of these sympathomimetic amines is directly related to their stability in an alkaline aminophylline solution (pH 8.6–9.0) for injection. Little information has appeared regarding the stability of these sympathomimetic amines (I–VI) when combined with aminophylline solutions. This may be due in part to the lack of a rapid, specific, stability-indicating assay for I–VI in the presence of their degradation products and theophylline.

Over the years, catecholamines (I–V) have been assayed using UV or spectrofluorometric methods. However, the poor specificity of these commonly employed methods for I–V in the presence of quinone degradation products raises doubts about their validity.

One significant advantage of chromatographic assays is that they are stability-indicating (2, 3). This is of importance when determining the rate and extent of degradation of potent, potentially lifesaving pharmacologic agents such as sympathomimetic drugs which are diluted in parenteral solutions for clinical administration, sometimes in combination with other drugs.

Several HPLC procedures for the assay of catecholamines and related drugs or metabolites have employed UV detection (4–7), fluorescence detection with precolumn derivatization (8), or electrochemical detection (9–11). Finally, other less expedient chromatographic procedures have been applied to the analysis of some of these drugs (12, 13). Apparently, neither the HPLC assay for terbutaline and methyldopate, nor the use of fluorometric detection requiring no derivatization has been reported for the HPLC analysis of VI or the catecholamines (I–V) in solutions intended clinically for injection. The present report describes a rapid, sensitive, and stability-indicating method for I–VI in the presence of aminophylline and the superiority of fluorometric over UV detection.

Table I—Conditions for the High-Performance Liquid Chromatographic Assay of Terbutaline and Five Catecholamines in 5% Dextrose in Water Injection in the Absence and Presence of Aminophylline

Drug	Methanol in Eluant, % (v/v)	Fluorometer Sensitivity[a]	Volume Injected, μl
Dopamine Hydrochloride (I)	25	2 × 0.3	20
Epinephrine Hydrochloride (II)	25	4 × 3	20
Isoproterenol Hydrochloride (III)	35	4 × 10	20
Methyldopate Hydrochloride (IV)	35	4 × 1	15
Norepinephrine Bitartrate (V)	15	4 × 10	15
Terbutaline Sulfate (VI)	35	4 × 10	40
Aminophylline (VII)	—	—	—

[a] Fluorescence detector P.M. gain × sensitivity range. Maximum sensitivity = 4 × 10.

EXPERIMENTAL

Apparatus—A high-pressure liquid chromatograph[1] equipped with a septumless injector[2] and a variable wavelength spectrofluorometric[3] or a variable wavelength UV detector[4] was employed in conjunction with a microparticulate C_{18} column[5] for separation.

Reagents—All chemicals and reagents were analytical grade unless otherwise indicated. Dopamine hydrochloride[6], epinephrine hydrochloride[7], isoproterenol hydrochloride[8], methyldopate hydrochloride[9], norepinephrine bitartrate[7,8], terbutaline sulfate[10], aminophylline[7], and glass-distilled HPLC grade methanol[11] were used without further purification. Sodium heptanesulfonate[12] was recrystallized from an acetone–water mixture.

[1] M-6000A solvent delivery system, Waters Associates, Milford, Mass.
[2] U6K injector, Waters Associates, Milford, Mass.
[3] Model 204A Spectrofluorometer, Perkin-Elmer, Norwalk, Conn.
[4] Model LC-55 Spectrophotometer, Perkin-Elmer, Norwalk, Conn.
[5] Catalog No. 27324, Waters Associates, Milford, Mass.
[6] Arnar-Stone, McGaw Park, Ill.
[7] Sigma Chemical, St. Louis, Mo.
[8] Sterling-Winthrop, Rensselaer, N.Y.
[9] Merck, Sharp and Dohme, West Point, Pa.
[10] Ciba-Geigy, Summit, N.J.
[11] Fisher Scientific Co., Fairlawn, N.J.
[12] Eastman Chemicals, Rochester, N.Y.

0022-3549/82/0800-0956$01.00/0

Table II—Retention Time and Analysis of the Catecholamines and Terbutaline in Known Mixtures

Drug		t_R[a] min	t_R, min[a] aminophylline	Concentration, mg/liter Added	Concentration, mg/liter Found ± CL[b]	Error, %
I	(80–900)[c]	4.08	ND[d]	800.0	802.53 ± 7.09	0.32
II	(0.4–4.2)[c]	4.13	3.50	4.0	3.98 ± 0.02	0.50
III	(0.2–2.4)[c]	4.33	3.17	2.0	2.02 ± 0.06	1.0
IV	(120–1100)[c]	4.50	2.75	1000.0	996.92 ± 5.30	0.31
V	(0.8–8.5)[c]	4.75	5.33	8.0	8.05 ± 0.09	0.63
VI	(0.4–4.2)[c]	5.33	2.75	4.0	3.96 ± 0.05	1.0

[a] Flow rate = 1.6 ml/min. t_R for aminophylline is for theophylline. [b] 95% confidence limits. [c] Concentration range (μg/ml) used for preparing standard curves. [d] Not detected, sensitivity too low.

Analytical Procedure—Standard solutions (Table I) of each drug were freshly prepared by diluting a stock solution with 5% dextrose in water for injection, USP[13]. The mobile phase was 0.35 *M* acetic acid and 0.005 *M* sodium heptanesulfonate in different concentrations of methanol (Table II). The flow rate was 1.6–2.0 ml/min at ambient temperature. The injection volumes for the standard or sample solution are reported in Table II. The catecholamines (I–V) were detected using an excitation wavelength (λ_{ex}) of 285 nm and an emission wavelength (λ_{em}) of 315 nm. The values of λ_{ex} and λ_{em} for terbutaline were 280 and 310 nm, respectively. The UV detector was operated at 280 nm. The concentrations of I–VI were determined from their respective peak areas by comparison to a standard curve. Peak areas were calculated from the product of peak height times half-height peak widths. Good reproducibility was obtained without the use of an internal standard.

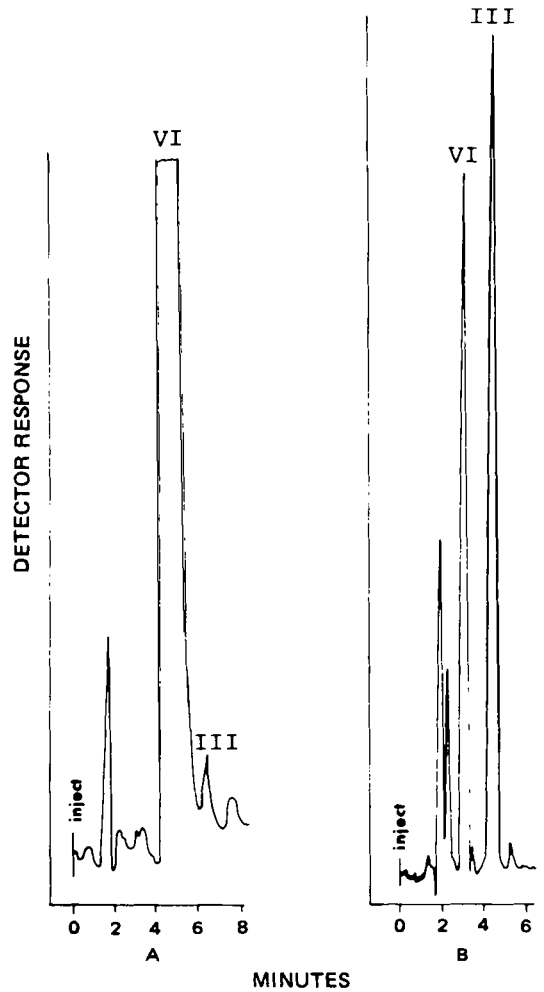

Figure 1—*(A) Chromatogram of isoproterenol hydrochloride (III), 2 μg/ml and aminophylline (VII), 500 μg/ml. UV detector = 280 nm, flow rate = 1.6 ml/min, 0.005* M *1-heptanesulfonate sodium and 0.35* M *acetic acid in 20% (v/v) methanol. The recorder was set at 1 mV; detector response = 0.004 aufs, and injection volume = 20 μl. (B) Chromatogram of isoproterenol hydrochloride (III), 2 μg/ml and aminophylline (VII), 500 μg/ml. Fluorometric detector (λ_{ex} = 285 nm, λ_{em} = 315 nm), flow rate = 1.6 ml/min, 0.005* M *1-heptanesulfonate sodium and 0.35* M *acetic acid in 35% (v/v) methanol. The recorder was set at 10 mV, and a chart speed of 0.5 cm/min; detector sensitivity and injection volume are given in Table I.*

[13] Abbott Laboratories, North Chicago, Ill.

RESULTS

The chromatogram peaks attributed to aminophylline actually represent theophylline, because at pH 2.4–3.0 of the mobile phase, aminophylline dissociates into theophylline and the ethylenediammonium ion. The high concentration and strong UV absorbance of theophylline makes the accurate HPLC analysis for dilute solutions of II, III, V, and VI difficult and poorly reproducible with a UV detector. Increasing the injection volume and/or retention time produced unsatisfactory results. The advantage of fluorescence detection in this study was to provide decreased response to theophylline concurrently with increased sensitivity and selectivity towards II, III, V, and VI without pre- or postcolumn derivatization. A typical chromatogram illustrating the advantage of using fluorescence detection is shown for III in Fig. 1. For I and IV, fluorescence detection offered no advantage over UV detection because of their much higher concentrations, relative to II, III, V, and VI (Table III). Theophylline may not be detectable when using the fluorescence detector at its lower sensitivity, which was the case for dopamine. The sensitivity of the fluorescent method permits quantitation of II, III, V, and VI to <0.1 μg/ml. The methanol concentration in the mobile phase needed to achieve the desired separation for I–VI from aminophylline is given in Table I, and their respective retention times, which are the mean of four replicate determinations, are reported in Table II. Standard curves of eight drug concentrations *versus* their peak height areas were constructed from triplicate injections for each drug in Table I. The linear plots showed that the peak height area of each drug was directly related to its concentration.

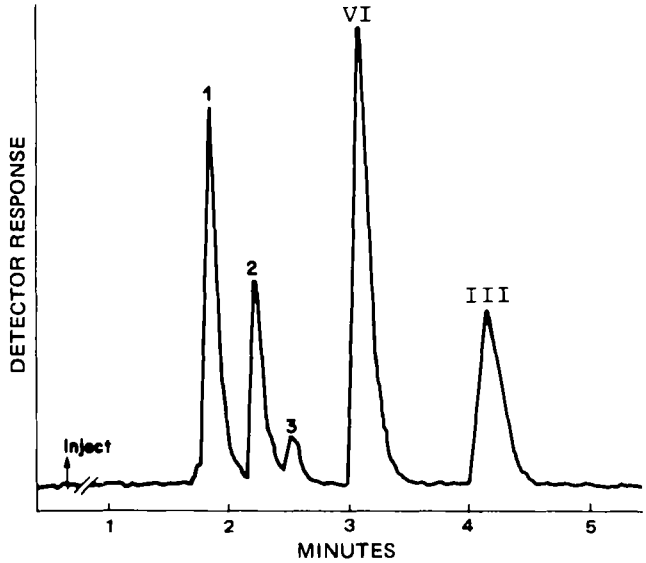

Figure 2—*Chromatogram of isoproterenol hydrochloride (III), 2 μg/ml and aminophylline (VII), 500 μg/ml, after 17 hr of exposure to fluorescent light. Fluorometric detector (λ_{ex} = 285 nm, λ_{em} = 315 nm), flow rate 1.6 ml/min 0.005* M *1-heptanesulfonate sodium and 0.35* M *acetic acid in 35% (v/v) methanol. The recorder was set at 10 mV and a chart speed of 4 cm/min; detector sensitivity and injection volume are given in Table I. Peaks 1 and 3 are degradation products, and Peak 2 is unidentified.*

The correlation coefficient for the standard curves over the concentration range used in this study was >0.997 (n = 22 or 25). The analysis of the concentrations of I–VI in the experimental samples compared favorably with the theoretical values of the drug formulations (Table II) with <1% error.

The described HPLC assay has been successfully applied as a stability-indicating method for determining the rate and percentage of autoxidation of commercially supplied injectable solutions of I–VI which had been diluted in 5% dextrose in water injection, with and without aminophylline (500 μg/ml) (14). The chromatographic separation of I–VI from their autoxidation products is illustrated in Fig. 2, with III, which has undergone 70% decomposition after being exposed to 17 hr of fluorescent light. Peak 1 represents the principal degradation product and Peak 3 a minor product. The unidentified Peak 2 is present in unexposed samples and does not appear to change after exposure to light. Similarly, the degradation products of I, II, and IV–VI elude within 2–3 min following injection. Figure 1B represents III before exposure to fluorescent light and decomposition has already commenced. This method has demonstrated the capability of separating degradation products from the analysis of pure I–VI.

The application of ion-pair HPLC for the analysis of formulations containing VI or IV has not been previously described. This reported method is applicable for the accurate determination of these drugs, using either UV or fluorescence detectors.

DISCUSSION

Various analytical methods have been used in stability studies for catecholamines but suffered from their inability to quantitate or separate degradation products from the parent substance. The combination of fluorescence detection with reversed-phase ion-pair HPLC yields a rapid and selective method for the quantitative separation and determination of I–VI in the presence of their autoxidation products and pharmaceutical adjuvants. The lowest concentration at which the sympathomimetic amines used in this study could be detected was 0.1 μg/ml.

The described method is applicable for the content uniformity and quality control of products containing VI and IV.

Despite the widespread use of UV detection in HPLC assays, the sensitive fluorescence measurement for low concentrations of sympathomimetic amines in pharmaceutical preparations has not been extensively reported except for their clinical analysis in biological fluids. The sensitive measurement of I–VI will allow studies of the purity of dosage forms and investigation of their degradation kinetics and pharmacokinetics. Without the use of a fluorescence detector, II, III, V, or VI could not be quantitatively measured in the presence of aminophylline. Also, the presence of pharmaceutical adjuvants should not interfere, because of their poor fluorescent properties.

REFERENCES

(1) A. Lund, *Acta Pharmacol. Toxicol.*, **5**, 75 (1949).

(2) J. B. Johnson and V. S. Venturella, *Bull. Parenter. Drug Assoc.*, **25**, 239 (1971).

(3) L. Chafetz, *J. Pharm. Sci.*, **60**, 335 (1971).

(4) A. G. Ghanekar and V. D. Gupta, *ibid.*, **67**, 1247 (1978).

(5) K. E. Rasmussen, F. Tonnesen, and S. N. Rasmussen, *Medd. Nor. Farm. Selsk.*, **39**, 128 (1977).

(6) G. A. Scratchley, A. N. Mosaud, S. J. Stohs, and D. W. Wingard, *J. Chromatogr.*, **169**, 313 (1979).

(7) B. A. Persson and B. L. Karger, *J. Chromatogr. Sci.*, **12**, 521 (1974).

(8) T. P. Davis, C. W. Gehrke, C. W. Gehrke, Jr., T. D. Cunningham, K. C. Kuo, K. O. Gerhardt, H. D. Johnson, and C. H. Williams, *Clin. Chem.*, **24**, 1317 (1978).

(9) G. M. Kochak and W. D. Mason, *J. Pharm. Sci.*, **69**, 897 (1980).

(10) T. P. Moyer and N. S. Jiang, *J. Chromatogr.*, **153**, 365 (1978).

(11) T. M. Kenyhercz and P. T. Kissinger, *J. Pharm. Sci.*, **67**, 112 (1978).

(12) J. R. Watson and R. C. Lawrence, *ibid.*, **66**, 560 (1977).

(13) F. N. Minard and D. S. Grant, *Biochem. Med.*, **6**, 46 (1972).

(14) D. W. Newton, E. Y. Y. Fung, and D. A. Williams, *Am. J. Hosp. Pharm.*, **38**, 1314 (1981).

ACKNOWLEDGMENTS

Abstracted in part from a thesis submitted by Esther Yin Yee Fung to the Graduate Council of the Massachusetts College of Pharmacy and Allied Health Sciences in partial fulfillment of the M.S. degree requirements.

The authors thank Arnar-Stone Laboratories, Ciba Pharmaceutical, Merck, Sharp and Dohme, and Sterling-Winthrop for their donation of analytical quality drug samples, and Mrs. Gail Williams for typing the manuscript.

Enhanced Entrapment of a Quaternary Ammonium Compound in Liposomes by Ion-Pairing

MICHAEL JAY ˣ and GEORGE A. DIGENIS

Received September 9, 1981, from the *Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Kentucky, Lexington, KY 40536.* Accepted for publication November 10, 1981.

Abstract □ The encapsulation of a quaternary ammonium compound by multilamellar liposomes was enhanced by formation of ion-pairs with a counterion. Thus, [^{14}C]methantheline bromide was synthesized and paired with a 25 M excess of trichloroacetate. Under these conditions, the amount of radioactivity entrapped by phosphatidylcholine liposomes was three times greater than when no trichloroacetate was present. The increased liposomal loading was probably due to the solubilization of the ion-pair in the lipid membrane of the liposome.

Keyphrases □ Liposomes—quaternary ammonium compound, entrapment, ion pairing □ Ion-pairing—quaternary ammonium compound, entrapment, liposomes □ Ammonium—quaternary compound, entrapment in liposomes, ion-pairing

Liposomes are microscopic lipid vesicles originally used to study the structure and function of biological membranes (1). In recent years, liposomes have demonstrated potential as carriers and transporters of biologically active compounds (2). A wide variety of compounds have been encapsulated in liposomes, ranging from RNA (3) and insulin (4) to small molecules like histamine (5). Liposomes have been administered intravenously and orally (6), and a recent report describes the delivery of drugs *via* liposomes by the topical route (7). Liposomes elicit no immunological or toxicological responses and are completely biodegradable. For these reasons, liposomes have been viewed as an attractive mechanism for drug delivery, especially for biologically unstable compounds, but not without certain drawbacks. Special techniques have been developed in an attempt to direct liposomes to specific targets or organ systems by immunological methods (8) and by producing pH-sensitive liposomes (9). Polymerized

0022-3549/82/0800-0958$01.00/0