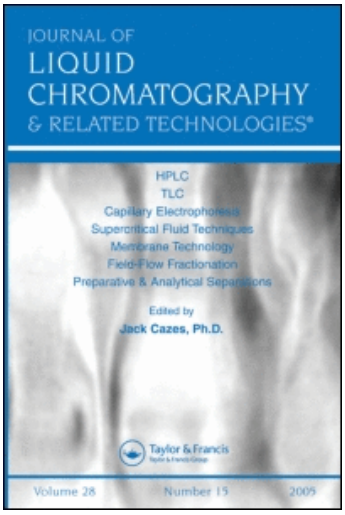

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Dimitri C. Tsilifonis[a]; Karen Wilk[a]; Robert Reisch Jr.[a]; Robert E. Daly[a]
[a] Analytical Development Laboratories Parke-Davis Division The Warner Lambert Company 170, New Jersey

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF VERAPAMIL HYDROCHLORIDE IN DOSAGE FORMS

Dimitri C. Tsilifonis,* Karen Wilk,
Robert Reisch, Jr., and Robert E. Daly
Analytical Development Laboratories
Parke-Davis Division
The Warner Lambert Company
170 Tabor Road
Morris Plains, New Jersey 07950

ABSTRACT

A stability indicating high-performance liquid chromatographic (HPLC) method for determining verapamil hydrochloride in dosage forms is described. The assay affords baseline separation of the drug from its synthesis impurities and from photolytic degradation products, as well as from formulation excipients. The drug was extracted in 0.05 **N** hydrochloric acid, chromatographed on a C_{18} reverse-phase column, eluted with methanol-water-acetic acid-triethylamine (55:44:1:0.1) and the effluent was detected at 280 nm. Linearity studies were carried out using peak height or peak area measurements and the detector response to the concentration of verapamil hydrochloride was confirmed. Excellent interlaboratory precision and recovery data were obtained by the spiked placebo method. This procedure was rapid and selective for the assay of the cardiotonic drug. Application of the method for the assay of verapamil hydrochloride in representative dosage forms is described.

*Author to whom correspondence and reprint requests should be addressed.

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INTRODUCTION

Verapamil hydrochloride, 5-[(3,4-dimethoxyphenethyl) methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile monohydrochloride, a calcium antagonist, is an important therapeutic agent for the management of angina and atrial tachyarrhythmias (1,2). The drug is marketed as 40 mg, 80 mg and 120 mg tablets and 0.25% I.V. solution. Dosage forms of the drug are currently assayed by the non-specific UV spectrophotometric procedure described in the British Pharmacopoeia (3). This method cannot distinguish verapamil from likely photolytic degradation products and/or process contaminants since these compounds exhibit absorption at the wavelength of interest. Spectrophotometry correlated with TLC was employed to test for drug decomposition or chemical incompatibilities in solution (4,5). McAllister developed a spectrofluorometric procedure for the determination of verapamil in biological specimens (6). GLC, HPLC and TLC for the detection of the drug and separation from related compounds have been reported (7). A literature search for analytical methodology revealed that procedures based on GLC (8) and HPLC (9-13) were employed to determine verapamil in biological fluids. However, a selective assay for verapamil hydrochloride in dosage forms has not been published. This manuscript describes the development of a simple, rapid, accurate and precise HPLC assay for the coronary vasodilator in dosage forms. The method is stability indicating for verapamil hydrochloride in the presence of possible photolysis products and separates the drug from synthesis impurities, if present.

MATERIALS AND METHODS

Apparatus - The liquid chromatograph included a pump[1], an automatic injector[2] with a fixed volume 20 µl loop, a stainless steel

1 Waters Associates Model 6000A

2 Perkin-Elmer LC-420

4 mm x 30 cm column packed with octadecylsilane on silica[3] (10 µm particles), a variable wavelength detector[4] set at 280 nm and a computing integrator[5]. The attenuation and chart speed on the integrator were set at AT = 16 and 0.5 cm per minute, respectively. The temperature was ambient and the flow rate of the mobile phase was 1.2 ml per minute. Photolysis studies were carried out in a Model 50 Sunlighter[6].

Materials - All reagents and solvents were of analytical grade and used as received. Verapamil hydrochloride BP[7] (I) was 99.9% pure. The synthesis intermediates, 3,4-dimethoxy-phenyl acetonitrile (II), 2-(3,4-dimethoxyphenyl)-3-methylbutyronitrile (III), N-methyl-(3, 4-dimethoxyphenyl)ethylamine (IV) and N-3-chloropropyl-N-methyl-(3,4-dimethoxyphenyl) ethylamine hydrochloride (V) were obtained in house[8].

Mobile phase - Fifty five milliliters of methanol was mixed with 44 ml of water, 1 ml of glacial acetic acid and 0.1 ml of triethylamine, and filtered through a 0.5 µm solvent resistant filter[9].

NOTE: Low-actinic glassware was used throughout the procedure except if indicated otherwise.

System suitability - When 20 µl of a solution containing approximately 0.16 mg of I and approximately 0.08 mg of II in 1-ml of mobile phase is injected into the chromatograph using the parameters described under **Apparatus**, the resolution factor (R) should be not less than 1.5 and the number of theoretical plates (N) should be not

3 µ-Bondapak C_{18}, Waters Associates

4 Perkin-Elmer LC-75

5 Spectra Physics SP-4100

6 Mercury vapor lamp, G.E. RS-4; Test Lab Apparatus Company, P.O. Box 55, Milford, New Hampshire 03055

7 Recordati, Industrie Chimica e Pharmaceutica, Milano, Italy.

8 Warner-Lambert/Parke-Davis Pharmaceutical Research Division, Holland, Michigan.

9 Millipore FH, 0.5 µm

less than 2370. Six successive injections of the Standard preparation should provide a relative standard deviation that does not exceed 2%. A plot of detector response versus concentration of I (~ 90 - 300 μg/ml) results in a straight line. If system suitability is not attained, adjust the chromatographic parameters, cleaning or replacing the column, if necessary, to obtain it.

Standard preparation - About 25 mg of I was weighed accurately, transferred to a 25 ml volumetric flask and dissolved in water. The solution was diluted to volume, and 4.0 ml of this solution was diluted to 25 ml with the mobile phase, in a volumetric flask, to obtain a concentration of ~ 0.16 mg/ml.

ASSAY PREPARATION

a) Solid state stability - (bulk drug under thermal and light stress). Approximately 1 g of I was transferred to the appropriate container and stored under the experimental conditions described in Table I. After the specified time, any physical changes were observed, the powder was mixed and ~ 25 mg was accurately weighed into a 25 ml volumetric flask. The solution was diluted to volume, and 4.0 ml of this solution was diluted to 25 ml with the mobile phase in a volumetric flask to obtain a concentration of ~ 0.16 mg/ml.

b) Solution phase stability - (thermal stress). Solutions of I were prepared in 0.1 N hydrochloric acid, water and 0.1 N potassium hydroxide in methanol, respectively, at a concentration of ~ 1 mg/ml. A volume of exactly 25.0 ml of the appropriate solution was transferred to a 250 ml round bottom flask and refluxed for ~ 4 hours. The contents were allowed to cool to room temperature, transferred quantitatively to a 50 ml volumetric flask and diluted to volume with the corresponding solvent. A volume of exactly 8.0 ml was then diluted to 25.0 ml with the mobile phase.

TABLE I

Effect of Stress Conditions on the Stability of Verapamil Hydrochloride (Bulk Drug)

Stress Condition	Appearance	Assay (%) Peak Area	Assay (%) Peak Height
100°C One week in dark	Off white powder	100.6	99.8
SUNLIGHTER (open amber glass vial, 24 hours)	White powder (No change)	100.2	100.9
SUNLIGHTER (closed amber glass vial, 24 hours)	White powder (No change)	101.4	102.0
SUNLIGHTER (closed clear glass vial, 24 hours	White powder (No change)	100.9	101.8
SUNLIGHTER (open clear glass vial, 24 hours)	White powder (No change)	100.8	101.8

c) Solution phase stability - (light stress). The photoreactions of I at a concentration of ~ 1 mg/ml in 0.1 **N** and 0.01 **N** hydrochloric acid and 0.1 **M** acetate buffer solutions pH 3 to pH 7 were carried out on exactly 25.0 ml volumes contained in transparent stoppered 25 ml volumetric flasks. The UV source (290 - 360 nm) was mounted above the samples and the cabinet temperature was maintained at 37° ± 0.1 C. (Control samples contained in 25 ml low actinic volumetric flasks were exposed to identical experimental conditions). After irradiation for ~ 24

hours, the samples were removed from the cabinet, allowed to reach room temperature and observations were made for any physical changes. A volume of exactly 4.0 ml from each sample was diluted to 25.0 ml in a volumetric flask with the mobile phase.

d) Tablets - Not less than 20 tablets were weighed and pulverized. A portion of the powder equivalent to about 80 mg of I was weighed, transferred to a 200 ml volumetric flask, suspended in ~ 100 ml of 0.05 **N** hydrochloric acid and heated at steam bath temperature for ~ 10 min. The suspension, while still warm, was mixed on a mechanical shaker for ~ 10 min, allowed to cool to room temperature, diluted to the mark with the solvent and mixed. A portion of the suspension was centrifuged at 2000 r.p.m. for ~ 5 min. and a 10.0 ml volume of the clear supernatant was pipeted to a 25 ml volumetric flask and diluted to the mark with the mobile phase.

e) I.V. solution - An exact volume of the solution equivalent to ~ 4 mg of I was pipeted to a 25 ml volumetric flask and diluted to the mark with the mobile phase.

Procedure - The mobile phase was pumped through the column at a flow rate of 1.2 ml/min. until a stable baseline was obtained. A 20 µl volume of the assay preparation was injected by means of a precise loop injector and the chromatogram was allowed to develop for ~ 15 min. The procedure was repeated using the standard preparation. The peak corresponding to I eluted at ~ 6 min. The quantity of verapamil hydrochloride in the bulk drug under stress conditions, in tablets and injections was calculated from 25 C (R_u/R_s)1/W), 80 C (R_u/R_s)1/W), and 4 C (R_u/R_s)1/V), respectively, where C is the exact amount in mg/ml of I taken in the standard

preparation, R_u and R_s are the peak heights for I obtained from the chromatograms of the assay preparation and the standard preparation, respectively, W is the weight, in mg, and V is the volume, in ml, of sample taken for analysis.

RESULTS AND DISCUSSION

System suitability - Baseline separation of the peaks corresponding to I and II was obtained. The elution times were 6.1 and 3.6 min., respectively. The resolution factor (R) and the theoretical plates of the column (N) calculated according to the procedure described in the USP XX (14), were found to be 6.4 and 3386, respectively. The precision of six replicate injections of the standard preparation was $\pm$ 0.52%.

Linearity - The peak height of verapamil hydrochloride in the chromatogram varied linearly with drug concentration. A typical standard curve obtained by assaying samples with 0, 91, 137, 183, 228, and 274 µg/ml had a linear regression coefficient of 0.9999.

Recovery - The recovery of verapamil hydrochloride was determined by adding known amounts of I to placebo tablets and assaying by the described procedure. An average recovery of 100.0% (80 mg tablets) and 101.0% (120 mg tablets) of the added amount, with relative standard deviations of 0.25% and 0.80%, respectively, was obtained. The method was demonstrated to be linear at 80% and 120% of label claim. These data are presented in Table II.

In each case a placebo blank was also run which showed no interfering peaks.

Sensitivity - A linear relationship was obtained between peak height and concentration of I at levels of ~ 6 - 23 µg/ml (integrator sensitivity was increased to AT = 1). A 2 µg/ml standard preparation in the mobile phase should produce a peak ~ 25 mm in height under the experimental conditions. This limit of detection can be increased by using a larger volume injection loop.

TABLE II

Recovery of I at 80%, 100% and 120% Label Claim[a]

% of Label Claim (Added)	Percent Recovered	
	80 mg Tablets	120 mg Tablets
80	100.6	99.0
80	98.9	100.5
100	100.2	101.1
100	100.0	101.7
100	99.6	99.4
100	100.3	101.8
100	99.7	101.2
100	100.0	100.6
120	98.7	99.2
120	98.4	99.8
Average Recovery ± RSD	100.0 ± 0.25	101.0 ± 0.80

[a] Recoveries at 100% label claim were used to calculate the average and RSD (%)

Assay of verapamil hydrochloride formulations - The method was tested by assaying five lots of tablets and an injection formulation. Six samples were assayed from each lot according to the described procedure. The results (Table III) indicate that the method gives accurate results for the formulations tested, since there is good agreement with the label claim amount and in addition satisfactory precision for all cases.

Separation of verapamil from synthesis precursors - The synthesis impurities most likely to be present are the intermediates (II), (III), (IV) and (V). A chromatogram was run under the described conditions using 172 μg (I), 11.6 μg (II), 9.3 μg (III), 1.3 μg (IV) and 9.4 μg (V) per ml in mobile phase. All four synthesis impurities were resolved from verapamil as shown in Figure 1. The five peaks in the chromatogram at retention volumes of 2.5, 2.8, 3.6, 6.1 and 8.3 min correspond to (IV), (V), (II), (I) and (III), respectively. Since the HPLC

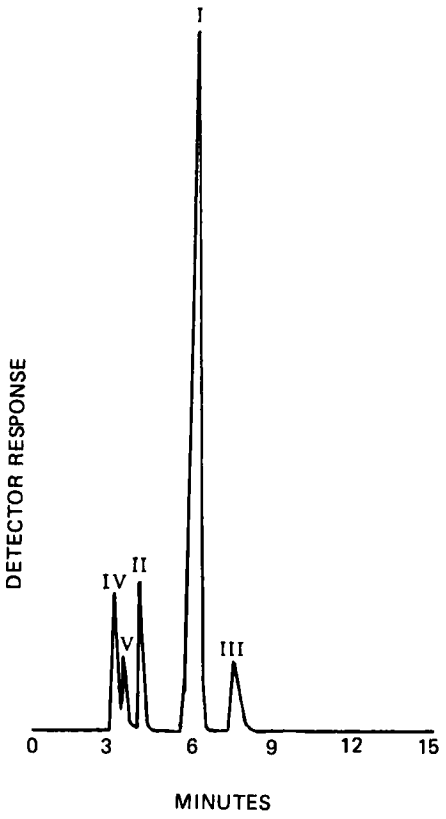

FIGURE 1 - Liquid chromatogram of verapamil hydrochloride and synthesis impurities. Key: (I) verapamil hydrochloride; (II) 3,4-dimethoxy-phenyl acetonitrile; (III) 2-(3,4-dimethoxyphenyl)-3-methylbuty-ronitrile; (IV) N-methyl-(3,4-dimethoxyphenyl)ethylamine; (V) N-3-chloropropyl-N-methyl-(3,4-dimethoxyphenyl)ethylamine hydrochloride

system separates all the synthesis impurities it can also be used for the analysis of the bulk drug.

Bulk drug stability - The HPLC chromatograms obtained for (I) placed at 100°C in an open vial and protected from light for one week or in simulated sunlight in open or closed clear glass or amber glass vials for 24 hours, exhibited only the main peak. Assay results which ranged from 100.2 to 101.4% are provided in Table I.

TABLE III

Sextuplicate Assays of Verapamil Hydrochloride Formulations

Dosage Form	Declared	Found[c]	± Sd	% Rsd
Conventional tablets	80 mg	79.9	0.90	1.10
Conventional tablets	120 mg	119.6	0.85	0.71
Conventional tablets[b]	80 mg	80.0	0.71	0.89
Conventional tablets[b]	120 mg	120.7	0.85	0.70
Sustained-Action tablets	160 mg	159.1	0.57	0.36
Ampoules[b]	2.5 mg/ml	2.485	0.04	0.84

[b]Isoptin[R]; Knoll Pharm. Co.
[c]Average of six trials

Solution stability - Acidic, basic and neutral reflux studies of I showed no degradation. The HPLC chromatogram in each case exhibited only the main peak at ~ 6 min. All three solutions showed no physical change from their initial state and the recovery of the drug was quantitative, indicating the stability of verapamil hydrochloride under severe thermal conditions. The most prominent drug degradation occured in the solutions contained in transparent flasks and exposed to simulated sunlight for 24 hours (1 hour in the sunlighter is equivalent to ~ 2 days in natural sunlight). Irradiation of solutions of I in 0.1 N and 0.01 N hydrochloric acid and in buffer solutions of pH 3 to pH 7 resulted in ~ 30% decomposition (based on recovered I) in all solutions. Besides the main peak at the elution time of ~ 6.1 min, additional peaks appeared in the chromatograms. Peaks with a detector response greater than 1% of the total peak area appeared at eluting times of 2.7, 3.3, 4.0 and 4.7 min. in each chromatogram thus indicating that the photolysis process under the experimental conditions produced the same degradation products regardless of the hydrogen ion concentration of the solutions studied. A typical chromatogram of a solution of verapamil hydrochloride at pH 6 exposed to UV radiation for 24 hours is shown in Figure 2. It

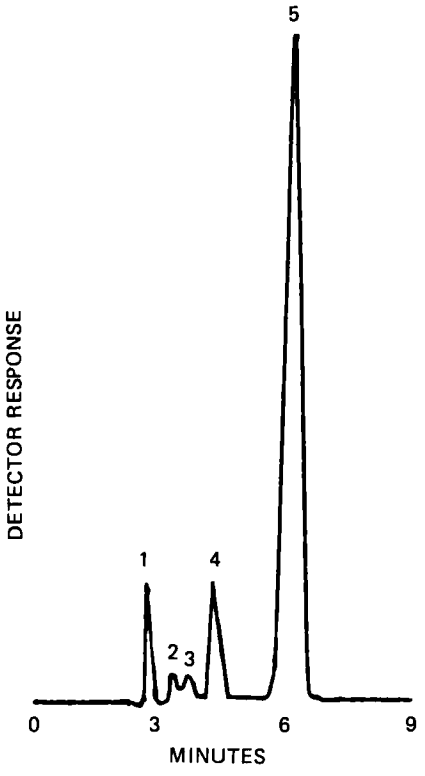

FIGURE 2 - Liquid chromatogram of photodecomposed verapamil hydrochloride. Key: 1–4, unidentified peaks; and 5, verapamil hydrochloride

exhibits the four unidentified peaks, along with the peak for undegraded drug. The photodegraded solutions changed in appearance from their initial state. Solutions in 0.1 N and 0.01 N hydrochloric acid remained clear but developed a light yellow coloration. The solutions in pH 3 to pH 7 remained colorless but developed haziness which progressively intensified with increase in pH. The control samples, contained in amber flasks, showed no physical change from their original state, their respective HPLC chromatograms exhibited only the main peak and assayed ~ 100% of initial drug. No attempt

was made to postulate a photolytic degradation mechanism and/or identify the decomposition products.

Selectivity of the Assay - Evidence of the validity of the HPLC procedure as stability indicating was obtained by spiking a photolytically degraded sample with a known quantity of verapamil hydrochloride and obtaining quantitative recovery of the drug by applying the procedure described in this paper.

In summary, verapamil hydrochloride shows excellent stability in its solid state under thermal and light stress. However, solutions of the drug, although thermally stable when protected from light, undergo photodecomposition when exposed to UV radiation. The HPLC method described here was designed to quantitate verapamil hydrochloride only in its dosage forms. It is stability indicating in so far as the drug is separated from its photolysis products and specific, since it provides adequate separation from its synthesis impurities.

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