High-Performance Liquid Chromatography Method for Assay of Diltiazem Hydrochloride and Its Related Compounds in Bulk Drug and Finished Tablets

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Received May 9, 1988, from the *Bureau of Drug Research, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A OL2, Canada.* Accepted for publication September 26, 1988.

Abstract □ The method provides for the resolution of *trans*-diltiazem and seven known and several unknown related compounds from diltiazem HCl. Minimum detectable amounts were <0.1%, except for an intermediate which originates early in the synthetic process, for which the sensitivity is ~2%. The relative standard deviation of the assay procedure is 0.15%. Total related compounds in four bulk drug and four tablet samples were <0.25%. The specific rotation of four samples of diltiazem HCl analyzed in duplicate was between +112 and +114°. The UV absorption spectra of all compounds exhibited two maxima, one between 203 and 213 nm and the other between 230 and 244 nm.

This report describes an HPLC procedure for the determination of diltiazem HCl and its related compounds in bulk drug and finished tablets. Diltiazem HCl [(2*S*-*cis*)-3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one hydrochloride] is an important coronary vasodilator, marketed as Apo-Diltiaz (Apotex) in Canada and as Cardizem in the United States (Marion) and Canada (Nordic).

A number of synthetic intermediates[1–3] and degradation products[4,5] of diltiazem HCl are listed in Table I. The HPLC methods for the determination of the drug and related compound 7,[6] and for the drug and its metabolites in plasma,[5–9] have been reported. The optical isomers of diltiazem HCl have also been determined by HPLC.[10,11]

A method suitable for use as a bulk drug purity and potency standard, which is the objective of this work, should provide for resolution of drug-related compounds, including *d*- and *l*-trans-diltiazem HCl, from the drug. None of the methods in the literature has been shown to achieve this resolution. The enantiomeric purity of the drug, *d*-*cis*-diltiazem HCl, is readily determined by measurement of the specific rotation. The diltiazem-related compounds listed in Table I were available for method development. Of these, **1**–**6**, **8**, and **10** are potential reaction intermediates or byproducts, **7** may be an intermediate or a degradation product, **11** may be a byproduct or a degradation product, and **12** is a degradation product.

Table I—Chemical Names of Diltiazem Related Compounds

Compound	Chemical Name
1	*trans*-3-(4-Methoxyphenyl)-oxiranecarboxylic acid, methyl ester
2	(±)-(*R**,*R**)-β-[(2-Aminophenyl)thio]-α-hydroxy-4-methoxybenzenepropanoic acid, methyl ester
3	(±)-(*R**,*R**)-β-[(2-Aminophenyl)thio]-α-hydroxy-4-methoxybenzenepropanoic acid
4	(±)-(*R**,*R**)-β-[(2-Aminophenyl)thio]-α-hydroxy-4-methoxybenzenepropanoic acid; compound with *R*(+)-1-phenylethylamine (1:1)
5	*S*-(*R**,*R**)-β-[(2-Aminophenyl)-thio]-α-hydroxy-4-methoxybenzenepropanoic acid
6	(2*S*-*cis*)-2,3-Dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one
7	(2*S*-*cis*)-5-[2-(Dimethylamino)ethyl]-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one
8	Diltiazem: (2*S*-*cis*)-3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one
9	Diltiazem hydrochloride
10	*trans*-Diltiazem hydrochloride
11	(2*S*-*cis*)-3-(Acetyloxy)-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one
12	Diltiazem-1-oxide hydrochloride

Experimental Section

Equipment—The liquid chromatograph used was a Varian Vista 5560 equipped with a variable wavelength detector set at 240 nm, a Rheodyne model 7126 10-μL loop injector, a Varian Vista 402 data processor, a Varian 8085 autosampler, and a Waters μ-Bondapak C-18 column (3.9 × 300 mm). Other equipment used was as follows: centrifuge, International Equipment, model K; ultrasonic bath, Mettler, model ME 4.6; UV/VIS spectrophotometer, Varian DMS90 connected to a HP85 computer with plotter and disk drive; autotitrator, Mettler DL 40 RC memotitrator equipped with a 5-mL burette and a glass calomel electrode; polarimeter, Perkin-Elmer, model 241 MC.

Chemicals—Acetonitrile and methanol (Baker, Phillipsburg, NJ), sodium acetate (Fisher, Fair Lawn, NJ), and acetic acid (Caledon, Georgetown, Ontario, Canada) were HPLC grade. 1-(*s*)-(+)-Camphorsulfonic acid (99%; Aldrich, Milwaukee, WI) was reagent grade. Related compounds **1**, **3**, **6**, **7**, and **10** were from ABIC Chemical and Pharmaceutical Industries, Ramat-Gan, Israel. Industria Chimica Profarmaco S.p.a., Milan, Italy, supplied **1**–**7**. The PMR and FTIR spectra of these compounds were determined and found to be consistent with their respective structures.

Method for Related Compounds in Diltiazem HCl Bulk Drug—*Solutions*—The 0.1 M sodium acetate buffer containing 5 mM *d*-camphorsulfonic acid (99%), was adjusted to pH 6.2 with 0.1 M sodium hydroxide. Standard solution I contained 12 μg/mL of **7** and 12 μg/mL of diltiazem HCl reference standard in methanol. The test solution contained ~1.2 mg/mL (accurately known) diltiazem HCl in methanol. The mobile phase, consisting of buffer:methanol:acetonitrile (50:25:25, v/v), was used at a flow rate of ~1.6 mL/min.

System Suitability—All system suitability calculations were based on six 10-μL aliquots of standard solution I. Chromatograms adequate for quantitation by the procedure described below were obtained when the resolution between **7** and diltiazem HCl, calculated by the procedure defined in the USP,[12] was between 3.0 and 6.3, the efficiency of the column calculated on the diltiazem HCl peak[12] was between 4000 and 13,900 plates/m, and the relative standard deviation of the diltiazem response was <7%. This value is higher than those typically quoted because of the low level of drug injected.

0022-3549/89/0300-0243$01.00/0

Column characteristics depended on the particular column and its age. The retention times of 7 and diltiazem HCl were typically 12.5 to 16.0 min and 18.0 to 25.0 min, respectively.

Procedure—Aliquots (10 μL) of standard solution I and the test solution were injected separately, and the chromatograph was run for 30 min. The amount of each impurity as a percentage of the total amount of drug was calculated from 100 $(A_u/A_s)(C_s/C_u)$, where A_u is the peak area due to the individual impurity, A_s is the area of the diltiazem HCl peak in standard solution I, and C_u and C_s are the concentrations of diltiazem HCl in the test solution and standard solution I, respectively.

Method for Related Compounds in Diltiazem HCl Tablets—The buffer, standard solution I, mobile phase, system suitability, and procedure were as described above in the *Method for Related Compounds in Diltiazem HCl.*

Test solution—Twenty tablets were powdered and quantitatively transferred to a 500-mL volumetric flask. Methanol (200 mL) was added and the flask was sonicated for 1 h. The flask was cooled to room temperature, and methanol was added to volume and mixed. Two 25-mL aliquots were transferred to individual 70-mL centrifuge tubes and the tubes were centrifuged at 3500 rpm for 15 min. If necessary, portions of the clear supernatant were diluted with methanol to obtain a solution having a concentration of ~1.2 mg/mL, based on label claim.

Assay of Diltiazem HCl Bulk Drug and Tablets—The buffer, standard solution I, test solution, mobile phase, and system suitability are as described above under *Methods for Related Compounds.*

Standard Solution II—A solution was prepared to contain 1.2 mg/mL of diltiazem HCl reference standard in methanol.

Procedure—Aliquots (10-μL) of standard solution II and the test solution were injected into the chromatograph and run for 30 min. The percentage of diltiazem HCl was estimated from 100 $(A_u/A_s)(C_s/C_u)$, where A_u and A_s are the areas of the diltiazem HCl peak in the test solution and standard solution II, respectively, and C_u and C_s are the concentrations of diltiazem HCl in the test solution and standard solution II, respectively.

Other Techniques—*Ultraviolet Spectra*—Diltiazem HCl and related compounds dissolved in methanol were scanned from 210 to 340 nm.

Specific Rotation—Specific rotation ($[\alpha]_D^{25°}$) was determined on freshly prepared 1% solutions of diltiazem HCl in water.

Assay of Bulk Drug by Nonaqueous Titration—Bulk drug dissolved in glacial acetic acid was titrated against standardized 0.1 M perchloric acid in glacial acetic acid to an electrochemical end point.

Results and Discussion

A chromatogram showing the resolution of six related compounds from diltiazem HCl is presented in Figure 1. The response of the HPLC system to the related compounds was determined over the range from 0.005 to 5.0% related compounds in diltiazem HCl. A minimum of two weighings with dilutions, all in methanol, were made for each compound. The results were analyzed by linear regression of the peak area response against the weight on column (Table II). The ultraviolet absorbance maxima, the absorbance at 240 nm, and the absorbance relative to diltiazem HCl of the related compounds are listed in Table III.

The linearity of response to diltiazem HCl was determined over the assay concentration range. Solutions of diltiazem HCl in methanol were prepared over the range of 0.4 to 1.6 mg/mL (33 to 133% of the assay concentration, based on injection of 12 μg of diltiazem HCl). Linear regression of the response (area counts) versus weight of diltiazem HCL on column (μg) gave a slope of 5.4×10^5 area counts/μg, an intercept of -1.35×10^4 area counts, and a correlation coefficient (r^2) of 0.999.

The reproducibility of repeat injections, the precision of the system, was determined by making six replicate injections of standard solution II (1.1962 mg/mL methanol) and determining the relative standard deviation of the peak responses. The mean area for the diltiazem HCl peak was 6.09×10^6 counts, with a relative standard deviation of 0.1%. A value of 0.58 has been proposed as suitable for an assay acceptance range of 98.5–101.5%,[13] for duplicate determinations of the drug. The precision of the method was 0.16% (Table IV).

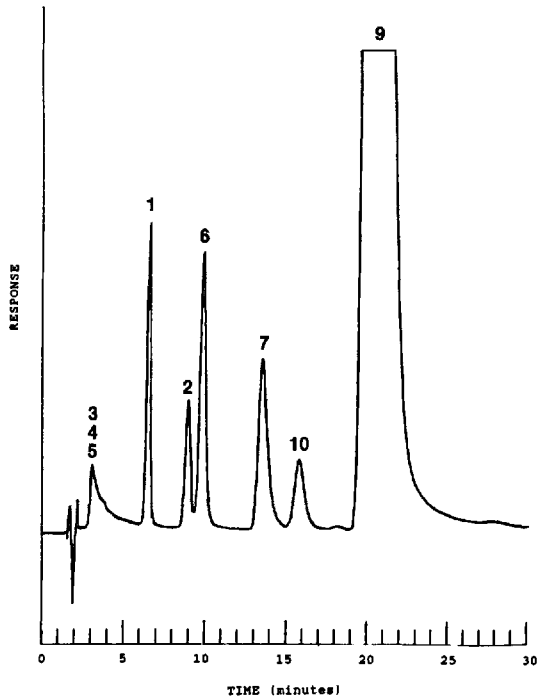

Figure 1—*Chromatogram showing the resolution of related compounds from diltiazem HCl. The compounds and their concentration (mg/mL) in the injected solution (10 μL), with the relative retention times in parentheses, are:* **1**, *0.0116 (0.32);* **2**, *0.0107 (0.43);* **3–5**, *0.0099, 0.0115, 0.0106 (0.15);* **6**, *0.0106 (0.49);* **7**, *0.0137 (0.68);* **10**, *0.0118 (0.81);* **9** *(diltiazem HCl), 1.003 (1.00).*

Table II—Linearity Data for Diltiazem Related Compounds

Compound	Slope[a]	Intercept[b]	r^2[c]	Minimum[d]	RRT[e]
Diltiazem HCl	545	−804	0.999	0.04%	1.00
1	639	601	0.999	0.02%	0.32
2	320	426	0.999	0.04%	0.43
3[f]	590	[g]	0.996	1.7%	0.15
6	894	220	0.999	0.01%	0.49
7	662	−1127	0.999	0.04%	0.68
10	379	−924	0.999	0.08%	0.81

[a] Area counts/ng. [b] Area counts. [c] The square of the correlation coefficient. [d] Minimum amount quantifiable, in percent, based on an injection of 12 μg of diltiazem HCl. [e] Retention times relative to diltiazem HCl at 19.7 min. [f] Compounds **3–5** all have the same retention time and produce a severely tailing peak. [g] The tailing peak produced by **3** resulted in a large negative value for the intercept.

Stability of Solutions—Diltiazem HCl in solution degrades rapidly to **7**. The rate of appearance of **7** from diltiazem HCl in several solvents was determined using the HPLC method described in this paper (Table V). On the basis of the results, methanol was chosen as the solvent for standard and test solutions.

Compounds **2–7** and **10** are relatively stable in methanol solutions. Compound **1** decomposed slowly in methanol to give a product having a retention time of 0.18 relative to diltiazem HCl.

Attempts were made to synthesize **11** and **12**,[4] both degradation products of diltiazem HCl. The reaction to prepare **12** yielded three peaks with retention times between 6 and 9 min, while that to prepare **11** gave a product with the main

Table III—Ultraviolet Absorbance of Diltiazem Related Compounds

Compound	Concentration, μg/mL[a]	Maxima, nm	Absorbance at 240 nm	Relative Absorbance[b]
Diltiazem HCl	10.0	208,240	0.458	1.0
1	11.6	207,240	0.747	1.2
2	10.2	204,233	0.481	1.0
3	10.9	203,230	0.374	0.8
4	10.3	204,230	0.274	0.6
5	10.0	203,231	0.362	0.8
6	9.4	208,240	0.722	1.7
7	10.8	212,244	0.572	1.2
10	10.3	213,244	0.305	0.6

[a] In methanol. [b] Relative to diltiazem HCl at 240 nm.

Table IV—Diltiazem HCl Assay (Potency) Results

Dosage Form	Manufacturer	Lot No.	Diltiazem HCl, %
Bulk drug	A	a	99.2, 99.6
Bulk drug	B	a	98.6, 99.0
Bulk drug	C	a	99.4, 98.9
Bulk drug	C	b	99.6, 100.4
Tablets (30 mg)	D	c	102.5, 102.7, 102.8
Tablets (30 mg)	D	d	101.0, 100.9, 101.2
Tablets (60 mg)	D	e	102.0, 102.4, 102.8
Tablets (60 mg)	D	f	101.2[a]

[a] Mean of six assays, duplicate injection: RSD is 0.16%.

Table V—Formation of Compound 7 from Diltiazem HCl[a]

Solvent	Concentration of Diltiazem HCl, mg/mL	Period Monitored, h	Rate of Formation of **7**	
			counts/h	%/h
Mobile phase	1.0096	0.4 to 7	2429	0.04
Methanol	1.0432	0.2 to 12	121	0.002
Water	1.0240	0.1 to 9.6	1209	0.02
Methanol:water (5:1)	1.0373	1.0 to 10	669	0.01

[a] In a closed vessel and at room temperature under fluorescent lighting.

peak around 15.5 min. None of these compounds was isolated or identified.

Ruggedness—To evaluate column-to-column variability, a test solution of 1.0 mg/mL of diltiazem HCl and ~0.01 mg/mL of each of the related compounds was injected on three columns from the same manufacturer. Two of the columns were new; the other had been previously used with other mobile phases. The two new columns met the system suitability requirements and gave an excellent separation of diltiazem HCl and the related compounds. The previously used column did not meet the system suitability requirements. On this column, resolution between **7** and the drug was 2.65, the efficiency of the column, calculated using the diltiazem HCl peak, was 4080 plates/meter, and retention times were generally shorter.

Lowering the pH of the buffer component of the mobile phase from 6.2 to 6.0 caused a loss of resolution between **6** and **7**. An increase in pH to 6.5 increased the retention times of the drug, **7**, and **10**.

The film coating on the tablets analyzed was soluble in water but not in methanol. To minimize degradation (Table V), tablets were powdered to break the film coating, and the drug was dissolved in methanol. Using 100 mL instead of 200 mL of methanol to sonicate the ground tablets, or sonicating for 2 h instead of 1 h, had little effect on the assay results, but did result in an increase in the degradation product **7**. Sonicating for 15 min resulted in lower assay results, probably due to incomplete dissolution of the drug.

Four samples of drug bulk (Table VI) and four samples of diltiazem HCl tablets (Table VII) were analyzed for related compounds. In all cases the total detected impurities were <0.25%.

Table VI—Percent Related Compounds Found in Diltiazem HCl Bulk Drugs

Manufacturer	Lot No.	Relative Retention Time[a]		
		0.55[b]	0.69[c]	0.93[b]
A	a	trace[d]	0.13	trace
		trace	0.15	trace
B	a	—	trace	—
		—	trace	—
C	a	trace	0.19	—
		trace	0.20	—
C	b	trace	0.18	—
		trace	0.20	—

[a] Relative to diltiazem HCl at 20.9 min. [b] Unidentified impurity. [c] Compound **7** identity based on retention time. [d] Trace is less than the minimum quantifiable amount.

Table VII—Percent Related Compounds Found in Diltiazem HCl Tablet Formulations[a]

Manufacturer	Lot No.	Dose, mg	Relative Retention Time[b]	
			0.47[c]	0.73[d]
D	c	30	0.07	0.12
D	d	30	0.06	0.09
D	e	60	0.05	0.14
D	f	60	0.07	0.12

[a] Obtained commercially and stored at room temperature. [b] Relative to diltiazem HCl at 19.7 min. [c] Unidentified impurity; it is not **2**. [d] Compound **7**: identity based on retention time only.

Table VIII—Results Obtained by a Second Analyst

Manufacturer	Lot No.	Form	Assay, %	Related Compounds at Relative Retention Times, %[a]		
				0.38	0.47	0.64[b]
C	b	Drug Substance	99.2	—	—	0.16
			99.2	—	—	0.16
D	e	Tablet (60 mg)	98.1	0.06	—	0.12
			97.7	0.06	—	0.12
D	d	Tablet (30 mg)	101.0	—	0.11	0.09
			101.0	—	0.11	0.10

[a] Relative to diltiazem HCl at 19.7 min. [b] Compound **7**: identity based on retention time only.

Diltiazem HCl bulk drug samples Aa and Ba were assayed in duplicate by nonaqueous titration. Mean results were 100.3 and 100.5%, respectively. Sample Aa was used as a standard in the HPLC assay of bulk drugs and tablets. Results of the HPLC assays are given in Table IV.

The specific rotation, $[\alpha]_D^{25°}$, was determined in duplicate from 1% solutions of each of the four bulk drug samples that

were freshly prepared in water. Prior to making the measurements the polarimeter was checked against a known sucrose standard. All results for diltiazem HCl bulk drugs were between +112.0 and +114.0°.

The methods described in this report were checked by a second analyst who was not involved in their development. The results (Table VIII) were similar to those obtained during method development for the samples analyzed, recognizing the fact that a different column and equipment were used.

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Acknowledgments

Thanks are expressed to Mr. H. Avdovich and Mr. H. Beckstead for PMR and FTIR spectra, respectively.