High-Pressure Liquid Chromatographic Analysis of Polythiazide in Pharmaceutical Tablet Formulations

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Abstract □ A high-pressure liquid chromatographic method was developed for the quantitative determination of polythiazide in pharmaceutical tablet formulations after extraction of the powdered tablets with a water–acetonitrile solvent. Analysis is accomplished by comparison of peak areas after chromatography.

Keyphrases □ Polythiazide—high-pressure liquid chromatographic analysis, pharmaceutical formulations □ High-pressure liquid chromatography—analysis, polythiazide in pharmaceutical formulations □ Diuretics—polythiazide, high-pressure liquid chromatographic analysis in pharmaceutical formulations □ Antihypertensives—polythiazide, high-pressure liquid chromatographic analysis in pharmaceutical formulations

Polythiazide {6-chloro-3,4-dihydro-2-methyl - 3 - [[(2,2,2-trifluoroethyl)thio]methyl] -2*H*- 1,2,4- benzothiadiazine-7-sulfonamide 1,1-dioxide}, an orally effective, nonmercurial diuretic and antihypertensive agent, is supplied as a tablet, alone and in combination with reserpine (1). Methods for the determination of polythiazide include TLC (2), spectrophotometry (3–5), column chromatography (6), and polarography (7, 8). However, these methods do not have the rapidity, simplicity, sensitivity, and accuracy of the high-pressure liquid chromatographic (HPLC) methodology.

Recently, a quantitative HPLC method for polythiazide involving reversed-phase partition chromatography was published (9). This method utilized a Bondapak phenyl/Corasil column[1], methanol–water (35:65 v/v) as the mobile phase, and peak height quantitation. The system, however, does not appear optimum since the absolute recovery of added polythiazide was reported as 106%, and baseline separation was not achieved between vanillin, a formulation excipient, and polythiazide, and polythiazide and the reported internal standard, quinoline. The subject of this report is the development of a simple, direct, extremely rapid, and precise HPLC procedure for the quantitation of polythiazide in several pharmaceutical tablet formulations.

EXPERIMENTAL

Apparatus—A high-pressure liquid chromatograph[2], operated at ambient temperature, was equipped with a UV detector for monitoring the column effluent at 254 nm. The column[3] was 25-cm × 4.6-mm (i.d.) stainless steel packed with Partisil 10-ODS. An electronic computing integrator[4] was used to obtain peak areas. A 10-μl loop injection valve[5] was used to introduce samples into the chromatographic system.

Reagents—Polythiazide NF reference standard was dried in vacuum at 60° for 2 hr. Reagent ACS grade acetonitrile[6] and distilled water were used in preparing the mobile phase.

Mobile Phase—Add 20 ml of distilled water to 980 ml of acetonitrile and shake vigorously. Degas prior to use.

Standard Solution—Accurately weigh approximately 50 mg of polythiazide reference standard, transfer quantitatively to a 250-ml volumetric flask, and dissolve in and dilute to volume with the mobile phase.

Sample Solution—Accurately weigh and finely powder 20 polythiazide tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2 mg of polythiazide, to a glass-stoppered 30-ml centrifuge tube. Add 10 ml of the mobile phase, shake for 30 min, centrifuge, and filter the supernate through a 1-μm membrane filter[7]. Use the filtered supernate for analysis.

Chromatography—Condition the column for 24 hr with the mobile phase at a flow rate of 0.5 ml/min. This procedure is necessary for new columns; conditioning is not required for previously used columns. Inject 10 μl of the standard solution and adjust either the pressure or flow rate so that the polythiazide exhibits a retention time of about 3 min.

The approximate chromatographic conditions are a flow rate of 1 ml/min (at an inlet pressure of 300 psig), a chart speed of 1.3 cm (0.5 in.)/min, and a detector sensitivity of 0.32 absorbance unit full-scale (aufs). Data acquisition parameters for the electronic computing integrator were optimized according to the manufacturer's instructions.

For the analysis of a sample, two 10-μl aliquots of the standard solution are injected followed by two 10-μl injections of the sample solution. The peak areas obtained or peak heights may be used for the calculations.

RESULTS AND DISCUSSION

Initial studies concerned the selection of an appropriate column for the separation and quantitation of polythiazide. The adsorption mode of chromatography was investigated using a silica gel-type column[8]. With methanol as the mobile phase, a sharp and symmetrical peak was observed for polythiazide. As additional injections of polythiazide were made onto the column, peak broadening and finally peak splitting were observed. This phenomenon may be attributed either to a nonuniform adsorption of polythiazide on the column or to elution of residual polythiazide retained on the column from previous injections.

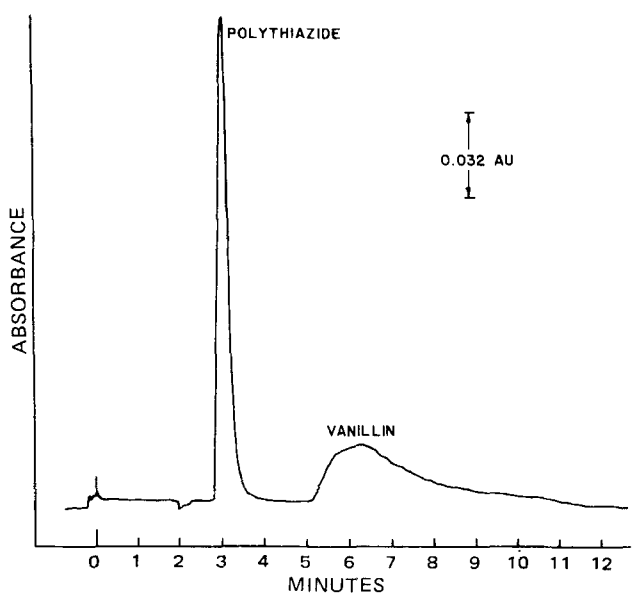

Figure 1—*Representative chromatogram of a polythiazide tablet formulation.*

[1] Catalog No. 27283, Waters Associates.
[2] DuPont model 840.
[3] Catalog No. PXS-1025-ODS, Whatman Inc.
[4] Autolab System I, Spectra-Physics Corp.
[5] Catalog No. 204590, DuPont.
[6] Eastman Kodak.
[7] Catalog No. FALP 02500, Millipore Corp.
[8] Pellosil-HS, catalog No. A011, Whatman Inc.

Table I—Percent Recovery of Polythiazide from Spiked Placeboes by HPLC

Formulation	Day	Weight	Injection Number 1	2
2 mg	1	1	102.3	102.3
		2	100.9	100.6
		3	101.0	103.6
	2	1	101.6	101.6
		2	99.7	99.7
		3	102.0	102.5
	3	1	100.4	99.0
		2	101.2	98.0
		3	99.2	99.7
Average = 100.8% (95% confidence limits of 99.8–101.8%)				
4 mg	1	1	101.0	100.9
		2	100.2	100.3
		3	99.8	100.6
	2	1	98.0	98.3
		2	98.7	100.3
		3	98.7	98.0
	3	1	102.1	101.6
		2	99.9	99.9
		3	100.1	99.9
Average = 99.9% (95% confidence limits of 99.0–100.8%)				
Combination[a]	1	1	99.5	100.0
		2	98.7	99.1
		3	101.2	101.7
	2	1	99.4	100.0
		2	100.9	100.6
		3	100.2	99.9
	3	1	101.8	102.2
		2	101.8	102.1
		3	101.1	102.1
Average = 100.6% (95% confidence limits of 99.8–101.4%)				

[a] Contained 2 mg of polythiazide and 0.25 mg of reserpine.

The addition of water to the methanolic mobile phase (50% v/v) produced a sharp and reproducible peak for polythiazide. However, the detector response was not linear and an interference was observed from some tablet excipients. Due to the problems encountered, adsorption chromatography was not investigated further.

Reversed-phase partition chromatography with a Partisil 10-ODS column was then investigated. With methanol as the mobile phase, the observed peak for polythiazide was sharp and the response was linear. However, vanillin, a tablet excipient, was not resolved from polythiazide. The addition of water to the methanolic mobile phase up to 50% (v/v) resulted in no observed improvement in resolution.

Because of vanillin's known solubility in methanol, acetonitrile was substituted for methanol in the mobile phase. A sharp peak was observed for polythiazide while no peak was observed for vanillin; it appeared to be retained by the column. However, continued injections of a polythiazide–vanillin solution resulted in tailing of the polythiazide peak, which may be attributed to the retained vanillin acting as a partition substrate. The addition of methanol (2:98 v/v) to the acetonitrile mobile phase resulted in elution of the vanillin while maintaining adequate resolution for polythiazide. However, continued injections of solutions prepared from spiked tablet placeboes resulted in peak broadening for polythiazide and decreased resolution between polythiazide and vanillin.

Table II—Estimates of Precision for Determination of Polythiazide in Pharmaceutical Tablet Formulations by HPLC

Weights per Day	Injections per Weight	Estimates of Precision[a], % 2 mg	4 mg	Combination[b]
1	1	±3.0	±2.6	±2.3
1	2	±2.6	±2.5	±2.2
2	2	±2.3	±2.2	±1.8
3	2	±2.2	±2.2	±1.6
Injection to injection within a weight		±2.0	±1.0	±0.7

[a] Ninety-five percent of the individual results or averages of two, four, or six results will not vary from each other by more than the percentages quoted. These estimates include variability due to days, weights, and injections. The estimates of precision for injection to injection within a weight exclude variability due to days and weights. [b] Contained 2 mg of polythiazide and 0.25 mg of reserpine.

Table III—Comparison of HPLC and Compendial Methods for Determination of Polythiazide in Pharmaceutical Tablet Formulations

Formulation	Day	Sample	Analysis, mg/Tablet HPLC	Spectrophotometric	TLC
2 mg	1	1	2.07	2.07	1.97
		2	2.07	2.09	1.96
		3	2.05	2.10	1.95
	2	1	2.09	2.05	2.00
		2	2.09	2.09	2.00
		3	2.08	2.05	1.97
	Average		2.08	2.08	1.98
4 mg	1	1	4.18	3.96	4.06
		2	4.18	4.03	3.99
		3	4.22	3.87	4.03
	2	1	4.18	4.12	3.99
		2	4.18	4.12	3.96
		3	4.22	4.18	4.03
	Average		4.18	4.06	4.03
Combination[a]	1	1	2.07	2.12	2.05
		2	2.07	2.12	1.99
		3	2.09	2.11	2.01
	2	1	2.06	2.07	1.97
		2	2.06	2.10	1.95
		3	2.06	2.10	1.97
	Average		2.07	2.10	1.99

[a] Contained 2 mg of polythiazide and 0.25 mg of reserpine.

The addition of water to the mobile phase was investigated and, after evaluating various combinations of acetonitrile, methanol, and water, optimum separation and column stability were obtained with a water–acetonitrile (2:98 v/v) system. With this system, baseline separation was obtained between polythiazide and vanillin and tablet placeboes did not interfere. Reserpine was retained on the column, and peak broadening or changing of retention time was not observed, indicating long-term column stability. The detector response for polythiazide was linear up to a concentration of 300 μg/ml when dissolved in the mobile phase. In addition, the column could be regenerated with water, the retained reserpine being washed off under this condition. A typical chromatogram of polythiazide is shown in Fig. 1.

The accuracy and precision of the HPLC method were determined by the following experiments. Three weights of the placeboes for the 2- and 4-mg tablets and 2-mg combination with 0.25 mg of reserpine, in which known quantities of polythiazide were added, were assayed each day for 3 consecutive days. The average percent recoveries of polythiazide were 100.8, 99.9, and 100.6, respectively (Table I).

Estimates of precision (Table II) were obtained using the analysis of variance statistical technique. Ninety-five percent of the individual results will not vary from each other (*i.e.*, from the mean) by more than ±3.0, ±2.6, and ±2.3% for the 2- and 4-mg tablets and 2-mg combination with reserpine, respectively. The standard errors for the average of two injections per sample were ±1.3, ±1.3, and ±1.1% for the 2- and 4-mg tablets and 2-mg combination with reserpine, respectively.

The proposed HPLC method was compared to the compendial TLC and colorimetric assays (2), and the results (Table III) were in agreement. All samples were within the requirements of NF XIV for polythiazide tablet formulations by HPLC methodology (2). Because of the speed, accuracy, and precision of the proposed procedure, it represents an alternative to present compendial and published methodologies.

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Quantitative High-Pressure Liquid Chromatographic Determination of Thimerosal in Pharmaceutical Formulations

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Abstract □ A quantitative high-pressure liquid chromatographic method using an anion-exchange resin column and an aqueous perchlorate solution as the mobile phase is employed for the determination of thimerosal in pharmaceutical formulations. With a liquid formulation containing large amounts of edetate disodium, calcium chloride is used for complexation to eliminate the interference from edetate disodium.

Keyphrases □ Thimerosal—high-pressure liquid chromatographic analysis, pharmaceutical formulations □ High-pressure liquid chromatography—analysis, thimerosal in pharmaceutical formulations

Thimerosal is an organomercurial chemical with antibacterial activity. It is used as an antiseptic for applications such as skin disinfection, urethral irrigation, and preservation of ophthalmic formulations (1). Compendial analytical methods using atomic absorption spectroscopy lack specificity (2). Another analytical method employing colorimetry of a dithizone complex (3) is tedious, and the presence of metal ions in the reagents causes interference.

Described here is a high-pressure liquid chromatographic (HPLC) analysis of thimerosal using an anion-exchange resin column with buffered sodium perchlorate as the mobile phase. The method is specific and simple.

EXPERIMENTAL

Apparatus—A high-pressure liquid chromatograph[1] equipped with a pump (7000 psig maximum), dual-channel UV detectors at 254 and 280 nm, and a 3 × 500-mm stainless steel column packed with anion-exchange resin[2] was used.

Reagents—The mobile phase was 0.35% perchloric acid[3] in 0.001 *M* dibasic sodium phosphate with pH adjusted to 7.0 with 1 *N* sodium hydroxide.

Standard Solution—Dissolve 8, 10, and 12 mg of thimerosal, separately, in purified water and dilute to 1 liter. These solutions represent thimerosal concentrations of 0.0008, 0.001, and 0.0012%, respectively.

Sample Solution—Dilute the sample solutions with purified water to make a final thimerosal concentration of 0.001%.

Chromatographic Separation—The procedure is run at ambient temperature, and the solvent flow is 1.6 ml/min. The UV monitor is set at 254 nm with a sensitivity of 0.02 absorbance unit. The samples and standards are injected with a 30-μl loop. The concentration of thimerosal is calculated from a standard curve using peak area (*i.e.*, height times width at half-height) for quantitation.

[1] Spectra Physics isocratic model 3500B, Santa Clara, Calif.
[2] Vydac, Applied Science Laboratories, State College, Pa.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.

RESULTS AND DISCUSSION

A typical chromatogram of thimerosal is shown in Fig. 1*a*. The chromatogram of one sample was complete in less than 5 min. Areas were used for the calculation of thimerosal concentration. Linear response over the concentration range from 0 to 15 μg/ml was obtained.

Table I shows the results using both the HPLC and the dithizone complex colorimetric methods. A solution containing edetate disodium, a common ingredient in ophthalmic formulations, and thimerosal in the ratio of less than 10:1 was separated and analyzed by this method without further sample treatment. However, an overlap of peaks was observed if a formulation contained edetate disodium and thimerosal in a ratio of more than 10:1. In such cases, calcium chloride was added to the sample solution to suppress the edetate disodium peak. With this step, complete separation and analysis of thimerosal were obtained.

Figure 1*b* illustrates a typical chromatogram of a sample solution containing 0.02% edetate disodium and 0.001% thimerosal. Figure 1*c* shows the chromatogram of the sample solution after treating with calcium chloride (to each 5 ml of sample solution was added 0.1 ml of 2%

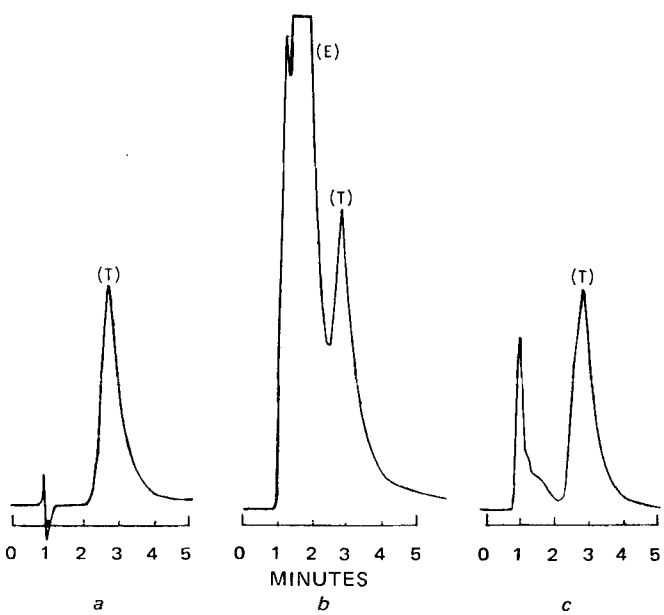

Figure 1—*Typical chromatograms of* (a) *thimerosal standard solution at 0.001%,* (b) *sample solution containing 0.02% edetate disodium and 0.001% thimerosal, and* (c) *0.1 ml of 2% $CaCl_2$ solution with 5 ml of sample solution* (b). *Key: T, thimerosal; and E, edetate disodium.*