Determination of Polythiazide in Pharmaceutical Dosage Forms by High-Pressure Liquid Chromatography

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Abstract □ A method is presented for the quantitative analysis of polythiazide tablets by high-pressure liquid chromatography. The powdered tablets are extracted with methanol, containing quinoline as the internal standard, and assayed by comparison of peak heights after liquid chromatography. The chromatographic conditions employed may be adapted for the determination of many other thiazide and nonthiazide diuretics.

Keyphrases □ Polythiazide—high-pressure liquid chromatographic analysis in pharmaceutical dosage forms □ High-pressure liquid chromatography—analysis, polythiazide dosage forms □ Diuretics—high-pressure liquid chromatographic analysis of polythiazide dosage forms

The NF XIII procedure for the analysis of polythiazide tablets requires a time-consuming TLC separation prior to spectrophotometric measurement of the isolated material (1). A similar approach using column chromatography was proposed recently as the official method; however, vanillin commonly added to tablet formulations of this drug interferes with the assay (2). Other available methods include a polarographic procedure (3) and several techniques involving hydrolysis and colorimetric measurement after diazotization and coupling with compounds such as *N*-(1-naphthyl)ethylenediamine dihydrochloride (4), thymol (5), and potassium guaiacolsulfonate (6).

High-pressure liquid chromatography (HPLC) allows for the direct analysis of polythiazide in pharmaceutical dosage forms not only in the presence of the excipient vanillin but also in formulations containing reserpine. The method reported here is simple, rapid, specific, and free of the analytical problems associated with the aforementioned procedures. Furthermore, the proposed assay is sufficiently sensitive that it can be carried out on individual tablets and therefore can serve equally well for the content uniformity determinations of this diuretic in pharmaceutical dosage forms. The application of this technique to the determination of polythiazide and its major metabolite in urine will be the subject of a subsequent publication.

EXPERIMENTAL

Apparatus—A high-pressure liquid chromatograph[1], equipped with a dual detector system, capable of operating at inlet pressures up to 6000 psig was used. The UV detector (254 nm) was also utilized.

Column—The column was purchased from the instrument manufacturer and consisted of a monomolecular layer of diphenyldichlorosilane bonded to a pellicular packing[2] [0.6 m × 0.3 cm (2 ft × 0.125 in.) o.d.].

Chromatographic Conditions—The chromatographic solvent was methanol–water (35:65 v/v). The temperature was ambient, and the solvent flow was approximately 0.4 ml/min (at an inlet pressure of 200 psig). The precision photometer detector (254 nm) was set at a sensitivity of 0.08 absorbance unit full-scale. The injection volume was 5 μl, and the chart speed was 0.5 cm (0.2 in.)/min.

Materials and Reagents—The chromatographic internal standard was quinoline[3]. Polythiazide reference material[4] was used without further purification. The methanol was ACS grade. All other reagents and solvents were of the best grade commercially available and were used without further purification.

Solutions—*Internal Standard Solution*—The quinoline (230 mg) was dissolved in methanol and diluted with methanol to a final volume of 1000 ml.

Polythiazide Standard Solution—Approximately 20 mg of polythiazide reference material was weighed accurately into a 100-ml volumetric flask. The polythiazide was dissolved in the internal standard solution and diluted to 100 ml with this solution.

Preparation of Standard Curve—Two, four, six, and eight milliliters of the polythiazide standard solution were accurately transferred into four 10-ml volumetric flasks and diluted to volume with the internal standard solution. Three injections (5 μl) of each of these solutions and of the undiluted polythiazide standard solution were made into the chromatographic system. The heights of the peaks were measured, and the ratios of the peak height of the polythiazide to that of the internal standard were calculated for each injection. The average peak height ratio for each dilution was plotted against the quantity of polythiazide in the solution.

HPLC Analysis of Polythiazide in Tablets—Twenty tablets were weighed and finely powdered. An accurately weighed portion, equivalent to one tablet weight, was transferred to a 30-ml glass-stoppered centrifuge tube. Ten milliliters of the internal standard solution was added, and the sample was shaken[5] mechanically for 30 min (for the 2-mg tablets, 20 ml of internal standard solution was added). The sample was then centrifuged[6] at 1500 rpm for 15 min, and three 5-μl injections of the clear supernate were made into the chromatographic system. The ratios of the heights of the peaks were calculated for each injection and then averaged. The quantity of polythiazide in the sample then was determined graphically.

NF XIII Analysis of Polythiazide in Tablets—The three different samples of polythiazide tablets were assayed following the compendial procedure (1).

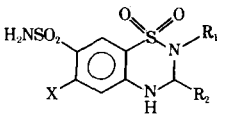
Colorimetric Analysis of Polythiazide in Tablets—The BP 1973 assay for bendrofluazide[7] tablets (7) was adapted for this determination, the method being essentially that described under content uniformity for polythiazide tablets in NF XIII (1).

RESULTS AND DISCUSSION

Column and Mobile Phase Selection—Preliminary experiments indicated that qualitative separation of many thiazide as well as nonthiazide diuretics could be obtained on the diphenyldichlorosilane-bonded column[2], using methanol–water as the mobile phase. The retention times for 12 thiazide and 10 nonthiazide diuretics under the chromatographic conditions described in the *Experimental* section are given in Tables I and II, respectively.

[1] Waters ALC 202/401.
[2] Bondapak phenyl/Corasil, Waters Catalog No. 27283.
[3] Reagent grade, bp 235–237°, Fisher Scientific Co.
[4] Donated by Pfizer Co. Ltd., Montreal, Canada.
[5] Eberbach two-speed utility shaker (set at low speed), Canlab Catalog No. S1105.
[6] International centrifuge, model CS, International Equipment Co.
[7] British Approved Name (BAN) for bendroflumethiazide.

Table I—Chromatographic Behavior[a] of Some Thiazide Diuretics



Compound	X	R_1	R_2	Retention Time, min
Hydrochlorothiazide	Cl	H	H	3.5
Hydroflumethiazide	CF_3	H	H	3.3
Chlorothiazide	Cl	H	($\Delta^{3,4}$)	3.1
Flumethiazide	CF_3	—	($\Delta^{2,3}$)	2.7
Trichlormethiazide	Cl	H	$CHCl_2$	3.5
Methyclothiazide	Cl	CH_3	CH_2Cl	4.3
Cyclopenthiazide	Cl	H		7.5
Cyclothiazide	Cl	H		6.7
Althiazide	Cl	H	$CH_2SCH_2CH{=}CH_2$	5.0
Bendroflumethiazide	CF_3	H	$CH_2C_6H_5$	6.7
Polythiazide	Cl	CH_3	$CH_2SCH_2CF_3$	7.0
Benzthiazide	Cl	H	$CH_2SCH_2C_6H_5(\Delta^{3,4})$	4.9

[a] Chromatographic conditions as described under *Experimental.*

These retention times could be varied considerably by changing the proportions of methanol and water in the mobile phase. Very little resolution was obtained using a similar type of column bonded with octadecyltrichlorosilane[8]. Honigberg *et al.* (8) found that some antihistaminic, antitussive, and analgesic drugs were separated better on the phenyl[2] than on the C_{18}[8] column.

In general, sharp symmetrical peaks were obtained, the only exception being triamterene, which gave a relatively broad peak after a comparatively long retention time. The selection of 35% methanol in water as the mobile phase for this work was dictated by the presence of the interfering excipient vanillin in the commercially available tablet formulations. Maximum separation of the polythiazide and vanillin peaks was obtained by employing this ratio of solvents. Under these chromatographic conditions, vanillin showed a retention time of 3.9 min compared to 7.0 min for polythiazide.

Internal Standard—To minimize injection and apparatus errors, a study was undertaken to find a suitable internal standard. Many compounds in Tables I and II with short retention times could have been selected. For example, by using acetazolamide (3.3 min) as the internal standard, a highly satisfactory quantitative relationship was obtained when analyzing pure polythiazide. However, since vanillin is extracted along with the polythiazide from the powdered tablets and since it is eluted after 3.9 min, a significant overlap of peaks occurs.

After screening a large number of compounds, quinoline (retention time 10.0 min) was found to be an excellent internal standard.

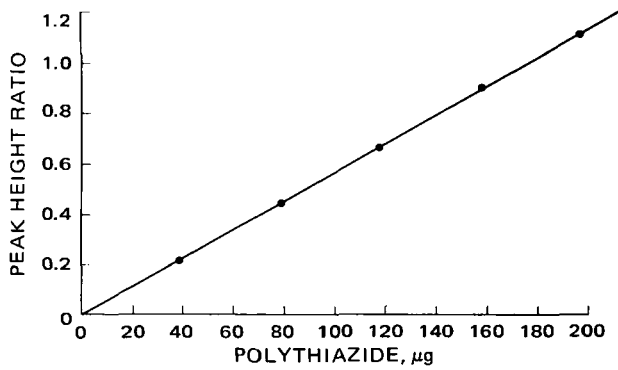


Figure 1—*Standard curve for polythiazide with quinoline as the internal standard.*

[8] Bondapak C_{18}/Corasil, Waters Catalog No. 98159.

Isoquinoline and carbazole, with retention times similar to quinoline, could have been employed, but their peaks were not as well developed.

Response and Linearity—The response *versus* concentration curve for polythiazide demonstrated that sample concentration was linear with peak height and that elution times were reproducible. Peak height rather than peak area was chosen as the method of measurement because previous studies (9, 10) had shown that peak height varies less with fluctuations in inlet pressure (flow rate) than does peak area.

Five different concentrations of polythiazide, ranging from 40 to 200 μg/ml, were injected. Figure 1 shows the linear relationship resulting from plotting varying concentrations of polythiazide against the ratios of the heights of the polythiazide peaks to those of the internal standard.

Analysis of Dosage Forms—Replicate assays were performed, as described under *Experimental,* on three different commercially available polythiazide preparations. Figure 2 illustrates a typical chromatogram obtained from each formulation. As is evident from the chromatograms, vanillin is separated sufficiently from polythiazide and does not interfere in the analysis. The 2-mg tablets (Product II) and the 1-mg tablets with reserpine (Product III) contain a second excipient which is extracted into the methanol, but it is also eluted well before the polythiazide and likewise shows no interference. Reserpine is present in a very low concentration in Product III; this fact, coupled with the limited solubility of reserpine in methanol, likely explains why it is not detected in the chromatogram and, accordingly, why it does not interfere with the analysis of polythiazide.

The assay results for three formulations are given in Table III.

Table II—Chromatographic Behavior[a] of Some Nonthiazide Diuretics

Compound	Retention Time, min
Acetazolamide	3.3
Amisometradine	3.9
Chlorthalidone	4.4
Clopamide	4.3
Dichlorphenamide	3.5
Ethacrynic Acid	3.1
Ethoxzolamide	6.9
Furosemide	3.1
Quinethazone	4.1
Triamterene	11.0[b]

[a] Chromatographic conditions as described under *Experimental.* [b] Very broad peak. Compound is only very slightly soluble in methanol.

Table III—Comparative Analyses of Three Commercially Available Polythiazide Tablet Preparations

Product	Components	Analyses, % of Labeled Claim			
		NF XIII	Colorimetric[a]	Manufacturer's Data	Proposed Method (HPLC)[a]
I[b]	Polythiazide, 1 mg	113.2	106.4	105.0[c]	110.2 ± 0.4
II[b]	Polythiazide, 2 mg	113.0	101.8	105.0[c]	108.1 ± 0.7
III[d]	Polythiazide, 1 mg Reserpine, 0.25 mg	—[e]	—[e]	105.5[f]	112.0 ± 0.6

[a] Average of four determinations. [b] Renese, Pfizer Co. Ltd. [c] TLC method. [d] Renese-R, Pfizer Co. Ltd. [e] Erratic results, reserpine interferes. [f] Spectrophotometric method.

The values obtained by the NF XIII procedure and by a colorimetric method are also given, along with the values received from the manufacturer for these lot numbers; the proposed method gives results that are slightly lower than the NF XIII procedure but somewhat higher than those found by the manufacturer. The standard deviations are quite comparable to those normally obtained with other related analytical techniques.

This procedure was repeated for Product I, using 10 ml of the internal standard solution containing 0.20 mg of added polythiazide reference material. The absolute recovery of the added polythiazide was calculated to be 0.212 ± 0.003 mg based on four replicate determinations.

CONCLUSION

HPLC provides a convenient and efficient method for the quantitative determination of polythiazide in its dosage forms. There was no interference from excipients in the products examined, so no additional extraction or separation procedures are required. The method is rapid and sensitive enough to be used for single-tablet assays.

Although the detailed chromatographic conditions were not explored for the quantitative determination of the other thiazide and nonthiazide diuretics, this method may provide a suitable starting point for the estimation of these diuretics in dosage forms and, possibly, in biological fluids.

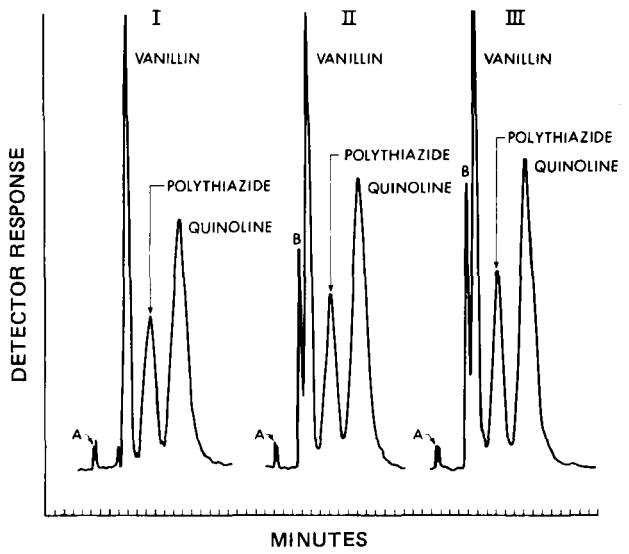


Figure 2—*Typical chromatograms of polythiazide dosage forms. Key: I, polythiazide 1-mg tablets; II, polythiazide 2-mg tablets; III, tablets containing 1 mg of polythiazide and 0.25 mg of reserpine; A, sample injection; and B, unknown excipient. Liquid chromatographic conditions are described under* Experimental.

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