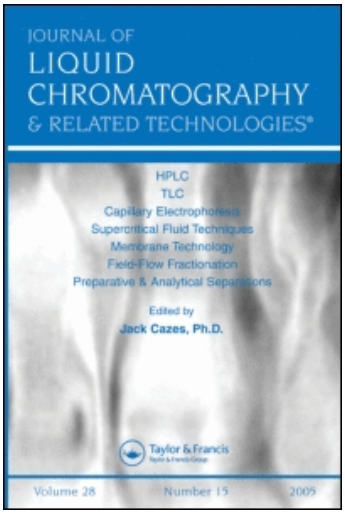

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Performance Liquid Chromatographic Determination of Diuretic-Antihypertensive Combination Products. I. Prazosin and Polythiazide

William J. Bachman[a]
[a] Food and Drug Administration, Atlanta, Georgia

To cite this Article Bachman, William J.(1986) 'High Performance Liquid Chromatographic Determination of Diuretic-Antihypertensive Combination Products. I. Prazosin and Polythiazide', Journal of Liquid Chromatography & Related Technologies, 9: 5, 1033 — 1049
To link to this Article: DOI: 10.1080/01483918608076688
URL: http://dx.doi.org/10.1080/01483918608076688

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(5), 1033-1049 (1986)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF DIURETIC-ANTIHYPERTENSIVE COMBINATION PRODUCTS. I. PRAZOSIN AND POLYTHIAZIDE

William J. Bachman
Food and Drug Administration
60 Eighth Street, N.E.
Atlanta, Georgia 30309

ABSTRACT

A high performance liquid chromatographic method for the simultaneous determination of prazosin and polythiazide in capsules is presented. Prazosin, polythiazide and benzophenone (internal standard) were separated by reverse phase chromatography on cyano-bonded phases from four manufacturers using acetonitrile-phosphate buffer mobile phases. Detection was by ultraviolet absorbance at 268 nm. Recoveries from synthetic formulations were 100.2 ± 0.7% for prazosin and 99.7 ± 0.7% for polythiazide. The method may also be used as a stability indicating assay for each drug substance and its impurities.

INTRODUCTION

Numerous diuretic-antihypertensive combination products are currently available to the physician for prescription in

 0148-3919/86/0905-1033$3.50/0

the treatment of cardiovascular disease. Combination drug products are not indicated for initial treatment of hypertension. However, if no single drug is effective alone, then two or more are used in combination. Drug therapy titrated to the individual is required with re-evaluation as conditions warrant. If a fixed combination product is equivalent to the dosage determined by titration, its use may be more convenient in patient management.

The benzothiadiazine (thiazide) drugs are among the most widely used prescription drugs. They are usually the first drug to be employed in the treatment of hypertension. Thiazide antihypertensive-diuretic agents are frequently combined with a variety of adrenergic blocking agents, vasodilators, aldosterone antagonists and rauwolfia alkaloids. Thiazides may be additive or potentiative of the action of other antihypertensive drugs.

High performance liquid chromatography (HPLC) has been successfully used for the analysis of a wide variety of pharmaceutical combination dosage forms including analgesics, cough-cold preparations, antibacterials, and contraceptives. Honigberg et al. investigated the chromatographic properties of seven widely prescribed antihypertensive drugs on two different bonded phase pellicular packings using fourteen different mobile phases (1). In a separate paper, a two-step HPLC procedure was used for the determination of reserpine and chlorothiazide from a simulated dosage form (2). HPLC methods have been reported for dosage forms containing hydrochlorothiazide in combination with reserpine (3), triamterene (4), amiloride hydrochloride (5), timolol maleate (6), and methyldopa (7). Additionally, the HPLC analysis of methyldopa in combination with hydrochlorothiazide or chlorothiazide in dosage

forms has been collaboratively studied (8). The official method for chlorthalidone, a non-thiazide diuretic, and clonidine hydrochloride in tablets also uses HPLC (9).

No methods have previously been reported for the simultaneous determination of dosage forms containing polythiazide and prazosin. Two HPLC methods for the analysis of single component polythiazide have been reported (10, 11). The U.S.P. assay procedure for polythiazide tablets employs preparative thin layer chromatography with ultraviolet absorbance quantitation (12). An HPLC method is used in the official assay of single component prazosin hydrochloride tablets (13). None of the HPLC methods for single component dosage forms has been shown to be applicable to the combination capsules. The only reported HPLC method for the determination of both polythiazide and prazosin was from human plasma rather than dosage forms (14). However, two different HPLC systems were used to chromatograph the plasma extract. Polythiazide was determined on a normal phase system with UV detection and prazosin was determined on a reverse phase system with fluorescence detection. A single isocratic system for the analysis of the two-component capsules would be faster and simpler.

As part of an on-going study of diuretic-antihypertensive multicomponent dosage forms in this laboratory, this paper reports the simultaneous HPLC separation and quantitation of fixed combination capsules containing 0.5 mg polythiazide and prazosin hydrochloride equivalent to 1, 2, or 5 mg prazosin base. In this procedure, prazosin, benzophenone (internal standard), and polythiazide are separated by reverse phase chromatography on a cyanopropyl-bonded silica column and detected by ultraviolet absorbance at 268 nm. Use of the HPLC system for stability indicating assays for each of the individual drug substances and their impurities was also examined.

EXPERIMENTAL

Materials:

Prazosin hydrochloride, polythiazide, 4-amino-2-chloro-6,7-dimethoxyquinazoline, 1,4-bis(2-furoyl)piperazine, 4-amino-6,7-dimethoxy-2-(1-piperazinyl)quinazoline, 1-(2-furoyl)piperazine, 1,4-bis(4-amino-6,7-dimethoxy-2-quinazolinyl)piperazine, 4-amino-6-chloro-1,3-benzenedisulfonamide and 4-amino-6-chloro-N^3-methyl-m-benzenedisulfonamide are all available from the United States Pharmacopeial Convention, Rockville, MD 20852. Samples of prazosin hydrochloride and polythiazide bulk drug substances used as working standards were obtained from the manufacturer. The monosodium dihydrogen phosphate monohydrate and phosphoric acid were purchased from J.T. Baker Chemical Co., Phillipsburg, NJ 08865. Acetonitrile, UV grade, was from Burdick & Jackson Laboratories, Inc., Muskegon, MI 49442. Benzophenone, certified grade, was from Fisher Scientific Co., Fairlawn, NJ 07410.

Solutions:

a. 0.05 M monobasic sodium phosphate:

6.9g monosodium dihydrogen phosphate monohydrate was diluted to 1 liter with water.

b. HPLC mobile phase:

A solution was prepared by mixing 650 ml 0.05M monobasic sodium phosphate solution and 350 ml acetonitrile. After the mixture equilibrated to room temperature, the apparent pH was adjusted to 3.0 with phosphoric acid. The solution was filtered through a Millipore solvent filtration apparatus using a 0.45 μ pore size nylon 66 membrane (Alltech Associates, Inc., Deerfield, IL 60015).

c. Internal standard solution:
A solution containing 30 mg benzophenone in 1 liter of HPLC mobile phase was used.

d. Prazosin stock solution:
Approximately 27.4 mg prazosin hydrochloride (equivalent to 25 mg prazosin base) was accurately weighed and dissolved in 100 ml of HPLC mobile phase. Ten minutes in an ultrasonic bath was required for dissolution.

e. Polythiazide stock solution:
Approximately 10 mg polythiazide was accurately weighed and dissolved in 100 ml HPLC mobile phase.

f. Mixed standard solutions:
An aliquot of prazosin stock solution (4.0, 8.0 or 20 ml - equivalent to 1, 2 or 5 mg prazosin base), 5.0 ml polythiazide stock solution and 25 ml internal standard solution were pipetted into a 50 ml volumetric flask and diluted to volume with HPLC mobile phase as required.

Instrumentation:

The liquid chromatograph consisted of a Laboratory Data Control Constametric I pump, Spectromonitor II variable wavelength detector and Rheodyne Model 7125 loop injector. (LDC/Milton Roy, Riviera Beach, FL 33404.) Operating parameters-flow rate 1.7 ml/min., absorbance range 0.01, detection wavelength 268 nm, 10 µl loop, ambient temperature.

Integrator:

HP 3380A Integrator (Hewlett-Packard Co., Avondale, PA 19311). Operating parameters-attenuation 128, slope sensitivity 3 mV/min., start delay off, area reject off, stop time 10 min., chart speed 0.5 cm/min.

HPLC Columns:

A: IBM Cyano, 5μ spherical particles, 4.5 mm (id) x 250 mm, (IBM Instruments Inc., Wallingford, CT 06492).

B: Nucleosil 10 CN, 10μ spherical particles, 4.6 mm (id) x 250 mm (Macherey-Nagel, Duren, Germany, in-house packed, packing available from Alltech Associates, Inc.)

C: Bondapak CN, 10μ irregularly-shaped particles, 3.9 mm (id) x 300 mm (Waters Associates, Milford, MA 01757).

D: Spherisorb CN, 5 μ spherical particles, 4.6 mm (id) x 250 mm (Phase Separations, Inc., Norwalk, CT 06850, custom-packed by Alltech Associates, Inc.)

Analysis of Working Standards:

Polythiazide was assayed by the official ultraviolet spectrophotometric procedure (15). Prazosin hydrochloride was assayed by comparison of its ultraviolet spectrum in methanolic 0.01N hydrochloric acid (6.5μ g/ml) versus a similarly prepared solution of the USP reference standard. Additionally, the stock solutions of both working standards were assayed chromatographically versus stock solutions prepared from the USP reference standards under the same HPLC conditions employed in the capsule assay. In both cases, comparable results (within 1.0%) were obtained by both the ultraviolet spectrophotometric and HPLC methods.

Analysis of Capsules:

Composites were prepared by manually grinding the contents of 20 capsules to pass a No. 60 mesh sieve. An acccurately weighed portion of the powder equivalent to one capsule was transferred to a 50 ml volumetric flask. A 25 ml aliquot of

internal standard solution was added. Flask was placed in an ultrasonic bath for 5 minutes. The sample was diluted to volume with HPLC mobile phase and mixed. A portion of the mixture was filtered through a stainless steel Swinney filter (Millipore Corp., Bedford, MA 07130) containing a 0.45μ pore size, 13 mm dia. nylon 66 membrane (Alltech Associates, Inc.) and a glass fiber pre-filter (Micro Filtration Systems, Dublin, CA 94566). Duplicate 10 μl injections of each sample were bracketed with injections of the appropriate mixed standard solution. Quantitation was by the area response ratio for each active ingredient to the internal standard.

Calculations:

mg prazosin base per capsule found =

$$\frac{\text{AR SPL}}{\text{AR STD}} \times \frac{\text{STD WT}}{\text{SPL WT}} \times \text{AVG. CAP. WT.} \times \frac{383.41}{419.87}$$

where:

AR SPL = sample area response ratio to internal standard

AR STD = standard area response ratio to internal standard

STD WT = weight of prazosin hydrochloride in the mixed standard solution in mg

SPL WT = weight of the sample in mg

AVG. CAP. WT. = average capsule content weight in mg

383.41 = molecular weight of prazosin base

419.87 = molecular weight of prazosin hydrochloride

Mg polythiazide per capsule found was calculated in a similar manner.

RESULTS AND DISCUSSION

Chromatographic separations were developed on four different cyano-bonded phases from four manufacturers. However, all recovery data, sample assays and system suitability tests were performed using Column A. Similar separations were obtained on two columns from different batches from this manufacturer. To obtain the separations on the four different columns, modification of the organic concentration and/or the pH of the mobile phase was required. See Figure 1 for typical separations obtained on each of the four columns. Note reversal of elution order with Column D.

Prazosin has a nearly three-fold greater molar absorptivity than polythiazide and is present in the capsules in 2 to 10 times greater concentration. Therefore, ultraviolet absorbance detection at 268 nm, the absorbance maxima of polythiazide, was selected in order to minimize the disparity of response between polythiazide and prazosin and to allow recording of peaks without programmed attenuation.

Benzophenone was selected as a convenient internal standard because its retention volume in this system lies between the components of interest. Use of phenones as internal standards has been previously reported (16). They are particularly suited for use as internal standards because as neutral compounds, neither solvent modifiers nor acidic/basic buffers will affect their retention volumes. Both polar and non-polar solvent systems can be used due to their wide solubility range.

The chromatographic system (Column A) also separated prazosin from the five impurities which have established limits

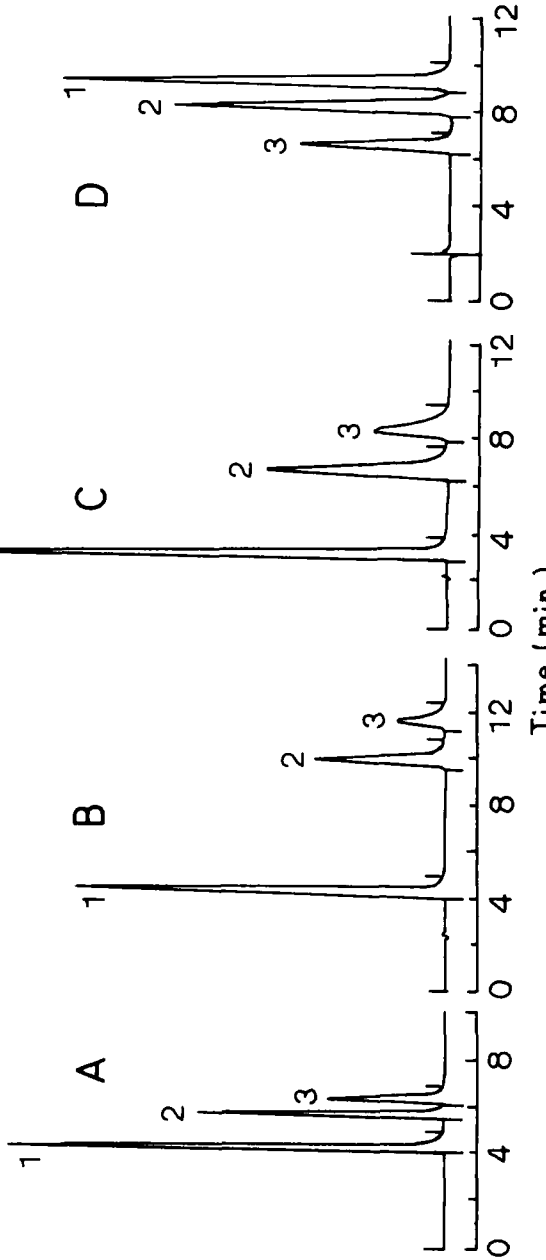

FIGURE 1. Typical capsule assay chromatograms obtained on each of the four cyano-bonded phase columns: Column A - 35% CH_3CN, pH 3.0, attn 128; Column B - 30% CH_3CN, pH 4.8, attn 128; Column C - 30% CH_3CN, pH 4.8, attn 64; Column D-16% CH_3CN, pH 5.5, attn 64. Remaining chromatographic conditions are given in the text. Key: 1, prazosin; 2, benzophenone; 3, polythiazide.

in the official monograph for prazosin hydrochloride bulk drug substance (17). Figure 2A shows a chromatogram of a prazosin solution which was spiked with 0.4% by weight of each of the five impurities. Prazosin concentration in the spiked solution was comparable to that of the prazosin stock solution. None of the five impurities was detected in either the working standard or USP reference standard prazosin hydrochloride. Detection of the impurities at 254 nm was selected because the sensitivity at 254 nm was twice that at 268 nm. The USP monograph employs five separate thin layer chromatographic limit tests for these impurities with visual comparison at the 0.5% level.

A chromatogram of polythiazide stock solution is shown in Figure 2B. Approximately 0.1% 4-amino-6-chloro-N^3-methyl-m-benzenedisulfonamide was detected in both the USP and working standards. No 4-amino-6-chloro-1,3-benzenedisulfonamide was detected in either standard. Analysis of a polythiazide solution spiked with both compounds showed that each impurity was detectable at the 0.1% level for each compound. The USP monograph for polythiazide drug substance uses a Bratton-Marshall colorimetric test for total diazotizable substances which determines both impurities with a combined limit of 1.0%. Additionally, three other trace impurities were detected in both standards. Two of the impurities have been tentatively identified as epithiazide and its dimethyl derivative based on synthetic and chromatographic considerations. The retention times of the drug substances and impurities are listed in Table 1.

Reproducibility of replicate injections was determined by injecting six 10 µl aliquots of mixed standard solution.

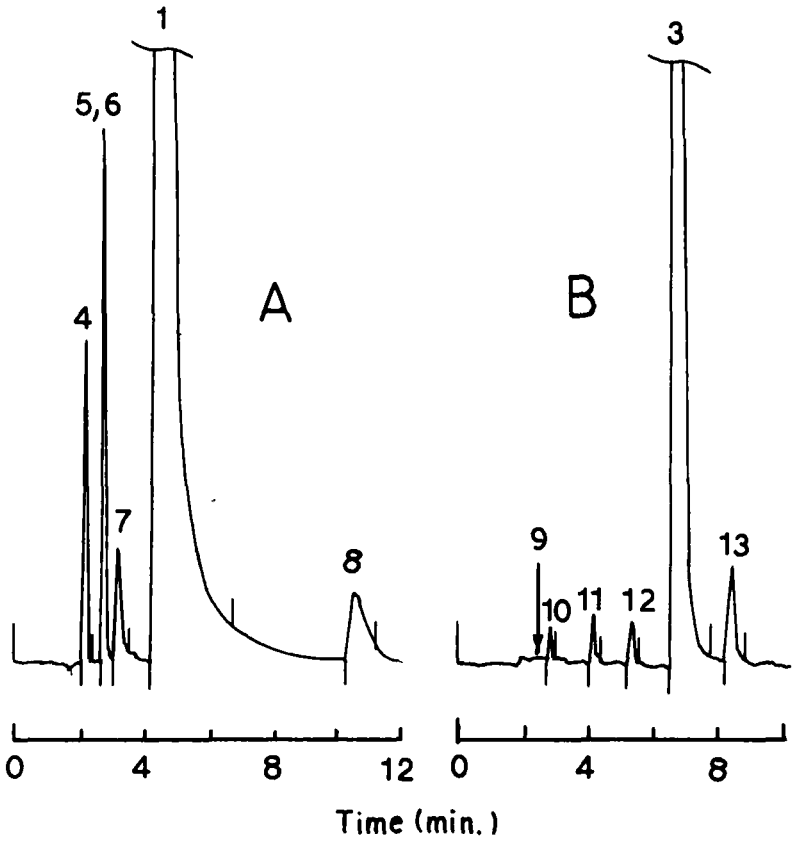

FIGURE 2. Typical chromatograms: A. Prazosin and impurities, 254 nm; B. Polythiazide and impurities, 268 nm. Same chromatographic conditions as capsule assay (column A, attn 32). Key: Same as Table 3.

Relative standard deviations were 0.4% for prazosin and 0.6% for polythiazide, respectively.

Linear response was determined by making duplicate injections of a series of 9 mixed standard solutions corresponding to 23% of the lowest prazosin dosage level (1 mg) to 116% of the highest dosage level (5 mg) and from 25% to 625% of the

TABLE 1

Retention Times of Drug Substances and Impurities

Key	Compound	Time (min.)
1	prazosin	4.23
2	benzophenone (internal standard)	5.54
3	polythiazide	6.46
4	1-(2-furoyl)piperazine	2.11
5	1,4-bis-(2-furoyl)piperazine	2.63
6	2-chloro-4-amino-6,7-dimethoxyquinazoline	2.64
7	4-amino-6,7-dimethoxy-2-(1-piperazinyl)quinazoline	3.04
8	1,4-bis-(4-amino-6,7-dimethoxy-2-quinazolinyl)piperazine	10.08
9	4-amino-6-chloro-1,3-benzenedisulfonamide	2.37
10	4-amino-6-chloro-N-methyl-m-benzenedisulfonamide	2.65
11	unidentified peak	3.98
12	epithiazide	5.11
13	dimethyl derivative of epithiazide	8.06

polythiazide dosage level. Linearity was observed from 50 ng to 1250 ng prazosin hydrochloride with a coefficient of correlation of 0.9999. Linearity of polythiazide response was observed from 25 ng to 625 ng with a coefficient of correlation of 0.9999. The minimum detectable levels, defined as signals twice the noise levels, were determined to be 0.6 ng prazosin and 1.0 ng polythiazide.

Freedom from sample matrix interferences was demonstrated by injecting a placebo formulation and sample solutions prepared without addition of the internal standard.

Capsules containing 0.5 mg polythiazide and 1, 2 or 5 mg prazosin were simultaneously assayed for both components by reverse phase HPLC. The method requires minimal sample preparation. Results of the capsule assays are presented in Table 2. All samples were well within the manufacturer's specifications for the product. The relative standard deviation calculated on the basis of six replicate determinations for each sample was, in all cases, less than 1.6% for polythiazide and less than 0.8% for prazosin.

Recovery data were obtained for synthetic formulations at each of the three dosage levels placebo and are found in Table 3. Prazosin was added to synthetic placebo formulations prepared to duplicate the sample formulations in amounts corresponding to 82.2% to 114.1% of the theoretical label claims. The mean recovery of 9 determinations was 100.2% ± 0.7%. Polythiazide was added to the synthetics placebos in amounts corresponding to 87.5% to 112.4% of the theoretical label claims. The mean recovery of 9 determinations was 99.7% ± 0.7%.

TABLE 2

Results Of Capsule Assays*

Formulation	Prazosin			Polythiazide		
	Label Claim (mg)	% Found	RSD	Label Claim (mg)	% Found	RSD
1	1	98.8	0.30	0.5	96.8	1.58
2	2	100.5	0.44	0.5	104.0	1.58
3	5	100.4	0.79	0.5	101.8	0.90

* Calculated on the basis of 6 determinations for each sample.

TABLE 3

Recovery Data From Synthetic Formulations

	Prazosin			Polythiazide		
Synthetic	Theoretical Formulation Level (mg)	% of Formulation Level Added	% Recovery	Theoretical Formulation Level (mg)	% of Formulation Level Added	% Recovery
1a	1	91.3	100.6	0.5	100.0	99.6
1b	1	114.1	101.4	0.5	90.0	100.4
1c	1	100.0	100.5	0.5	112.4	98.7
2a	2	91.3	100.1	0.5	100.0	98.9
2b	2	79.9	99.4	0.5	87.5	100.0
2c	2	100.0	100.7	0.5	112.4	98.9
3a	5	91.3	99.0	0.5	100.0	99.8
3b	5	82.2	99.7	0.5	100.0	100.6
3c	5	100.0	100.3	0.5	112.4	100.5
MEAN			100.2			99.7
RSD			0.73			0.74

A rapid, precise and accurate method for the analysis of prazosin and polythiazide in capsules was developed. The chromatographic system may also be employed for stability indicating assays of both drug substances and their impurities. The method could potentially be used for content uniformity assays as well as the analysis of single component prazosin hydrochloride or polythiazide dosage forms.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. Eric B. Sheinen, Drug Standards Research Branch, Food and Drug Administration, for supplying the prazosin impurity standards. The author is also indebted to Dr. James T. Stewart, College of Pharmacy, University of Georgia, and Linda J. Pennington, Food and Drug Administration, for their helpful comments in reviewing this manuscript.

REFERENCES

1. Honigberg, I. L., Stewart, J.T., Smith, A. P., and Hester, D.W., Liquid Chromatography in Pharmaceutical Analysis III: Separation of Diuretic-Antihypertensive Mixtures, J. Pharm. Sci., 64, 1250, 1975.

2. Honigberg, I. L., Stewart, J.T., Smith, A.P., Plunkett, R.D., and Hester, D.W., Liquid Chromatography in Pharmaceutical Analysis II: Determination of a Reserpine-Chlorothiazide Mixture, J. Pharm. Sci., 63, 1762, 1974.

3. Butterfield, A.G., Lovering, E.G., and Sears, R.W., Simultaneous Determination of Reserpine and Hydrochlorothiazide in Two-Component Tablet Formulations by High Performance Liquid Chromatography, J. Pharm. Sci., 67, 650, 1978.

4. Menon, G. N., and White, L.B., Simultaneous Determination of Hydrochlorothiazide and Triamterene in Capsule Formulations by High Performance Liquid Chromatography, J. Pharm. Sci., 70, 1083, 1981.

5. The United States Pharmacopeia, Twenty-first Revision, United States Pharmacopeial Convention, Inc., Rockville, MD, 1985, Mack Publishing Co., Easton, PA 18042, Amiloride Hydrochloride and Hydrochlorothiazide Tablets, p.36.

6. Ibid, Timolol Maleate and Hydrochlorothiazide Tablets, p. 1064.

7. Ibid, Methyldopa and Hydrochlorothiazide Tablets, p. 675.

8. Ting, S., Liquid Chromatographic Determination of Methyldopa and Methyldopa - Thiazide Combinations in Tablets: Collaborative Study, J. Assoc. Off. Anal. Chem., 67, 1118, 1984.

9. U.S.P. XXI, Clonidine Hydrochloride and Chlorthalidone Tablets, p. 235.

10. Moskalyk, R.E., Locock, R.A., Chatten, L.G., Veltman, A.M., and Bielech, M.F., Determination of Polythiazide in Pharmaceutical Dosage Forms by High-Pressure Liquid Chromatography, J. Pharm. Sci., 64, 1406, 1975.

11. Wong, C.K., Tsau, D.Y.J., Cohen, D.M., and Munnelly, K.P., High-Pressure Liquid Chromatographic Analysis of Polythiazide in Pharmaceutical Tablet Formulations, J. Pharm. Sci., 66, 736, 1977.

12. U.S.P. XXI, Polythiazide Tablets, p. 851.

13. Ibid, Prazosin Hydrochloride Tablets, p. 869.

14. Dokladova, J., Coco, S.J., Lemke, P.R., Quercia, G.T., and Korst, J.J., Determination of Polythiazide and Prazosin in Human Plasma by High Performance Liquid Chromatography, J. Chromatogr., 224, 33, 1981.

15. U.S.P. XXI, Polythiazide, p. 850.

16. Kikta, E.J., and Stange, A.E., Phenones: A Family of Compounds Broadly Applicable to Use As Internal Standards in High-Performance Liquid Chromatography, J. Chromatogr., 138, 41, 1977.

17. U.S.P. XXI, Prazosin Hydrochloride, p. 868.