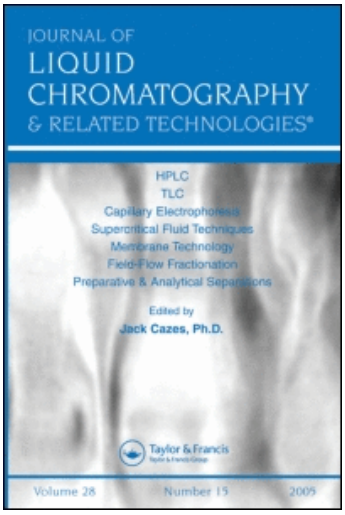

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Bachman, William J.(1986) 'High Performance Liquid Chromatographic Determination of Diuretic-Antihypertensive Combination Products. II. Polythiazide and Reserpine', Journal of Liquid Chromatography & Related Technologies, 9: 7, 1463 — 1478
To link to this Article: DOI: 10.1080/01483918608076696
URL: http://dx.doi.org/10.1080/01483918608076696

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(7), 1463-1478 (1986)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF DIURETIC-ANTIHYPERTENSIVE COMBINATION PRODUCTS. II. POLYTHIAZIDE AND RESERPINE

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ABSTRACT

A high performance liquid chromatographic method for the simultaneous determination of polythiazide and reserpine in tablets is described. Polythiazide, reserpine, and vanillin (an excipient) are separated isocratically on an octadecylsilane column using a methanol-water-acetic acid (55:44:1) mobile phase. The column effluent was monitored by ultraviolet absorbance at 254 nm. Recoveries from synthetic formulations were 100.2 ± 1.7% for polythiazide and 98.7 ± 1.1% for reserpine.

INTRODUCTION

Polythiazide and reserpine, two anti-hypertensive agents with complementary properties, are commonly

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prescribed in a combination tablet dosage form for the treatment of hypertension. Polythiazide, an orally effective long-acting diuretic of the benzothiadiazine (thiazide) class is indicated in the management of hypertension either as the sole therapeutic agent or to enhance the effectiveness of other antihypertensive drugs in the more severe forms of hypertension. Polythiazide alone has demonstrated clinical effectiveness in lowering elevated blood pressure in patients with or without visible edema (1).

Reserpine, an alkaloid of Rauwolfia serpentina, has several complementary actions of benefit in hypotensive therapy including a calming effect and a slowing of the pulse rate (1). It is used alone or in combination with thiazide diuretics for the management of mild, labile hypertension and in conjunction with potent hypotensive agents for the management of essential hypertension and hypertension associated with toxemia of pregnancy. Oral reserpine alone is considered to be of little value for severe hypertension but it is useful to augment or to prolong the action of potent hypotensive agents, to reduce dosage and side effects (2). Since polythiazide reduces or eliminates the sodium and fluid retention frequently associated with hypertension, it enhances the efficacy of reserpine in lowering elevated blood pressure. The combination often has been found to be more effective than equivalent doses of either agent alone (1).

Use of high performance liquid chromatography (HPLC) for the analysis of diuretic-antihypertensive

combination dosage forms has been the subject of a continuing study in this laboratory. The analysis of capsules containing prazosin hydrochloride and polythiazide was reported in a prior paper in this series (3). No HPLC methods have been reported for the determination of polythiazide and reserpine in combination.

Moskalyk et al. reported an HPLC method for polythiazide tablets (4). However, the method did not determine reserpine, had high recoveries, exhibited incomplete resolution of vanillin, polythiazide and quinoline (the reported internal standard), and, as reported by a second laboratory, was not reproducible (5). Wong et al. described an HPLC method which successfully separated polythiazide and vanillin in single component tablets but was not applicable to combinations containing reserpine which was retained on the column (6).

Vincent and Awang presented a reverse phase HPLC method for single component reserpine tablets using UV detection (7). The method requires preliminary partitioning and centrifuging of tablet extracts prior to chromatography. The chromatographic system resolves and permits the UV detection of 3-isoreserpine and fluorometric detection of 3,4-dehydroreserpine and 3,4,5,6-tetradehydroreserpine but not at the levels present in dosage forms.

Cieri reported a normal phase HPLC method with fluorometric detection for the determination of reserpine in commercial tablets containing reserpine

alone or in combination with chlorothiazide, hydrochlorothiazide or hydralazine hydrochloride (8). Only reserpine is determined by the method and tablets containing one or more of the other drug substances require a preliminary partitioning into chloroform and evaporation of the chloroform extract on a steam bath prior to the chromatographic determination. The possibility of reserpine oxidation in chloroform solution and instability to heat and light are well known (9).

A normal phase HPLC procedure for the simultaneous determination of hydrochlorothiazide and reserpine which used polythiazide as an internal standard was described by Butterfield, Lovering, and Sears (10). The authors suggested that the procedure should be applicable to commercial single component polythiazide tablets and polythiazide-reserpine tablets. However, the method did not resolve an impurity of polythiazide which appeared as a small shoulder on the front of the polythiazide peak. Also the chromatographic behavior of vanillin, a strongly ultraviolet absorbing excipient which is present in both formulations, was not determined.

The official USP assay procedure for single component polythiazide tablets uses preparative thin layer chromatography with ultraviolet spectrophotometric determination (11). The official methods for reserpine tablets (12), reserpine-hydrochlorothiazide tablets (13), and reserpine-hydralazine hydrochloride-hydrochlorothiazide tablets (14) all employ procedures requiring a preliminary partitioning step

and derivatization to reserpine oxidation products, mainly 3,4-dehydroreserpine, which are determined by visible absorbance at 390 nm. A blank determination is used as a correction for other alkaloids and degradation products present prior to analysis.

In a continuation of the study of HPLC methods for the analysis of multicomponent diuretic antihypertensive dosage forms, this paper reports the simultaneous determination of polythiazide and reserpine in tablets.

EXPERIMENTAL

Materials:

Polythiazide, reserpine, 4-amino-6-chloro-1,3-benzenedisulfonamide, and 4-amino-6-chloro-N^3-methyl-m-benzenedisulfonamide reference standards were all obtained from the United States Pharmacopeial Convention, Rockville, MD 20852. Polythiazide bulk drug substance used as a working standard was obtained from the manufacturer. Reserpine used as a working standard was obtained from K&K Laboratories, Inc., Plainview, NY 11803. 3-isoreserpine was synthesized by the procedure of MacPhillamy et al. (15). 3,4-dehydroreserpine was generated in solution by the nitrous acid oxidation of reserpine as per the official assay method (12) and injected directly onto the chromatograph. Glacial acetic acid was purchased from Fisher Scientific Co., Fairlawn, NJ 07410. Methanol was from Burdick & Jackson Laboratories, Inc., Muskegon, MI 49442.

Solutions:

a. HPLC mobile phase:

A solution was prepared by mixing 550 ml methanol, 440 ml water, and 10 ml glacial acetic acid. After equilibration to room temperature, the solution was filtered through a Millipore solvent filtration apparatus using a 0.45 pore size, nylon 66 membrane (Alltech Associates, Inc.,Deerfield, IL 60015).

b. Polythiazide stock solution:

Approximately 20 mg polythiazide was accurately weighed and dissolved in 50 ml of HPLC mobile phase.

c. Reserpine stock solution:

Approximately 25 mg reserpine was accurately weighed and dissolved in 500 ml of HPLC mobile phase. Solution was stored protected from light.

d. Mixed standard solution:

A 5.0 ml aliquot of polythiazide stock solution and a 5.0 ml aliquot of reserpine stock solution were pipetted into a 25 ml volumetric flask and diluted to volume with HPLC mobile phase. Solution was stored protected from light. A portion of the solution was transferred to an amber glass vial (Alltech Associates, Inc.) to protect it from light while in use.

Instrumentation:

The liquid chromatograph consisted of a Laboratory Data Control Constametric III pump, Spectromoni-

tor III variable wavelength detector, and Rheodyne Model 7125 loop injector (LDC/Milton Roy, Riviera Beach, FL 33404). Operating parameters - flow rate 1.5 ml/min., absorbance range 0.002, detection wavelength 254 nm, 10 µl loop, ambient temperature.

Integrator:

HP 3380A Integrator (Hewlett Packard Co., Avondale, PA 19311). Operating parameters - attenuation 256, slope sensitivity 3.0 mV/min, start delay off, area reject 10^4, stop time 15 min, chart speed 0.5 cm/min.

HPLC columns:

A: µBondapak C18, 10µ irregularly-shaped particles, 3.9 mm (i.d.) X 300 mm, (Waters Associates, Milford, MA 01757).

B: ASI C18, 10µ irregularly-shaped particles, 3.9 mm (i.d.) X 300 mm, (Analytical Sciences Inc., Santa Clara, CA 95050).

Analysis of Tablets:

Composites were prepared by manually grinding 20 tablets to pass a No. 60 mesh sieve. An accurately weighed portion of the powder equivalent to one tablet was transferred to a 25 ml volumetric flask and 15 ml of HPLC mobile phase was added. The 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 containing a 0.45µ pore size, 13 mm dia. nylon 66 membrane (Alltech

Associates, Inc.) and a glass fiber prefilter (Macro Filtration Systems, Dublin, CA 94566) into an amber glass vial to protect the sample from light. Duplicate 10 µl injections of each sample were bracketed with 10 µl injections of the mixed standard solution. Quantitation was by comparison of the average sample area response for each drug substance to the corresponding average standard area response.

Calculations:

mg polythiazide per tablet found =

$$\frac{\text{AR SPL}}{\text{AR STD}} \times \frac{\text{STD WT.}}{\text{SPL WT.}} \times \text{AVG. TAB. WT.}$$

where:

AR SPL = average sample area response for polythiazide

AR STD = average standard area response for polythiazide

STD WT. = weight of polythizaide in the mixed standard solution in mg

SPL WT. = weight of the sample in mg

AVG. TAB. WT. = average tablet weight in mg

Mg reserpine per tablet found was calcuated in a similar manner.

RESULTS AND DISCUSSION

Similar octadecylsilane columns from two different manufacturers were evaluated for use in the pro-

posed procedure. System suitability tests performed on both columns were equivalent and both columns were found to be suitable for the method. See Figure 1 for typical sample chromatograms obtained on each column.

Both columns resolved polythiazide and reserpine from tablet excipients including vanillin, polythiazide impurities, and reserpine impurities. Polythiazide impurities included 4-amino-6-chloro-1,3-benzenedisulfonamide and 4-amino-6-chloro-N^3-methyl-m-benzenedisulfonamide. Reserpine impurities resolved included 3,4-dehydroreserpine, the major oxidation product of reserpine, and 3-isoreserpine, its major epimerization product (16). Retention times of the drug substances, excipients and related compounds obtained on Column A are given in Table 1. No internal standard was employed because the retention times of the various known impurities prohibited selection of a compound which would not co-elute with a potential impurity on either column.

Ultraviolet detection at 254 nm was selected because the advantages of increased sensitivity and greater specificity offered by fluorescence detection were offset by those of UV detection - the convenience of simultaneous detection of both components and the simplicity of using only one detection system. The level of reserpine present in the tablets did not warrant the added complexity of a second detection mode. The minimum detectable levels, defined as signals twice the noise level, were 3 ng polythiazide and 5 ng reserpine. Both the proposed UV detec-

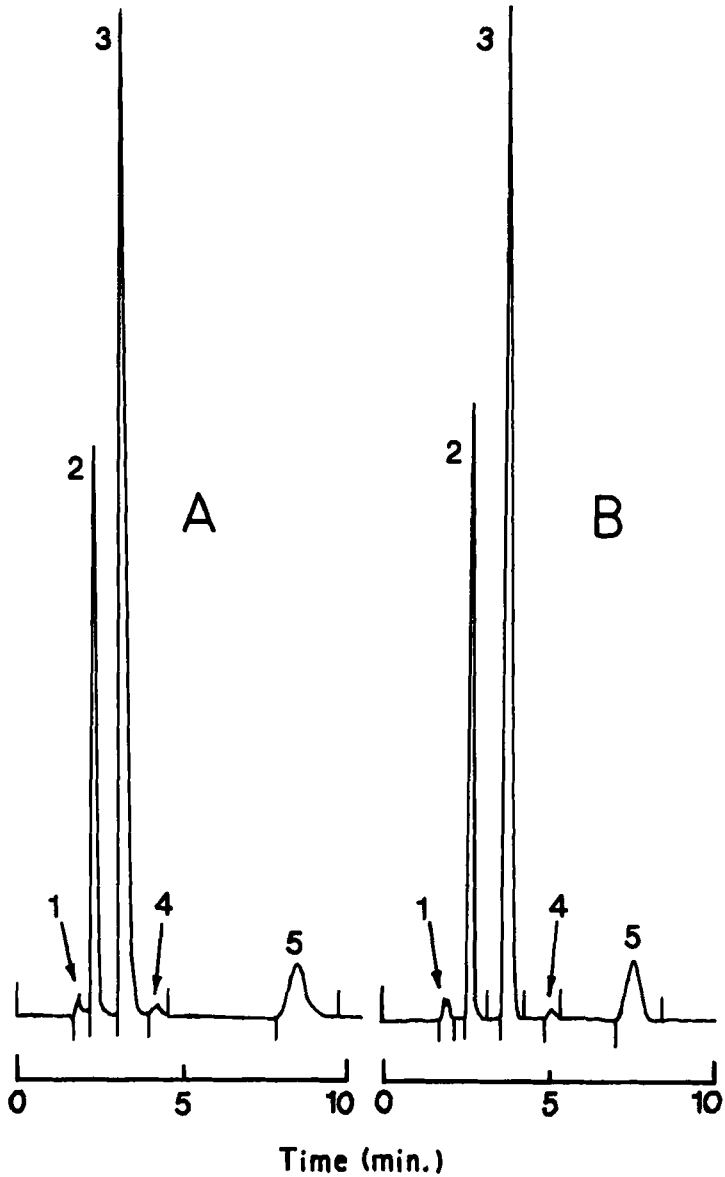

Figure 1. Typical sample chromatograms obtained on A: μBondapak C18, B. ASI C18. Key: Same as Table 1.

TABLE 1

Retention Times of Drug Substances and Related Compounds

Key	Compound	Retention Time(min.)
1	excipients	1.98
2	vanillin	2.52
3	polythiazide	3.46
4	polythiazide impurity	4.49
5	reserpine	8.86
6	4-amino-6-chloro-1,3-benzenedisulfonamide	1.89
7	4-amino-6-chloro-N^3-methyl-m-benzenedisulfonamide	1.98
8	3-isoreserpine	6.77
9	3,4-dehydroreserpine	12.06
10	rescinnamine	10.64
11	methychlothiazide	2.51
12	benzthiazide	3.53

tion method and the reported fluorescence detection method for reserpine determined the drug at the 100 ng level (8).

Linearity of response was determined by making duplicate 10 µl injections of a series of seven mixed standard solutions corresponding to 20% to 200% of

the theoretical label claims for both components. Linearity was observed from 160 ng to 1600 ng polythiazide with a coefficient of correlation of 0.9999. Linearity of reserpine response was observed from 20 ng to 200 ng with a coefficient of correlation of 0.9999.

Reproducibility of replicate injections was determined by making six 10 μl injections of mixed standard solution. Relative standard deviations were 1.5% for polythiazide and 1.7% for reserpine.

Polythiazide and reserpine were added to six synthetic placebo formulations, prepared to duplicate the sample formulation, in amounts corresponding to 90.0% to 110.0% of the theoretical label claims of each active principle. Recovery data obtained from the analyses of the synthetic formulations are given in Table 2. The mean recovery of polythiazide was 100.2% ± 1.7%. The mean reserpine recovery was 98.7%± 1.1%. Lack of interferences from sample excipients was demonstrated by injection of a placebo formulation.

Tablets containing 2 mg polythiazide and 0.25 mg reserpine were simultaneously assayed for both components by reverse phase HPLC. The method requires no preliminary partitioning, evaporation or centrifuging of tablet extracts. The minimal sample preparation entails only dilution, sonication and filtration prior to chromatographic determination. Two representative samples were analyzed. Sample 1 was a current product batch with 27 months before its labeled expi-

TABLE 2

Recovery Data From Synthetic Formulations

	Polythiazide		Reserpine	
Synthetic	% of Formulation Level Added[a]	% Recovery	% of Formulation Level Added[b]	% Recovery
1	100.0	97.5	90.0	98.9
2	90.0	100.7	90.0	99.8
3	100.0	102.5	100.0	98.1
4	90.0	100.7	110.0	97.5
5	110.0	99.5	90.0	98.0
6	100.0	100.0	100.0	100.1
Mean		100.2		98.7
RSD		1.65		1.06

[a] Theoretical formulation level 2.0 mg per tablet

[b] Theoretical formulation level 0.25 mg per tablet

TABLE 3

Results of Tablet Assays[a]

	Polythiazide		Reserpine	
Sample	% of Label Claim Found[b]	RSD	% of Label Claim Found[c]	RSD
1	104.5	0.74	100.0	2.04
2	102.0	1.03	98.0	1.58

[a] Calculated on the basis of 6 determinations for each sample

[b] Label claim 2.0 mg per tablet

[c] Label claim 0.25 mg per tablet

ration date. Sample 2 was analyzed 8 months after the labeled expiration date. Both samples were found to be within the manufacturer's specifications for the product. Results of the tablet assays and relative standard deviations calculated on the basis of six replicate determinations for each sample are presented in Table 3.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation 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.

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