REFERENCES

(1) E. Pawelczyk and R. Wachowiak, *Diss. Pharm. Pharmacol.*, **21**, 491 (1969).

(2) *Ibid.*, **22**, 475 (1970).

(3) W. Awe and H. J. Kienert, *Pharm. Acta Helv.*, **38**, 805 (1963).

(4) J. Slingsby and D. A. Zuck, *Can. J. Pharm. Sci.*, **7**, 115 (1972).

(5) E. Pawelczyk and R. Wachowiak, *Acta Pol. Pharm.*, **26**, 425 (1969).

(6) E. Pawelczyk and R. Wachowiak, *Diss. Pharm. Pharmacol.*, **20**, 653 (1973).

(7) E. Pawelczyk and M. Zajac, *Acta Pol. Pharm.*, **27**, 104 (1970).

(8) H. D. Beckstead, K. K. Kaistha, and S. J. Smith, *J. Pharm. Sci.*, **57**, 1952 (1968).

(9) D. V. C. Awang, A. Vincent, and F. Matsui, *ibid.*, **62**, 1973 (1973).

(10) D. V. C. Awang and A. Vincent, *ibid.*, **65**, 68 (1976).

(11) H. G. Ibrahim, F. Pisano, and A. Bruno, *ibid.*, **66**, 669 (1977).

(12) D. Barrett and J. J. Fell, *ibid.*, **64**, 335 (1975).

(13) "Annual Book of ASTM Standards," Part 35, American Society for Testing and Materials, Philadelphia, Pa., 1975, p. 852.

(14) J. R. Watson, F. Matsui, R. C. Lawrence, and P. M. J. McConnell, *J. Chromatogr.*, **76**, 141 (1973).

(15) E. G. Lovering and C. A. Mainville, *Can. J. Pharm. Sci.*, in press.

(16) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975.

Simultaneous Determination of Reserpine and Hydrochlorothiazide in Two-Component Tablet Formulations by High-Performance Liquid Chromatography

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Received March 7, 1977, from the *Drug Research Laboratories, Health Protection Branch, Ottawa, Ontario, K1A 0L2, Canada.* Accepted for publication August 22, 1977.

Abstract □ A high-performance liquid chromatographic procedure is presented for the simultaneous determination of reserpine and hydrochlorothiazide in two-component tablet formulations. An aliquot of a tetrahydrofuran extract of the tablet, containing polythiazide as an internal standard, is chromatographed on a microparticulate silica gel column using a mobile phase of 0.01% (v/v) diethylamine, 5% (v/v) chloroform, and 18% (v/v) 2-propanol in *n*-hexane. The relative standard deviations are 1.2 and 0.6% for the simultaneous determination of reserpine and hydrochlorothiazide, respectively. Seven commercial tablet formulations were found to contain 92.7–101.0% and 98.3–101.4% of the labeled amounts of reserpine and hydrochlorothiazide, respectively.

Keyphrases □ Reserpine—high-performance liquid chromatographic analysis, simultaneously with hydrochlorothiazide, in tablets □ Hydrochlorothiazide—high-performance liquid chromatographic analysis, simultaneously with reserpine, in tablets □ High-performance liquid chromatography—simultaneous analyses, hydrochlorothiazide and reserpine in tablets □ Diuretics—hydrochlorothiazide, high-performance liquid chromatographic analysis, simultaneously with reserpine, in tablets □ Antihypertensives—reserpine, high-performance liquid chromatographic analysis, simultaneously with hydrochlorothiazide, in tablets

Reserpine–hydrochlorothiazide formulations are marketed in Canada by several manufacturers. A rapid, accurate procedure, applicable to all formulations, was required for the simultaneous determination of both drugs in these preparations. Literature methods are based on many different techniques for the analysis of the individual compounds (1–5), but only an automated procedure (6) simultaneously determines both compounds. This automated procedure is useful for content uniformity purposes but is cumbersome for occasional use and is not specific for the determination of reserpine or hydrochlorothiazide since known impurities may interfere.

High-performance liquid chromatography (HPLC) has been used extensively for the analysis of mixtures (7–9) because of high specificity, speed, and sensitivity. The technique has been applied to the analysis of alkaloids (10–13) and thiazide diuretics (14–16), including the quantitative analysis of reserpine–chlorothiazide mixtures (16). This report describes an HPLC procedure for the simultaneous determination of reserpine and hydrochlorothiazide in two-component tablet formulations.

EXPERIMENTAL

Materials—Reserpine[1], hydrochlorothiazide[2], polythiazide[3], and 1-amino-3-chloro-4,6-benzenedisulfonamide[4] (I) were used as received. Both drug substances were essentially identical to the corresponding reference standards[5] when retention times, impurities, and response factors were compared by HPLC and when drug content was compared by the USP XIX assay procedure (5). Solvents and reagents were commercial analytical reagent grade, except for tetrahydrofuran[6] and *n*-hexane[6] which were UV grade. Tetrahydrofuran was stored under nitrogen.

Apparatus—A liquid chromatograph[7] fitted with a septumless injection port[7], a fixed-wavelength UV detector[8] (254 nm), and a computing integrator[9] was used. The detector was attenuated to 0.01 absorbance unit full scale (aufs).

Column—A 250 × 2.1-mm i.d. column[10] packed with 5-μm diameter silica gel[11], using a balanced density slurry technique similar to that described by Majórs (17), was used at ambient temperature and at a mobile phase flow rate of 90 ml/hr (210 bar).

Mobile Phase—A solution of 0.01% (v/v) diethylamine, 5% (v/v) chloroform, and 18% (v/v) 2-propanol in *n*-hexane was prepared as required. It was degassed (refluxed for 5 min) and stored in the solvent reservoir of the instrument.

Internal Standard Solution—A solution of polythiazide in tetrahydrofuran (0.1 mg/ml) was used.

Preparation of Standard Curves—Stock solutions of reserpine (0.10 mg/ml), hydrochlorothiazide (25 mg/ml), and polythiazide (0.5 mg/ml)

[1] Aldrich Chemical Co., Montreal, Canada.
[2] Merck Sharp and Dohme, Kirkland, Canada.
[3] Pfizer Co., Arnprior, Canada.
[4] Pfaltz and Bauer, Flushing, N.Y.
[5] United States Pharmacopeial Convention, Rockville, Md.
[6] Burdick and Jackson Laboratories, Muskegon, Mich.
[7] Model 4100, Varian Aerograph, Palo Alto, Calif.
[8] Model 440, Waters Associates, Milford, Mass.
[9] Autolab System I, Spectra-Physics, Santa Clara, Calif.
[10] Li-Chroma I.D., Alltech Associates, Arlington Heights, Ill.
[11] LiChrosorb SI 60, British Drug Houses, Toronto, Canada.

Table I—Retention Time and Capacity Factor of Reserpine, Hydrochlorothiazide, and Related Compounds

Compound	Retention Time[a], sec	Capacity Factor[b] (k')
Reserpine[c]	68	1.0
3-Isoreserpine[d]	50	0.5
3,4-Dehydroreserpine[e]	—[l]	—
3,4,5,6-Tetrahydroreserpine[e] (lumireserpine)	—[l]	—
Deserpidine[f]	61	0.8
Rescinnamine[g]	82	1.4
3,4,5-Trimethoxybenzoic acid[c]	—[l]	—
3,4,5-Trimethoxycinnamic acid[c]	—[l]	—
1-Amino-3-chloro-4,6-benzenedisulfonamide[h]	210	5.2
Hydrochlorothiazide[i]	320	8.5
Chlorothiazide	200	4.9
Methyclothiazide[f]	114	2.4
Bendroflumethiazide[j]	59	0.7
Polythiazide[k]	108	2.2

[a] Using 0.01% diethylamine, 5% chloroform, and 18% 2-propanol in n-hexane; 1.5 ml/min; and 250 × 2.1 mm LiChrosorb SI 60 (5 μm). [b] The $k' = (t_r - t_0)/t_0$, where t_r = retention time and t_0 = retention time of n-hexane. [c] Aldrich Chemical Co., Montreal, Canada. [d] Prepared by the method of MacPhillamy *et al.* (18). [e] Prepared by carrying out the "assay" for reserpine in USP XIX (5). [f] Abbott Laboratories, Montreal, Canada. [g] NF Reference Standard. [h] Pfaltz and Bauer, Flushing, N.Y. [i] Merck Sharp and Dohme, Kirkland, Canada. [j] Frank W. Horner, Ltd., Montreal, Canada. [k] Pfizer Co., Arnprior, Canada. [l] Retained peak.

in tetrahydrofuran were prepared. Standard solutions were prepared by pipetting 5 ml of the polythiazide stock solution into each of eight 25-ml volumetric flasks along with 1.0, 3.0, 5.0, 6.0, 7.0, 8.0, 9.0, or 10.0 ml of both the reserpine and hydrochlorothiazide stock solutions. Each solution was made up to volume with tetrahydrofuran, if necessary, and duplicate 7-μl aliquots of each standard solution were chromatographed. Samples were stored at 0° until they could be analyzed.

Preparation of Calibration Standard Solutions—Approximately 25 and 50 mg of reserpine were accurately weighed into separate 25-ml volumetric flasks, dissolved, and made up to volume with tetrahydrofuran. Each solution was diluted by pipetting 1.0 ml of the initial solution into a 10-ml flask and bringing to volume with tetrahydrofuran. Similarly, approximately 25 and 50 mg of hydrochlorothiazide were accurately weighed into separate 10-ml volumetric flasks. One milliliter of each diluted reserpine stock solution was pipetted into the flask containing the corresponding level of hydrochlorothiazide along with 2.0 ml of polythiazide internal standard solution (0.5 mg/ml). Each solution was made up to volume with tetrahydrofuran.

Duplicate 7-μl aliquots of the resulting solutions were chromatographed periodically to determine the slopes of the calibration curves. When not in use, the standards were stored in the dark at 0°. Calibration solutions were prepared daily.

Analysis of Pharmaceuticals—*Single-Tablet Assay*—A tablet was placed in a 16 × 125-mm screw-capped culture tube[12] and crushed to a fine powder with a glass rod. Then 10.0 ml of internal standard solution (0.1 mg/ml) was added to the tube, which was closed, tumbled on a rotator[13] at 30 rpm for 20 min, and centrifuged[14] at 2000 rpm for 5 min. Duplicate 7-μl aliquots of the supernate were chromatographed.

Tablet-Composite Assay—A tablet composite was prepared by grinding 20 tablets in a mechanical mill[15]. A quantity of powdered tablet material equivalent to one tablet was weighed into a 16 × 125-mm screw-capped culture tube. The sample was treated as previously described.

Calculations—*Calibration*—The slopes of the standard curves were calculated from the following equation, using data obtained by chromatographing the calibration solutions:

$$S = \frac{N_D C_I}{N_I C_D} \qquad \text{(Eq. 1)}$$

where S is the slope of the standard curve for the drug, either reserpine or hydrochlorothiazide; N_D is the peak height (millimeters) for reserpine or the peak area for hydrochlorothiazide; C_I is the concentration of the internal standard (milligrams per milliliter); N_I is the integrator counts

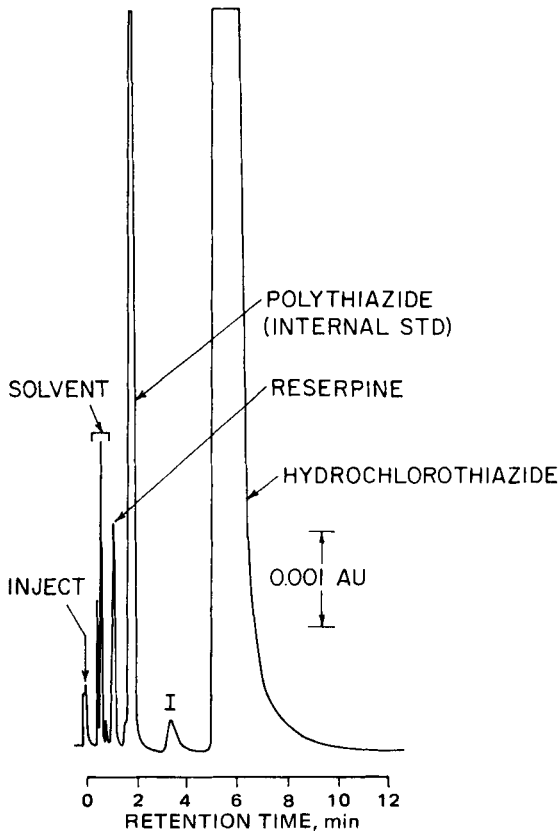

Figure 1—*High-performance liquid chromatogram of a tablet extract containing approximately 0.0125 mg of reserpine/ml, 0.1 mg of polythiazide/ml (internal standard), 2.5 mg of hydrochlorothiazide/ml, and a trace amount of I. Chromatographic conditions are given in the text.*

for the internal standard; and C_D is the concentration (milligrams per milliliter) of reserpine or hydrochlorothiazide.

Quantitation—The percent label claim for reserpine or hydrochlorothiazide was calculated from:

$$\%\ \text{label claim} = \frac{N_D C_I}{N_I \bar{S} W} \times 1000 \qquad \text{(Eq. 2)}$$

where W is the weight (milligrams) of reserpine or hydrochlorothiazide claimed to be present in the tablet or tablet composite analyzed and $\bar{S}$ is the mean slope of the appropriate standard curve.

RESULTS AND DISCUSSION

Chromatography—Previous reports on the HPLC analysis of reserpine–thiazide diuretic mixtures (14, 16) were based upon reversed-phase chromatography. Initial attempts to utilize this mode, employing a microparticulate reversed-phase column[16], were not pursued because the major component, hydrochlorothiazide, eluted ahead of the minor component, reserpine. Since peak shape and column efficiency were not satisfactory, no attempt was made to reverse this order of elution. Instead, a change was made to a forward-phase system with a microparticulate silica gel column. Mobile phase constituents were adjusted to elute reserpine immediately after the solvent front and to elute hydrochlorothiazide later. This system was desirable to maximize the response for reserpine.

The specificity of the procedure for reserpine was tested by chromatographing samples of several related compounds (Table I). Deserpidine[17] and rescinnamine[18], when present, appeared as shoulders on the reserpine peak. The presence of 3-isoreserpine and 3,4-dehydroreserpine in formulations has been reported (19). The former was well separated from reserpine, and the latter was retained on the column. If necessary, formulations can be tested for 3,4-dehydroreserpine by TLC (19).

[12] Canadian Laboratory Supplies, Montreal, Canada.
[13] Multi-Purpose rotator, Scientific Industries, Springfield, Mass.
[14] Model K, International Equipment Co., Needham Heights, Mass.
[15] Micro mill, Chemical Rubber Co., Cleveland, Ohio.
[16] μBondapak C_{18}, Waters Associates, Milford, Mass.
[17] Abbott Laboratories, Montreal, Canada.
[18] NF reference standard.

Table II—Analysis of Synthetic Solutions

Sample	Reserpine			Hydrochlorothiazide		
	Calculated, μg/Injection	Found, μg/Injection	Recovery, %	Calculated, μg/Injection	Found, μg/Injection	Recovery, %
1	0.151	0.152	100.7	15.48	15.69	101.3
2	0.045	0.045	99.7	30.08	30.08	100.0
3	0.090	0.091	100.9	26.64	26.12	98.0
4	0.045	0.044	98.4	25.27	24.89	98.5
5	0.090	0.089	98.9	24.88	25.41	102.1
Mean recovery, %			99.7			100.0
RSD, %			1.1			1.8

Table III—HPLC Analysis of Reserpine–Hydrochlorothiazide Tablet Formulations [a]

Formulation	Reserpine		Hydrochlorothiazide	
	Label, mg	Found [b], %	Label, mg	Found [b], %
A	0.2	96.1	50	101.4
B	0.125	100.1	25	97.8
C	0.125	98.0	25	100.1
D	0.125	101.0	50	98.3
E	0.10	92.7	25	99.2
F	0.20	99.3	50	98.5
G	0.125	100.6	25	98.6

[a] Tablet-composite assay. [b] Percent of label claim.

Trimethoxybenzoic acid[1] and trimethoxycinnamic acid[1], the hydrolysis products of reserpine and rescinnamine, respectively, either did not elute or eluted but were not detected. Samples of reserpic acid and methyl reserpate were not available, but, based on structural considerations, reserpic acid probably would be retained on the column and methyl reserpate would elute ahead of reserpine.

A common impurity in hydrochlorothiazide formulations, 1-amino-3-chloro-4,6-benzenedisulfonamide (I), was observed in several reserpine–hydrochlorothiazide tablet formulations. Comparison to authentic material showed that there was less than 0.5% I relative to the hydrochlorothiazide level in all cases. Figure 1 shows the separation of reserpine, polythiazide, I, and hydrochlorothiazide obtained when an aliquot of a tablet extract was chromatographed. The small shoulder on the front of the polythiazide peak was due to an impurity in the drug substance.

When commercial analytical reagent grade solvents capable of dissolving hydrochlorothiazide at the 5-mg/ml level, such as ethanol, methanol, and tetrahydrofuran, were used for extraction, the solvent front interfered with the reserpine peak. With tetrahydrofuran, this interference proved to be due either to the presence of butylated hydroxyanisole preservative or to tetrahydrofuran decomposition products. When preservative-free tetrahydrofuran[6] was used, satisfactory results were obtained if care was taken to store the solvent under nitrogen.

It may be necessary to adjust the proportion of mobile phase constituents slightly to obtain the desired separation with a particular column. The retention times of hydrochlorothiazide and polythiazide can be lengthened appreciably by increasing the proportion of diethylamine to 0.02–0.03%, or they can be shortened by increasing the proportion of 2-propanol, with only a marginal effect on reserpine retention. The reserpine retention time can be decreased by an increase in the proportion of chloroform, which only slightly affects the retention times of hydrochlorothiazide and polythiazide.

Linearity and Standard Curves—A plot of peak area *versus* the amount of polythiazide (internal standard) injected was linear up to about 1.4 μg/injection (0.2 mg/ml). A 0.1-mg/ml level was used in the analysis.

A standard curve for reserpine was constructed by plotting the ratio of the reserpine peak height to the internal standard peak area *versus* the ratio of the weight of reserpine to the weight of the internal standard. Similarly, a standard curve for hydrochlorothiazide was constructed by plotting the ratio of the hydrochlorothiazide peak area to the internal standard peak area *versus* the corresponding weight ratio.

The standard curve for reserpine, examined over the range of 28–280 ng/injection (4–40 μg/ml), was linear with a negligible intercept up to at least 280 ng/injection. The mean slope of the linear portion of the curve was 0.023 with a relative standard deviation of 1.2%. Similarly, the standard curve for hydrochlorothiazide, examined over the range of 7–70 μg/injection (1–10 mg/ml), was linear with a negligible intercept up to at least 50 μg/injection. The mean slope of the linear portion of the line was 1.08 with a relative standard deviation of 0.6%. Therefore, the standard curves were applicable to tablets containing from 0.04 to 0.40 mg of reserpine and from 10 to 100 mg of hydrochlorothiazide.

Duplicate injections of calibration standard solutions were chromatographed daily to determine the slopes of the standard curves. A check sample also was analyzed daily to confirm that the calibration of the system was accurate and to monitor system stability. This sample was drawn from a homogeneous tablet composite previously analyzed in quintuplicate. The slopes obtained from calibration standards varied over a range of approximately 2.5% during the assay. However, the working relative standard deviations obtained over the same period from check sample data were 1.2% for reserpine and 0.6% for hydrochlorothiazide.

Sample Preparation—The time required for constant extraction of reserpine and hydrochlorothiazide from the tablet mass was determined by tumbling crushed single tablets of a representative formulation for 5, 10, and 15 min in 10 ml of the internal standard solution. In each case, the sample was centrifuged briefly after the appropriate time interval and a 7-μl aliquot was chromatographed. The sample to internal standard peak height or area ratios were identical in all three cases, indicating constant extraction of both compounds in 5 min or less.

Extraction appeared to be complete based on response factor data and the label claim of the formulation examined. This result was proven using a geometric dilution procedure. Tablet-composite samples, equivalent to 0.25-, 0.5-, and 1.0-mg tablets, were prepared from a formulation containing 0.2 mg of reserpine and 50 mg of hydrochlorothiazide and analyzed using a 20-min tumbling time. The concentrations of both reserpine and hydrochlorothiazide in the three composite samples were in the ratio 1:2:4. These data showed that constant and complete extraction of the compounds of interest was effected in 20 min or less.

Repeated injection of an extracted sample showed that, at 22°, reserpine isomerized readily to 3-isoreserpine; cooling to 0° allowed samples to be stored for several hours with negligible isomerization. Accordingly, all samples were prepared only as required and were cooled to 0° as soon as possible after preparation. Because of the known sensitivity of reserpine to photooxidation, all work was performed in a darkened laboratory, and all samples were covered with aluminum foil.

Quantitation—Reproducibility of the chromatographic system was shown by chromatographing six 7-μl aliquots of an extract of a formulation labeled to contain 25 mg of hydrochlorothiazide and 0.125 mg of reserpine. The relative standard deviations of the sample to internal standard peak area ratios for hydrochlorothiazide and reserpine were ±0.64 and ±2.3%, respectively. However, since the relative standard deviation of the reserpine peak height to internal standard peak area ratios was only ±1.2%, this latter approach was used for quantitation of reserpine. The use of peak height for quantitation has the added advantage that accuracy can be maintained in the presence of the small, partially resolved, peaks (20), which occur if related alkaloids are present (*e.g.*, deserpidine and/or rescinnamine).

Five solutions of reserpine and hydrochlorothiazide, prepared in the same manner as the calibration standard solutions, were analyzed. The mean recoveries for the two compounds were 99.7 ± 1.1 and 100.0 ± 1.8%, respectively (Table II).

Five replicate samples of a composite, prepared from tablets labeled to contain 0.125 mg of reserpine and 25 mg of hydrochlorothiazide, were assayed. The relative standard deviations of the assay were ±0.64% for hydrochlorothiazide and ±0.70% for reserpine.

Seven commercial formulations of reserpine–hydrochlorothiazide tablets were analyzed in duplicate. Drug content, expressed as a percentage of the label claim, ranged from 92.7 to 101.0% for reserpine and from 98.3 to 101.4% for hydrochlorothiazide (Table III).

The minimum detectable level of I is approximately 25 ng/injection (2× noise). Therefore, as little as 0.05% of I could be detected in a formulation containing 50 mg of hydrochlorothiazide.

The described procedure is applicable to the determination of reserpine and hydrochlorothiazide in single tablets and in tablet composites. The procedure is unlikely to be suitable for the analysis of commercial reserpine–chlorothiazide formulations because of the poor solubility of chlorothiazide in tetrahydrofuran. However, the procedure should be suitable for the analysis of commercial polythiazide–reserpine formulations and deserpidine–methyclothiazide formulations. It may also be applicable to the analysis of single-component formulations of most of the drugs listed in Table I.

REFERENCES

(1) C. R. Szalkowski and W. J. Mader, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 613 (1956).

(2) W. J. Mader, R. P. Haycock, P. B. Sheth, and R. J. Connelly, *J. Assoc. Offic. Agr. Chem.*, **43**, 291 (1960).

(3) T. Urbányi and H. Stober, *J. Pharm. Sci.*, **59**, 1824 (1970).

(4) A. I. Cohen, B. T. Keeler, N. H. Coy, and H. L. Yale, *Anal. Chem.*, **34**, 216 (1962).

(5) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. 235, 438.

(6) H. Kala, *Pharmazie*, **16**, 297 (1961).

(7) A. G. Butterfield and R. W. Sears, *J. Pharm. Sci.*, **66**, 1117 (1977).

(8) T. L. Sprieck, *ibid.*, **63**, 591 (1974).

(9) A. Menharth, F. P. Mahn, and J. E. Heveran, *ibid.*, **63**, 430 (1974).

(10) R. Verpoorte and A. B. Svendren, *J. Chromatogr.*, **100**, 227 (1974).

(11) R. A. Heacock, K. R. Langille, J. D. MacNeil, and R. W. Frei, *ibid.*, **77**, 425 (1973).

(12) M. H. Stutz and S. Sass, *Anal. Chem.*, **45**, 2134 (1973).

(13) G. H. Jolliffe and E. J. Shellard, *J. Chromatogr.*, **81**, 150 (1973).

(14) I. L. Honingberg, J. T. Stewart, A. P. Smith, and D. W. Hester, *J. Pharm. Sci.*, **64**, 1201 (1975).

(15) R. E. Moskalyk, R. A. Locock, L. G. Chatten, A. M. Veltman, and M. F. Bielech, *ibid.*, **64**, 1406 (1975).

(16) I. L. Honingberg, J. T. Stewart, A. P. Smith, R. D. Plunkett, and D. W. Hester, *ibid.*, **63**, 1762 (1974).

(17) R. E. Majors, *Anal. Chem.*, **44**, 1722 (1972).

(18) H. B. MacPhillamy, C. F. Huebner, E. Schlittler, A. F. St. André, and P. R. Ulshafer, *J. Am. Chem. Soc.*, **77**, 4355 (1955).

(19) G. E. Wright and T. Y. Tang, *J. Pharm. Sci.*, **61**, 299 (1972).

(20) L. R. Snyder, *J. Chromatogr. Sci.*, **10** (4), 200 (1972).

Determination of Serum Nadolol Levels by GLC–Selected Ion Monitoring Mass Spectrometry: Comparison with a Spectrofluorometric Method

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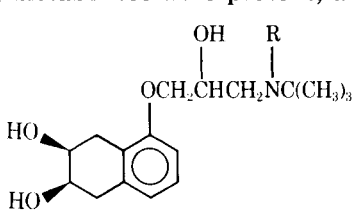
Abstract □ A method to determine the serum concentration of the β-adrenergic receptor blocking agent, nadolol, by GLC-selected ion monitoring mass spectrometry of the tri(trimethylsilyl) ether derivative is described. A basic solution of serum was extracted, known amounts of internal standard were added to the extract, and the extract was back-extracted into acidic media and lyophilized. The resulting solids were reacted with *N*-trimethylsilylimidazole. Coded serum samples of 12 subjects, given nadolol alone or in combination with a second drug, were analyzed. The ions at *m/e* 86 and 100 were monitored to establish the relative concentration ratio of nadolol and the internal reference *N*-methylnadolol. No interferences from blood components or other administered drugs were observed. A detection level of 6.95 ng/ml of serum was found.

Keyphrases □ Nadolol—GLC–mass spectrometric analysis in serum □ GLC–mass spectrometry—analysis, nadolol in serum □ Antiadrenergic agents—nadolol, GLC–mass spectrometric analysis in serum

Nadolol, 2,3-*cis*-1,2,3,4-tetrahydro-5-[2-hydroxy-3-(*tert*-butylamino)propoxy]-2,3-naphthalenediol (I), is a potent β-adrenergic receptor blocking agent (1, 2) that has no detectable metabolites (3, 4). With the assumption that no interfering metabolites were present, a fluorescence method previously was developed to determine serum nadolol concentrations (5). The GLC–mass spectrometric method was developed to monitor serum levels by selected ion monitoring, using a modification of the extraction procedure developed for the fluorescence method (5).



I: R = H

II: R = CH_3

EXPERIMENTAL

Reagents—Methanol, nitric acid, hydrochloric acid, and potassium chloride were reagent grade. Sodium hydroxide[1], *n*-butyl acetate[2], and petroleum ether (bp 30–60°) were used without further purification. Both nadolol[3] (I) and *N*-methylnadolol[3] (II) were used without further purification.

A solution of dimethyldichlorosilane[4] was used to condition the glassware used in the extraction and lyophilization procedures. The compounds were derivatized and the GLC column was conditioned with *N*-trimethylsilylimidazole in pyridine[5] (1.5 mEq/ml), obtained in 1-ml sealed glass ampuls.

Standard Solutions—Just prior to use, a solution of 40 ng of II/ml was prepared in *n*-butyl acetate. A stock solution of 4000 ng of I/ml in 0.1 *N* HCl was used to prepare the standard serum samples.

Glassware—All glassware used in the extraction procedure (150-mm screw-capped test tubes fitted with conical polypropylene liners[6] in plastic caps; 5- and 10-ml serological pipets[7]; 2- and 10-ml volumetric pipets[7]; and 10-, 25-, 50-, and 100-ml and 1- and 2-liter volumetric flasks[7])

[1] Aristar, BDH Chemicals Ltd., Poole, England.

[2] Gold Label-spectrophotometric grade, Aldrich Chemical Co., Milwaukee, Wis.

[3] E. R. Squibb & Sons, Princeton, N.J.

[4] Supelco, Inc., Bellefonte, Pa.; Sylon-CT is dimethyldichlorosilane in toluene (5%).

[5] Tri Sil Z, Pierce Chemical Co., Rockford, Ill.

[6] Catalog No. 24-420, Polyseal Corp., Jersey City, N.J.

[7] Fisher Scientific Co.