High-Performance Liquid Chromatographic Analysis of Nadolol and Bendroflumethiazide Combination Tablet Formulations

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Abstract □ A reverse-phase high-performance liquid chromatographic (HPLC) method was developed for the simultaneous assay of nadolol and bendroflumethiazide in tablet formulations. The tablets were extracted with methanol and, after centrifugation, were chromatographed. A phenyl column was used with a mobile phase of aqueous acetate buffer with sodium chloride–methanol (60:40); detection was at 270 nm. Linearity of both drugs was satisfactory. The procedure can be automated and also applied to bendroflumethiazide formulations and bulk material.

Keyphrases □ Nadolol—high-performance liquid chromatography with bendroflumethiazide, combination tablet formulations □ Bendroflumethiazide—high-performance liquid chromatography, with nadolol, combination tablet formulations

One of the principal uses of β-adrenergic-blocking drugs is for the control of hypertension (1). Accordingly, the β-blocker nadolol (2) has been formulated with the diuretic bendroflumethiazide since they work synergistically and need to be administered only once a day (3). A reverse-phase high-performance liquid chromatographic (HPLC) method for analyzing nadolol and other β-adrenergics has been developed in this laboratory (4). The USP methods for assaying bendroflumethiazide include UV spectrophotometry (for tablet content uniformity), nonaqueous titration (for bulk material), and Bratton–Marshall colorimetry (for tablet assay) (5).

The HPLC method for bendroflumethiazide described by Moskalyk *et al.* (6) uses an aqueous methanol mobile phase and may not be stability indicating since no mention is made of the disulfonamide degradation product. To simultaneously assay nadolol and bendroflumethiazide, a method was needed that would be rapid, stability indicating, and amenable to automation for unattended overnight analyses. Such a method is described in this report.

EXPERIMENTAL

Materials—Nadolol[1], bendroflumethiazide[1], and timolol[2] were used as received. Water was double-distilled and stored in glass. HPLC-grade methanol[3] and ACS reagent-grade sodium chloride[4], glacial acetic acid[4], and anhydrous sodium acetate[4] were obtained commercially.

Apparatus—A pump[5] capable of maintaining a constant flow at 200–3000 psig delivered deaerated mobile phase at a flow rate of 1.5–2.5 mL/min. A precision loop injector[6] with a nominal volume of 20 μL was attached to a prepacked reverse-phase medium-polarity column[7] (see *Discussion* section), containing 5- or 10-μm particles and 250–300 mm long × 4.6-mm i.d., through an inlet filter[8]. A saturator column, 50 × 0.2 cm packed with 37-μm silica, preceded the loop injector. This column saturates the mobile phase with silica and thus protects the analytical column. The analytical column was maintained above ambient temperature[9] (32°C) to avoid variations in retention time.

A variable-wavelength detector[10] was used, set at 270 nm. The 25-cm strip-chart recorders[11] had inputs compatible with the detector output. One recorder (or channel) was set to trace the nadolol and bendroflumethiazide peaks on scale. The other was set to be ~10 times more sensitive to detect possible impurities or degradation products. For automated, unattended analysis, an autosampler[12] was used in conjunction with a computing integrator[13].

The mobile phase was aqueous acetate buffer (0.029 M acetic acid, 0.01 M sodium acetate, 0.04 M sodium chloride)–methanol (6:4).

Tablet Analysis—Currently, there are three formulations containing 40, 80, or 160 mg of nadolol and 5 mg of bendroflumethiazide per tablet. These were extracted with 50, 100, and 150 mL of methanol, respectively, ultrasonicated for 15 min, and centrifuged for 15 min at 3000 rpm (~1200×*g*). For content uniformity, the whole, unground tablet was extracted; for average content (assay), 10–20 tablets were ground and the weight equivalent to one tablet of the powder was taken. Methanolic standard solutions were prepared by pipetting the appropriate amount of a stock methanolic bendroflumethiazide standard solution (1 or 0.5 mg/mL) into a volumetric flask containing a quantity of nadolol similar to that of the sample. All glassware containing bendroflumethiazide was low-actinic or covered with aluminum foil. A standard solution of the disulfonamide precursor (and degradation product) of bendroflumethiazide containing 2 μg/mL of methanol was also prepared.

System Suitability Test—A standard solution containing disulfonamide, nadolol, and bendroflumethiazide was injected into the system. The resolution of disulfonamide from the solvent front and of nadolol from disulfonamide were each >1.4, while the resolution of bendroflumethiazide from nadolol was >1.7. The resolution, R_s, is defined as $2(t_2 - t_1)/(W_1 + W_2)$, where t_1 and t_2 are the retention times and W_1 and W_2 are the respective peak widths of tangents to the base line expressed in the same units. Any column which passes this system suitability test can be used.

Solvent Suitability Test—Bendroflumethiazide has been shown to

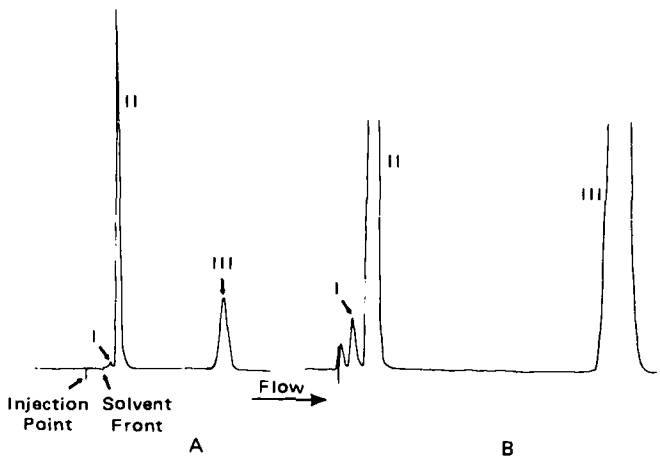

Figure 1—*Chromatogram of disulfonamide (I), nadolol (II), and bendroflumethiazide (III); Key: (A) on scale; (B) off scale. See text for details of the separation.*

[1] E. R. Squibb and Sons.
[2] Merck & Co.
[3] Baker.
[4] Fisher Scientific.
[5] Beckman-Altex 110A.
[6] Rheodyne 7010.
[7] Phenyl (Waters Associates or E.S. Industries).
[8] Rheodyne Model 7362.
[9] Bioanalytical Systems.
[10] Perkin-Elmer LC-75 or LC-85, or Schoeffel 770.
[11] Linear, Inc.
[12] Perkin-Elmer Model 420.
[13] Perkin-Elmer Sigma 10B.

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Table I—Recoveries from Placebo Formulation of Added Nadolol (80 mg), Bendroflumethiazide (5 mg), and Disulfonamide (0.05 mg) Using the Recommended Procedure

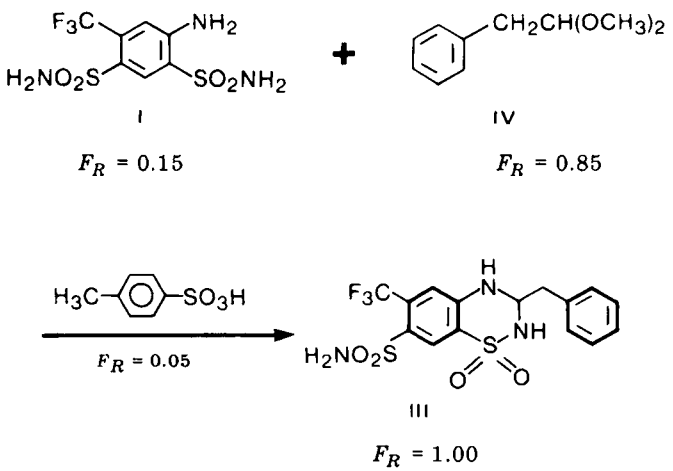
Compound	Recoveries, %	Mean	*RSD*, %
Nadolol	99.8, 99.0, 99.0 99.8, 99.8, 99.8	99.5	0.4
Bendroflumethiazide	100.4, 100.4, 100.2	100.3	0.1
Disulfonamide	100.2, 100.2, 100.0	100.1	0.1

react with even minute amounts of formaldehyde (7); thus, the methanol used to extract the tablets and to prepare the mobile phase must be free of formaldehyde. Therefore, when a fresh bottle of methanol is opened, it should be tested by preparing a standard solution of nadolol and bendroflumethiazide, as described above, injecting it after 24 h, and checking for the presence of a new peak eluting after bendroflumethiazide. If this peak is present, the methanol should not be used in the assay.

RESULTS AND DISCUSSION

Bendroflumethiazide, on hydrolysis or exposure to light, yields 2,4-disulfonamide-5′-trifluoromethylaniline, also referred to as free amine or (more correctly), as disulfonamide. Most compendia set limits on the disulfonamide content of bendroflumethiazide raw material. The USP (5) and BP (8) allow 1.5 and 1%, respectively. Thus, the system developed for assaying nadolol–bendroflumethiazide must be able to detect disulfonamide at these and lower concentrations. Furthermore, the sample preparation conditions must be designed so as to minimize or eliminate *in situ* formation of disulfonamide. The recommended system gave good separation of nadolol from bendroflumethiazide for all octadecylsilane columns tested (5–15% coverage). Separation of the disulfonamide peak from the solvent front was difficult. Accordingly, the system suitability test described in the *Experimental* section was devised. Any column that passes this test can be used. The mobile phase as described above has been optimized for high-efficiency phenyl columns (Fig. 1). Other columns which can be used on the basis of this test include trimethylsilane[14] and Partisil[15] 5 C8. However, the mobile phase would require slight modification to be optimized for these (and possibly other) columns. Changing the extracting solvent from methanol to mobile phase would, of course, eliminate the solvent front peak, but this has been shown to promote the degradation of bendroflumethiazide to disulfonamide at a rate of 40 μg/h/tablet.

The stability of bendroflumethiazide in methanol (in the dark) was confirmed by observing no change in the disulfonamide content after 60 h. A solution containing nadolol and bendroflumethiazide exposed to laboratory-intensity light showed increases in disulfonamide content corresponding to an average rate of formation of ~1.2 μg/h. In addition, the methanol must be free of formaldehyde which reacts with bendroflumethiazide (7) (*cf*, solvent suitability test in the *Experimental* section).



Scheme I—Synthetic intermediates in a synthesis of bendroflumethiazide showing relative retention times (F_R).

[14] Zorbax, DuPont.
[15] Whatman, Inc.

Table II—Assays of Typical Nadolol–Bendroflumethiazide (80:5) Tablets

Formulation	Nadolol, mg/tablet	Bendroflumethiazide, mg/tablet	Disulfonamide, mg/tablet
1	78.9	5.06	All
	80.1	5.08	<0.005
	78.2	5.08	
	82.4	4.92	
	79.3	5.08	
	81.8	5.04	
	80.5	5.08	
	80.6	5.02	
	80.9	4.93	
	81.2	5.07	
Mean	80.4	5.04	
RSD	1.3%	1.2%	
2	81.2	5.14	All
	78.9	5.07	<0.005
	79.4	5.14	
	81.1	5.13	
	79.4	5.07	
	80.6	5.07	
	83.1	5.00	
	81.8	5.04	
	79.0	5.11	
	81.2	4.85	
Mean	80.6	5.06	
RSD	1.7%	1.7%	

The precision of the method was confirmed by performing six injections of nadolol, bendroflumethiazide, and disulfonamide both alone and in the presence of each other. The relative standard deviation was always <0.7%. The plots of response *versus* concentration for all three compounds were linear and passed through the origin. The ranges for concentrations tested were 0–2000 μg/mL for nadolol, 0–250 μg/mL for bendroflumethiazide, and 0–200 μg/mL for disulfonamide. Table I lists the recoveries from spiked placebos of the three compounds both alone and combined, using amounts equivalent to the contents of a typical tablet. When placebo tablets were extracted and analyzed, no peak was seen corresponding to nadolol, bendroflumethiazide, or disulfonamide. Thus, the assay appears to be both accurate and precise. The potencies of two typical lots of tablets are shown in Table II. Disulfonamide results for various samples are all <0.005 mg/tablet (0.1%). The limit of detection is considered to be 0.005 (0.1%); better detectors give lower limits. The disulfonamide values for several experimental lots of bulk bendroflumethiazide as determined by this HPLC method are 0.15–0.25%.

The wavelength of 270 nm was selected because the peak heights of nadolol and bendroflumethiazide at the specified concentrations are relatively similar. If bendroflumethiazide raw material is being assayed, then 220 nm should be used and the samples and standards diluted accordingly. A synthetic route to bendroflumethiazide (9) (III) is shown in Scheme I. The relative retention times of the intermediates disulfonamide (I) and 1,1-dimethoxy-2-ethylbenzene (IV) and the catalyst, *p*-toluenesulfonic acid, indicate that they are resolved from each other and from bendroflumethiazide (III).

A commercially available formulation of timolol with bendroflumethiazide was injected into the system described herein. Both components and the disulfonamide were resolved from each other, indicating the potential of this method.

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Relative Bioavailability of a Commercial Trifluoperazine Tablet Formulation using a Radioimmunoassay Technique

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Abstract □ The relative bioavailability of a new conventional tablet formulation (5 mg) of trifluoperazine dihydrochloride was studied in 24 healthy volunteers. Using a sensitive radioimmunoassay technique, plasma trifluoperazine concentrations were measured up until 24 h following ingestion of single 5-mg doses of trifluoperazine. The mean ± *SD* for the peak concentration (C_{max}), time to C_{max}, area under the curve from 0 to 24 h (AUC_0^{24}), and terminal elimination half-life following the administration of the test formulation were 2.15 ± 1.07 ng/mL, 4.10 ± 1.38 h, 21.04 ± 11.92 ng·h/mL, and 9.5 ± 7 h, respectively. Following the ingestion of the original trifluoperazine tablet formulation (5 mg) these same parameters were estimated to be 1.92 ± 0.88 ng/mL, 4.02 ± 1.10 h, 18.03 ± 10.11 ng·h/mL, and 9.3 ± 7 h, respectively. Large intersubject variations in C_{max} and AUC_0^{24} were observed. The relative bioavailability of the test formulation was calculated to be 106.5 ± 25.5%.

Keyphrases □ Trifluoperazine—relative bioavailability, commercial tablet formulation, RIA technique □ Bioavailability—relative, commercial trifluoperazine tablet formulation, RIA technique □ Radioimmunoassay—relative bioavailability, commercial trifluoperazine tablet formulation

Trifluoperazine is an orally administered phenothiazine antipsychotic agent that has been in clinical use since 1958. Bioavailability studies of this drug have not been hitherto reported for a number of reasons. Trifluoperazine undergoes extensive metabolism to many metabolites which are formed from attack on both the phenothiazine ring and the side chain (1). Trifluoperazine is also known to undergo pronounced presystemic biotransformation in animals following oral administration (2). Phenothiazine drugs in general undergo significant first-pass effects in humans which contribute to large intersubject variability (3–5). Therefore, bioavailability studies require sensitive and specific analytical procedures. Recent analytical methods for trifluoperazine in plasma include GC–NPD (nitrogen-phosphorus detection) (6–8), GC–MS (9, 10), and radioimmunoassay (RIA) (11). Of these the GC–MS and RIA procedures have been used in single-dose pilot studies, where it was found that the bioequivalency parameters as determined by RIA were similar to those determined using GC–MS (12).

This study describes the estimation of the bioavailability of a new conventional trifluoperazine tablet formulation (5 mg)[1] relative to the original product[2]. Following single oral doses of 5 mg, the plasma concentration–time profiles of trifluoperazine were examined up to 24 h using RIA, which is sensitive to 0.25 ng/mL using a 200-μL plasma sample (11).

Table I—*In Vitro* Tablet Test Results

Test	Method	Test Product	Reference Product
Assay, mg (% potency)	USP XX	4.88 (97.6%)	5.23 (104.6%)
Disintegration[a], min	USP XX	5.5	7.0
Content uniformity, % (*RSD*, %)	USP XX	95.7 (3.04)	100.5 (1.69)
Dissolution, % (*RSD*, %)	(Gastric test solution, no enzyme)	100.7 (1.50)	80.7 (30.13)

[a] Gastric test solution.

EXPERIMENTAL

Tablet samples from production lots of two formulations of trifluoperazine were assigned as test[1] and reference[2]; standard *in vitro* tests were performed on both tablet formulations. The dissolution test was carried out on six individual tablets using apparatus 1, as described (13). The basket was rotated at 50 rpm, and the dissolution medium (900 mL) was 1% HCl (v/v) at 37 ± 0.5°C. At the end of 30 min, a suitable portion of the dissolution fluid was filtered. After discarding the first 20 mL of the filtrate, the absorbance of the standard and dissolution test preparations were determined in 1-cm cells at 255 (the wavelength of maximum absorbance) and 278 nm (the wavelength of minimum absorbance) using 1% HCl (v/v) as the blank.

Twenty-four healthy adult male volunteers, from whom written informed consent was obtained, were included in this study. With one exception, all were nonsmokers. The fitness of each subject was assessed by an independent physician who conducted complete physical examinations, reviewed medical histories and the results of clinical laboratory tests (hematology, SMA 12 biochemistry screen, and urinalysis), and monitored the health of the subjects throughout the study period. All subjects were drug free 30 d prior to the study and were asked to refrain from taking any drugs during the study, including abstaining from alcohol for 24 h, prior to and 24 h following each dose. The subjects were assigned randomly to receive the test or reference formulation for the first dose

[1] Trifluoperazine hydrochloride, lot #79-082, Cord Laboratories, Ltd., Broomfield, Colo.

[2] Stelazine, lot #2129S06, Smith Kline & French Ltd., Philadelphia, Pa.

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