Propildazine degradation in water was monitored by following the change in absorbance (A) at 254 nm as a function of time. Two isosbestic points at 233 and 279 nm were observed (Fig. 1). The logarithms of the normalized difference in the absorbance ($A_t - A_\infty / A_0 - A_\infty$, A_∞ being calculated after about 10 half-lives) were plotted against time (Fig. 2). The resulting profiles are clearly concentration (curves 5, 6, and 8), pH (curves 2–4), and temperature (curves 7 and 8) dependent. Moreover, the behavior at pH 7.4, using water bubbled with nitrogen and maintained under nitrogen, showed that oxygen also plays a significant role in the hydrolytic degradation of I (curves 1 and 3).

Propildazine is stable in acidic methanolic solution (0.1 N methanolic HCl, λ_{max} 254 nm unvaried after 2 days), while it is rapidly degraded in methanolic trimethylamine. Moreover, in methanol concentrations of 10^{-5} M, I showed a UV spectrum that rapidly changed its maximum from 259 to 280 nm. This variation was due to trace amounts of acetone present in the spectroscopic grade methanol which reacted with I. In fact, I is a very strong carbonyl group scavenger, giving rise to the corresponding hydrazone 3-isopropylidenehydrazine-6-[(2-hydroxypropyl)methylamino]pyridazine (V) (see *Experimental*).

It is worthwhile to remember the importance of *in vivo* hydrazone formation (14, 15) to explain the prolonged antihypertensive effect of hydralazine-like compounds.

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High-Pressure Liquid Chromatography of Nadolol and Other β-Adrenergic Blocking Drugs

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Abstract □ A high-pressure liquid chromatographic assay was developed for the analysis of the β-adrenergic blocking agent nadolol as a bulk material or formulated in a tablet. Other β-adrenergic blocking drugs such as acebutolol, alprenolol, atenolol, metoprolol, oxprenolol, pindolol, practolol, propranolol, sotalol, and timolol can be chromatographed in this system. An ethylsilane column and a mobile phase consisting of 35% methanol–65% aqueous 0.0005 M hydrochloric acid–0.05 M sodium chloride are used. Detection is either at 254 nm with a fixed UV wavelength detector or at the peak maximum of 220 nm with a variable-wavelength detector. As exemplified by nadolol, the drug content can be quantitated with or without atenolol as an internal standard.

Keyphrases □ Nadolol—high-pressure liquid chromatographic analysis □ β-Adrenergic blockers—high-pressure liquid chromatographic analysis □ High-pressure liquid chromatography—analysis, nadolol and 10 other β-adrenergic agents

β-Adrenergic blocking drugs are presently being used for the treatment of diseases characterized by excess sympathetic nervous activity (1). These illnesses range from cardiac arrhythmia, sinus tachycardia, angina pectoris, hypertension, and migraine to anxiety, essential tremor, alcoholism, and drug dependence.

A high-pressure liquid chromatographic (HPLC) assay was developed for nadolol, *cis*-5-[3-((1,1-dimethylethyl)amino)-2-hydroxypropoxyl] - 1,2,3,4 - tetrahydro-2,3-naphthalenediol, a potent β-adrenergic blocking drug, both as bulk material and in tablets. The chromatographic system also elutes other β-adrenergic blocking agents such as acebutolol, alprenolol, atenolol, metoprolol, oxprenolol, pindolol, practolol, propranolol, sotalol, and timolol at convenient retention times. An analytical profile on nadolol was published previously (2). Nadolol was determined in tablets using colorimetry (3) and in serum using GLC–mass spectrometry (4) or fluorometry (5).

The reported chromatographic procedures are mainly for β-adrenergic blocking agents in body fluids: propranolol in plasma (6–11), atenolol in plasma (12, 13), alprenolol in serum (14), oxprenolol in blood or plasma (15, 16) and in liver tissue (17), acebutolol in serum (18), and practolol in plasma and urine (19). Presumably, these methods could be modified for bulk and formulated material.

EXPERIMENTAL

Materials—Acebutolol[1], alprenolol[2], atenolol[3], metoprolol[4], nadolol[5], oxprenolol[4], pindolol[6], practolol[7], propranolol[5], sotalol[8], and timolol[9] were

[1] May and Baker.
[2] Astra Pharmaceuticals.
[3] Stuart Pharmaceuticals.
[4] Ciba-Geigy.
[5] E. R. Squibb and Sons.
[6] Sandoz Pharmaceuticals.
[7] Ayerst Laboratories.
[8] Mead Johnson.
[9] Merck & Co.

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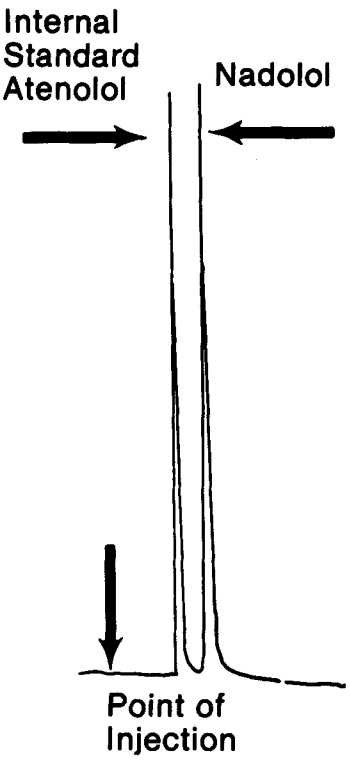


Figure 1—*High-pressure liquid chromatogram of nadolol with atenolol as an internal standard. The void volume was at ~3 min, atenolol eluted in ~4.5 min, and nadolol eluted in ~6 min. Detection was at 254 nm.*

used as received. Distilled water was double distilled and stored in glass. HPLC grade methanol, ACS reagent grade sodium chloride, and concentrated hydrochloric acid were obtained commercially[10].

Apparatus—A pump[11] capable of maintaining a constant flow at 200–2000 psig delivered degassed mobile phase at a flow rate of 1.2 ml/min. A precision loop injector[12], with a nominal capacity of 20 μl, was attached to a reversed-phase, ethylsilane (RP-2), 10-μm, 250-mm long × 4.6-mm i.d. prepacked column[13] through an inlet filter[14]. The column outlet was connected to a fixed-wavelength detector[15] with the peak maximum at 254 nm or to a variable-wavelength monitor[16] set at 220 nm. The strip-chart recorders[17], 25 cm, had input capability for receiving the detector output. The system was washed with methanol–water (1:1) at the end of the day.

Mobile Phase—The mobile phase was 35% methanol–65% aqueous 0.0005 *M* hydrochloric acid–0.05 *M* sodium chloride. One milliliter of 0.1 *M* HCl was added to ~1200 ml of double-distilled water in a 2-liter glass-stoppered graduated cylinder, and then 5.84 g of sodium chloride was added. The solution was mixed to dissolve the salt, and then 700 ml of methanol was added and mixed. The volume was adjusted with additional double-distilled water, and the solution was mixed and then filtered through a medium-porosity sintered-glass funnel. The apparent pH was ~4.5.

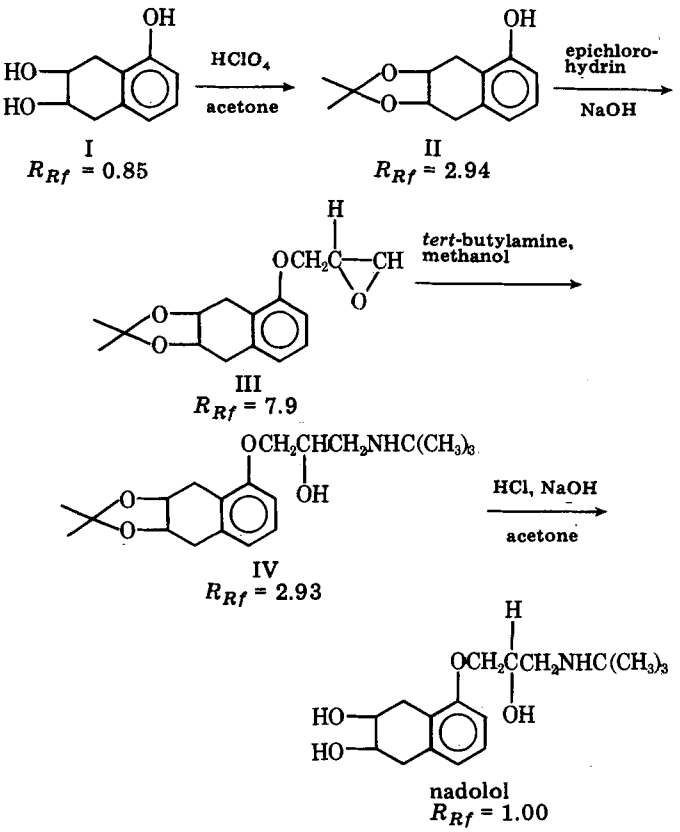
Standard or Bulk Drug Solution—A solution containing 0.2 mg of nadolol standard or bulk drug/ml of mobile phase was used.

Tablet Analysis—A ground tablet, equivalent to ~50 mg of drug, was weighed accurately into a 250-ml volumetric flask. Nadolol was extracted into 200 ml of the mobile phase by ultrasonicating the flask for 15 min. The volume of the solution was adjusted to the 250-ml mark with additional mobile phase. The solution was filtered through a 0.45-μm solvent inert filter or was centrifuged.

Tablet Analysis Using Internal Standard—Each 20.0 ml of tablet extract and standard solution, containing 0.5 mg of nadolol/ml, was fortified with 5.0 ml of atenolol solution (1.2 mg/ml in mobile phase) as an internal standard.

System Suitability Test—A solution containing nadolol and atenolol (0.4 and 0.24 mg/ml, respectively, in the mobile phase) was injected. The resolution, R_s, should be above 1.7 for the two peaks and is calculated using the formula $R_s = 2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 are the retention times and w_1 and w_2 are the respective peak widths expressed in the same units.

[10] Fisher Scientific.
[11] Altex model 110A or Waters M6000.
[12] Rheodyne model 7010.
[13] LiChrosorb, E. Merck.
[14] Rheodyne model 7362.
[15] Altex model 153 or Chromatronix model 220.
[16] Schoeffel model 770 or Varian Varichrome.
[17] Linear Inc.



Scheme I—Relative retention times (R_{Rf}) or α of the intermediates in the synthesis of nadolol.

RESULTS AND DISCUSSION

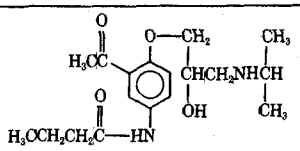
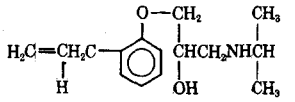
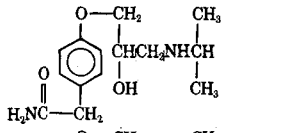
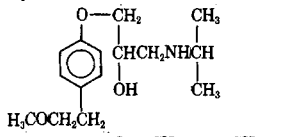
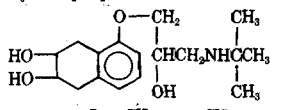
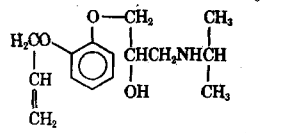
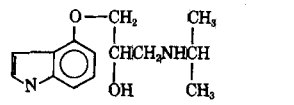
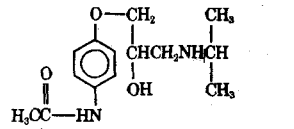
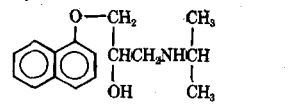
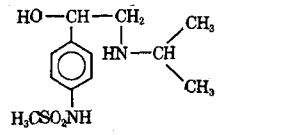
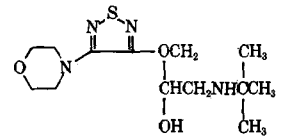
Nadolol tablets with potencies of 40–320 mg were analyzed with and without an internal standard. Figure 1 shows a sample chromatogram of nadolol when atenolol was used as an internal standard. The mean peak heights of atenolol and nadolol, using the recommended procedure, were 12.98 and 13.25 cm with relative standard deviations of 0.5 and 0.4%, respectively, for seven repetitive injections. When the ratios of peak heights of nadolol to atenolol were calculated, the series gave a relative standard deviation of 0.4%, which was insignificant.

The use of an internal standard is not necessary if the chromatograph is equipped with a precision loop injector. However, the nadolol–atenolol solution is recommended for the system suitability test. Nadolol tablets were analyzed using HPLC as well as TLC on silica gel plates developed with acetone–chloroform–2 *M* ammonium hydroxide (8:1:1). The results obtained by the two techniques were similar with an average difference of 1% and served to validate the two methods. When the tablets were analyzed with or without atenolol as an internal standard, the results did not vary by more than 1%. Recoveries of nadolol added to placebo formulations were 100.4 and 100.6%. UV detector responses for nadolol at 220 and 254 nm were linear for concentrations of 0.025–0.2 μg/ml and passed through the origin. Detection at 220 nm was ~25 times more sensitive than at 254 nm, but no impurities were detected in the standard at either wavelength.

Scheme I gives the structures of the synthetic intermediates and their relative retention times (R_{Rf} or α of nadolol = 1.0), demonstrating the selectivity of the chromatographic system. Several possible impurities were synthesized. When added to a solution of nadolol and injected into the chromatographic system, all were completely resolved, showing that the method is stability indicating.

The retention times relative to nadolol for 10 other β-adrenergic blocking drugs are presented in Table I. If desired, minor modifications can be made in the mobile phase and flow rate to optimize the retention time and peak shape. For example, to quantitate propranolol (as the hydrochloride), 15% more methanol was added to the mobile phase to halve the retention time to ~8 min. Five repetitive injections gave an

Table I—Retention Times of β-Adrenergic Blocking Agents Compared to Nadolol

Compound	Structure	R_{Rf}
Acebutolol		1.50
Alprenolol		4.68
Atenolol		0.65
Metoprolol		1.75
Nadolol		1.00
Oxprenolol		2.7
Pindolol		1.29
Practolol		0.80
Propranolol		2.7
Sotalol		1.38
Timolol		1.45

[a] Retention time of agent divided by nadolol retention time.

average peak height of 9.47 cm (RSD = 0.5%). The response to various concentrations from 0.025 to 0.2 μg/ml was linear and passed through the origin. The relative standard deviation of the normalized response was 0.5%. The experimentally observed relative retention times (Table I) can be used to calculate Hansch π values. A straight line was found, as was predicted previously (20), for atenolol, practolol, metoprolol, oxprenolol, and alprenolol.

Although use of an ethylsilane (RP-2) column is recommended, a reasonable peak shape for nadolol may be obtained with an octylsilane (RP-8) column. A broader peak with longer retention was obtained with an octadecylsilane (RP-18) column. Although RP-18 columns give higher theoretical plate counts with nadolol, the peaks are sharper and more symmetrical with RP-2 columns.

In conclusion, nadolol and 10 other β-adrenergic blocking agents are analyzable by one HPLC system. One agent may be used as an internal standard for another.

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