3100–3000 cm^{-1} (secondary amine salt), and strong bands at 1700 and 1570 cm^{-1} (amide, I and II). Compound II revealed broad multiple bands at 3200–3100 cm^{-1} (NH stretching) and strong bands at 1700 (amide-I), 1620 (tertiary amide), and 1540 cm^{-1} (amide-II) (20).

Parasitological Screening—Compounds III, V, and VIII were tested for anthelmintic efficacy in mice naturally infected with *H. diminuta* and were active at the dose level (100 mg/rat, single dose) that showed no activity for VI and VII. Only VIII was active at 20 mg/rat (single dose); a further dose reduction abolished the activity.

Preliminary test revealed the activity of II against *Oxyuris* in naturally infected rats (100 mg/rat, single dose).

All of the drugs tested showed no clinical toxic manifestations even at the highest dose level.

CONCLUSION

The experimental results demonstrate that the replacement of the nitro group in 2-imino-3-[(*N*-4-nitrophenylcarbamoyl)methyl]-2,3,4,5-tetrahydrothiazole (VIII) by ethoxycarbonyl (V) or bromo (VI) substituents results in partial or complete loss of anthelmintic activity. Electronegativity alone cannot explain the crucial role played by the nitro group, and *in vivo* drug bioactivation may be involved.

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Quantitative High-Pressure Liquid Chromatographic Determination of Epinephrine in Pharmaceutical Formulations

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Abstract □ A quantitative high-pressure liquid chromatographic method, using a cationic exchange resin column and an aqueous phosphate buffer as the mobile phase, was employed for the determination of epinephrine in liquid pharmaceutical preparations. The method is stability determining and can differentiate epinephrine in the presence of oxidative and other degradation products.

Keyphrases □ Epinephrine—high-pressure liquid chromatographic analysis, liquid pharmaceutical preparations □ High-pressure liquid chromatography—analysis, epinephrine, liquid pharmaceutical preparations □ Adrenergics—epinephrine, high-pressure liquid chromatographic analysis, liquid pharmaceutical preparations

Epinephrine solutions are widely used for the treatment of open angle glaucoma and are also used as cardiac stimulants and vasoconstrictors. Air oxidation is a major degradation process involved in epinephrine pharmaceutical preparations (1). Antioxidants such as bisulfite are used in many commercial liquid formulations. Bisulfite causes a degradation of epinephrine to form 1-(3,4-dihydroxyphenyl)-2-methylaminoethanesulfonic acid (2).

A stability-indicating method that differentiates epinephrine from its oxidation products as well as sulfonic acid derivatives is needed. The USP XIX assay method requires the formation of the triacetyl epinephrine derivative and is very lengthy (3). Several spectrofluorometric methods have the disadvantage of interference from the antioxidants (4). The addition of antioxidants is necessary to stabilize the final fluorescence in these assay methods.

This report describes a high-pressure liquid chromatographic (HPLC) assay method for epinephrine in liquid pharmaceutical preparations which will differentiate epinephrine from these major degradation products.

Table I—Comparison of Spectrofluorometric and HPLC Methods for Epinephrine Determination

Assay Method	Epinephrine Found, Product A[a], %	Percentage of Label Claim	Epinephrine Found, Product B[a], %	Percentage of Label Claim
Spectrofluorometric[b]	0.262	105	2.14	107
HPLC	0.255	102	2.10	105

[a] Label claim of Product A was 0.25%; label claim of Product B was 2%. [b] See Footnote 4.

EXPERIMENTAL

Apparatus—A high-pressure liquid chromatograph[1], composed of a pump (7000 psig maximum) and equipped with dual-channel UV detectors at 254 and 280 nm, was used. A 3 × 500-mm stainless steel column was packed with cationic exchange resin[2]. The mobile phase consisted of 0.05 *M* KH_2PO_4 at pH 4.5, which was degassed by using vacuum suction.

Reagents—1-(3,4-Dihydroxyphenyl)-2-methylaminoethanesulfonic acid was prepared according to a literature method (5).

Preparation of Epinephrine Standard Solutions—Dissolve epinephrine bitartrate[3], 181.8, 272.7, and 363.6 mg, in water and dilute to 100 ml. These solutions represent concentrations of 0.1, 0.15, and 0.2% of epinephrine base, respectively.

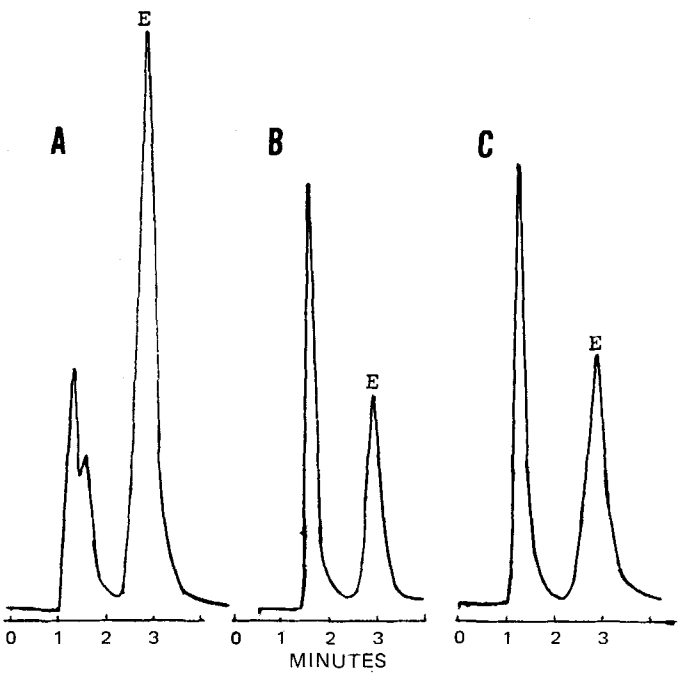

Figure 1—*Chromatographic separation of epinephrine (E) from degradation products. Key: A, air oxidation of 0.1% epinephrine at pH 7; B, hydrogen peroxide oxidation of epinephrine solution at pH 5; and C, mixture of epinephrine and its sulfonic acid derivative.*

[1] Spectra Physics Isocratic model 3500B.
[2] Vydac, Applied Science Laboratories, State College, Pa.
[3] 3M Laboratories, Loughborough, England.

Degradation of Epinephrine—*Method A*—Dissolve 181.8 mg of epinephrine bitartrate in water, adjust the pH to about 7 with 0.02 *N* NaOH, and dilute to 100 ml. Bubble air through this solution overnight.

Method B—Dissolve 181.8 mg of epinephrine bitartrate in water, adjust the pH to about 5 with 0.02 *N* NaOH, and dilute to 100 ml. To 50 ml of this solution, add 2 ml of 30% hydrogen peroxide and heat the mixture in an 80° water bath for 1 hr.

Preparation of Sample Solution—Dilute the liquid sample with water to obtain a concentration of 0.1% epinephrine.

Chromatographic Separation—The procedure was run at ambient temperature, and the solvent flow was 1.6 ml/min. The UV monitor was set at 280 nm with a sensitivity of 0.32 absorbance unit. The samples and standards were injected at 10 μl in duplicate. Peak heights were used for calculating epinephrine concentrations; the concentration of epinephrine samples was obtained from a standard curve.

RESULTS AND DISCUSSION

Excellent linearity of peak height response over the concentration range from 0 to 2 mg/ml was obtained. Figure 1 contains three individual chromatograms and shows the different retention times of epinephrine, the oxidation products, and the sulfonic acid derivative. The difference in chromatograms A and B is probably the result of the complexity of the epinephrine oxidation process. Epinephrine eluted at about 3 min; therefore, the degradation products did not interfere with the epinephrine assay.

The results of adding known amounts of epinephrine to a commercially available formulation[4] in two different concentrations were assayed by both HPLC and spectrofluorometric (4) methods. The results are given in Table I.

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