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High-Performance Liquid Chromatographic Stability-Indicating Assay for Naphazoline and Tetrahydrozoline in Ophthalmic Preparations

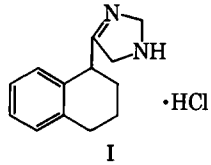
JOHN BAUER ˣ and SUZANNE KROGH

Received July 12, 1982, from the *Pharmaceutical Products Division, Abbott Laboratories, North Chicago, IL 60064.* Accepted for publication October 5, 1982.

Abstract □ A high-performance liquid chromatographic (HPLC) analysis for tetrahydrozoline and naphazoline in ophthalmic solutions is presented. The analysis allows a more reproducible, direct stability-indicating assay than the colorimetric methods generally employed. The HPLC system is so designed that a variety of ophthalmic solutions containing either naphazoline or tetrahydrozoline can be analyzed concomitantly.

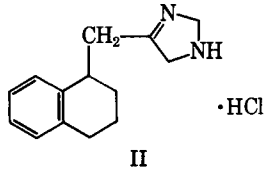
Keyphrases □ High-performance liquid chromatography—stability-indicating assay, ophthalmic preparation, naphazoline, tetrahydrozoline □ Naphazoline—high-performance liquid chromatographic stability-indicating assay, ophthalmic preparation □ Tetrahydrozoline—high-performance liquid chromatographic stability-indicating assay

Tetrahydrozoline (I) and naphazoline (II) are sympathomimetic agents used in the majority of commercially available occular decongestants. The current analytical methodologies are either colorimetric or ultraviolet. Tetrahydrozoline analysis involves color development with either sodium nitroprusside (1, 2) or bromophenol blue (2). Naphazoline is presently assayed by either UV-absorption spectroscopy (3) or colorimetry (1).



I

2-(1,2,3,4-Tetrahydro-1-naphthyl)-2-imidazoline, monohydrochloride



II

2(1-Naphthylmethyl)-2-imidazoline, monohydrochloride

These methods generally involve isolation steps as well as color development times and are subject to interferences. The high-performance liquid chromatographic (HPLC) procedure developed in our laboratory is a direct stability-indicating assay for tetrahydrozoline and naphazoline in ophthalmic solutions.

EXPERIMENTAL

Materials—Methanol[1], citric acid[2], sodium citrate[3], and perchloric acid[4] were used as received. Tetrahydrozoline and naphazoline were USP reference standards. A high-performance liquid chromatograph[5] and a UV visible spectrophotometer[6] were used. A microparticulate octadecylsilane column[7] was used. The temperature was ambient and the flow rate 2.0 ml/min. The analytical wavelength was 265 nm. Injection volume was 20 μl.

Mobile Phase—Six grams of sodium citrate dihydrate and 4 g of anhydrous citric acid were added to 700 ml of water and mixed until dissolved; 7 ml of perchloric acid was added and the pH determined. The pH was adjusted to 2.2 ± 0.2 by further addition of perchloric acid. A 300-ml volume of methanol was added, the solution mixed thoroughly, filtered through a 0.45-μm filter, and deaerated for ~10 min.

Tetrahydrozoline Internal Standard Solution—A solution containing ~150 mg of tetrahydrozoline hydrochloride was prepared by dissolving and diluting to volume with distilled water to 100 ml (~1.5 mg/ml).

Naphazoline Internal Standard Solution—A preparation of ~40 mg of naphazoline in 100 ml of water was prepared and diluted 1/10 with water for use as the internal standard solution (~0.04 mg/ml).

Naphazoline Standard—Approximately 120 mg of naphazoline hydrochloride was weighed accurately into a 100-ml volumetric flask and dissolved and diluted to volume with distilled water. A 5.0-ml volume of this solution was pipetted into a 50-ml volumetric flask and diluted to volume with distilled water (~0.12 mg/ml); 5.0 ml of this solution was pipetted into a 10-ml volumetric flask and diluted to volume with tetrahydrozoline internal standard solution.

[1] Methanol, Burdick and Jackson or equivalent spectrophotometric grade.
[2] Citric acid anhydrous AR Grade.
[3] Sodium citrate dihydrate AR Grade.
[4] Perchloric acid (60%), Fisher Scientific.
[5] Waters Model 6000A Liquid Chromatography Pump; Rheodyne Model 7120 Injector with 20-μl loop; DuPont variable-wavelength detector; Hewlett-Packard 3385A Recording Integrator.
[6] Varian/Cary 219 UV Spectrophotometer.
[7] Waters μBondapak C_{18} liquid chromatographic column.

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Table I—Comparison of Tetrahydrozoline Assays

Sample No.	Run No.	HPLC Analysis	Colorimetric Analysis (Sodium Nitroprusside)
1	1	99.5	99.5
	2	99.0	96.7
2	1	97.4	99.5
	2	97.6	99.9
3	1	98.4	99.5
	2	99.0	101.3
4	1	97.3	97.5
	2	97.7	98.1

Table II—Comparison of Naphazoline Assays

Sample No.	Run No.	HPLC Analysis	Colorimetric Analysis (Sodium Nitroprusside)
1	1	100.8	106.8
	2	100.1	97.8
2	1	103.8	102.3
	2	103.4	107.8
3	1	98.2	104.5
	2	99.9	104.5
4	1	102.4	106.1
	2	102.5	105.2

Table III—Precision of Naphazoline and Tetrahydrozoline Analyses

	Naphazoline, mg/ml Found	Tetrahydrozoline, mg/ml Found
	0.1210	0.524
	0.1210	0.524
	0.1200	0.508
	0.1180	0.518
	0.1204	0.508
	0.1228	0.515
	0.1218	0.515
	0.1228	0.510
	0.1225	0.513
	0.1228	0.506
Mean	0.1212	0.514
SD	±0.0016	±0.006
RSD	±1.33%	±1.2%

Tetrahydrozoline Standard—Approximately 100 mg of tetrahydrozoline was accurately weighed into a 100-ml volumetric flask, dissolved, and diluted to volume with distilled water. A 25.0-ml volume of this solution was pipetted into a 50-ml volumetric flask and diluted to volume with distilled water (~0.5 mg/ml); 5.0 ml of this solution was pipetted into a 10-ml volumetric flask and diluted to volume with naphazoline internal standard solution.

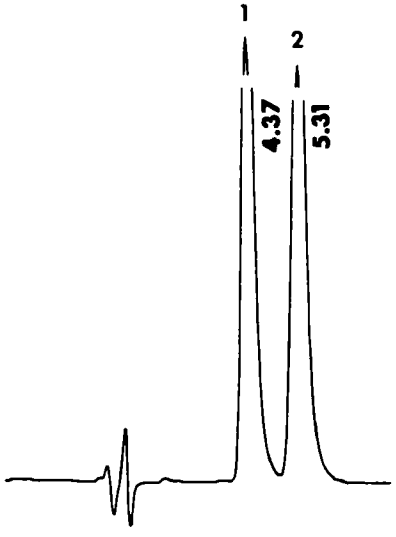


Figure 1—*Typical chromatogram of (1) naphazoline and (2) tetrahydrozoline.*

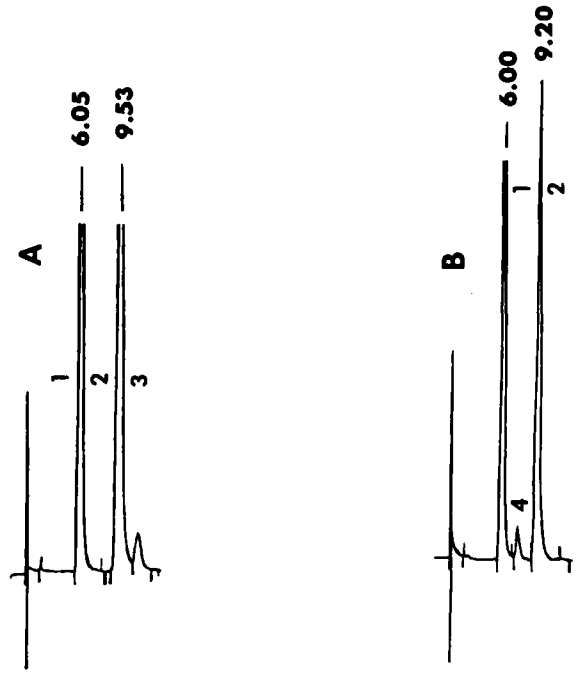


Figure 2—*Separation of degradation products of (A) naphazoline preparation and (B) tetrahydrozoline preparation. Key: (1) tetrahydrozoline, (2) naphazoline, (3) N-(2-aminoethyl)-1,2,3,4-tetrahydro-1-naphthylamine, and (4) N-(2-aminoethyl)-1-(1-naphthyl)acetamide.*

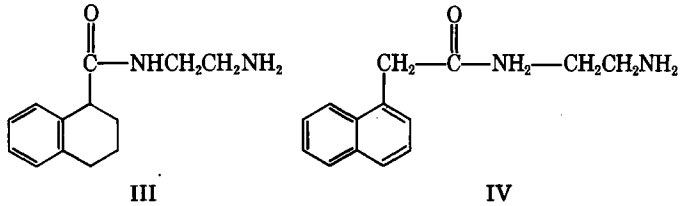
Sample Procedure—A 5.0-ml volume of the sample was pipetted into a 10-ml volumetric flask and diluted to volume with the appropriate internal standard solution.

RESULTS AND DISCUSSION

The HPLC analysis is, in the majority of cases, more reproducible than the sodium nitroprusside colorimetric methods for tetrahydrozoline and naphazoline (Tables I and II). In addition the use of naphazoline as the internal standard for tetrahydrozoline and tetrahydrozoline as the internal standard for naphazoline allows direct analysis of a variety of ophthalmic solutions using a single eluant system.

The precision data for the HPLC analysis (Table III) and the comparative study (Tables I and II) demonstrate that the HPLC analysis is better than the colorimetric techniques, which in our laboratories gave standard deviations of 1.5% for naphazoline and 2.8% for tetrahydrozoline.

A typical chormatogram is shown in Fig. 1. Both tetrahydrozoline and naphazoline can be separated from their respective degradation products, *N*-(2-aminoethyl)-1,2,3,4-tetrahydro-1-napthylamine (III) and *N*-(2-aminoethyl)-1-(1-napthyl)acetamide (IV) (Fig. 2). These components can be quantitated separately if desired, although appropriate corrections for extinction coefficient differences must be used.



III IV

Both compounds III and IV have been isolated from the HPLC system and identified spectroscopically. The response for both tetrahydrozoline and naphazoline is linear. The naphazoline response from 0.02 to 0.1 mg/ml gave a correlation coefficient of 0.9999, n = 1.0019 (4). Tetrahydrozoline response gave a correlation coefficient of 0.9999 between 0.1 and 0.5 mg/ml, n = 1.0029. The analysis is applicable to a variety of ophthalmic solutions[8].

The HPLC assay presented constitutes a direct, stability-indicating

[8] Clear Eyes, Naphcon, 20/20, Murine Plus, Visine, Visine AC, Soothe.

analysis for either of the two major sympathomimetic agents in use in topical ocular decongestants. The technique is more reproducible than the present colorimetric assay and does not suffer from the time restraints and interferences possible in colorimetric assays. The HPLC method presented can be easily automated and allows analysis of a variety of ophthalmic solutions on a single HPLC system.

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Stability-Indicating Assay, Dissolution, and Content Uniformity of Sodium Levothyroxine in Tablets

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Received July 19, 1982, from the *Stability Laboratory, Pharmaceutical Basics, Inc., Denver, CO 80223.* Accepted for publication October 14, 1982.

Abstract □ A reverse-phase high-performance liquid chromatographic (HPLC) method for determining sodium levothyroxine in tablet formulations is described. The sodium levothyroxine was extracted from tablets using a mobile phase consisting of 60% acetonitrile and 40% aqueous buffer. After centrifugation 200 μl of the solution was chromatographed on a 10-μm C_{18} column. The method gave accurate results when tested against the USP method, by the standard additions method, and by the spiked-placebo method. The method can also be used to determine content uniformity and dissolution of sodium levothyroxine tablets.

Keyphrases □ Sodium levothyroxine—stability-indicating assay, dissolution, content uniformity, tablets □ Dissolution—sodium levothyroxine, stability-indicating assay, content uniformity, tablets □ Content uniformity—sodium levothyroxine, stability-indicating assay, dissolution, tablets □ Stability—assay of sodium levothyroxine in tablets, dissolution, content uniformity

Sodium levothyroxine (I) tablets are widely prescribed in thyroid replacement therapy, with a wide range of doses available (25–300 μg/tablet). The USP XX method of assay consists of a lengthy ignition and oxidation to iodate followed by titration of the liberated iodine (1). The method is neither stability indicating nor sensitive enough to be used for content uniformity and dissolution determinations.

A number of other assay procedures based on liquid chromatography have appeared in the literature (2–13). This report describes a new reverse-phase liquid chromatographic (HPLC) assay method that adequately separates I from degradation products and can be used for the identification, content uniformity analysis, and dissolution testing of I in tablets.

EXPERIMENTAL

Reagents and Materials—Sodium levothyroxine[1] (I) was assayed by the USP XX procedure (14); sodium liothyronine[1] and 3,5-diiodo-L-thyronine[2] were used as received. Reagents used were analytical reagent grade. The levothyroxine sodium tablets (USP) were obtained commercially from four sources[3–6].

Apparatus—The high-performance liquid chromatograph[7] was equipped with a variable-wavelength UV detector[8], a strip-chart recorder[9], an electronic integrator[10], and a 200-μl loop-type injector[11]. Commercial 10-μm C_{18} columns[12] (30 cm × 4 mm i.d.) were used at am-

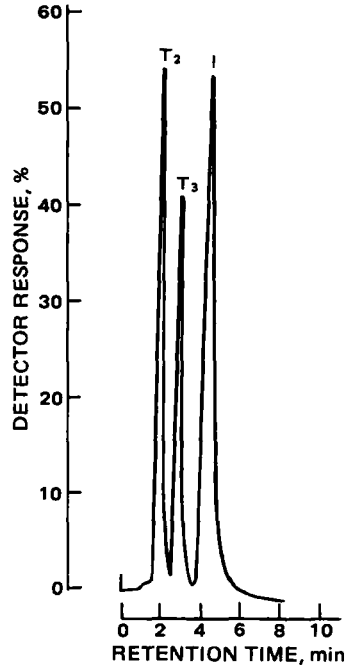

Figure 1—*Chromatogram of sodium levothyroxine (I, 20 μg/ml) with added sodium liothyronine (T_3, 10 μg/ml), and 3,5-diiodo-L-thyronine (T_2, 10 μg/ml).*

[1] Sanabo Gesellschaft, Kundle, Austria.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Armour Pharmaceutical Co., Phoenix, Ariz.
[4] Flint, Division of Baxter-Travenol, Morton Grove, Ill.
[5] Lederle Laboratories, Pearl River, N.Y.
[6] Pharmaceutical Basics, Inc., Denver, Co.
[7] Model 5020, Varian Associates, Palo Alto, Calif.
[8] Model UV-50, Varian Associates, Palo Alto, Calif.
[9] Model 1005, Beckman Instruments, Fullerton, Calif.
[10] Model CDS-111L, Varian Associates, Palo Alto, Calif.
[11] Model CV-6-UHPa-N60, Valco Instruments Co., Houston, Tex.
[12] Model MCH-10, Varian Associates, Palo Alto, Calif.; μBondapak C18, Waters Associates, Milford, Mass.

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