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Note

Stability-indicating determination of tetrahydrozoline hydrochloride in ophthalmic solutions by high-performance liquid chromatography

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Tetrahydrozoline hydrochloride is a sympathomimetic agent with marked alpha adrenergic activity. Its action is similar to naphazoline hydrochloride. Spectrophotometric methods have been utilized for tetrahydrozoline analysis[1], but the degradation products interfere in the determination.

Today, the only published method to estimate tetrahydrozoline with sufficient specificity in pharmaceutical preparations is high-performance liquid chromatography (HPLC)[2]. This method fails to distinguish N-(2-ethylamino)-1,2,3,4-tetrahydro-1-naphthamide (I) which might be a decomposition product of tetrahydrozoline[2]. For studies of the stability of pharmaceutical preparations containing tetrahydrozoline a sensitive method for the independent detection of tetrahydrozoline and I is preferable.

Here we report an improved HPLC procedure, useful in monitoring the overall purity of tetrahydrozoline in pharmaceutical preparations, as well as in assessing the degradation product of the drug.

EXPERIMENTAL

Instrumentation

The HPLC system comprised a Waters 6000A constant-flow pump, a Waters automatic injector system WISP 7103 with a 20-μl loop, a μBondapak C_{18} column (10 μm), 30 × 3.9 mm, a Schoeffel 750 detector set at 254 nm, a Waters data module M730 and a Waters system controlled M720. The mobile phase was filtered on a 0.22-μm filter and degassed with helium.

Reagents

Mobile phase. Exactly weighed amounts 10.061 g sodium tetraborate ($Na_2B_4O_7$) and 13.609 g potassium dihydrogenphosphate (KH_2PO_4), were placed in two separate 1000-ml volumetric flasks. The solids were dissolved in 18-MΩ purified water. Using the second solution, 500 ml of the sodium tetraborate solution were adjusted to pH 7.0 and then diluted to 1000 ml. A 600-ml volume of the resulting solution was filtered through a 0.2-μm cellulose filter, and 400 ml acetonitrile for HPLC (which had been filtered through a 0.2-μm PTFE filter) were added.

Standard tetrahydrozoline hydrochloride solutions. A sample of 50 mg tetra-

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hydrozoline hydrochloride was weighed exactly into a 100-ml volumetric flask, dissolved and made up to the mark with purified water. This 50 mg/100 ml solution was then used to prepare the following solutions: 30, 20, 10, 5 and 2.5 mg/100 ml.

Degraded tetrahydrozoline hydrochloride solution. A sample of 50 mg tetrahydrozoline hydrochloride was weighed exactly into a 100-ml volumetric flask and dissolved in 20 ml purified water. Five millilitres of 10 *M* NaOH were added and the solution was placed in an oven at 50°C for 24 h. The pH was then adjusted to *ca.* 7.0 with 12.5 *M* HCl, and the volume made up to the mark with purified water.

RESULTS AND DISCUSSION

Figs. 1, 2 and 3 illustrate chromatograms obtained from mixtures of tetrahydrozoline, degraded tetrahydrozoline and tetrahydrozoline 0.05% ophthalmic solu-

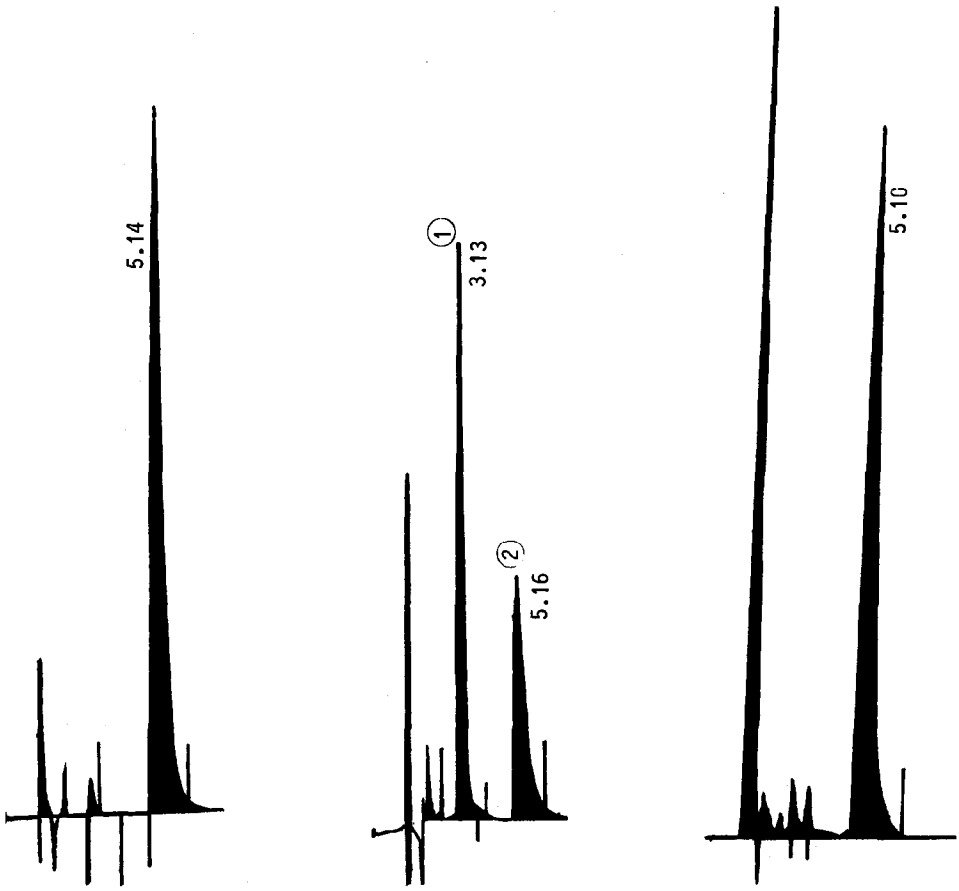

Fig. 1. Typical chromatogram of tetrahydrozoline hydrochloride solution.

Fig. 2. Typical chromatogram of a degraded tetrahydrozoline solution. Peaks: 1 = N-(2-ethylamino)-1,2,3,4-tetrahydro-1-naphthamide; 2 = tetrahydrozoline hydrochloride.

Fig. 3. Typical chromatogram of a tetrahydrozoline 0.05% ophthalmic preparation stored at room temperature for 36 months.

tion. The tetrahydrozoline hydrochloride (retention time: 5.14) is separated completely from its degradation product (retention time: 3.13).

Standardization and sensitivity of assay

Analyses were carried out with the following tetrahydrozoline hydrochloride solutions: 50, 30, 20, 10, 5 and 2.5 mg/100 ml. A 20-μl aliquot of each solution was injected four times successively.

The standardization curve thus obtained shows a correlation coefficient of 0.9996, in the range 0.5–10 μg injected. The equation of the linear regression line is $y = 198{,}427x - 12{,}050$, where y is the peak area at 254 nm expressed in integration units and x the amount (μg) of tetrahydrozoline hydrochloride injected. The sensitivity of this assay is 198,427 surface integration units per microgram of tetrahydrozoline hydrochloride injected.

Sensitivity limit

The sensitivity limit of this assay is 300 ng tetrahydrozoline injected, *i.e.* 20 μl of a 1.5 mg/100 ml solution.

TABLE I

REPRODUCIBILITY OF HPLC ASSAY FOR TETRAHYDROZOLINE HYDROCHLORIDE (50 mg/100 ml)

Day	*Tetrahydrozoline hydrochloride peak area at 254 nm (integration units)*		
	Mean (n = 10)	*Standard deviation*	*Relative standard deviation (%)*
1	1958	29	1.4
2	1980	29	1.4
3	2025	36	1.7

TABLE II

INTERLABORATORY COLLABORATIVE STUDY ON TETRAHYDROZOLINE HYDROCHLORIDE

In each case, $n = 6$.

Tetrahydrozoline hydrochloride injected (μg)	*254 nm peak area*		
	Chemist A	*Chemist B*	*Chemist C*
10	1960 ± 28	1845 ± 6	1888 ± 94
6	1194 ± 21	1118 ± 2	1155 ± 46
2	376 ± 7	395 ± 5	315 ± 20
1	187 ± 15	159 ± 16	145 ± 19
Correlation coefficient, *r*	0.9995	0.9994	0.9972

Reproducibility

The reproducibility was determined by injecting 20 μl of a 50 mg/100 ml solution, ten times successively on three consecutive days. The results shown in Table I are highly reproducible (relative standard deviation = 1.5%). Interanalyst variations, with three different chemists, were determined at four levels of tetrahydrozoline. Each gave rectilinear results, Table II.

CONCLUSION

The method described provides a sensitive and quantitative assay for tetrahydrozoline in pharmaceutical dosage forms. It is sufficiently sensitive to permit simultaneous detection of tetrahydrozoline in the presence of its possible degradation product. A significant advance over the previous HPLC method[2] of determining tetrahydrozoline is thus achieved.

REFERENCES

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