A simple, selective and rapid liquid chromatographic method is described for the identification of degradation product (naphthylacetylethylenediamine) of naphazoline in nasal drops. The placebo, standard and sample solutions were chromatographed using a μ Bondapac C18 column and eluent pH 2.2 phosphate buffer/Methanol (7/3, v/v) at a flow rate of 1 ml/min and detected at 280 nm. The peak purities were checked by photodiode array (PDA) facility.

Keywords: Naphazoline HCl; 1-Naphthylacetylethylenediamine; RP-HPLC; Photodiode array

Introduction

Naphazoline is a sympathomimetic agent with marked alpha-adrenergic activity. It is used in nasal preparations as decongestant(1). The methods applied for the analysis of naphazoline have been reviewed(2) and colorimetry, TLC and HPLC have been represented as methods of analysis for this compound in various preparations. A liquid chromatographic method for the determination of naphazoline in rat plasma has also been described(3). The HPLC methods(4-8) mentioned above have been summarized in Table 1.

Table 1. HPLC methods mentioned in reference 2 for the analysis of naphazoline HCl in nasal preparations

<table>
<thead>
<tr>
<th>Column</th>
<th>Mobile phase</th>
<th>Detection system</th>
<th>Preparation</th>
<th>Lit No</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 μm octadecysilane (3.9 x 300 mm)</td>
<td>0.08 M HClO4 (pH:2.2)-MeOH (7/3, v/v)</td>
<td>UV256 nm</td>
<td>Ear and eye drop</td>
<td>4</td>
</tr>
<tr>
<td>10 μm octadecysilane (4 x 250 mm)</td>
<td>MeOH-Water (2/3, v/v)</td>
<td>UV279 nm</td>
<td>Ophthalmic formulation</td>
<td>5</td>
</tr>
<tr>
<td>5 μm cyano (4.6 x 150 mm)</td>
<td>Dilute phosphate solution (pH:3)-acetonitrile (3/2, v/v)</td>
<td>UV225 nm</td>
<td>Ophthalmic formulation</td>
<td>6</td>
</tr>
<tr>
<td>5 μm octadecysilane (4.6 x 250 mm)</td>
<td>0.05 M phosphate solution (pH:6.6)-acetonitrile (4/1, v/v) containing 0.07 M triethylamine</td>
<td>UV270 nm</td>
<td>Ophthalmic formulation</td>
<td>6</td>
</tr>
<tr>
<td>10 μm phenyl (4 x 300 mm)</td>
<td>Water-methanol-glacial acetic acid(55/44/1, v/v/v) containing 0.005 M heptane sulfonic acid sodium salt</td>
<td>UV254 nm</td>
<td>Tablets and capsules</td>
<td>7</td>
</tr>
<tr>
<td>10 μm phenyl (4 x 30mm)</td>
<td>Water-acetonitrile-glacial acetic acid (74/25/1,v/v/v) containing0.005 M heptane sulfonic acid sodium salt</td>
<td>UV280 nm</td>
<td>Nasal solution</td>
<td>8</td>
</tr>
</tbody>
</table>

+Correspondence
Naphazoline has been shown to be relatively stable in acidic or neutral solutions but can easily be hydrolyzed in alkaline media, the main degradation products being 1-naphthylacetylenediamine and 1-naphthylacetic acid(9). Thus identification of these products in preparations is an important issue in stability tests. The European(10) and British Pharmacopoeias 1988 (11) give a TLC method for the identification of 1-naphthylacetylenediamine in the presence of naphazoline. Other methods reported for the analysis of 1-naphthylacetylenediamine include column chromatography followed by UV(9,12) and HPLC(6) assays.

In this study we developed a simple, selective and rapid RP-HPLC method for identifying degradation products of naphazoline in nasal preparations by using photodiode array detector.

Materials and Methods

**Instruments:** Waters Liquid Chromatograph, U6 K universal injector HP H404M (photodiode array detector, PDA) with Column µ-Bondapack C-18, 5μ (300x4.6 mm id), pH meter (Sesa, Model 1400)

**Operating conditions:** Isocratic mobil phase: pH 2.2 phosphate buffer-MeOH (7/3 v/v), flow rate: 1 ml/min, pressure: 1000 psi, temperature : 25°C, detection 280 nm : PDA (190-600 nm), sample conc. : 400 µg/ml, injection volume : 5µl.

**Chemicals:** The chemicals used for the preparation of the mobile phase were from E. Merck (Darmstadt, Germany) and from Sigma (USA). All solvents used were of analytical grade. USP naphazoline standard was obtained from Refik Saydam Central Institute of Hygiene and placebo nasal solution was prepared in our laboratory.

**Preparation of the phosphate buffer pH 2.26:** 28.5 ml 2N NaOH, 9.5 ml o-phosphoric acid and 3.275 ml hexylamine were made up to volume (1 lt) with water and the pH was adjusted to pH 2.26.

**Preparation of placebo nasal solution:** 10 ml of the placebo nasal solution was made up to 25 ml with water.

**Preparation of sample solution:** 10 ml of the nasal preparation was made up to 25 ml with water.

**Precision of the system:** 5 replicates of standard naphazoline solution were injected successively and the retention times of each run was recorded. Precision of the method was determined by measuring the retention times of the sample solutions corresponding to the naphazoline standard.

Results and Discussion

Naphazoline hydrochloride (I) is prone to hydrolysis at alkaline pH and easily gives 1-naphthylacetylenediamine hydrochloride (II) (Fig.1) due to the opening of the imidazoline ring. Thus, the pH of the nasal solution is important for the preservation of the product.

So far few methods (6,9,12) have been described in the literature for the identification of 1-naphthylacetylenediamine in presence of naphazoline.

![Fig. 1. Naphazoline HCl (I) and 1-naphthylacetylenediamine HCl (II)](image)

Due to similar UV profiles of naphazoline HCl and 1-naphthylacetylenediamine HCl (Fig. 2), spectral methods are not useful to identify this degradation product. In addition to the colorimetric method given in BP 1988, two methods are described in the literature which use column chromatography for the separation of the two compounds (9,12) while another method applies RP-HPLC with cyanopropyl column (12).

In this study we developed a more practical and cheaper method for the identification of 1-naphthylacetylenediamine HCl in nasal drops without prior separation.
Fig. 2. The UV spectra of naphazoline HCl (I) (0.019 mg/ml in ethanol) and 1-naphthylacetylene-diamine HCl (II) (0.02 mg/ml in ethanol)

Table 2. The retention times and precision of commercial sample solution

<table>
<thead>
<tr>
<th>Sample No</th>
<th>$R_I$ naphazoline</th>
<th>$R_I$ degradation product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.704</td>
<td>11.166</td>
</tr>
<tr>
<td>2</td>
<td>8.682</td>
<td>11.025</td>
</tr>
<tr>
<td>3</td>
<td>8.625</td>
<td>10.698</td>
</tr>
<tr>
<td>4</td>
<td>8.574</td>
<td>10.682</td>
</tr>
<tr>
<td>5</td>
<td>8.497</td>
<td>10.910</td>
</tr>
<tr>
<td>x</td>
<td>8.6164</td>
<td>x: 10.8962</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.1190</td>
<td>S.D.: 0.2090</td>
</tr>
<tr>
<td>R.S.D.</td>
<td>1.38%</td>
<td>R.S.D.: 1.91%</td>
</tr>
</tbody>
</table>
The selectivity of the method was assessed by injecting the placebo nasal solution and peak purity test by PDA in the wavelength range of 190-600 nm. The chromatogram (Fig.3) thus obtained has not shown any remarkable peak at the retention times of naphazoline standard and peak purity check has indicated lack of interference.

The reproducibility of both instrument and the method was assessed with satisfaction on the basis of the precision values given in Table 2.

The method was applied to 5 commercial products produced at different dates, and drawn from the market due to stability problems. As can be seen in Fig.4., the HPLC chromatogram of the products show two resolved peaks [R= 2.74 (n=5), S.D. 0.013, R.S.D.=0.47%] one of which belongs to naphazoline (Rt= 8.61) [compared with UV spectra of standard naphazoline (Fig.4 a,b)] while the other, to the degradation product (Rt=10.89) and the PDA-UV spectrum (Fig.4 c,d,e,f,g) is completely in accordance with the one given for 1-naphthylacetyleneamine (Fig.2) in the literature(2).

Thus, it can be concluded that this method is rapid, reproducible and selective for testing the existance of degradation in nasal drops containing naphazoline.
Fig. 4. The HPLC chromatogram and UV-PDA spectra of a-g.

a: Standard naphazoline HCl in placebo nasal solution, b: Standard naphazoline HCl in water (400μg/ml),
c: Sample 1, d: Sample 2, e: Sample 3, f: Sample 4, g: Sample 5.
References

5. Al-Kaysi, H.N., Salem, M.S., Al-Khalili, N.: Dirasat 12, 101 (1985); (Ref.2)
6. Alcon Laboratories Inc.: Unpublished Data on File; (Ref.2)

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