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Determination of Naphazoline in Rat Plasma Using Column Liquid Chromatography with Ultraviolet Detection

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A sensitive (1 ng/mL) and rapid method for the determination of naphazoline in rat plasma is described. Following extraction, the compound is analysed by reversed phase high performance liquid chromatography and ultraviolet detection at 214 nm.

INTRODUCTION

Naphazoline (NPZ), a potent vasoconstrictor, can be used in topical decongestants (nasal drops, sprays, opthalmic preparations) for treatment of allergic rhinitis (Bryant and Cormier, 1983). The chemical structure of the drug is shown in Fig. 1.

Several spectrophotometric and chromatographic methods are described for assaying NPZ in several pharmaceutical preparations (Jane *et al.*, 1985; Salam *et al.*, 1986; Santoni *et al.*, 1989; Korany *et al.*, 1990)

However, until now, the determination of NPZ in biological fluids had not been published. Because of excessive and prolonged use of nasal decongestants, cardiovascular and central nervous system effects have been reported (Mueller, 1983; Bale *et al.*, 1984; Chaplin, 1984; Saunders, 1986).

The present report describes an analytical procedure using high performance liquid chromatography (HPLC) for the quantification of NPZ in biological fluids and, in particular, in plasma.

EXPERIMENTAL

Chromatographic system. The liquid chromatographic system consisted of a 110A Beckman pump, a 160 absorbance detector (Beckman Instruments, Berkeley, CA, USA) with a Zn lamp and a 214 nm filter, a sample injector with a 20 µL loop (Beckman) and a recorder (Kipp and Zonen, The Netherlands). The analyses were performed on a 25 cm × 4.6 mm i.d. stainless-steel column packed with 5 µm Hypersil-Phenyl (Prolabo, Paris, France). The mobile phase consisted of methanol and aqueous 0.025 M sodium phosphate buffer (pH 5) containing 0.005 M sodium 1-heptanesulphonate (44:56, v/v). The mixture was filtered through a 0.45 µm filter and degassed, before use, 30 min at 25 °C under vacuum and gentle stirring (magnetic and heated stirring rod, 500 revolutions/min; Bioblock, Strasbourg, France). The flow rate was set at 1.5 mL/min.

Chemicals and reagents. Methanol and dichloromethane were of HPLC grade (Carlo Erba, Milan, Italy). The distilled water used for all solutions and the mobile phase was purified through a Milli-Q water purification system (Millipore, Waters, Milford, MA, USA).

Sodium 1-heptanesulphonate was purchased from Aldrich (Steinheim, Germany), sodium dihydrogen phosphate from E. Merck (Darmstadt, Germany) and NPZ from Roussel Uclaf (Paris, France).

Sample preparation. In one experiment, a male Sprague-Dawley rat (Charles River, MA, USA) weighing 350 g was used for study. NPZ was dissolved in 0.025 M sodium phosphate buffer (pH 7), diluted in saline and administered intravenously at a dose of 50 µg/kg. Total blood was drawn by cardiac puncture 2 min after intravenous administration, centrifuged for 20 min and the plasma carefully removed.

In a second step, a male rat weighing 490 g was injected with 10 µg/kg NPZ by i.v. injection (after anaesthesia by i.p. injection of pentobarbital). Blood samples were collected at various time intervals after the injection.

To 0.5 mL of each plasma sample, 1 mL of perchloric acid (0.6 M) was added. The mixture was vortexed and allowed to stand for 10 min. The samples were centrifuged (at 2000 g, room temperature) for 15 min and the supernatant was collected. An aliquot (0.8 mL) was transferred to a 10 mL screw-capped tube and mixed with 0.5 mL of 1.2 M potassium hydrogen carbonate. After a few minutes, 0.5 mL of 2 M sodium hydroxide and 4 mL of dichloromethane were added and the tube shaken gently with neutralizing supernatant for 15 min and then centrifuged for 10 min (500 g).

After removal of the aqueous phase, 3.5 mL of the organic phase was transferred to a conical tube, and the solvent was evaporated under nitrogen gas (heating block, ca. 40 °C), The residue was dissolved in 100 µL of 0.025 M sodium phosphate buffer (pH 5) and 20 µL was injected into the chromatograph.

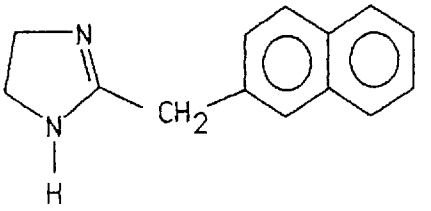

Figure 1. The chemical structure of NPZ.

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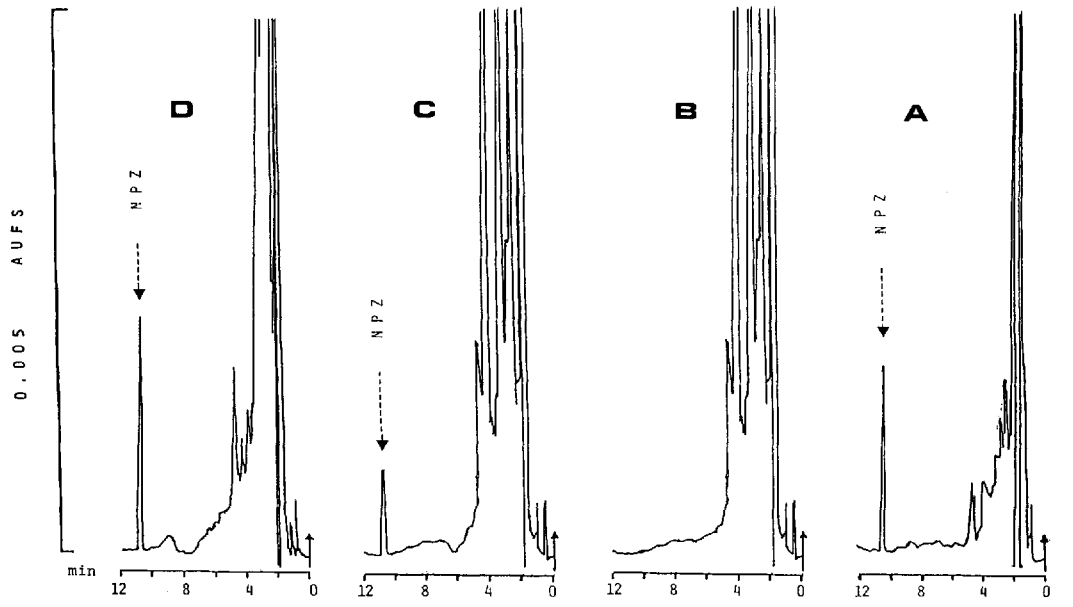

Figure 2. Chromatograms of NPZ in phosphate buffer (0.25 mg/mL; A), rat pool plasma (B), rat pool plasma spiked with the drug (0.05 mg/L; C) and rat plasma injected with an elevated dose of the drug (50 μg/kg; D), 2 min after intravenous administration.

RESULTS AND DISCUSSION

Extraction and recovery

Plasma proteins were removed by perchloric acid precipitation. Excess acid present in the supernatant was neutralized with potassium hydrogen carbonate and sodium hydroxide was added to alkaline pH. NPZ, like many basic drugs, could be extracted in its uncharged form by an organic solvent.

The solvents studied were chloroform, diethyl ether, ethyl acetate, acetonitrile, dichloromethane and solvent mixtures. With many solvents, we found emulsions, impurities or bad recovery. Dichloromethane gave the best recovery of 98% for 25 ng/mL ($n=4$; CV = 3.6%) in one extraction step. To prevent the formation of emulsions during the extraction of plasma samples, the extractions were carried out on a platform shaker under gentle shaking.

Chromatographic conditions

To achieve maximum sensitivity, several columns, the composition of the mobile phase and the wavelengths were tested.

A moderately polar phenyl-bonded phase was adopted and heptanesulphonic acid was used in the mobile phase (acid pH) as an ion pairing agent. By changing the counterion from heptanesulphonic acid to pentanesulphonic acid, the NPZ retention was reduced.

Monitoring of 254 nm and 280 nm, which have been used by previous investigations, did not give sensitivity enough for biological fluids. At 214 nm, NPZ showed maximum absorbance; phosphate was chosen for the buffer instead of acetate because its absorbance was negligible. For the same reason, methanol was used instead of acetonitrile. Degassing was necessary, before using the mobile phase, to eliminate any trace of oxygen absorbed at this wavelength as it gives a very bad baseline for the low detection range used in biological fluids.

Under the conditions described, NPZ has a retention time of ca. 11 min.

Standard curve and precision

Calibration graphs were constructed by spiking samples of rat plasma with different concentrations of NPZ and analysing them according to the method. The resulting peak areas were plotted versus the concentrations of NPZ (0–200 ng/mL range). The curves were linear and passed through the origin (correlation coefficient, $r=0.997$; $n=6$). The coefficient of variation (CV) was determined to be 1.2% ($n=8$) for the 50 ng/mL level of the drug. However, with low levels (1 ng/mL) the CV

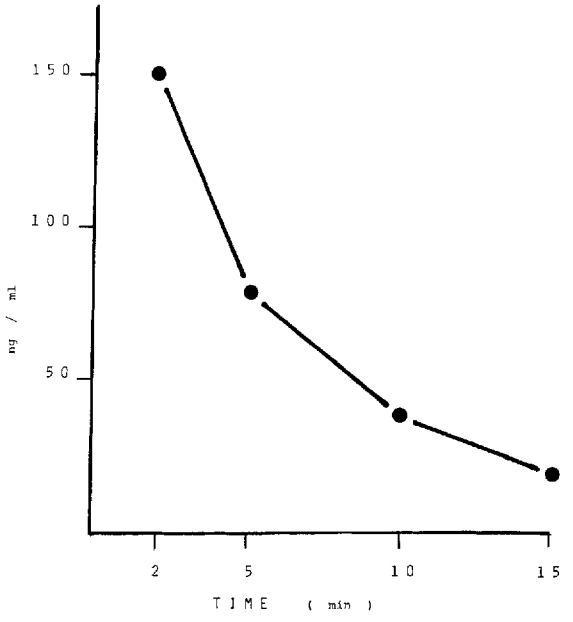

Figure 3. Concentration of NPZ in plasma from a rat injected with the drug (10 μg/kg i.v.).

was about 4.5% ($n=8$). The detection limit, at a signal-to-noise ratio of 2, was ca. 1 ng/mL. The day-to-day repeatability of the method was determined by analysing the same sample (single operator) on four consecutive days (CV = 1%).

Analysis of rat plasma after intravenous administration of NPZ

Figure 2 shows chromatograms of rat pool plasma (B; no peak that could interfere with the determination of NPZ was seen), rat pool plasma spiked with the drug (C) and rat plasma, 2 min after a high intavenous dose of naphazoline (50 μg/kg; D).

Figure 3 gives the results obtained in another rat after intravenous administration of 10 μg/kg.

An extension study of these experimental data, in particular in human plasma samples collected after administration of a nasal spray or drops, will be the subject of a further publication.

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