Journal of Chromatography, 222 (1981) 241—248
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 731

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF NITROXOLINE IN PLASMA AND URINE

R.H.A. SOREL*, C. SNELLEMAN and A. HULSHOFF

Department of Analytical Pharmacy, University of Utrecht, Pharmaceutical Laboratory, Catharijnesingel 60, 3511 GH Utrecht (The Netherlands)

(Received July 10th, 1980)

SUMMARY

A high-performance liquid chromatographic method has been developed for the determination of nitroxoline in 50-μl plasma and urine samples.

A structural analogue of nitroxoline, 8-hydroxyquinoline, was added to the eluent in order to suppress peak asymmetry. Several parameters of the eluent were studied for the optimisation of the chromatographic system.

Plasma concentration—time curves were constructed for three volunteers after they had received an oral dose of 100 mg of nitroxoline. Plasma half-life was about 1 h. Within 12 h, about 1% of the dose was excreted in the urine as free nitroxoline and about 30% as conjugated metabolite of the parent compound.

INTRODUCTION

Nitroxoline (8-hydroxy-5-nitroquinoline) is used in the treatment of urinary tract infections of Gram-negative and Gram-positive microorganisms.

In the literature only ultraviolet—visible spectrophotometric determinations of nitroxoline in plasma [1] and in urine [1, 2] have been reported. Pharmacokinetic data of nitroxoline in man are scarce. Recently some data have been published concerning nitroxoline levels in urine, of both the conjugated and the unconjugated drug [2].

The aim of this study was to develop a high-performance liquid chromatographic (HPLC) method for the analysis of nitroxoline in plasma and urine, of sufficient sensitivity to allow the analysis of small plasma samples collected from finger-pricks. The usefulness of the method was tested by analyzing plasma and urine samples of three volunteers who had received an oral dose of nitroxoline.

0378-4347/81/0000—0000/$02.50

EXPERIMENTAL

Apparatus

Chromatography was performed on a component system consisting of a Waters Assoc. (Milford, MA, U.S.A.) Model 6000A solvent delivery system, Model U6K injector and Model 440 absorbance detector operated at 436 nm.

A μBondapak C_{18} column (Waters Assoc.), 30 cm × 3.9 mm I.D., particle size 10 μm, was used.

Chemicals

Nitroxoline was a kind gift from Roussel Laboratories B.V. (Hoevelaken, The Netherlands).

Chloroform, methanol, 8-hydroxyquinoline, disodium phosphate and picric acid (all of "pro analysis" grade) were purchased from Merck (Darmstadt, G.F.R.).

Extraction of plasma and urine

To 50 μl of plasma (or urine) in a 7-ml centrifuge tube were added 15 μl (or 4 μl) of 4 *M* hydrochloric acid. The resulting solution was extracted with 2 ml of a solution of the internal standard in chloroform (picric acid, 5 μg ml^{-1}). After mixing on a Vortex mixer for 30 sec and subsequent centrifugation at 2500 *g* for 2 min, the chloroform phase was transferred to another centrifuge tube. The chloroform layer was evaporated to dryness under a stream of nitrogen at 30° C. The residue was reconstituted in 50 μl of eluent by mixing on a Vortex mixer for 15 sec; 20 μl of this solution were injected into the HPLC system.

Chromatography

The eluent consisted of 8-hydroxyquinoline (0.1%, w/w), aqueous phosphate buffer (0.2 *M*, pH 7.4); 8%, w/w), methanol (35%, w/w) and water. The flow-rate was 1.5 ml min^{-1}. Chromatography was performed at ambient temperature.

In vivo studies

Three volunteers received an oral dose of 100 mg of nitroxoline each. Blood samples were collected by finger-prick into 1-ml plastic vials containing heparin sodium after 1, 2, 3, 4, 6, 8, 12, and 24 h. The samples were spun down at 2500 *g*; the plasma was collected and stored at −20° C prior to analysis. Conjugated nitroxoline in urine was determined after refluxing the samples for 1 h at pH 0.5. The samples were then extracted and analyzed as described above.

RESULTS AND DISCUSSIONS

With some frequently used chromatographic systems — reversed-phase, ion-exchange and dynamic ion-exchange — strongly tailing peaks of nitroxoline were observed. Typical chromatograms are presented in Fig. 1a—c. This tailing is possibly caused by a strong interaction of nitroxoline with the free silanol

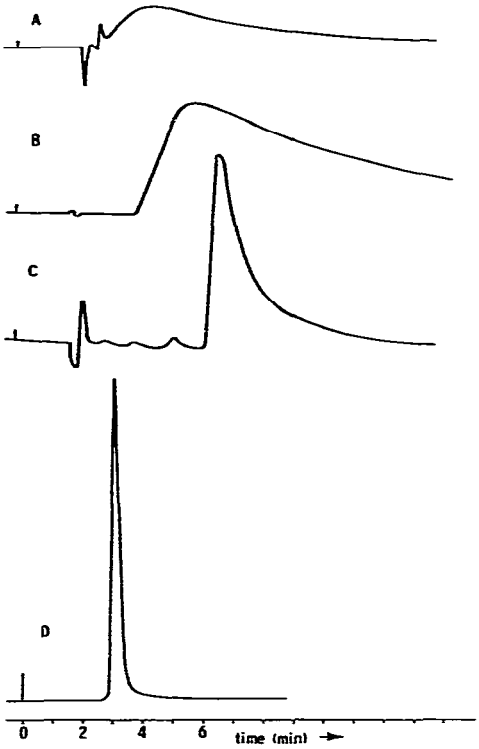

Fig. 1. Chromatograms of nitroxoline in different chromatographic systems. (A) Strong anion exchange. Column Partisil SAX, 25 cm × 4.6 mm I.D.; particle size 10 μm (Whatman, Clifton, NJ, U.S.A.);.eluent, aqueous phosphate buffer (pH 7.2, 0.05 *M*) 75% (w/w), methanol 25% (w/w). (B) Dynamic ion exchange. Column, μBondapak C_{18}; eluent, cetrimide 0.125% (w/w), aqueous phosphate buffer (pH 7.7, 0.2 *M*) 4% (w/w), methanol 62.5% (w/w) and water. (C) Dynamic ion exchange. Column, μBondapak C_{18}; eluent, cetrimide 0.1% (w/w), 1,2-diaminocyclohexane-N,N,N¹,N¹-tetraacetic acid (Titriplex IV, Merck) 0.1% (w/w), aqueous phosphate buffer (pH 7.4, 0.2 *M*) 7.3% (w/w), methanol 55% (w/w) and water. (D) Column, μBondapak C_{18}; eluent, 8-hydroxyquinoline 0.1% (w/w), aqueous phosphate buffer (pH 7.4, 0.2 *M*) 8% (w/w), methanol 50% (w/w) and water.

groups of the column packing material. This tailing was suppressed by the addition of 8-hydroxyquinoline, a structural analogue of nitroxoline (Fig. 1d). Another cause for the strongly tailing peaks could be the presence of trace elements, which are well-known impurities in silica gel [3]. When Titriplex IV, a complexing agent for metal ions, was added to the eluent in the dynamic ion-exchange system, a significant decrease of tailing was observed (Fig. 1b and c). It is therefore possible that the complex-forming properties of 8-hydroxyquinoline are at least partly responsible for the improved symmetry of the nitroxoline peaks.

The composition of the eluent — pH, ionic strength and 8-hydroxyquinoline concentration — was optimized by considering peak symmetry. For the determination of nitroxoline a phosphate concentration of 16 mmol was chosen, because at this concentration the curve of phosphate concentration versus peak symmetry is almost flat (Fig. 2). At higher concentrations the risk of precipitation is evident. Fig. 3 shows the effect of the pH of the phosphate

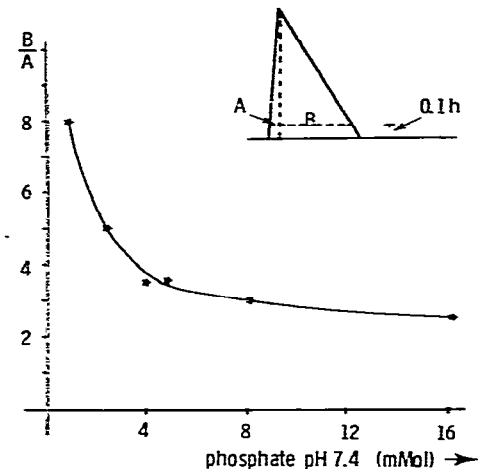

Fig. 2. Influence of the phosphate concentration in the eluent on the peak symmetry of nitroxoline. Eluent: 8-hydroxyquinoline 0.1% (w/w), methanol 35% (w/w) and water.

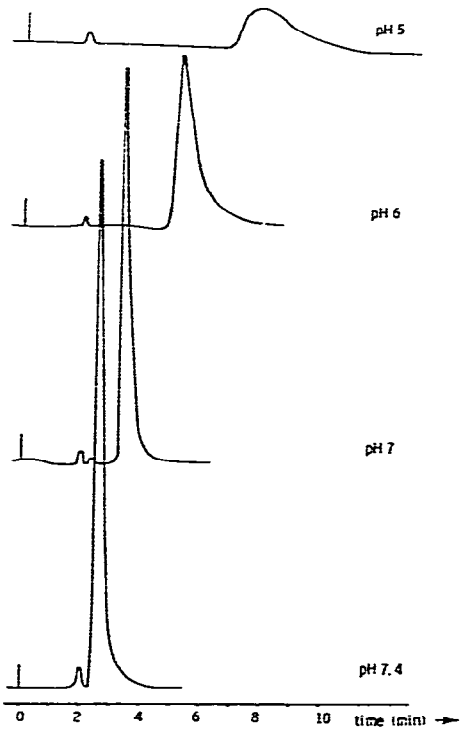

Fig. 3. Influence of the pH of the aqueous phosphate buffer on the chromatographic behaviour of nitroxoline. Eluent: 8-hydroxyquinoline 0.1% (w/w), aqueous phosphate buffer (0.2 *M*) 8% (w/w), methanol 35% (w/w) and water.

buffer on the chromatographic behaviour of nitroxoline. For the determination pH 7.4 was chosen, because at this pH the peak symmetry is acceptable and the detector response at 436 nm is close to the maximum response of the anionic form of the drug. At higher pH values the column life is the limiting factor.

A concentration of 0.1% 8-hydroxyquinoline in the eluent was sufficient to reduce the tailing to a minimum (results not shown). The concentration of methanol in the eluent did not affect the peak symmetry, so this parameter was used to optimize the analysis time. A concentration of 35% (w/w) methanol was chosen for the determination. The chromatographic analysis was performed within 5 min at this methanol concentration. The best day-to-day reproducible chromatograms were obtained when, at the end of each day, the column was washed with methanol containing 1% glacial acetic acid.

A very simple method for the determination of nitroxoline in plasma would be the direct injection of the plasma supernatant after precipitation of the proteins with acetonitrile. However, chromatograms obtained in this way showed a peak at the dead time and a negative peak after about 12 min (k' = 6.3). Apparently acetonitrile stripped some of the 8-hydroxyquinoline off the column; the negative peak is then caused by re-loading of the column resulting in a temporary decrease in the concentration of 8-hydroxyquinoline in the eluent. This effect could not be suppressed by adding 8-hydroxyquinoline to the acetontrile. Although these peaks did not interfere with the nitroxoline and internal standard peaks in the chromatogram, the analysis time was much prolonged. Moreover, the nitroxoline peaks showed more tailing in the presence of the acetonitrile. Obviously, the chromatographic system is disturbed by the addition of acetonitrile. It was therefore decided to include an extraction step in the procedure, which created the possibility of reconstituting the nitroxoline in the eluent after evaporation of the organic layer.

Picric acid was chosen as the internal standard. The stability of this compound under the preceding conditions was studied; no decomposition or loss of picric acid was observed, not even when after the evaporation of the chloroform layer the residue was heated for 2 h at 40°C. Fig. 4a shows the absolute recovery of nitroxoline and picric acid from serum after the addition of different volumes of 4 *M* hydrochloric acid. In Fig. 4b the pH values of serum are shown as a function of the amount of added 4 *M* hydrochloric acid. The absolute recovery was found to be 95% for nitroxoline and 85% for picric acid, when 15 μl of 4 *M* hydrochloric acid were added to 50 μl of serum. With different batches of serum and plasma no significant differences in pH were found upon addition of hydrochloric acid.

Fig. 5 shows chromatograms obtained from the in vivo experiment. Following the procedure (50-μl samples, 20-μl injection volumes) the detection limit (signal-to-noise ratio is 3) was 80 ng ml^{-1} plasma. For the in vivo experiment two calibration curves (concentration range 0.0792—39.6 μg ml^{-1}) were constructed by analyzing serum or urine samples with varying amounts of nitroxoline. The results were, for serum, $y = 0.0412x + 0.0020$ ($r^2 = 0.9996$, $n = 14$) where y = peak height ratio of nitroxoline/internal standard and x = concentration of nitroxoline in μg ml^{-1}, and for urine $y = 0.0406x - 0.0093$ ($r^2 = 0.9998$, $n = 4$). The reproducibility of the method was examined at two

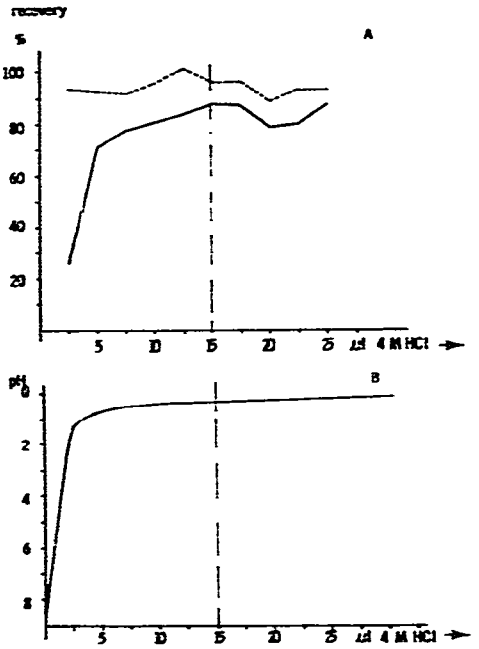

Fig. 4. (A) Recovery of nitroxoline (— — —) and picric acid (———) from 50 μl of serum, and (B) pH, both as a function of the added volume of 4 *M* hydrochloric acid.

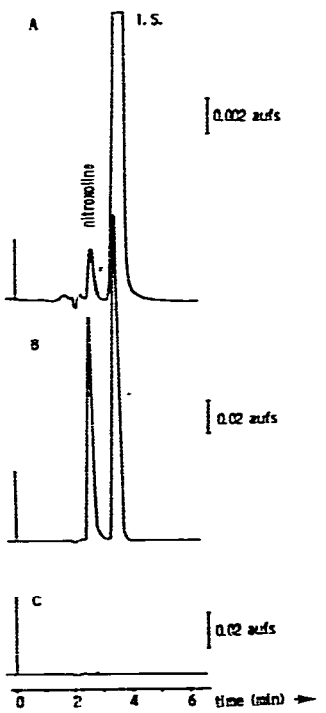

Fig. 5. Chromatograms obtained from (A) a volunteer plasma sample (0.4 μg ml^{-1} nitroxoline), (B) a spiked serum sample (15.8 μg ml^{-1} nitroxoline) and (C) a volunteer plasma blank. I.S. = internal standard.

concentrations, 0.2 μg ml^{-1} and 20 μg ml^{-1}, by analyzing eight serum samples at each concentration. The coefficients of variation were 7.6% at 0.2 μg ml^{-1} and 3.5% at 20 μg ml^{-1}.

Fig. 6 shows the plasma concentration—time curves of nitroxoline for three volunteers. The plasma half-life of nitroxoline was about 1 h. After 8—12 h the plasma concentration of nitroxoline was decreased to the detection limit; for a more exact kinetic study of the elimination phase the sensitivity of the determination must be increased. This can be achieved, for example, by using 200-μl plasma samples (also obtainable by finger-prick) and injection of a larger portion of the final solution into the chromatographic system.

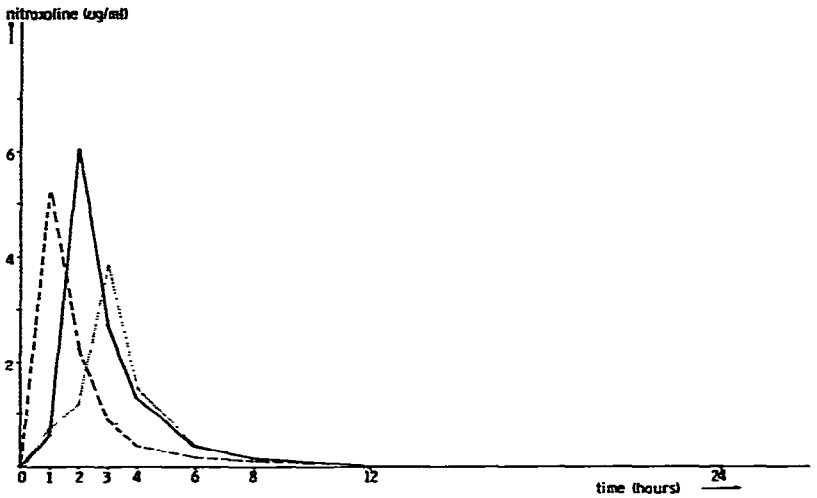

Fig. 6. Plasma concentration—time curves for three volunteers after an oral dose of 100 mg of nitroxoline.

Table I shows the nitroxoline recovery from the 12-h urine samples collected from the three volunteers, and the values for free and conjugated nitroxoline reported in the literature [2].

TABLE I

RECOVERY OF NITROXOLINE IN URINE (12 h) AFTER ORAL ADMINISTRATION OF 100 mg

Volunteer	Nitroxoline (free) (%)	Nitroxoline (conjugated) (%)
A	1.2	23.3
B	0.3	41.1
C	0.3	24.1
Literature [3]	1.5*	48*

*Oral dose, 200 mg.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. Dr. A.W.M. Indemans for helpful discussions.

REFERENCES

1 M.R. Jacobs, R.G. Robinson and H.J. Koornhof, S. Afr. Med. J., 54 (1978) 959.
2 A. Mrhar, Z. Kopitar, F. Kozjek, V. Presl and R. Karba, Int. J. Clin. Pharmacol., 17 (1979) 476.
3 M. Verzele, M. De Potter and J. Ghyssels, High Resolut. Chromatogr. Chromatogr. Commun., 2 (1979) 151.