Journal of Chromatography, 163 (1979) 92—95
Biomedical Applications

CHROMBIO. 301

Note

Rapid gas chromatographic method for the determination of nalidixic acid in plasma

H. ROSEBOOM, R.H.A. SOREL, H. LINGEMAN and R. BOUWMAN

Pharmaceutical Laboratories, University of Utrecht, Catharijnesingel 60, Utrecht (The Netherlands)

(First received September 26th, 1978; revised manuscript received December 19th, 1978)

Nalidixic acid is mostly used for the treatment of urinary tract infections, but in some cases it is administered intravenously for the treatment of general infections [1]. In those cases it is necessary to determine the plasma concentration of nalidixic acid, preferably by means of a rapid, simple method. In the literature, fluorimetric [2, 3] and liquid chromatographic [4, 5] methods have been described. The fluorimetric methods have the disadvantage of not being specific and the recently described high-performance liquid chromatographic (HPLC) method involves a time consuming derivatization step.

In this paper we shall describe a gas chromatographic (GC) method that is specific and sensitive enough to determine therapeutic levels in a 1 ml plasma sample within 30 min of receipt of the sample.

EXPERIMENTAL

Apparatus

A Packard-Becker Model 419 gas chromatograph, equipped with flame ionisation detectors was used. The glass column (1.5 × 6 mm O.D.) was packed with 10% OV-17 on Chromosorb W HP, 80—100 mesh (Chrompack, Middelburg, The Netherlands). The operating conditions were: injection port temperature, 290°; column temperature, 270°; detector temperature, 290°; carrier gas (nitrogen) flow-rate 20 ml/min; hydrogen flow-rate 30 ml/min; air flow-rate 300 ml/min.

Quantitation was carried out both by means of peak height measurements and peak area measurements with the chromatography Data Analyser System IV (Spectra Physics, Santa Clara, Calif., U.S.A.).

Chemicals

Toluene and dimethylacetamide (analytical grade) were obtained from Merck (Darmstadt, G.F.R.), nalidixic acid and butyliodide from Fluka (Basel, Switzerland), phenprocoumon from Hoffman-La Roche (Basel, Switzerland), flufenamic acid from Parke-Davis (Detroit, Mich., U.S.A.) and tetramethylammoniumhydroxide solution (20% in methanol) from Aldrich Europe (Beerse, Belgium). Hydroxymethylnalidixic acid was a friendly gift from Winthrop Labs. (New York, N.Y., U.S.A.).

Procedure

To 1 ml of plasma in a centrifuge tube was added 50 μl of 4 *M* hydrochloric acid and 2 ml toluene, containing flufenamic acid (2 mg/ml) and phenprocoumon (25 μg/ml) as internal standard. After shaking on a vortex mixer for 30 sec. and subsequent centrifugation at 2500 g for 2 min the toluene phase was transferred into another centrifuge tube. To this solution was added 25 μl of a 20% solution of tetramethylammoniumhydroxide in methanol and after thorough mixing and centrifugation 10 μl of the bottom layer was transferred into a glass capillary tube (5 cm × 3 mm I.D.) to which was added 25 μl dimethylacetamide and 7 μl butyliodide. After mixing and standing for 5 min the tubes were centrifuged for 5—10 min at 2500 *g* and 1—2 μl of the clear supernatant was injected into the gas chromatograph.

RESULTS AND DISCUSSION

Injection of solutions of underivatized nalidixic acid on various columns revealed that it was necessary to alkylate this drug before GC in order to obtain reasonably symmetrical peaks. Methylation proved to be unsatisfactory. Probably the methyl ester is still too polar a compound. The butyl ester gave better results. On a 3% OV-17 column there was still a significant tailing, which was also the case on 10% SE-30, so a 10% OV-17 column was used. Multiple injections of the butyl ester on this column revealed that there were still losses in the first injections. Therefore, flufenamic acid was added to the solution in a rather high concentration, resulting in a deactivation of the column. In this manner reproducible results were obtained.

At pH values below 4, nalidixic acid, flufenamic acid and phenprocoumon are well extracted with toluene, the back extraction into a small volume of tetramethylammoniumhydroxide solution is an easy way to obtain a very clean blank chromatogram (Fig. 1a) and to concentrate the sample.

Pre-column butylation based on the method described by Greeley [6] has some advantages over on-column butylation, for instance there is less risk of decomposition of the compounds due to the high temperature and the strongly alkaline medium during the alkylation process, the peaks are somewhat narrower and higher and column life is prolonged, because a neutral instead of a strongly alkaline solution is injected.

Under the described chromatographic conditions phenprocoumon and nalidixic acid give well resolved symmetrical peaks (Fig. 1b) with retention times of 7 and 8 min respectively. Flufenamic acid gives a peak at 1.5 min, so it is eluted in the solvent peak.

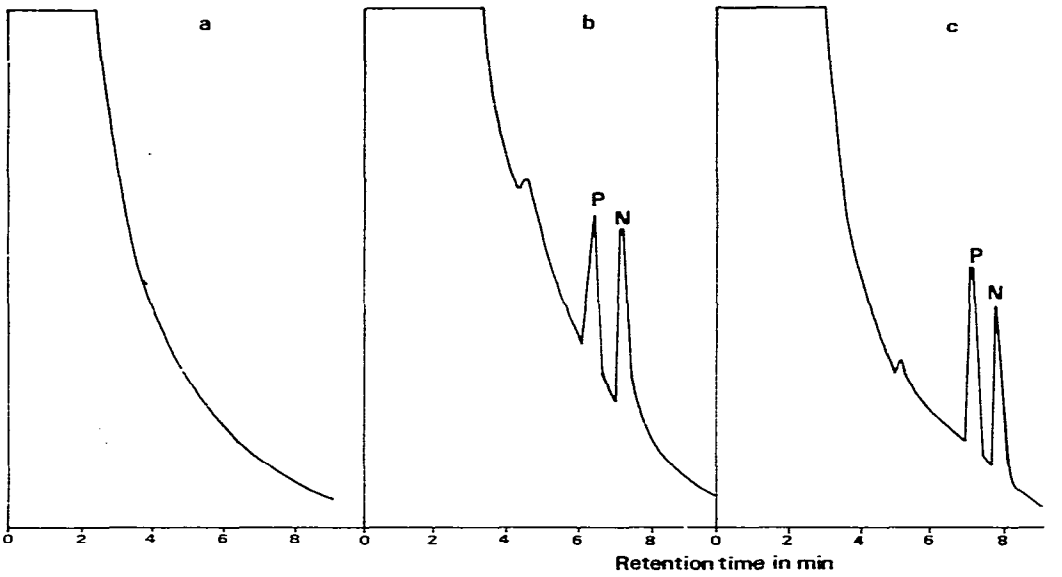

Fig. 1. Chromatogram of plasma samples (for conditions, see text). a = Blank plasma. b = Blank plasma spiked with nalidixic acid to a concentration of 26 μg/ml; N = nalidixic acid, P = phenprocoumon. c = Plasma sample obtained from a patient, treated with nalidixic acid.

Calibration curves were constructed by adding known amounts of nalidixic acid to blank plasma samples and treating these samples as described. They were straight lines in the range studied (5—100 μg/ml), which covers the therapeutic range of 10—60 μg/ml; there was a small negative intercept of 0.1—0.3 μg/ml.

The standard deviation was determined at 13 μg/ml and at 50 μg/ml by analyzing six plasma samples at each concentration and calculated to be 3.5%.

Recovery studies showed that with toluene, nalidixic acid is extracted completely from the plasma, the recovery of the back extraction with tetramethylammonium hydroxide is 77%, so the overall recovery of the method is 77%.

In order to evaluate the potential interference of the most important metabolite of nalidixic acid (hydroxymethyl nalidixic acid) this compound was treated as described for nalidixic acid. GC showed two very small peaks at longer retention times, so there is no interference of this metabolite with the quantitative determination of nalidixic acid. Analysis of a plasma sample of a patient, treated with nalidixic acid, using this method (Fig. 1c) and our previously described HPLC procedure [5] gave essentially the same result (23.8 ± 3.5% versus 26.4 ± 4% μg/ml).

Although the GC method is less sensitive than the HPLC method and needs a larger plasma volume, its speed and ease of operation make it attractive for routine monitoring of plasma levels.

REFERENCES

1 G. Schortgen, C. Tancrede, M.C. Eveleigh and R. Neday, Reanimation Med.,(1972) 321.
2 E.W. McChesney, E.J. Froelich, G.Y. Lesher, A.V.R. Crain and D. Rosi, Toxicol. Appl. Pharmacol., 6 (1964) 292.
3 P. Brühl, G. Gundlach, K. Wintjes, W. Eichner and H.P. Bastian, Arzneim. Forsch., 23 (1973) 1311.
4 L. Shargel, R.F. Koss, A.V.R. Crain and V.J. Boyle, J. Pharm. Sci., 62 (1973) 1452.
5 R.H.A. Sorel and H. Roseboom, J. Chromatogr., 162 (1979) 461.
6 R.H. Greeley, Clin. Chem., 20 (1974) 192.