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DETERMINATION OF NIFUROXAZIDE IN BIOLOGICAL FLUIDS BY AUTOMATED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH LARGE-VOLUME INJECTION

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SUMMARY

A high-performance liquid chromatographic method for the measurement of nifuroxazide in plasma is described. The technique is based on the single extraction of the drug from buffered plasma with chloroform, using nifuratel as internal standard. The chromatographic system consisted of a 15 cm × 4.6 mm I.D. stainless-steel column packed with Spherisorb ODS, 5 μm, and the mobile phase was acetonitrile–orthophosphoric acid (pH 2.5) (30:70). The method was able to measure accurately plasma nifuroxazide concentrations down to 2 ng·ml^{-1} using 2 ml of sample with no interference from endogenous compounds. The coefficients of variation of the method at 200 and 2 ng·ml^{-1} were 3% and 15%, respectively, and the calibration graph was linear in this range. The use of automatic injection makes the method suitable for the routine analysis of large numbers of samples.

INTRODUCTION

Nifuroxazide {4-hydroxybenzoic acid [(5-nitro-2-furanyl)methylene]hydrazide} is the active principle of Ercefuryl, which is used for the treatment of acute bacterial diarrhoea. Although this drug has been available for 15 years, no analytical method has yet been developed to determine "therapeutic" nifuroxazide concentrations in biological fluids.

In this study a method was developed involving a single extraction followed by high-performance liquid chromatography (HPLC). The different steps of the extraction and the stability of nifuroxazide were investigated.

EXPERIMENTAL

Solvents

Reagent grade chloroform used for the extraction, analytical-reagent grade orthophosphoric acid and LiChrosolv acetonitrile used for the mobile phase were purchased from Merck (Darmstadt, G.F.R.).

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Standards

Nifuroxazide was synthesized in the laboratories of Synthélabo (Bagneux, France). The internal standard, methylmercadone or nifuratel, 5-[(methylthio)-methyl]3-{[(5-nitro-2-furanyl)methylene]amino}-2-oxazolidone, was kindly supplied by Fumouse (Ile Saint-Denis, France). Standard solutions were prepared by dissolving nifuroxazide in 0.01 *M* sodium hydroxide solution and the internal standard in acetonitrile.

Equipment

Routine analyses were carried out on a liquid chromatographic system consisting of a Constametric II G pump (LDC, Riviera Beach, FL, U.S.A.), a Micromeritics 725 automatic injector and a Micromeritics Chromonitor UV detector.

Chromatographic analysis

The mobile phase (acetonitrile–orthophosphoric acid (pH 2.5), 3:7) was adjusted to a flow-rate of 1.0 ± 0.01 ml·min^{-1} through a stainless-steel column (15 cm × 4.6 mm I.D.), packed in the laboratory[1] with Spherisorb ODS, 5 μm (batch 17/49) (Phase Separations, Queensferry, Great Britain). The detector wavelength was set a 362 nm, which corresponded to the highest optical absorption of nifuroxazide dissolved in the mobile phase.

Extraction procedure

A 2-ml volume of plasma together with 1 ml of phosphate buffer, pH 5.0, was introduced into a conical tube containing 150 ng of the internal standard (30 μl of a 5 ng·μl^{-1} solution in acetonitrile). This mixture was shaken on a Vortex mixer and then extracted with chloroform (7 ml) on a "rock and roll" shaker for 30 min. The two phases were then separated by centrifugation (1000 *g*, 5 min at 4°C) and the aqueous phase was discarded; 6.5 ml of the chloroform extract were transferred into a second tube and evaporated to dryness at 60°C under a gentle stream of nitrogen. The drug extract was then dissolved in 800 μl of acetonitrile–water (10:90) by agitation on a Vortex mixer. This solution was transferred into an injection vial and injected onto the column by means of an automatic injector with a 500-μl loop.

RESULTS

Two chromatograms, one obtained from a blank plasma extract and the other from a plasma extract spiked with nifuroxazide and internal standard, are presented in Fig. 1b and a, respectively. The corresponding peaks for the two compounds were well resolved and no endogenous material extracted at the same time interfered in the analysis.

The retention times of nifuroxazide and methylmercadone were 5.2 and 8.8 min, respectively, with reduced plate heights of 3.4 and 3.5, respectively.

The procedure was quantified by the internal standard technique using the peak-height ratio method. The coefficient of variation of the slope of the calibration graph was 5.5%. The response was linear between 2 and 200 ng·ml^{-1} under the conditions described above. The accuracy and reproducibility of the method were determined by analysing spiked plasma samples of nifuroxazide at various concentrations. The mean values, the standard deviation and the coefficient of variation are given in Table I.

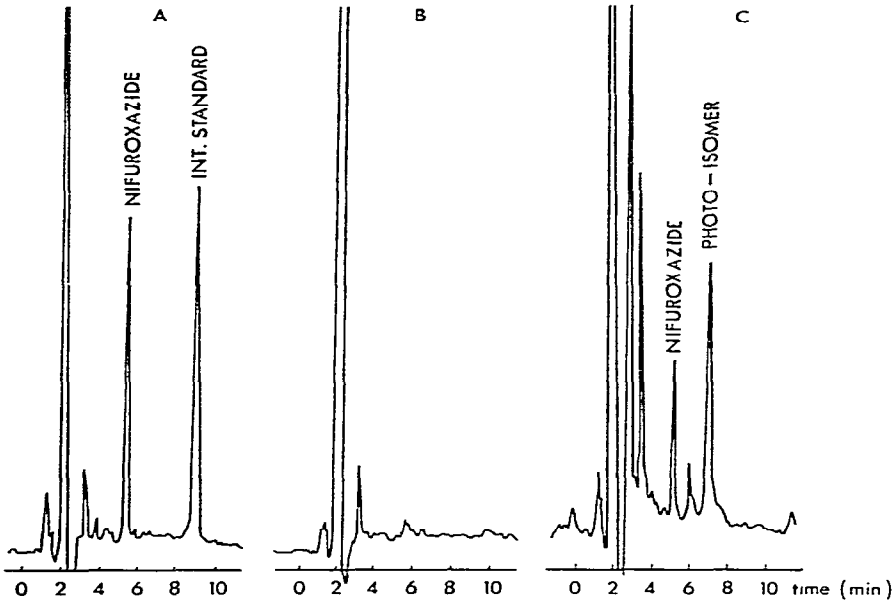

Fig. 1. Typical chromatograms of human plasma extracts for nifuroxazide determinations. A, Spiked plasma (100 ng·ml^{-1} of nifuroxazide and the internal standard); B, blank plasma; C, spiked plasma (nifuroxazide and its photo-isomer).

TABLE I

REPRODUCIBILITY OF THE HPLC METHOD FOR THE MEASUREMENT OF NIFUROXAZIDE AT DIFFERENT CONCENTRATIONS IN PLASMA

Concentration prepared (ng·ml^{-1})	*No. of measurements*	*Mean concentration obtained (ng·ml^{-1})*	*Standard deviation (ng·ml^{-1})*	*Coefficient of variation (%)*
2	6	1.8	0.3	15
5	6	5.6	0.6	10
10	11	9.9	0.8	8
20	18	20.5	1.0	5
100	10	100.5	5.3	5
200	4	199.5	6.7	3

All of the parameters involved in the extraction of nifuroxazide from plasma were investigated because there was no literature available on this subject. The stability of standard solutions was checked for nifuroxazide and the internal standard. Methylmercadone was stable in organic solvents whereas nifuroxazide exhibited rapid photodecomposition in methanol and acetonitrile. One of the main products of the light transformation was identified by IR and NMR spectroscopy as the imine double-bond isomer of nifuroxazide. This compound was chromatographed using the same conditions as nifuroxazide and had a retention time of 7.0 min (Fig. 1c). To prevent this isomerization, the nifuroxazide standard solutions were prepared in 0.01 *M* sodium hydroxide solution, which seemed to be stable if stored at 4°C.

In order to determine the optimal extraction pH, a series of plasma standards (1 ml) spiked with 200 ng of nifuroxazide and 300 ng of internal standard were extracted at different pHs (1 ml of buffer) with chloroform. The extract was dissolved in 100 μl of injection solvent and 50 μl were injected, the peak heights being measured at different pH values. The maximal extraction pH was found to be between 5.8 and 6.7 (Fig. 2).

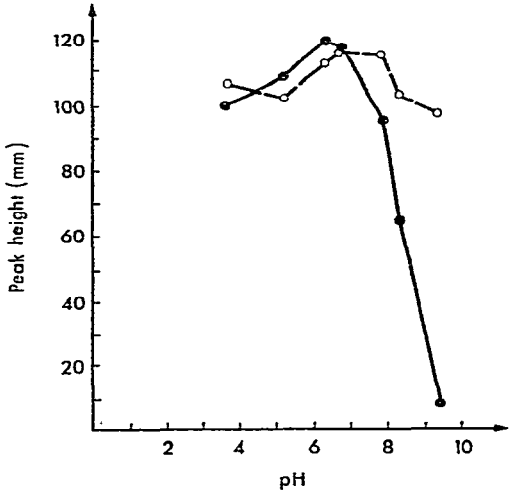

Fig. 2. Relative extraction (recovery) for nifuroxazide at different pH values. ●—●, Nifuroxazide; ○- - -○, internal standard.

Different extraction solvents were tried, using the conditions described above for chloroform. The recovery for nifuroxazide increased in the order *n*-hexane < chloroform < diethyl ether = ethyl acetate. Diethyl ether and ethyl acetate extracted a large amount of endogenous material which was trapped on the top of the column and not eluted. This created an increase in pressure drop and band broadening. Chromatograms of plasma extracted by diethyl ether or ethyl acetate exhibited interferences with nifuroxazide and the internal standard. These interferences did not appear with chloroform even after extraction for 4 h. The recovery of nifuroxazide extracted with chloroform was about 25% less than the recovery with diethyl ether; this 25% improvement in recovery with diethyl ether decreased the sensitivity from 2 to 1.5 $ng \cdot ml^{-1}$, which was relatively unimportant in comparison with the possibility of interference.

DISCUSSION

In other studies[2] it was demonstrated that reproducibility, sensitivity and efficiency of the analysis of a number of drugs by HPLC was greatly improved when the sample was injected dissolved in a large volume of a "non-eluting solvent" (*i.e.* a solvent acting as a relatively weak eluent for the compounds under analysis), compared with the more traditional method of injecting the sample dissolved in a

small volume of mobile phase or some other eluting solvent. The chromatographic processes appear not to be modified by the repeated injection of a large volume of a solvent that is not an eluent, providing this is made by using one of the components of the mobile phase[2]. This finding was also observed in the present method, where the mobile phase consisted of 30% acetonitrile in acidified water and the non-eluting solvent was 10% acetonitrile in pure water. Using automated injection it was possible to analyse at least 40–50 samples daily without any problem due to the repeated injection of relatively large volumes of the non-eluting solvent.

In conclusion, the HPLC method for nifuroxazide that has been developed is simple, sensitive, accurate and suitable for routine studies.

REFERENCES

1 M. Broquaire, *J. Chromatogr.*, 170 (1979) 43–52.
2 M. Broquaire and P. R. Guinebault, unpublished results.