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Note

High-performance liquid chromatographic determination of nifurtimox in human serum

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Nifurtimox, 4-[(5-nitrofurylidene)amino]-3-methylthiomorpholine-1,1-dioxide (Fig. 1), is a chemotherapeutic agent derived from nitrofuran, which has been successfully used in treatment of trypanosomiasis caused by T. Cruzi (Chagas' disease) [1]. Two methods have been published for the determination of nifurtimox in biological fluids, including colorimetry and thin-layer chromatography [2], but there is no method in the literature that is suitable for pharmacokinetic studies. Therefore we have developed a more convenient method for the analysis of nifurtimox in serum using high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Chemicals and standards

Dichloromethane, perchloric acid, sodium hydroxide (reagent grade) and methanol (HPLC grade) were obtained from Merck Química Chile. Nifurtimox ("Lampit", Bayer, Santiago, Chile) and the internal standard nitrofurazone (Profarma, Santiago, Chile) were donated by the respective companies. The standard solution of nifurtimox was protected from light and stored at 4°C.

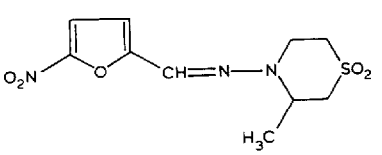

Fig. 1. Structure of nifurtimox.

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Instrumentation and chromatographic conditions

A Perkin-Elmer Series 10 liquid chromatograph was used with a Perkin-Elmer LC-75 spectrophotometric detector, operated at 400 nm. The column was a 15 cm × 3.9 mm I.D. μBondapak C_{18} (10 μm particle size). The mobile phase was methanol–Sorensen phosphate buffer, pH 7.0 (50:50, v/v). The mobile phase was filtered and pumped at a flow-rate of 1 ml/min at ambient temperature generating a back-pressure of 10.5 MPa.

Procedure

A 50-μl of nitrofurazone solution (30 μg/ml) was added to a serum sample (1 ml) in a glass tube. The mixture was shaken for 15 s. Plasma proteins were precipitated with 0.6 ml of 1 *M* perchloric acid, then centrifugated at 1000 *g* for 10 min. The liquid phase was separated and 200 μl of 1 *M* sodium hydroxide were added. Nifurtimox and the internal standard were extracted from plasma in a rotator tube at 0.2 *g* for 1 h with 3.5 ml of dichloromethane. A 3-ml volume of the organic phase was transferred and evaporated to dryness at 40°C in a thermostatted bath. The residue was dissolved in 200 μl of mobile phase, and 20 μl of the solution were injected.

Calibration and linearity

Calibration curves were prepared by analysing 1 ml of serum sample spiked with known amounts of the compounds (ranging from 0.077 to 2.301 μg/ml for nifurtimox). A 50-μl volume of internal standard (30 μg/ml), prepared as an aqueous solution, was added. Peak-height measurements were found to be reproducible. Calibration standards were run on each day of analysis. A straight line was obtained by plotting the concentration (μg/ml) of nifurtimox in plasma against the peak-height ratio of nifurtimox to the internal standard.

RESULTS

Linear regression analysis of calibration curve indicated no significant deviation from linearity ($r=0.9993$). In addition, intercept values did not significantly differ from zero (0.027).

Detection limit

Using 1.0 ml of plasma, the limit of detection for nifurtimox was 77.0 ng/ml. A 2.0-cm peak-height was obtained, at a signal-to-noise ratio of 2:1.

Reproducibility

Replicate analysis of pooled plasma sample containing nifurtimox at a concentration of 500 ng/ml gave an intra-day coefficient of variation of 3.5% ($n=10$, mean=11.2, S.D.=0.399). Assay of the same pooled plasma over a two-week period yielded an inter-day coefficient of variation of 4% ($n=10$, mean=11.5, S.D.=0.460).

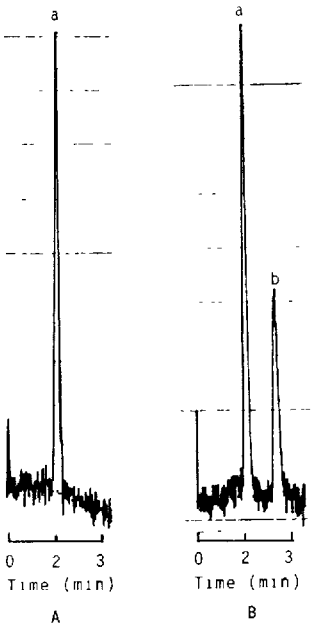

Fig. 2. Typical chromatogram of nifurtimox analysis. (A) Blank serum with internal standard (1.5 μg/ml). (B) Serum after administration of nifurtimox to a patient. Peaks: a = internal standard (1.5 μg/ml); b = nifurtimox (0.2 μg/ml).

Recovery

The analytical recovery was determined by comparing the peaks heights of each compound extracted from spiked solution with those obtained by direct injection of the compound. The recovery of nifurtimox added to a solution was 90.6%.

Selectivity

The separation of nifurtimox and nitrofurazone is shown in Fig. 2. The retention times were 3 and 2 min, respectively. No interfering peaks were present in drug-free plasma at the retention time corresponding to nifurtimox (Fig. 2A).

DISCUSSION

The ease of sample preparation (extraction with dichloromethane), the small sample volume required, the low limit of nifurtimox detection and the short retention time all contribute to making this HPLC method suitable for pharmacokinetic studies in patients with chronic renal failure.

The method is sensitive enough for the serum concentration after an oral dose of 10 mg/kg nifurtimox to be measured. The colorimetric method has a sensitivity of 0.5 μg/ml, which rules out pharmacokinetic studies owing to the low serum concentration range founded after oral administration of nifurtimox.

Nitrofurazone was found to be a good internal standard, as it showed reproducible extraction and an adequate retention time, and was well resolved from

the nifurtimox peak. We used nitrofurazone because it is structurally similar to nifurtimox [3]. The two drugs have the same solubility and UV absorption spectrum, and they may be extracted by organic solvent from aqueous acid or alkaline solutions.

ACKNOWLEDGEMENTS

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