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Letter to the Editor

High-performance liquid chromatographic determination of milrinone in biological tissues and fluids

Sir,

Milrinone (Fig. 1) is a recently developed cardioactive bipyridine [1]. Its pharmacological profile, a combination of positive inotropic and vasodilator properties, gives the drug an excellent potential for the treatment of congestive heart failure [2]. To examine the pharmacokinetics or tissue distribution of milrinone in humans or in laboratory animals, plasma and tissue concentrations of the drug must be determined accurately. As a result of the novel character of the drug, few methods are available for the quantitation of milrinone in biological matrices. Recently, high-performance liquid chromatographic (HPLC) techniques to determine plasma milrinone concentrations were reported, but these require one or more extraction steps [3,4] and are restricted to analysis in body fluids.

We present here a simple and versatile method to measure milrinone in both liquid and more solid matrices, such as liver, heart, brain, whole blood or other animal tissues. A high level of accuracy is achieved by avoiding extraction steps. Instead, a centrifuge-supported microfiltration technique following reversed-phase HPLC with UV detection is utilized to purify and analyse the samples.

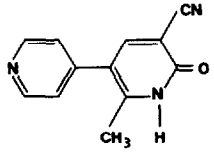

Fig. 1. Chemical structure of milrinone [1,6-dihydro-2-methyl-6-oxo-(3,4′-bipyridine)-5-carbonitrile].

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EXPERIMENTAL

Chemicals and reagents

Milrinone was kindly supplied by Sterling Winthrop (Guildford, Surrey, U.K.). All other chemicals were of analytical grade (pro analysi, Merck, Darmstadt, F.R.G.) and were used without further purification. All solutions were made with glass-distilled water.

Apparatus

Tissues were homogenized with an Ystral D7801 homogenizer. Microfiltration was carried out using Amicon Centricon-30 microconcentrators in a Sorvall RC-50 refrigerated superspeed centrifuge.

The chromatographic system consisted of a Varian Model 8500 pump, a 10 mm×3 mm I.D. RP-2 pre-column (Chrompack, Middelburg, The Netherlands), an RP Select B RT 250-4 column (Merck) and an LKB 2151 variable-wavelength monitor (LKB, Bromma, Sweden), operated at 331 nm. Samples were loaded with a Rheodyne Model 7210 loop injector, equipped with a 100-μl sample loop.

Chromatographic procedure

The mobile phase was methanol–25 m*M* ammonium acetate buffer, pH 3.75 (25:75, v/v). Tetramethylammonium hydroxide (final concentration 0.01%) was added for additional peak symmetry. The flow-rate was 45 ml/h. Analyses were carried out at room temperature.

Preparation of samples

Organs were cleaned of adipose tissue and weighed. Twice the mass of a solution of 10 m*M* $NaHCO_3$, containing 2.5% acetic acid (final pH 3) was added. The mixture was homogenized, using an Ystral D7801 tissue homogenizer. The crude homogenate was centrifuged at 20 000 *g* in a Sorvall RC-50 centrifuge for 30 min. For the analysis of milrinone in purified tissue or plasma, 0.1 ml of the supernatant or plasma was mixed with 0.375 ml of water. Then 0.025 ml of a solution of 50% acetic acid was added and the mixture was vortexed. After centrifugation in an Eppendorf centrifuge (7000 *g*, 5 min), the supernatant was transferred into a Centricon-30 microconcentrator. Centrifugation at 5500 *g* for 35 min in a Sorvall RC-50 refrigerated superspeed centrifuge yielded a clear and protein-free sample, suitable for HPLC analysis. Corrections for blood present in the investigated organs were made by estimating the haemoglobin content immediately after organ excision using a colorimetric method.

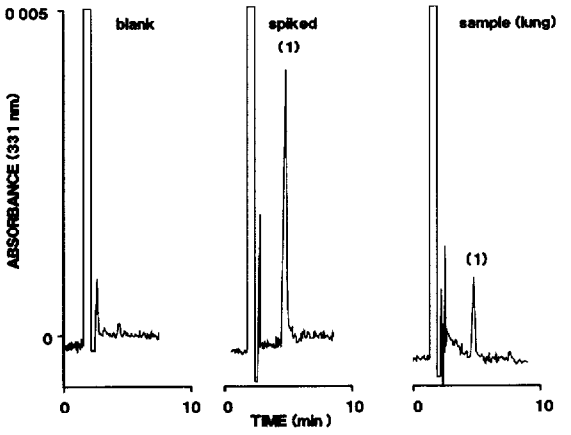

Fig. 2. Chromatograms of (left) a blank control sample, (centre) a sample spiked with 30 ng/ml milrinone and (right) a sample of lung tissue. Peak 1 = milrinone.

Calibration

Milrinone standard solutions were made by dissolving several different known amounts of milrinone, ranging from 5 ng/ml to 15 μg/ml, in samples from untreated rats. Samples containing more than 15 μg/ml milrinone were diluted ten-fold with mobile phase prior to injection.

RESULTS AND DISCUSSION

Calibration curves were linear ($r=0.998$) and reproducible over the range from 5 ng/ml to 15 μg/ml milrinone. The detection limit was 500 pg milrinone (at a signal-to-noise ratio of 3), corresponding to 5 ng/ml of plasma. The capacity factor of milrinone was 2.3. The recovery of milrinone (spiked sample, 100 ng/ml) was $99.2 \pm 0.7\%$ ($n=6$). Intra-assay and inter-assay variations were 3.1% ($n=6$) and 5.3% ($n=6$), respectively.

Fig. 2 shows three characteristic chromatograms from a control, a sample spiked with 30 ng/ml milrinone and a lung sample. HPLC analysis of a purified sample is accomplished within 10 min.

We conclude that this simple and rapid method of analysis of milrinone is a useful tool in distribution and pharmacokinetic experiments with milrinone.

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