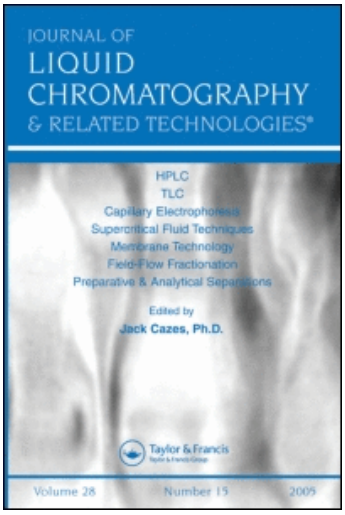

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Solid Phase Extraction and High Performance Liquid Chromatography for the Analysis of Milrinone in Rat Plasma

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SOLID PHASE EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE ANALYSIS OF MILRINONE IN RAT PLASMA

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ABSTRACT

A high performance liquid chromatographic method is presented for the determination of milrinone in rat plasma. The technique requires only 100 μl of sample volume, and is sensitive, rapid and reproducible. It has been applied to the measurement of milrinone in plasma of rats dosed orally or intravenously with milrinone. The oral bioavailability for milrinone was estimated at about 0.64 which is much lower than that reported in human.

INTRODUCTION

Milrinone [1,6-dihydro-2-methyl-6-oxo-(3,4'-bipyridine)-5 carbonitrile, Sterling-Winthrop Research Institute, Reusselaer, NY, USA] is a new non-glycoside inotropic agent presently under clinical investigation. The first published method for the determination of milrinone in plasma and urine requires a time-consuming ethyl-acetate extraction prior to the chromatographic

separative step (1). Also, since this technique was developed for human and dog plasma, the sample volume used was 1 ml, which can be a limiting factor for milrinone determination in plasma of small laboratory animals.

The present paper describes a procedure for the measurement of milrinone concentration in rat plasma samples (100 μl) based on the solid-phase extraction media used in SPE™ columns, extraction that is followed by a high-performance liquid chromatography (HPLC) and a UV detection. The technique is sensitive, rapid and reproducible. It has been applied to the measurement of milrinone in plasma of rats dosed orally or intravenously with milrinone.

During the development of our technique, a solid-phase procedure for milrinone extraction in human plasma has been published (2). Our technique differs from it in several aspects, mainly the sample volume required that is 10 to 20 times greater than ours.

MATERIALS

Milrinone and the internal standard [1,6-dihydro-2-ethyl-6-oxo-(3,4'-bipyridine)-5-carbonitrile, WIN 47,306] were generously supplied by Sterling-Winthrop Research Institute, Russelaer, NY, USA. Acetonitrile, tetrahydrofuran and methanol were of HPLC grade and the other reagents were of analytical grade. Water was double distilled. Disposable 1 ml SPE™ columns packed with reversed-phase octadecylsilane (C_{18}) bonded silica gel (Baker) were used on a Baker-10SPE™ system. The columns were conditioned by passage of 2 volumes of methanol followed by 2 volumes of double distilled water prior to use.

METHODS

Standard Preparation

Standard solutions of milrinone (1 mg/ml) and internal standard (1 mg/ml) were prepared in 0.01N HCl. Aliquots of 0.2 ml of these standards were kept in a freezer until the assay, at

which time 1.8 ml of 0.01N HCl were added to one of each milrinone and internal standard containing tubes. From these standard solutions, calibration standards (STD) of 40, 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 μg/ml of milrinone and of 2.5 μg/ml of internal standard were prepared in 0.01N HCl. Calibration standard samples for extraction were prepared in duplicate by mixing 0.1 ml of STD (10, 5, 2.5, 0.625 and 0.3125), 0.1 ml of internal standard (2.5 μg/ml) and 0.1 ml of drug-free rat plasma. Therefore, the actual amounts of drug to be applied to the C_{18} SPE™ columns were 1000, 500, 250, 62.5 and 31.25 ng. Control samples at 0.5, 1, 4 and 8 μg/ml were prepared in the same manner. A blank sample (0 ηg) was made by mixing 0.1 ml 0.01N HCl, 0.1 ml drug-free rat plasma and 0.1 ml internal standard (2.5 μg/ml).

Plasma Sample Preparation and Extraction

Arterial blood was collected in glass tubes containing potassium oxalate as anticoagulant and centrifuged at 2800 RPM for 15 min at 4°C. Plasma was kept in a freezer at -20°C until the assay. Volumes of 25, 50, 100 or 200 μl of plasma were used for extraction depending on the expected milrinone concentration. To this volume of plasma, 0.1 ml of internal standard (2.5 μg/ml) and 0.01N HCl were added to obtain a final volume of 0.3 ml. All samples were prepared in duplicate.

After the C_{18} SPE™ columns had been conditioned, the total volumes (0.3 ml) of calibration standards and plasma samples were applied to the columns and aspirated through using gentle suction. The columns were washed twice with 1 ml of double distilled water and allowed to dry for 2 min. Samples were then eluted with 1 ml of methanol. The extracts were dried at 55°C under gentle nitrogen stream and reconstituted in 0.2 ml 0.01N HCl. The samples were then filtered through 0.22 μm GS Millipore membranes.

Chromatography

The HPLC system consisted of a pump (Beckman, 112), a Supelcosil LC-18-DB (250 mm x 4 mm ID, 5 μm particle size, Supelco)

TABLE 3.

Mean Rat Plasma Milrinone Levels After a Single Oral or Intravenous Milrinone Dose (10 mg/Kg)

Time (h)	Mean Milrinone Concentration ± SEM (μg/ml) Intravenous	n	Oral	n
.0167	23.20 ± 2.82	5	0.63 ± 0.09	5
.0333	19.40 ± 2.07	5	-	
.0833	15.74 ± 1.81	4	2.33 ± 0.48	6
.1667	15.37 ± 1.26	5	5.29 ± 0.68	6
0.25	12.36 ± 1.28	5	4.16 ± 0.67	8
0.3333	17.11 ± 3.84	3	-	
0.5	12.27 ± 2.16	3	4.73 ± 0.84	9
0.75	10.87 ± 0.29	5	4.72 ± 1.01	7
1	8.73 ± 0.77	5	5.00 ± 0.91	6
1.5	-		3.60 ± 0.73	7
2	5.60 ± 1.80	4	2.55 ± 0.54	7
3	2.74 ± 1.59	4	3.25 ± 0.68	8
4	1.01 ± 0.25	5	0.87 ± 0.18	8
6	0.62 ± 0.10	4	2.05 ± 0.79	7
8	0.33 ± 0.14	5	0.46 ± 0.13	11
12	0.16 ± 0.03	3	0.22 ± 0.08	7
24	0.19 ± 0.04	2	ND	3

ND = not detectable

10 mg/kg milrinone; these levels correspond to major hemodynamic effects of the drug.

Although the oral bioavailability for milrinone that we have calculated (0.64) can only be a rough estimate, it seems that it is much lower than the one found by Stroshane et al. in human (4) (bioavailability is about 0.92).

performed by plotting peak height ratios (milrinone : internal standard) of the standards on the ordinate vs the concentration (μg/ml) of the calibration standards on the abcissa. The concentrations of milrinone in the samples were determined from the calibration curve.

The percentage of recovery of the extraction procedure for the internal standard and for milrinone were determined by comparing the peak heights of internal standard and of milrinone obtained from extracted samples with those obtained after injection of unextracted solutions.

Since only one to four blood samples were obtained from each rat, it is only possible to approximate the oral bioavailability. This was done by comparing the areas under the mean concentration-time curves (AUC) from 0 to 24h of oral and intravenous dosing. The trapezoid rule was used to determine AUC (3).

RESULTS

Typical chromatograms for plasma extracts are shown in Fig. 1. The retention times for milrinone and the internal standard are 3.8 and 4.8 min, respectively. The presence of a small peak that appears between milrinone and the internal standard does not interfere with peak heights calculation. This peak is also present in unextracted samples (containing no plasma; chromatogram not shown) and is therefore not due to the extraction procedure or to an interference from plasma constituents. The percentages of recovery for milrinone and for the internal standard after extraction ranged from 92.8 to 99.4 (mean ± SEM, 96.5 ± 1.25, n=5) and from 97.6 to 104.3 (mean ± SEM, 101.1 ± 1.2, n=5), respectively.

Linear regression analysis of the data of standard curves on five consecutive assays (done on different days) (Table 1) shows correlation coefficient of .9989 or greater. The CV of 4.7% represents the variability in the slope of standard curves made from extracted standard samples. Accuracy and precision of the assay were tested by using rat plasma spiked with 0.5 to 8 μg/ml

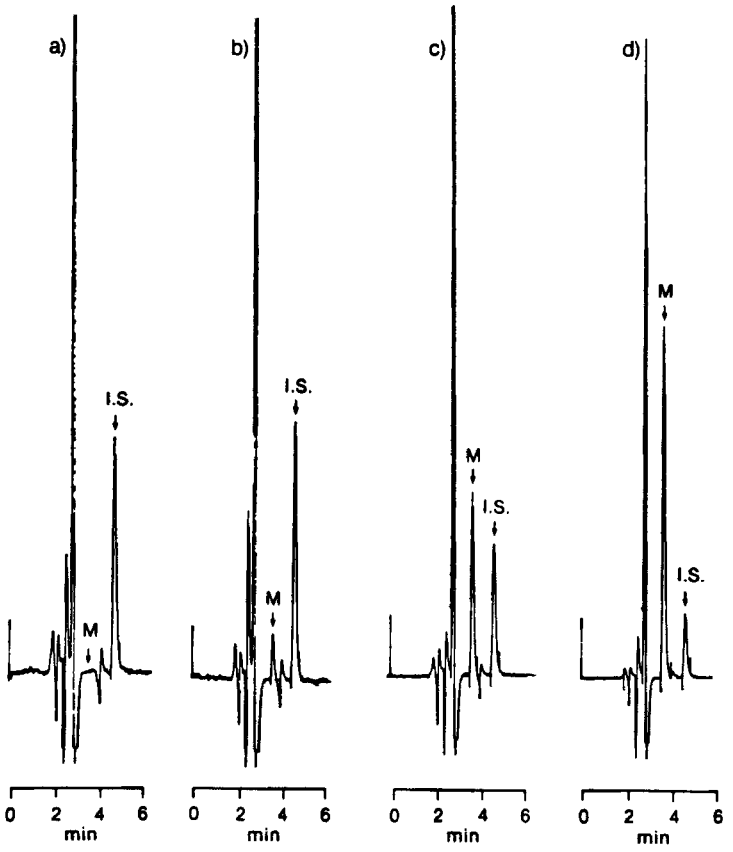

Figure 1. Typical chromatograms of extracted blank rat plasma (a), extracted rat plasma spiked with .3125 μg/ml (b), 2.5 μg/ml (c) or 10 μg/ml of milrinone (d). The gain attenuation factor of the recorder has been adjusted according to peak heights. The arrows indicate milrinone peaks (M) at 3.8 min and the internal standard peaks (I.S.) at 4.8 min.

TABLE 1.

Linearity and Between-Assay Precision of the Calibration Curves

Curve	Slope	Y Intercept	Correlation Coefficient
1	.532	.004	.9998
2	.513	.04	.9995
3	.569	0	.9998
4	.554	.014	.9999
5	.559	-.008	.9989
$\bar{x}$	.5454	.01	
SD	.0226	.0185	
CV(%)	4.15%		

of milrinone. The data presented in Table 2 show that the obtained concentrations varied from 96.6 (0.5 μg/ml) to 99.2% (1.0 μg/ml) compared to the theoretical concentrations, and that the coefficients of variation between-assays were from 4.70% to 6.34%. The method can detect milrinone at levels down to .3125 μg/ml with a CV of 8.7%. The sensitivity can be increased by increasing the sample volume.

Concentrations of milrinone in rat plasma after oral and intravenous dosing of a single dose of 10 mg/kg of milrinone are given in Table 3. After oral dosing a mean peak concentration of milrinone (5.2 μg/ml) was observed at 10 min following administration and the level of milrinone in plasma after 24h was nearly undetectable in the oral dosing group but was still 0.19 μg/ml in the intravenous dosing group.

The areas under the concentration vs time curves were approximately 20.46 (μg/ml).h and 32.01 (μg/ml).h for the oral and

TABLE 2.

Accuracy and Precision of the Assay

Added Concentration (μg/ml)	Obtained Concentration (μg/ml) (mean ± SD)	Between-Assay Precision (CV%)	# of Analysis
0.5	0.517 ± 0.026	5.03	5
1.0	1.008 ± 0.064	6.34	6
4.0	3.867 ± 0.222	5.73	6
8.0	7.861 ± 0.370	4.70	6

the intravenous dosing groups respectively (same dose, 10 mg/kg). Although different rats were used for the oral and the intravenous experiments, an estimate of the bioavailability can be obtained from these 2 independent studies by calculating the ratio of oral-parenteral AUCs, that is 0.64.

DISCUSSION

A solid-phase extraction procedure, using C_{18} bonded silica gel columns, has been applied successfully to the initial step of the extraction of milrinone in rat plasma. The recovery, precision and accuracy of the assay are comparable to those obtained with the conventional solvent extraction technique (1) or to those of a solid-phase extraction procedure requiring 1 or 2 ml of plasma (2).

Our technique can be used for rat plasma to estimate kinetics parameters. Our first experiment shows that peak plasma milrinone level occurs approximately 10 min after an oral dose of 10 mg/kg, and that significant amount remains in the blood for 12h. Very high levels are obtained in the blood after an intravenous dose of

To our knowledge, there is only one literature report concerning milrinone levels in rat blood (5). The peak level of radioactivity measured in the whole-blood after a single oral dose of 5 mg/kg corresponded to 3 μg/ml of milrinone and was observed 45 min after dosing. Since there are absolutely no details concerning the method that was used or the actual data, it is very difficult to compare our data to those mentioned by Baker et al. (5).

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REFERENCES

1. Edelson J., Koss R.F., Baker J.F. and G.B. Park High-performance liquid chromatographic analysis of milrinone in plasma and urine. J. Chromatogr., 276, 456, 1983.

2. Oddie C.J., Jackman G.P. and A. Bobik. Analysis of milrinone in plasma using solid-phase extraction and high performance liquid chromatography. J. Chromatogr., 374, 209, 1986.

3. Gibaldi M. and D. Perrier (Ed) Pharmacokinetics, 2nd edition, Marcel Dekker, New York 1982, p 445.

4. Stroshane R.M., Koss R.F., Biddlecome C.E., Luczkowec C. and J. Edelson. Oral and intravenous pharmacokinetics of milrinone in human volunteers. J. Pharm. Sci. 73, 1438, 1984.

5. Baker J.F., J. Edelson, In Milrinone Investigation of New Inotropic Therapy for Congestive Heart Failure. Braunwald E., Sonnenblick E.H., Chakrin L.W. and R.F. Schwarz (editors). Raven Press, New York, 1984, p 49.