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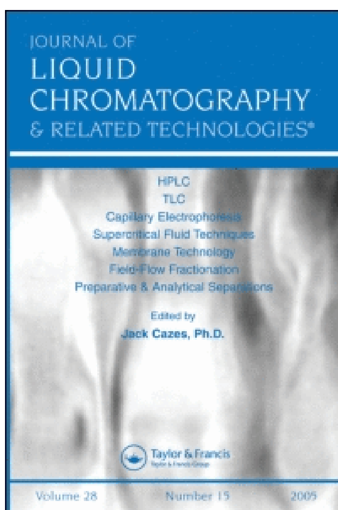

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Determination of Amrinone in Human Plasma by High-Performance Liquid Chromatography with Ultraviolet Detection

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DETERMINATION OF AMRINONE IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

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ABSTRACT

A specific, rapid and sensitive high pressure liquid chromatographic method is described for the determination of amrinone in human plasma. The method involves the use of a commercially available 4 μm particle size reverse phase Waters Nova pak Cyano Column, guard pak Cyano cartridges and a UV/VIS detector. The extraction was based on liquid - liquid single step method. Extraction is carried out with ethyl acetate on a 100 μl plasma sample. The residue is reconstituted with 100 μl of the solution 50:50 (Acetonitrile and 3.2 pH phosphate buffer) and 20 μl of the final solution is injected into the column. Elution is carried out at ambient temperature using acetonitrile and 3.2 pH phosphate buffer (70:30 by volume) as mobile phase at a flow rate of 2.0 ml/min at 1200 PSI. Detection is at 210 nm. Mean retention time (± SEM) was 4.18 ± 0.26 minutes. Separation requires 5 minutes and the sensitivity limit is 0.25 μg/ml - 10 μg/ml. This technique was used in 19 newborn and young infants receiving amrinone. Their plasma concentrations of amrinone were 1.48 ± 2.13 mg/L during a constant infusion of amrinone 5 to 10 micrograms/kg/minute indicating applicability of this technique in therapeutic drug monitoring.

INTRODUCTION

Amrinone is a synthetic bipyridine derivative (5-amino-3,4'-bipyridin-6-(IH)-one) with potent cardiac inotropic and pulmonary/systemic vasodilatory effects. This prototype of a new class of nonglycoside, noncatecholamine cardiotonic agents is currently used in the treatment of heart failure.

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Patients with congestive heart failure (CHF) have a high incidence of sudden death that may be due to either an acute exacerbation of pump function or arrhythmias (1,2). Maskin et al (3) reported that in patients with severe heart failure, the prevalence of complex ventricular arrhythmias is 92% and that of ventricular tachycardia is 71%. Drug aggravation of arrhythmias has occurred with many cardioactive drugs (4). Amrinone is also metabolized by the liver via acetylation and glucuronidation (5). Both amrinone and it's major metabolite (the physiologically inactive n-acetyl derivative) are excreted by the kidneys (5). Amrinone and its metabolites may, because of delayed acetylation or renal elimination, accumulate in some patients. This problem is of great relevance in the newborn and young infant wherein drug elimination and metabolism are also deficient (9). Amrinone is used for cardiovascular support in pediatric patients, thus therapeutic drug monitoring of amrinone concentrations would be beneficial.

A direct relationship between the plasma concentration of amrinone and its pharmacodynamic effect has been suggested (6), thus measurement of plasma amrinone may be useful in the clinical setting. One of the reported methods for detection of amrinone by HPLC was based on a more time consuming extraction procedure (7). Another method does not report the recovery (8). We, therefore, developed a microassay using HPLC and ultraviolet-detection method.

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MATERIALS AND METHODS

Patients and Adult Volunteers

Plasma samples (0.2 ml/sample) were obtained from 19 babies ages 3 days to 4 years. All babies received amrinone for cardiovascular support with a loading dose of 2 mg/kg for 10 minutes intravenously followed by a constant intravenous infusion of 5 to 10 microgram/kg/min. Venous blood samples were collected in a heparanized tube during this continuous intravenous infusion for amrinone assay. In addition, drug free venous blood (1-2 ml) was collected from healthy human volunteers (n=5) receiving no medications. Blood was collected into plastic tubes containing lithium heparin and centrifuged for 10 min. at 3000 rpm. Plasma was separated and stored at -80°C until the time of analysis.

Sample Preparations and Extraction Procedures

Extraction was performed in a silanized tube. Ethylacetate (1000 μl) was added to patients' plasma samples or spiked plasma standards (100 μl) containing amrinone in a silanized tube. The mixture was vortexed for 30 seconds. All samples were centrifuged at 2000 rpm for 5 minutes. The organic phase was transferred into a glass tube and the solvent was evaporated under nitrogen at 30°C for about 10 minutes. The residue was reconstituted in 100 μl of the (50:50 V/V) Acetonitrile and phosphate buffer at pH 3.2 and 20 μl was injected into a column.

ASSAY OF AMRINONE

Reagents and Solutions

All chemicals used were of analytical grade unless otherwise stated. Sodium dihydrogen orthophosphate, sodium phosphate monobasic, orthophosphoric acid, sodium hydroxide, ammonium hydroxide (analytical grade), ethyl acetate and acetonitrile (HPLC grade) were obtained from BDH (Pooled, U.K.). Sigmacote (silanising reagent) and amrinone were purchased from Sigma Chemical Company (U.S.A.). Only HPLC-grade de-ionized water was used. All glassware used during the sample preparation was silanized with Sigmacote, rinsed with toluene-methanol and several times with methanol and de-ionized water.

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Apparatus

The instruments included a PH-M-82 Standard pH meter, IEC Centra-8R Centrifuge, and Concentrator - Jouan RC 1010. A Waters HPLC System consisting of a 510 HPLC pump, model Waters 715 Ultra WISP autosampler, and 994 UV/VIS detector. Peak height measurements of the drug were integrated on a Waters model 820 integrator plotter. A 4 μm, 8 mm x 100 mm Waters Nova Pak reverse phase cyano column and guard-pak cyano cartridges (Waters, Milford, MA, USA) were used.

Chromatographic Conditions

For the analysis of amrinone, the mobile phase was phosphate buffer 5 mM, pH 3.2 (30%), acetonitrile (70%) (70:30 by volume). The mobile phase was filtered before use with a 0.45 μm

polyvinylidene difluoride membrane filter (Millipore) and degassed under suction. The column was operated at ambient temperature at the flow rate of 2.0 ml/minute and the column effluent was measured at the sensitivity of 1.0 or 0.05 absorbance units (AUFS). Wavelength was 210 nm and the run time was $<$ 10 minutes. The resolution of the chromatographic system was verified daily by injection of 20 μl of a solution containing amrinone. Amrinone has a UV maximum absorption with a retention time of 4.18 $\pm$ 0.26 minutes (Figures 1 and 2).

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Standard Solutions

Stock solution of amrinone (100 μg/ml) was made from powdered form after dissolution with 1 M hydrochloric acid and addition of water pH adjusted to 3-4. The stock solution of amrinone was diluted with plasma to prepare six calibration standards (0.25, 0.5, 1.0, 2.0, 5.0, and 10.0 μg/ml). All stock solutions were stored at room temperature and were protected from prolonged exposure to light.

Preparation of Validation Samples

Plasma samples (100 μl) were prepared in triplicate at each of five concentrations. One set of triplicate samples was assayed upon preparation. The other two were stored in a laboratory freezer for validation at a later date. The validation samples were analyzed under single-blind conditions after one week.

Recovery, Precision, Accuracy and Reproducibility

Extraction recovery was estimated by comparison of the peak heights of an extracted and an unextracted sample containing the same amount of the compound. Typical chromatograms of extracted and unextracted samples are shown in Figures 1 and 2. A chromatogram from a baby's blood sample is shown in Figure 3.

Five samples for each concentration ranging from 0.25 to 10 μg/ml were studied under the same chromatographic conditions in order to check the recovery, reproducibility and precision of the method. The average recovery for unextracted and extracted blood plasma samples was found to be 98 $\pm$ 2% and 88.9 $\pm$ 3%, respectively. Intra-assay precision was determined by analysis of spiked samples containing

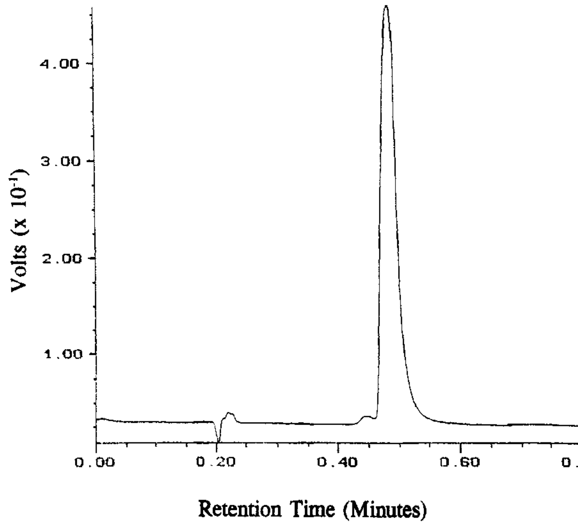

FIGURE 1: Chromatogram of amrinone from unextracted samples.

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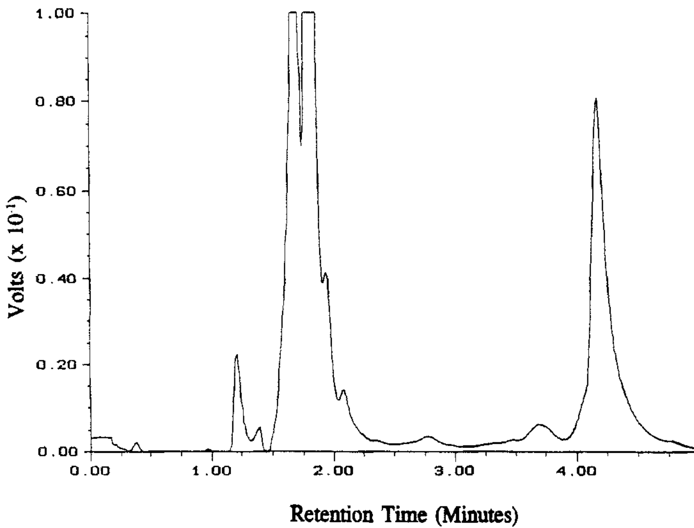

FIGURE 2: Chromatogram of amrinone from extracted plasma samples. Mean retention time (± SEM) is 4.18 ± 0.26 minutes.

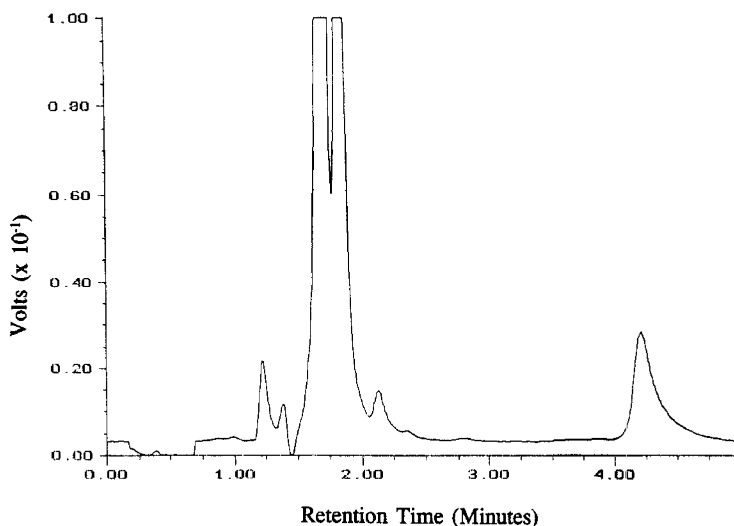

FIGURE 3: Chromatogram of amrinone from a newborn baby receiving a loading dose of 2 mg/kg via intravenous bolus followed by constant infusion of 5 μg/kg/hr.

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amrinone at five different concentrations (Table 1). Inter assay precision was determined by analysis of spiked samples on 5 consecutive days. Accuracy and reproducibility was calculated as the percentage difference between amount of drug added to drug-free plasma and amount of drug measured. The intra-assay coefficient of variation was < 5% and a day-to-day coefficient of variation was < 10% in the therapeutic range of amrinone.

Calculations

Standard curves and concentrations of amrinone were calculated from peak heights. Calibration curves were constructed after the addition of known concentrations of amrinone to plasma sample by linear regression analysis of peak height versus concentrations.

Statistical Analysis

When peak heights of plasma containing 0.25, 0.50, 1.0, 2.5, 5.0, 8.0 and 10.0 μg/ml of amrinone were plotted against concentration, the resulting line had a slope of 0.0012, a y-intercept of -

0.003, x-intercept of -0.038, a standard error of 0.009 and a correlation coefficient of 0.996. Correlation coefficient squared is 0.993. The standard error is < 0.0011 at 95%, confidence interval 0.090 to 0.096. The two tailed p value is < 0.001. The slope is very significantly different than zero.

RESULTS AND DISCUSSION

The present paper describes an HPLC method for the determination of amrinone in 100 μl of blood plasma. Liquid-liquid extraction on reversed phase has emerged as a fast and simple technique for recovery of amrinone. The present work shows that this technique may be used for the assay of amrinone. Previous methods for amrinone was based on a more time-consuming extraction procedure (9). In the method described here, the Nova pak Cyano columns were found to be most suitable as compared to the other methods, giving higher recoveries and requiring smaller volumes of elution solvent.

Using the extraction method described above, endogenous plasma components did not interfere with amrinone at plasma concentrations ranging from 0.25 μg/ml to 10 μg/ml. When the absolute peak height of amrinone were plotted against the concentration, the relationship was linear and passed through the origin. In the investigated concentration range, the regression line was linear (y = 0.999) with an intercept on the y-axis close to the origin (0.0032). Results of analysis of spiked serum for the determination of precision and accuracy of the method are given in Table 1.

Recovery of amrinone, assessed by the injection of known amounts onto the chromatographic column averaged 88.9 ± 3% (n=6) for amrinone. The inter and intra-assay precisions of the method for amrinone were < 5.0% and < 10% respectively. The precision of the method was evaluated in a blind study in the concentration range of 0.25 μg to 10.0 μg amrinone per ml. The experimentally determined concentrations were similar to the actual concentrations.

The applicability of the method for amrinone was demonstrated by the analysis of plasma samples from a patient after an intravenous dose of 2 mg amrinone per kg body weight. The concentration of amrinone in patient samples were 1.5, 1.0, 0.9, 0.75, 0.45 μg/ml after 1,2,3,4,5 minutes.

The precision and accuracy obtained with the present method make it suitable for blood plasma level measurements in patients receiving usual clinical doses of amrinone. In 19 newborn and young infants receiving amrinone with a loading dose of 2 mg/kg followed by a constant infusion of 5 to 10 micrograms/kg/minute, the mean plasma concentrations during steady state was 1.48 ± 2.13 mg/L.

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TABLE 1

Reproducibility, Precision, and Accuracy of the Method

Amount Spiked (μg/ml)	Amount found * (μg/ml)	Confidence Interval (95%)	Standard Error of Mean	Coefficient of variation (CV) (%)
0.25	0.26 ± 1.80	0.25-0.28	0.054	4.69
0.50	0.51 ± 0.79	0.48-0.54	0.102	4.48
1.00	1.02 ± 0.58	0.99-1.05	0.112	2.50
2.50	2.45 ± 2.49	2.41-2.50	0.168	1.52
5.00	4.97 ± 1.61	4.93-5.02	0.160	0.723

* Each value is expressed as Mean ± SD; (n=5)
Volume was 100 μl

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Amrinone is given together with other drugs, as was the case for the patients included in this study. In such cases, the specificity for this amrinone assay was tested by using those medications that can possibly be administered to patients who are also receiving amrinone. Fentanyl, furosemide, morphine, calcium chloride, dobutamine, dopamine, midozalam, ampicillin sodium, gentamicin, clafoxan, diazepam, phenytoin, pavulon, gentamicin, vitamin K did not interfere with the measurement of amrinone. The method described is precise, sensitive and selective for amrinone and is also rapid and simple to perform.

REFERENCES

1. Wilson JR, Schwartz JS, Sutton MSJ, Ferraro N, Horowitz LN, Reichek N, Josephson ME, Prognosis in severe heart failure: relation to hemodynamic measurements and ventrical or ectopic activity, JACC, 2:403-410 (1983)

2. Meinertz T, Hofman T, Kasper W, Treese N, Bechtold H, Stienen V, Pop T, Leitner ERV, Andersen D, Neyer J, Significance of ventricular arrhythmias in idiopathic dilated cardiomyopathy, Am J Cardiol, 53:902-907 (1984)

3. Maskin CS, Siskind SJ, LeJemtel TH, High prevalence of nonsustained ventricular tachycardia in severe congestive heart failure, Am Heart J 107:896-901 (1984)

4. Ruskin JN, McGovern B, Garan H, DiMarco JP, Kelly E, Antiarrhythmic drugs: a possible cause of out-of-hospital cardiac arrest, NEJM 309:1302-1305 (1983)

5. Ward A, Brogden R, Heil R, Speight T, Avery G, Amrinone - A preliminary review of it's pharmacological properties and therapeutic use, Drugs 26:468-502 (1983)

6. Lawless S, Buckart G, Diven W, Thompson A and Siewers R, Amrinone in neonates and infants after cardiac surgery, Critical Care Medicine 17(8):751-754 (1989)

7. Kullberg MP, Dorrbecker B, Lennon J, Rowe E and Edelson J, High-performance liquid chromatographic analysis of amrinone and it's N-acetyl derivative in plasma pharmacokinetics of amrinone in the dog, J Chromatogr 187:264-270 (1980)

8. Lawless S. Burekart G., Piccola G, Diven W, Miles M, and Zaritsky A, Simplified assay of amrinone in plasma by high-performance liquid chromatography, Therapeutic Drug Monitoring 12:570-573 (1990)

9. Aranda JV, Hales BF, Reider MF, "Developmental Pharmacology", in Neonatal-Perinatal Medicine, 5th Ed., A Fanaroff, RJ Martin, eds., Mosby Yearbook, St. Louis, MO, 1992, p. 123

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