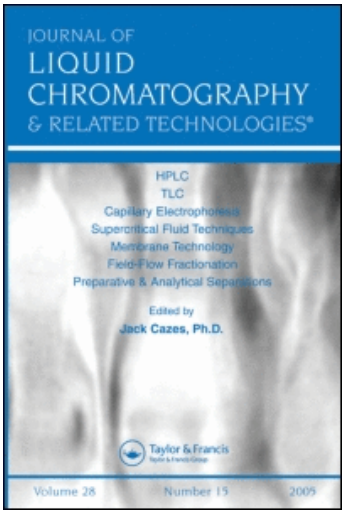

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AN IMPROVED ASSAY FOR ACADESINE (AICA-RIBOSIDE) IN HUMAN PLASMA USING LIQUID CHROMATOGRAPHY WITH AMPEROMETRIC ELECTROCHEMICAL DETECTION

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ABSTRACT

A LC method using amperometric electrochemical detection has been developed for the quantitation of the adenosine regulating agent acadesine (AICA-riboside) in human plasma. Ultrafiltrates of plasma were chromatographed under isocratic conditions on a reverse-phase (C18) LC column using a mobile phase of 1.5% methanol in phosphate buffer at pH 6.3. The column eluant was monitored with a working electrode potential of +750 mV vs Ag/AgCl. Using a 250 μL sample of plasma for analysis the method has a validated limit of quantitation (LOQ) for acadesine of 6.25 ng/mL with a run-time of 12 minutes/sample. This new method provides a 20-fold decrease in the detection limit for acadesine compared to a previous procedure which employed UV detection.

INTRODUCTION

Acadesine (AICA-riboside; 5-amino-4-imidazole carboxamide ribonucleoside, Figure 1A) is a purine nucleoside analog with anti-ischemic properties that has been tested for the prevention of adverse cardiovascular outcomes in patients undergoing coronary artery bypass graft (CABG) surgery (1). Since acadesine has been viewed as a novel site and event-specific adenosine-

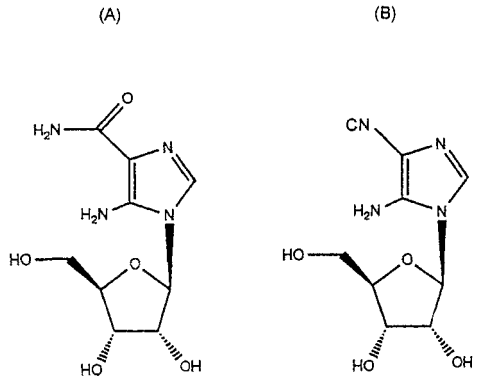

FIGURE 1. A: Structure of Acadesine (AICA-riboside)
B: Structure of compound A used as internal standard

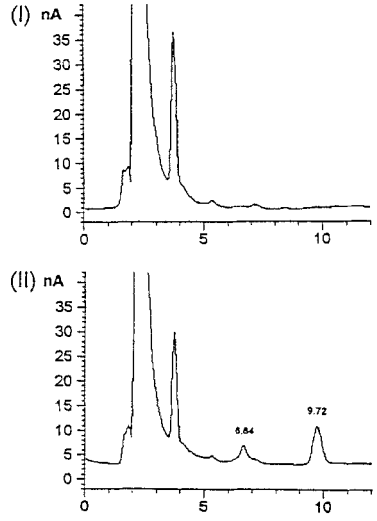

FIGURE 2. Chromatograms showing the retention time for acadesine and compound A (internal standard) in human plasma (I: Control; II: 50 ng/mL of acadesine Rt=6.64; compound A Rt=9.72)

regulating agent (ARA) that may be used for the treatment of certain cardiovascular disorders (2,3), the development of a sensitive method for the detection of acadesine in plasma is important.

A number of procedures have been described for the measurement of acadesine in biological fluids, including a high performance liquid chromatography method with spectrophotometric detection (4,5) and a colorimetric procedure (6). The limit of quantitation (LOQ) of both these assays is 125 ng/ml. In the present study we analyzed ultrafiltrates of plasma sample (4) in combination with amperometric electrochemical detection.

MATERIALS AND METHOD

All chemicals used were either HPLC or reagent grade. Methanol, water, and sodium hydroxide were obtained from Fisher (Fairlawn, NJ, USA). Phosphoric acid (85%, w/v) was purchased from Curtin Matheson Scientific (Houston, TX, USA). Acadesine (AICA-riboside) and GP115 (Figure 2) were obtained from Gensia, Inc.. (San Diego, CA).

The LC system consisted of a pump and autosampler (Hewlett-Packard HP1090, Wallbronn, Germany) and an amperometric electrochemical detector (HP1049A, Wallbronn, Germany). The working electrode potential was set at 750 mV vs Ag/AgCl (3 M KCl). Samples were chromatographed on a Beckman Ultrasphere C-18, 4.6 x 250 mm, 5 μ column (Beckman, Fullerton, CA, USA) with a Brownlee precolumn, 3.2 x 15 mm, 7 μ (Applied Biosystems, Foster City, CA, USA) and eluted with a mobile phase consisting of 0.01 N phosphoric acid and 1.5% v/v methanol adjusted to pH 6.3 with 10% w/v solution of sodium hydroxide. The mobile phase was filtered by vacuum through an HV-filter (Millipore, Bedford, MA, USA) and was continuously degassed with helium during sample injection.

The standard solutions were prepared by successive 1:1 dilutions of 1600 ng/mL of acadesine in human plasma down to 6.25 ng/mL. Low, medium and high *in vitro* quality control samples were prepared at 6.4, 128 and 1280 ng/mL. These quality controls were stored in aliquots at -20°C and used in the daily method validation (7). Since acadesine has negligible (~1%) plasma protein binding, approximately 250 μL of each plasma sample or

TABLE 1

Intra-day precision (n=4) of acadesine in human plasma.

Amount acadesine added (ng/mL)	Amount acadesine found (ng/mL)	RSD %	Relative error %
1600	1740	3.41	+8.75
800	900	3.27	+12.50
400	430	3.04	+7.50
200	211	4.11	+5.50
100	96	2.55	-4.00
50	43.3	4.77	-13.40
25	20.7	3.03	-17.20
12.5	11.50	4.76	-8.00
6.25	6.87	2.74	+9.92

TABLE 2

Inter-day precision (n=5) of acadesine in human plasma.

Amount acadesine added (ng/mL)	Amount acadesine found (ng/mL)	RSD %	Relative error %
1600	1700	4.40	+6.25
800	850	3.93	+6.25
400	421	5.21	+5.25
200	204	2.71	+2.00
100	97	4.53	-3.00
50	46.1	7.68	-7.80
25	22.5	6.24	+10.00
12.5	11.86	12.81	-5.12
6.25	6.62	6.70	+5.92

TABLE 3

Standard curve statistics (mean ± SD, n=5) for acadesine in human plasma.

Date	Slope	Y-intercept	Correlation coefficient
05/04/94	4.200	-0.001	0.9998
05/05/94	3.866	-0.006	0.9995
05/06/94	3.617	0.004	0.9995
05/07/94	4.320	-0.001	0.9999
05/08/94	3.981	-0.002	0.9999
mean	3.997	-0.001	0.9997
SD	0.277	0.003	0.0002
RSD %	6.94	-329.35	0.02

standard were filtered through an Amicon Centrifree Micropartition System (Amicon, Beverly, MA, USA) by centrifugation (Beckman, Model T-6 Centrifuge, Fullerton, CA, USA) at 2000 x g for 30 minutes at room temperature. An internal standard of compound A (Figure 1B) (25 μL of a 10 μg/mL stock solution) was added to 100 μL of ultrafiltrate. After vortexing for 5 sec, a 25 μL injection volume was injected into the LC. The data were collected and analyzed using ChemStation based software (HP, Kennet Square, PA, USA). Variance stabilized transformation regression analysis was used to fit the standard curve (8).

RESULTS

Acadesine was eluted at 6.64 min while the internal standard GP115 eluted at 9.72 min (Figure 2). The intra- (n=4) and inter-day (n=5) precision were <13% (RSD %) (Tables 1 and 2). The standard curves (n=5) were linear throughout the range tested (r>0.999) (Table 3). The relative standard deviation of the slope during the 5-day analysis was 6.94% (Table 3). The accuracy using *in vitro* quality control samples in high, medium, and low concentrations was between 94 and 105% (Table 4). The method was used to measure acadesine in human plasma with concentrations ranging from 1600 to 6.25 ng/mL.

TABLE 4

Accuracy for the determination of acadesine in quality control plasma samples (n=10).

Amount acadesine added (ng/mL)	Amount acadesine found (ng/mL)	RSD %	Accuracy %
1280	1340	4.51	104.77
128	121	4.97	94.14
6.40	6.47	14.62	101.14

CONCLUSIONS

We have developed a highly sensitive HPLC-amperometric electrochemical detection assay for the quantitation of acadesine in human plasma. The detection limit of the assay (LOQ = 6.25 ng/ml) is twenty times lower than previously reported methods (4,5,6). Another benefit is that volumes of plasma as low as 100 µl can be analyzed. In summary, the validated assay described has the advantage of being simple and exhibiting good precision, linearity, accuracy, and lower detection limit (9).

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