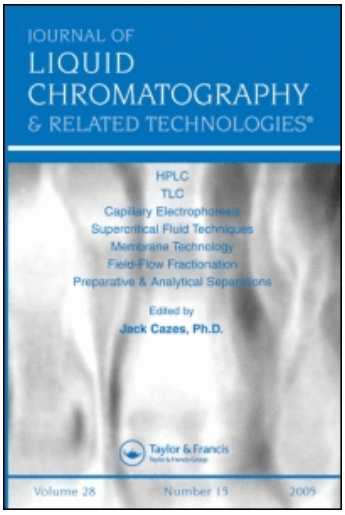

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Simultaneous Assay for Acadesine (Aica-Riboside) and Acadesine 5'-Monophosphate Using Ion-Pair Reverse Phase High Performance Liquid Chromatography

Tamie M. Sandoval[a]; James M. Fujitaki[a]; Long-Shiuh Chen[a]; Ross Dixon[a]
[a] Department of Pharmacokinetics and Drug Metabolism, Gensia, Inc., San Diego, California

To cite this Article Sandoval, Tamie M. , Fujitaki, James M. , Chen, Long-Shiuh and Dixon, Ross(1994) 'A Simultaneous Assay for Acadesine (Aica-Riboside) and Acadesine 5'-Monophosphate Using Ion-Pair Reverse Phase High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 17: 11, 2373 — 2381
To link to this Article: DOI: 10.1080/10826079408013486
URL: http://dx.doi.org/10.1080/10826079408013486

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(11), 2373-2381 (1994)

A SIMULTANEOUS ASSAY FOR ACADESINE (AICA-RIBOSIDE) AND ACADESINE 5'-MONOPHOSPHATE USING ION-PAIR REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

TAMIE M. SANDOVAL, JAMES M. FUJITAKI, LONG-SHIUH CHEN, AND ROSS DIXON
Department of Pharmacokinetics and Drug Metabolism
Gensia, Inc.
11025 Roselle Street
San Diego, California 92121

ABSTRACT

A reverse phase (C_{18}) high performance liquid chromatographic method using an ion-pair reagent has been developed for the simultaneous quantification of the cardioprotective drug, acadesine (AICA-riboside), and a major nucleotide metabolite, acadesine 5'-monophosphate (ZMP), in perchloric acid (PCA) extracts of mouse heart tissue. The limit of quantification (LOQ) was 0.5 μM for both acadesine and its 5'-monophosphate.

INTRODUCTION

The cardioprotective properties of acadesine (AICA-riboside) (Figure 1) are currently being evaluated in Phase 3 clinical trials involving patients undergoing coronary artery bypass graft surgery (CABG) (1). Acadesine is a substrate for adenosine kinase and acadesine 5'-monophosphate (ZMP) is a major cellular metabolite (2). Separate HPLC assays for acadesine and its 5'-monophosphate have been developed (3). This paper describes a

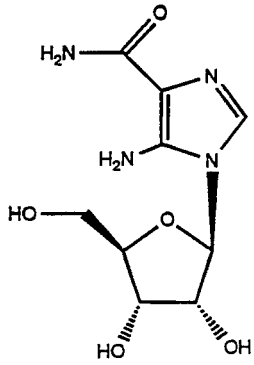

FIGURE 1. Structure of Acadesine (AICA-riboside)

HPLC assay for the simultaneous determination of both compounds in tissue extracts using ion-pair reverse phase chromatography.

MATERIALS

Tetrabutylammonium phosphate was purchased from Sigma Chemical (St. Louis, IL). Tetraethylammonium hydroxide and tetrapropylammonium hydroxide were purchased from Aldrich Chemical (Milwaukee, WI). The pH of the mobile phases was adjusted with phosphoric acid (Curtin Matheson Scientific, Houston, TX). All buffers were filtered through Millipore 0.45-μm HVAP-filters (Bedford, MA) and were degassed using a vacuum pump. Standards were prepared from acadesine (AICA-riboside) from Gensia (San Diego, CA) and reagent grade ZMP purchased from Sigma Chemical (St. Louis, IL) using Milli-Q (Millipore, Bedford, MA) deionized water. All other compounds were reagent grade.

METHODS

Thirty minutes after i.p. administration of 500 mg/kg acadesine to mice, the animals were killed, their hearts

removed, blotted dry of extraneous blood, and freeze-clamped using liquid nitrogen-cooled Wollenberger clamps. Each frozen heart was weighed and homogenized at 4° C in 3 ml ice-cold 0.6 N perchloric acid with a single 10 sec burst at setting 8 using a Brinkmann Model PT 10/35 Polytronic homogenizer. The homogenate was then centrifuged at 2000 x g for 10 min at 4° C and 1 ml of the supernatant was added to 75 μl of K_2CO_3. The neutralized sample (checked by full-range pH paper) was re-centrifuged and the supernatant passed through a 0.45 μm nylon filter (Western Analytical, Temecula, CA).

The extracts were analyzed using a Waters HPLC system consisting of a model 510 pump, satellite WISP auto injector Model 700 and an adjustable UV absorbance detector Model 484 set at 270 nm. The data were collected and analyzed using a Maxima 820 computer based software (Waters Millipore, Milford, MA). Samples were chromatographed on a Beckman Ultrasphere C-18, 4.6 x 150 mm, 5 μ column (Alltech Associates, Deerfield, IL) at a flow rate of 1.5 ml/min using an injection volume of 50 μl. The column was equilibrated for at least 30 minutes with mobile phase before sample analysis.

RESULTS & DISCUSSION

Three ion-pair reagents were tested to determine the optimal conditions for elution of acadesine and ZMP. Blank neutralized PCA extracts of mouse heart tissue and extracts spiked with both acadesine and ZMP were analyzed using each of the three ion pair buffers. The retention times of acadesine and ZMP using a mobile phase of 20 mM tetraethylammonium phosphate (pH 3.6) were 6.5 and 7.3 min, respectively. With a mobile phase consisting of 20 mM tetrabutylammonium phosphate pH 4.7, acadesine and ZMP had

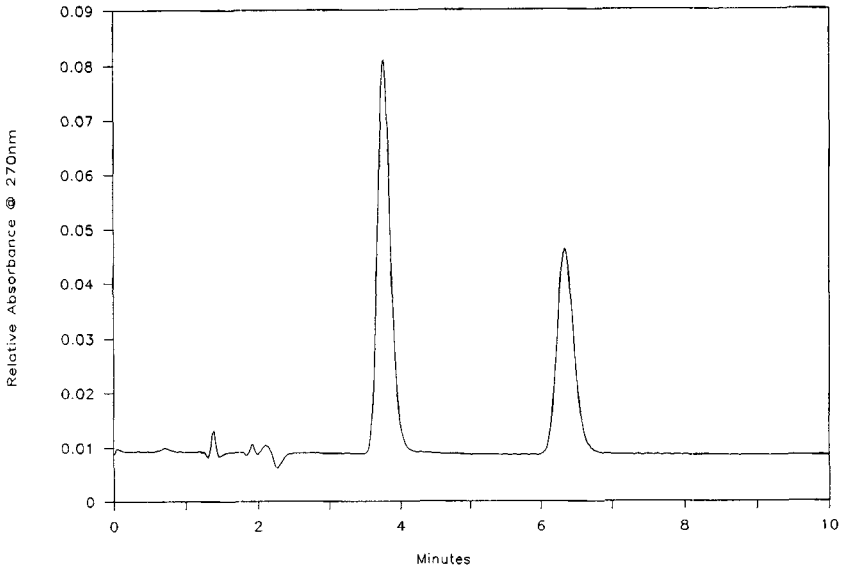

FIGURE 2. HPLC chromatogram of standard acadesine (retention time = 3.7 min) and standard ZMP (retention time = 6.3 min) using a 100 mM tetrapropylammonium phosphate pH 3.5 mobile phase

corresponding retention times of 3.4 and 26.1 min. The mobile phase selected consisted of 100 mM tetrapropylammonium phosphate pH 3.5 in which acadesine and ZMP were eluted in 3.7 and 6.3 min, respectively (Figure 2). The tetrapropylammonium phosphate buffer yielded the best results since the run time was 10 minutes with no endogenous peaks coeluting with either acadesine or ZMP. A typical chromatogram of acadesine and ZMP measured in heart tissue from a rat administered acadesine i.p. is shown in Figure 3.

Intra-assay precision (Table 1) for the analysis of ZMP from 0.5 to 100 μM had a mean %c.v.=1.71 (n=3) and the mean relative percent error was 3.1 (n=3). The inter-assay precision for ZMP

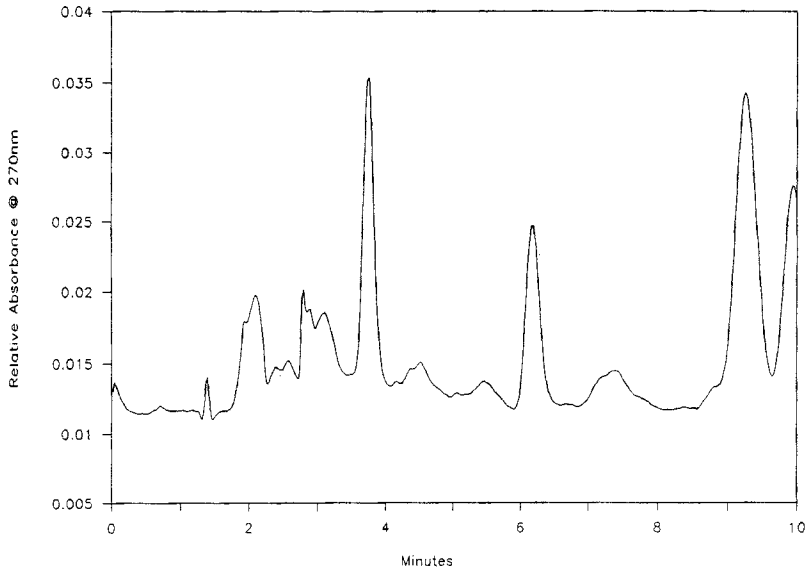

FIGURE 3. HPLC chromatogram of extract from heart of a mouse administered acadesine i.p. (acadesine retention time = 3.7 min; ZMP retention time = 6.3 min)

from 0.5 to 100 μM (Table 2) had a mean %c.v.=1.64 and the mean relative percent error was 2.7 (n=3).

Intra-assay precision (Table 1) for the analysis of acadesine from 0.5 to 100 μM had a mean %c.v.=3.27 and the mean relative percent error was 4.72 (n=3). The inter-assay precision (Table 2) had a mean %c.v.=2.47 and the mean relative percent error was 4.5 (n=3).

The limit of quantification (LOQ) for acadesine and ZMP was determined to be 0.5 μM.

The linearities of the concentration-time plots for both acadesine and ZMP were determined by variance stabilized transformation regression (4). The linearities of repeated runs of standards were found to be constant with a mean slope of 22308

TABLE 1

Intra-Assay Precision for the Analysis of Aqueous Acadesine and ZMP

Number of Runs	Amount Acadesine Added (μM)	Amount Acadesine Found (μM)	$\pm$s.d.	%c.v.	Relative Error %
3	0.5	0.51	0.04	8.55	+2.0
3	1	1.08	0.08	7.58	+8.0
3	5	5.20	0.11	2.12	+4.0
3	10	10.58	0.30	2.84	+5.8
3	25	24.12	0.03	0.13	-3.5
3	50	47.84	0.44	0.91	-4.3
3	100	94.62	0.70	0.74	-5.4
			mean = 3.27		4.72

Number of Runs	Amount ZMP Added (μM)	Amount ZMP Found (μM)	$\pm$s.d.	%c.v.	Relative Error %
3	0.5	0.49	0.01	2.34	-2.0
3	1	1.01	0.04	4.32	+1.0
3	5	5.19	0.10	1.93	+3.8
3	10	10.56	0.04	0.34	+5.6
3	25	23.97	0.22	0.90	-4.1
3	50	48.35	0.51	1.05	-3.3
3	100	98.07	1.04	1.06	-1.9
			mean =	1.71	3.1

TABLE 2

Inter-Assay Precision for the Analysis of Aqueous Acadesine and ZMP

Number of Runs	Amount Acadesine Added (μM)	Amount Acadesine Found (μM)	±s.d.	%c.v.	Relative Error %
3	0.5	0.49	0.01	2.04	-2.0
3	1	1.03	0.03	2.98	+3.0
3	5	5.30	0.11	2.09	+6.0
3	10	10.68	0.44	4.13	+6.8
3	25	24.10	0.55	2.28	-3.6
3	50	47.83	0.86	1.80	-4.3
3	100	94.43	1.85	1.96	-5.6
			mean =	2.47	4.5

Number of Runs	Amount ZMP Added (μM)	Amount ZMP Found (μM)	±s.d.	%c.v.	Relative Error %
3	0.5	0.49	0.01	1.17	-2.0
3	1	1.03	0.04	3.94	+3.0
3	5	5.13	0.07	1.35	+2.6
3	10	10.39	0.17	1.65	+3.9
3	25	24.46	0.33	1.33	-2.2
3	50	48.77	0.14	0.29	-2.5
3	100	97.17	1.73	1.78	-2.8
			mean =	1.64	2.7

TABLE 3

Standard Curve Statistics

Aqueous Acadesine

Date	Slope	Y-intercept	Correlation Coefficient
04/08/93	24448.228	3062.690	0.9999
04/08/93	24206.710	3008.784	1.0000
04/08/93	23771.813	5828.875	0.9999
04/09/93	24660.553	10901.537	0.9998
04/12/93	23831.858	513.597	1.0000
n =	5	5	5
mean =	24183.832	4663.097	0.9999
s.d. =	384.49	3962.12	0.000
C.V.% =	1.59	84.97	0.008

Aqueous ZMP

Date	Slope	Y-intercept	Correlation Coefficient
04/08/93	22275.137	309.267	0.9999
04/08/93	22118.305	-486.342	0.9999
04/08/93	21808.841	636.863	0.9999
04/09/93	23514.643	1008.210	1.0000
04/12/93	21824.880	-1124.333	1.0000
n =	5	5	5
mean =	22308.361	68.733	0.9999
s.d. =	702.78	865.27	0.000
C.V.% =	3.15	1258.88	0.005

± 703 (3.15% c.v.) for ZMP and a mean slope of 24184 ± 384 (1.59% c.v.) for acadesine (Table 3). The correlation coefficient was 0.9999 for ZMP and 0.9999 for acadesine (Table 3).

In summary, this HPLC assay for the simultaneous quantification of acadesine and ZMP eliminates the need for separate and time-consuming assays. The assay can be applied

toward measuring concentrations of acadesine and ZMP in biological samples.

ACKNOWLEDGEMENTS

The authors thank Mark Fox for preparing the mouse heart homogenates.

REFERENCE

1 J. Leung, T. Stanley, J. Mathew, P. Curling, P. Barash, M. Salmenpera, G. Reves, M. Hollenberg, and D. Mangano, J. Am. Coll. Cardiol., 19: 112A (1992)

2 R. L. Sabina, D. Patterson, and E. W. Holmes, J. Biol. Chem., 260: 6107-6114 (1985)

3 R. Dixon, J. Fujitaki, L. Chrastil, and J. Lee, Res. Comm. Chem. Pathol. Pharmacol., 65: 405-408 (1989)

4 A. M. McLean, D. A. Ruggirello, C. Banfield, M. A. Gonzalez, and M. Bialer, J. Pharm. Sci., 75: 1005-1008 (1990)

Received: December 6, 1993
Accepted: December 20, 1993