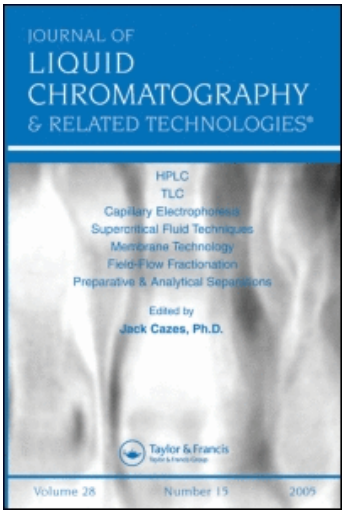

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPLC Measurement of the Novel Non Nucleoside Anti-HIV Agent, ( ) Dihydrocalanolide A

R. A. Newman[a]; W. Chen[a]; T. Madden[a]
[a] Clinical Pharmacology Resource Facility Department of Clinical Investigation, The University of Texas, M. D. Anderson Cancer Center, Houston, TX

To cite this Article Newman, R. A. , Chen, W. and Madden, T.(1997) 'HPLC Measurement of the Novel Non Nucleoside Anti-HIV Agent, ( ) Dihydrocalanolide A', Journal of Liquid Chromatography & Related Technologies, 20: 4, 651 — 659
To link to this Article: DOI: 10.1080/10826079708010951
URL: http://dx.doi.org/10.1080/10826079708010951

J. LIQ. CHROM. & REL. TECHNOL., 20(4), 651–659 (1997)

HPLC MEASUREMENT OF THE NOVEL NON-NUCLEOSIDE ANTI-HIV AGENT, (+)DIHYDROCALANOLIDE A

R. A. Newman,* W. Chen, T. Madden

The University of Texas, M. D. Anderson Cancer Center
Clinical Pharmacology Resource Facility
Department of Clinical Investigation, Box 52
1515 Holcombe Boulevard
Houston, TX 77030

ABSTRACT

An HPLC assay was established to determine levels of the promising anti-HIV agent (+)dihydrocalanolide A (DHCal-A; NSC 678323) in murine plasma. The structurally related compound costatolide (NSC 661122) was found to be a suitable internal standard. Drugs were extracted from plasma using a solid phase C_{18} cartridge. After injection onto an ODS analytical HPLC column, compounds were eluted using an acetonitrile: water mobile phase. Drugs were quantified over the assay range of 19.5 to 625 ng/mL with excellent within- and between-day reproducibility. Results are obtained from the application of the described method to determination of dihydrocalanolide A pharmacokinetics in mice. This is the first report of a validated HPLC assay for determining dihydrocalanolide A levels in biological fluids such as mouse plasma.

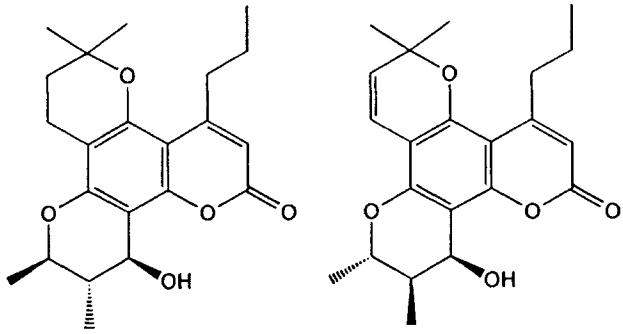

Figure 1. Structures of (+)dihydrocalanolide A (NSC 678323) and the internal standard, costatolide (NSC 661122).

INTRODUCTION

(+)Dihydrocalanolide A was produced by chemical modification of extracts of latex from the Malaysian plant *Calophyllum teysmannii var. ionphylloide* as part of a National Cancer Institute sponsored program to identify novel, natural product compounds with anti-HIV activity. While the structures of calanolide and costatolide related compounds have been known since 1964,[1] discovery of the anti-HIV activity of this class of agents is a relatively recent event.[2] In anticipation of potential clinical tests of these compounds, analytical assays were developed which would be suitable for evaluation of bioavailability as well as pharmacokinetic parameters of lead candidate compounds from this series of natural products.[3] Here we present a validated HPLC method for the determination of (+)dihydrocalanolide A in mouse plasma. Also presented is the application of this method towards determination of murine derived pharmacokinetic data.

MATERIALS AND METHODS

Reagents and Chemicals

DHCal-A as well as the internal standard, costatolide, were provided as dry powders by the National Cancer Institute (for structures, see Figure 1). Acetonitrile used in the mobile phase was of analytical grade and was obtained

from EM Science (Gibbstown, NJ, USA). Water was obtained using a Milli-Q system (Millipore, Bedford, MA, USA). Mouse plasma was purchased from Pel-Freeze (Rogers, AR, USA).

Stock solutions of DHCal-A and costatolide (1 mg/mL), were prepared in 100% acetonitrile and stored at -20°C. Under these conditions the solutions were stable for at least 1 month.

Extraction Procedure

A 25-μL volume of a stock solution of internal standard (25 μg/mL) was added to 1 mL of each plasma sample to be analyzed. After vortex mixing, the sample was loaded onto a conditioned C_{18} Sep-Pak solid-phase extraction cartridge (Millipore, Bedford, MA, USA). The cartridges were then washed with 5 mL of water and the eluate discarded. Drug was eluted with 2 mL of ethyl acetate and the eluants dried under nitrogen. Samples were reconstituted in 250 μL of 100% acetonitrile and transferred to an automatic injector vial for HPLC analyses.

Calibration Curve and Linearity

Calibration curves were prepared by spiking control mouse plasma with increasing amounts of DHCal-A and a constant amount of internal standard. Linearity in this matrix was assessed with drug concentrations ranging from 19.5 to 625 ng/mL (19.5, 39, 78, 156, 312 and 625 ng/mL). The ratios of the peak areas for DHCal-A were plotted against those of the DHCal-A concentration to check for linearity, and the correlation coefficient was calculated.

Chromatography

The isocratic liquid chromatography system (Waters Assoc., Div. of Millipore, Milford, MA, USA) consisted of a Model 510 solvent delivery pump, a Model 717 autosampler and a Model 990 photodiode array detector. A wavelength of 318 nm was used.

Chromatographic separations were performed on a 250 x 4.6 mm I.D. Spherisorb ODS analytical column, 5 μm particle size (Phenomenex, Torrence, CA, USA). DHCal-A and costatolide were eluted using a mobile phase of 80% acetonitrile in water.

No addition of buffer or adjustment of pH was necessary. The flow rate was 1.2 mL/min. The limit of detection was defined as the lowest concentration of extracted DHCal-A which resulted in a signal-to-noise ratio of 4.

Reproducibility and Accuracy

Both within- and between-day reproducibilities were determined. Two concentrations of drug (125 and 500 ng/mL) were included in these studies. For within-day reproducibility, six replicates of each sample were tested on the same day and the resulting percent relative deviation (reproducibility) and percent relative error (accuracy) determined. To measure between-day reproducibility, two concentrations of drug (125 and 500 ng/mL) were run in triplicate on each of three separate days. Percent relative deviation and percent errors were determined.

Calanolide Pharmacokinetics

Preliminary pharmacokinetics of DHCal-A were determined in male CD_2F_1 mice obtained from the National Cancer Institute (Bethesda, MD). Drug was prepared for injection by dissolving it in analytical grade dimethylsulfoxide. Mice were administered DHCal-A at a dose of 20 mg/kg by either oral gavage or intravenous routes. Samples of blood were obtained from groups of 3 mice each at 2, 5, 15, 30 min, 1 h, 2 h and 4 h after injection of drug. Plasma was obtained from the blood samples by centrifugation and then frozen at -20°C until analysis. Pharmacokinetic parameters were obtained from the data through the use of the computer program WinNonlin (Scientific Consulting, Inc., Apex, NC).

RESULTS AND DISCUSSION

This is the first report of an analytical method for determination of DHCal-A concentrations in biological matrices such as plasma. The assay has been successfully applied to determination of pharmacokinetic parameters of DHCal-A in mice following either oral or intravenous administration. Shown in Figure 2 are typical chromatograms obtained from analyses of DHCal-A in mouse plasma. Chromatograms of extracted blank plasma and of extracted plasma containing DHCal-A and costatolide as the internal standard are presented. As can be seen, both DHCal-A and costatolide are resolved from endogenous peaks. Retention times in extracts from mouse plasma were 7.3 min for costatolide and 8.5 min for DHCal-A.

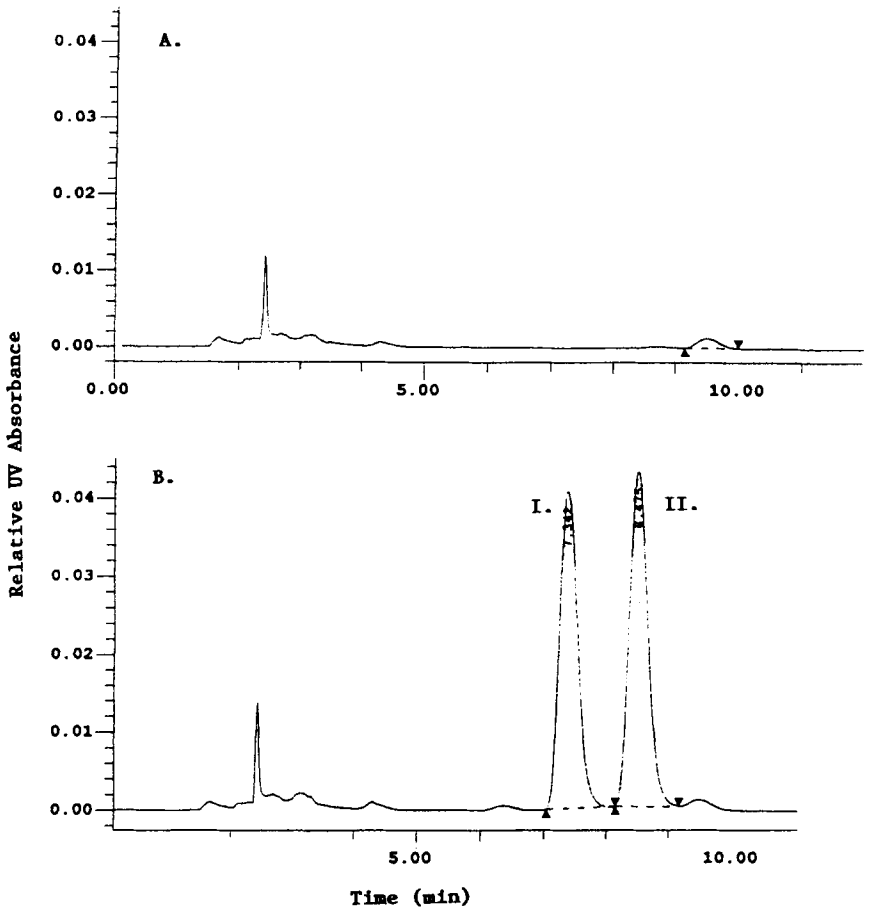

Figure 2. HPLC chromatograms of (A) blank murine plasma, (B) murine plasma containing the internal standard, costatolide (peak I: 335 ng) and (+)dihydrocalanolide A (peak II: 300 ng). Detector sensitivity, 0.001 AUFS.

Within-day and between-day reproducibilities for extracted plasma DHCal-A concentrations are shown in Tables 1 and 2, respectively. In Table 1, the relative standard deviations were less than 2%. Percent relative errors ranged from -1.2 to -1.4% in murine plasma. With between-day studies (Table 2), the relative standard deviations were low, ranging from 0.9 to 3.5. Accuracies (as percent relative errors) were also excellent, ranging from -3.5 to 2.4%.

Table 1

Within-Day Reproducibility and Accuracy: (+)Dihydrocalanolide A Assay

Nominal Conc. (ng/mL)	Calculated Conc. Mean ± S.D.* (ng/mL)	Percent Relative Deviation[a]	Percent Relative Error[b]
Mouse Plasma			
125	123.5 ± 1.1	0.9	-1.2
500	492.8 ± 8.3	1.7	-1.4

* n = 6

[a] % Relative deviation: [standard deviation (calculated mean)/calculated mean] • 100.

[b] % Relative error: [(calcualted mean - nominal value)/nominal value] • 100.

Table 2

Between-Day Reproducibility and Accuracy: (+)Dihydrocalanolide A Assay

Day	Nominal Conc. (ng/mL)	Calculated Con. Conc. Mean ± S.D.* (ng/mL)	Percent Relative Deviation[a]	Perecent Relative Error[b]
1	500	482.3 ± 9.5	2.0	-3.5
2	500	491.2 ± 7.8	1.6	-1.8
3	500	500.7 ± 4.4	0.9	0.1
1	125	128.0 ± 1.2	0.9	2.4
2	125	126.0 ± 4.4	3.5	0.8
3	125	125.9 ± 2.2	1.7	0.7

* n = 6

[a] % Relative deviation: [standard deviation (calculated mean)/calculated mean] • 100.

[b] % Relative error: [(calcualted mean - nominal value)/nominal value] • 100.

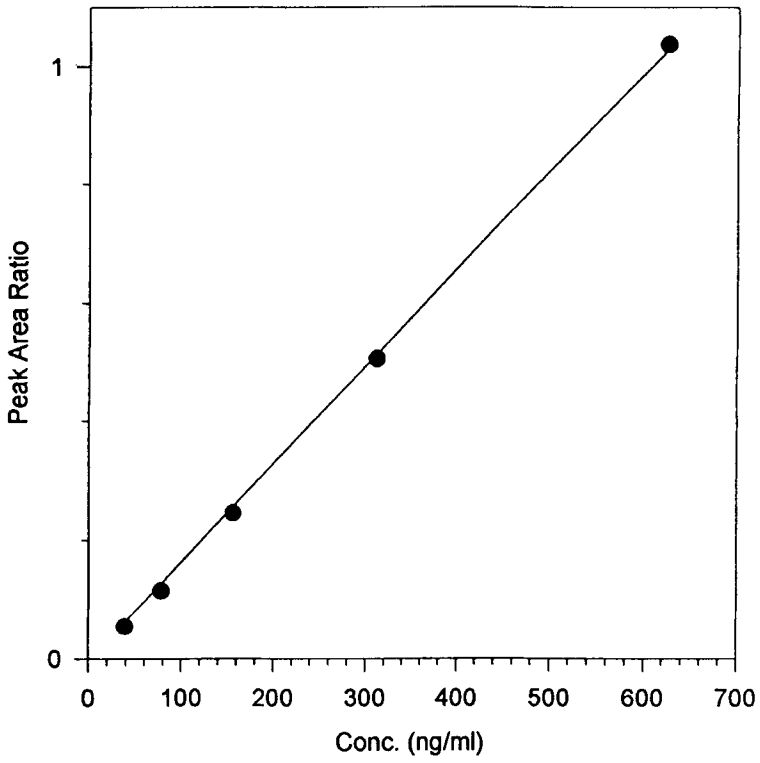

Figure 3. Standard curve (working range) for dihydrocostatolide. Data are presented as mean values of triplicate determinations per point; $r^2 = 0.999$.

Using mouse plasma as a matrix, the limit of detection of DHCal-A was 1.5 ng/mL while the useful limit of quantitation was 19.5 ng/mL. The linearity study was carried out over 3 days with concentrations ranging from 19.5 to 625 ng/mL. The coefficient of correlation between DHCal-A/costatolide peak-area ratios and DHCal-A concentrations in plasma was 0.999 (see Fig. 3). The equation resulting from the analysis of the regression plot for DHCal-A in mouse plasma was y *(conc.)* = 5.89 +546.4 x *(peak area ratio).*

The extraction recoveries (n= 5) from plasma samples spiked with DHCal-A (125 and 500 ng/mL) were 58 ± 7% and 84 ± 9% , respectively. The variances in recoveries observed for different concentrations of DHCal-A were controlled for by use of costatolide as an internal standard.

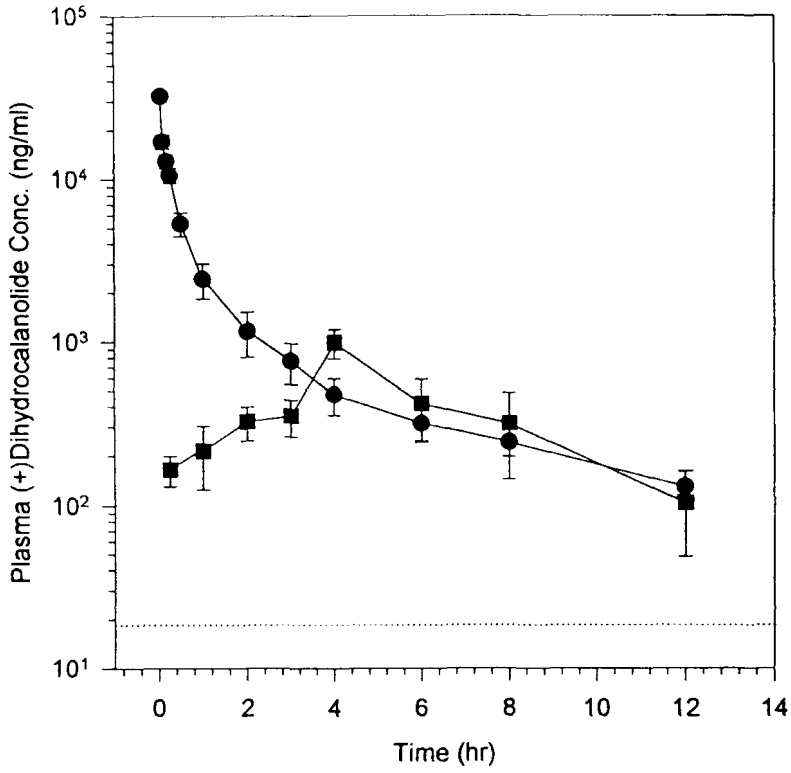

Figure 4. Plot of DC-A plasma concentrations versus time obtained with mice administered drug (20 mg/kg) by either oral (-●-) or intrav enous routes (-■-). Data are presented as the mean ± S.D. of determinations from 3 separate mice per time point. The dashed line indicates the lower limit of quantititation.

Drug concentration-time curves for DHCal-A after both i.v. and oral administration are presented in Fig. 4. Pharmacokinetic parameters of dihydrocostatolide after intravenous and oral administration of drug to mice were determined using the pharmacokinetic computer program WinNonlin. The initial estimates of plasma DHCal-A $t_{1/2}\alpha$ and $t_{1/2}\beta$ half-lives (derived from the obtained pharmacokinetic data are 0.013 hr and 2.26 hr, respectively, following i.v. injection and 1.25 hr and 6.10 hr, respectively, after oral administration. The data obtained permitted calculation of F, the fraction of drug absorbed, as a measure of oral bioavailability. This was a reasonable 36%.

ACKNOWLEDGEMENTS

This investigation was supported in part by N01-CM-57203 and CA-16672 from the National Cancer Institute.

REFERENCES

1. G. H. Stouts, K. L. J. Stevens, Org. Chem., **29**, 3604-3609 (1964).

2. Y. Kashman, K. R. Gustafson, R. W. Fuller, J. H. Cardelina, J. B. McMahon, M. J. Currens, R. W. Buckheit, S. H. Hughes, G. M. Cragg, M. R. Boyd, J. Med. Chem., **35**, 2735-2743 (1992).

3. R. A. Newman, W. Chen, T. Madden, Proc. Amer. Assoc. Cancer Res., **37**, 2787 (1996).

Received June 3, 1996
Accepted June 18, 1996
Manuscript 4204