CAPSULES notes and applications from BASi
December 2001
365

LC Coupled with Multi-Channel EC Detection for the Determination of Daidzin in Rat Blood Sampled by an Automated Blood Sampling System

Purpose

Determination of daidzin (**F1**) in rat blood by LC with multi-channel EC detector and evaluation of pharmacokinetics of daidzin with a Culex® automated blood sampling system.

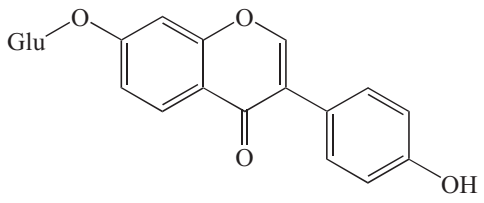

Figure 1. The structure of Daidzin (DIN).

Daidzin, a glucosylated isoflavone, is abundant in soybeans as well as in the root of *puerariae lobata*, a traditional Chinese medicinal plant. As one of the major isoflavone compounds, daidzin is reputed to have antioxidant and anticarcinogenic effects.

Existing Methods

LC with EC detection has been used to detect daidzin and related isoflavones *(1-3)*. In most studies, this glycosylated isoflavone is subjected to enzymatic hydrolysis and measured as daidzein *(4-5)*. Reports on the determination of intact daidzin in biological fluids are few.

Conditions

LCEC System: BAS PM-502e chromatograph with a multi-channel amperometric detector (epsilon™, BAS) and ChromGraph version 2.00 software
Electrode: 4, 2 mm glassy carbon working electrodes
Potential: +1100, 950, 850 and 750 mV vs. Ag/AgCl
Column: guard column: C18, 3 µm, 14 x 1.0 mm, BAS MF-8946; microbore analytical Column: C18, 3 µm, 100 x 1.0 mm, BAS MF-8949
Mobile phase: 20 mM sodium acetate, 0.25 mM EDTA, pH 4.3, 4 % methanol and 11 % acetonitrile (v/v)
Flow rate: 90 µl/min
Blood collecting system: freely moving rat containment device (Raturn®, BAS), automated blood sampler (Culex®, BAS) and refrigerated fraction collector (HoneyComb™, BAS).

Sample Preparation

A total of 150 µL of blood solution, which contained 75 µL of rat blood and 75 µL of saline containing heparin, comprised each sample. The solution was centrifuged at 2000 g for 10 min. Then two aliquots of plasma, 50 µL each, were transferred to 1.7 mL Eppendorf tubes, one for immediate analysis, the other for storage at - 20 °C as a backup. Ethyl acetate (0.8 mL) was added to the plasma, vortex-mixed for two min, and centrifuged at 5,600 g for six min. Following centrifugation, 700 µL of the clear supernatant was transferred to another centrifuge tube, dried under nitrogen and reconstituted with 20 µL of mobile phase. A volume of 10 µL of the solution was injected by autosampler.

Preliminary Animal Study

Male Sprague-Dawley rats weighting 280-350 g were implanted with a jugular vein catheter (CX-2010, BAS) and/or femoral vein catheter (CX-2020, BAS). After surgery, the rats were installed in the Raturn, and allowed to recover for one day with free access to food and water. The rats were dosed intravenously (i.v.) with daidzin through the femoral vein catheter. A 75 µL blood sample was withdrawn from the jugular vein into a vial containing an equal volume of heparine/saline and kept in a refrigerated fraction collector according to a preset schedule.

Two different daidzin dosing solutions were used, although both were administered at the same level of 5 mg/kg of body weight. The first was of standard daidzin in 20% DMSO/saline at a concentration of 2 mg/mL. The second solution was a botanical mixture prepared from a nutritional supplement in the following manner: 50 tablets were ground to a fine powder with a mortar and pestle, 12 g was added to 300 mL 80% methanol/water and sonicated for 30 min on ice bath.

2701 Kent Avenue West Lafayette, IN 47906 765-463-4527 fax 765-497-1102 basi@bioanalytical.com
www.bioanalytical.com

The filtered suspension was dried with a rotary evaporator and 100 mg of dried powder was dissolved in 1 mL of DMSO. Four milliliters of saline were added to give a 20 mg/mL solution in 20% DMSO/saline. Quantitation of this solution indicated that a 2 mL/kg dose of this solution delivered an equivalent amount of daidzin as the first dose.

Note

Chromatograms of daidzin standard (a), blank rat plasma (b), and plasma spiked with 50 ng/mL of daidzin (c) are shown in **F2**.

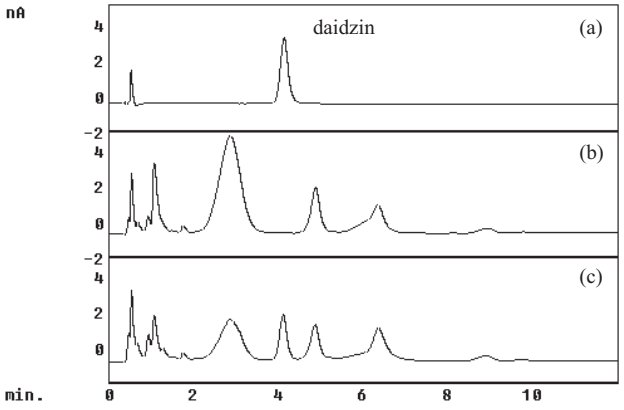

Figure 2. Chromatograms of daidzin standard (a), blank rat plasma (b), and plasma spiked with 50 ng/mL of daidzin (c). Applied potential: +850 mV vs. Ag/AgCl.

The limit of detection for daidzin in rat plasma was 5 ng/mL at a signal-to-noise ratio of 3:1. Linearity was obtained for the range of 25-1000 ng/ml. The intra- and inter-assay precision was in the range of 2.7-6.6 % and 1.9-3.7 %, respectively. This method is suitable to routine in vivo monitoring of daidzin in rat plasma.

The proposed method was used for the determination of daidzin in rat plasma *(6-7)*. **F3** illustrates data for a single intravenous dose administration of pure daidzin (filled circles) and daidzin-containing botanical mixture at an equivalent dose (open circles) to four different rats. It is interesting to see that daidzin given as a botanical mixture results in higher initial plasma concentration as compared to the pure compound. This observation is consistent with the report that crude kudzu extract potentates the bioavailability of daidzin administrated intraperitoneally to hamsters *(8)*. This impact of the complete botanical mixture on metabolism and elimination rates is not yet clear.

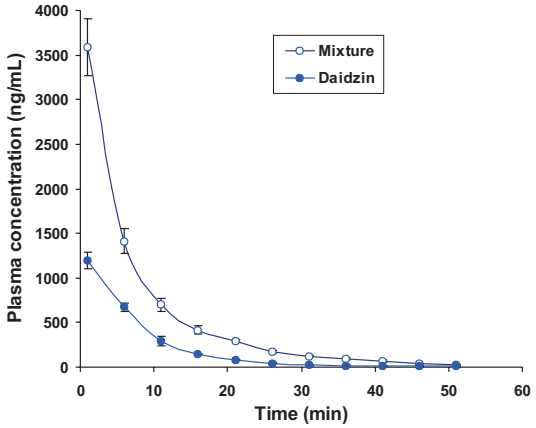

Figure 3. Mean (± S.D.) plasma daidzin concentration versus time profile in rats (n = 4) following a single intravenous administration. Filled circles: administration of pure daidzin at 5mg/kg. Open circles: administration of daidzin-containing botanical mixture, at a dose equivalent to 5mg/kg of standard compound.

References

1. *A. A. Franke, J. L. Custer, W. Wang, C. Shi, Proc. Soc. Exp. Bio. Med. 217 (1998) 263-273.*
2. *P. Gamache, E. Ryan, I. N. Acworth, J. Chromatogr. 635 (1993) 143-150.*
3. *K. D. Setchell, M. B. Welsh, C. K. Lim, J. Chromatogr. 386 (1987) 315-323.*
4. *S. Barnes, L. Coward, M. Kirk, J. Sfakianos, Proc Soc Exp Bio Med 217 (1998) 254-262.*
5. *R. A. King, D. B. Bursill, Am. J. Clin. Nutr. 67 (1998) 867-872.*
6. *Y. X. Zhu, T. H. Huang, C. Cregor, H. Long, C. B. Kissinger, P. T. Kissinger, J. Chromatogr. B, 740 (2000) 129-133.*
7. *H. Long, Y. X. Zhu, M. Cregor, F. F. Tian, L. Coury, C. B. Kissinger, P. T. Kissinger, J. Chromatogr. B, 763 (2001) 47-51.*
8. *W. M. Keung and B. L. Vallee Proc. Natl. Acad. Sci. USA 93 (1996) 14284-14288.*