CAPSULES notes and applications from BASi

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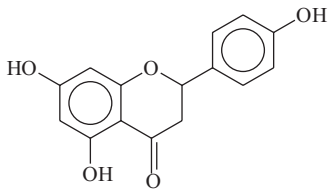
Determination of Naringenin in Rat Plasma with the Culex® Automated Blood Sampler Coupled with LCEC

Purpose

Develop an automated blood sampling LCEC method for pharmacokinetic studies of Naringenin (NG-E, ***F1***) in rat plasma.

NG-E is one of the active components in grapefruit juice that changes the pharmacokinetics of a variety of drugs. It also has the ability to activate polymorphonuclear (PMN) leucocytes to exhibit cytotoxic activity against tumor cells *in vitro*.



F1. Structures of NG-E.

Existing Methods

LCUV, LCEC, LCMS, GC/MS and capillary electrophoresis have been used to determine NG-E. Several of these methods suffer from inadequate lower limits of quantitation (LLOQ) and require tedious sample preparation steps. Our goal was a method suited to small blood volumes from rodent pharmacokinetic experiments.

Conditions

LCEC System: BASi 200B temperature control and solvent delivery system equipped with a LC-4C detector (BASi) for microbore chromatography
Electrode: glassy carbon working electrode, 3 mm (MF-1000)
Potential: +900 mV vs. Ag/AgCl
Guard Column: C18, 5 µM, 14 x 1.0 mm (MF-8945)
Analytical Column: C18, 5 µM, 150 x 1.0 mm (MF-8912)
Mobile Phase: 1 L of buffer containing 20 mM NaOAc and 27 µM EDTA (pH 6.2) plus 300 mL of CH_3CN and 50 mL of CH_3OH
Flow Rate: 100 µL/min
Injected Volume: 10 µL
Temperature: 50°C
Detection Limit: 0.5 pg of NG-E on column
Quantitation Limit: 1 ng/mL of NG-E in rat plasma at S/N of 5:1
Blood Collecting System: freely moving rat containment device (Raturn®, BASi), automated blood sampler (Culex®, BASi) and refrigerated fraction collector (HoneyComb™, BASi).

Sample Preparation

Heparinized rat blood samples were centrifuged at 2000 g for 10 min. Each 60 µL of plasma was transferred to a 1.7 mL centrifuge tube and extracted with ethyl acetate three times using 400 µL, 240 µL and 240 µL, respectively. The combined organic solvent was dried with nitrogen gas at room temperature and the residue dissolved in 60 µL mobile phase. Samples were diluted as needed to fall within the determined linear range. A 10 µL aliquot was injected into the LCEC system.

Primary Animal Study

For the automated blood sampling experiment, a male Sprague-Dawley (250g) was implanted with a jugular vein catheter (CX-2010, BASi) and a femoral vein catheter (CX-2020, BASi). After surgery, the rat was installed in the Raturn, and allowed to recover for two days with free access to food and water. The rat was dosed at 5mg/kg of NG-E through the femoral vein catheter at 2mg/mL of NG-E in 30% cyclodetrin. Successive 120 µL blood samples were collected through the jugular vein catheter into heparinized vials and kept refrigerated in a fraction collector according to a preset schedule.

Note

Chromatograms of NG-E standard (A), blank rat plasma (B) and plasma spiked with 10 ng/mL of NG-E (C) are shown in ***F2***.

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The recovery of NG-E spiked into rat plasma at a concentration of 10 ng/mL, 50 ng/mL, 100 ng/mL, and 1000 ng/mL (n=5) was determined to be 77.11%, 74.50, 74.43%, and 82.40%, respectively.

Rat plasma spiked with NG-E was linear in the range of 25-200 ng/mL. The regression equation is y=248386x-213486 with R^2=0.998.

The developed method was used for a pharmacokinetic study of NG-E in a rat. ***F3*** illustrates the results of a single intravenous dose of NG-E administered to a rat.

References

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3. *S.H. Lee, Y.B. Park, K.H. Bae, S.H. Bok, Y.K. Kwon, E.S. Lee and M.S. Choi., Ann. Nutr. Metab., 43 (1999) 173-180.*
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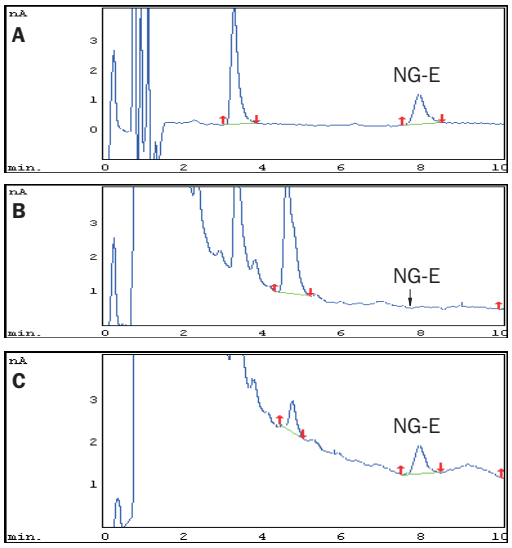
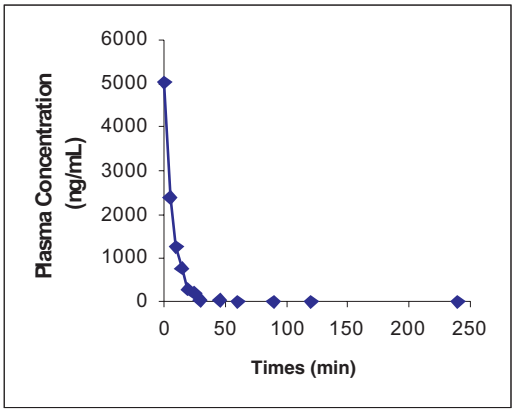


F2. Chromatograms of standard NG-E (10 ng/mL) (A), extracted blank rat plasma (B), and extracted plasma spiked with 10 ng/mL of NG-E (C).



F3. Rat plasma NG-E concentration vs. time profile in a rat following a single intravenous administration of NG-E.