CAPSULES notes and applications from BASi

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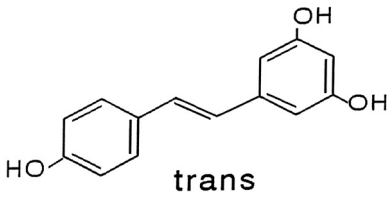
June 2000

Determination of Resveratrol in Rat Blood by Multi-Channel Electrochemical Detection with an Automated Blood Sampler

Purpose

Determination of the resveratrol (***F1***) in rat blood by multi-channel EC detector and evaluation of the pharmacokinetics of resveratrol.



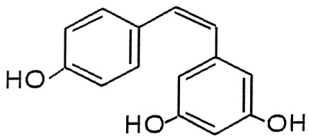


Figure 1. *The structures of trans and cis-resveratrol.*

Reseveratrol is an important phenolic antioxidant in wine that is considered to have possible value as a preventive medicine.

Existing Methods

LC with UV (1-6), most of them using gradient elution with low sensitivity.

Conditions

LCEC System: BAS 480e chromatogrph with a multi-channel amperometric detector (epsilon™, BAS)
Electrode: Four glassy carbon working electrode
Potential: +800, 700, 600, 500 mV vs. Ag/AgCl
Column: C18, 3 µm, 100 x 2.0 mm (BAS, MF-8957)
Mobile phase: 20 mM sodium acetate, 0.5 mM EDTA, pH 4.5, 21% acetonitrile (v/v)
Flow rate: 0.4 ml/min
Blood collecting system: An automated blood sampler (Culex™, BAS), including a rat containment (Raturn™, BAS) and a fraction collector (HoneyComb™, BAS)

Sample Preparation

A total of 150 µl of blood solution, which contained 75µl rat blood and 75 µl of physiological saline were transferred to a 1.7 ml centrifuge tube. A total of 150 µl of acetonitrile containing 1% of trichloroacetic acid were added, vortex-mixed, and centrifuged for 3 min at 10,000 rpm. Following centrifugation, a 75 µl aliquot of the clear supernatant was diluted with 75 µl of water and mixed. A volume of 20 µl of the solution was injected into the LC system.

Preliminary Animal Study

Sprague-Dawley rats weighting 280-330 g were used. For the automated blood sampling experiments, the rats were implanted with a jugular vein cannula (0.3 I.D. x 0.6 O.D. x 81.3 mm L, polyurethane, BAS). After surgery, the rats were installed in the Raturn™, then allowed to recover for one day with free access to food and water. The rats were dosed with resveratrol intraperitoneally (i.p.), orally or intravenously. The blood was automatically withdrawn from the jugular vein and followed by a heparin/saline flush. A total 150 µl of blood and saline (1:1) was collected by the fraction collector.

Notes

A typical chromatogram of blank rat blood (top) and rat blood spiked with 100 ng/ml resveratrol (bottom) is shown in ***F2***.

The calibration curve for reveratrol was linear over the concentration range of 5-1000 ng/ml in rat blood (**F3**). The detection limit of resveratrol in rat blood was determined at 2 ng/ml with a signal-to-noise ratio of 3. The limit of quantitation was 4 ng/ml. The precision values (% R.S.D.) at the three concentrations in the intra-assay study varied between 2.5 and 4.4% the inter-assay study varied between 1.2 and 4.3%. The accuracy (Bias %) values for all three concentrations deviated less than 6.2% from the corresponding nominal concentrations.

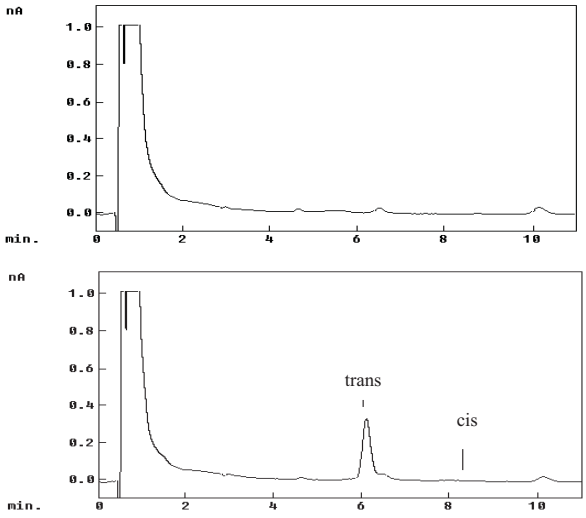


Figure 2. *Chromatogram of extract from blank rat blood (top) and blood spiked with 100 ng/ml of resveratrol (bottom). Applied potential: +700 mV vs. Ag/AgCl.*

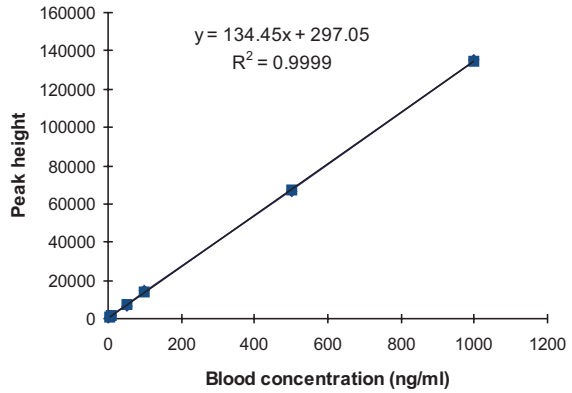


Figure 3. *Standard curve, extracted from rat blood.*

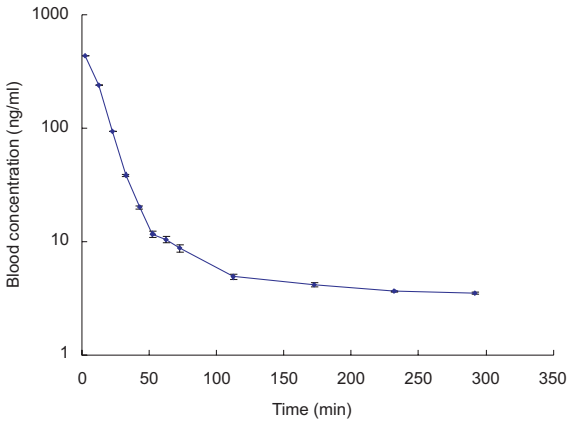


Figure 4. *Mean (± S.D.) blood concentration versus time profile of resveratrol in rats (n = 3) following a single 2 mg/kg intraperitoneal dose administration.*

Pharmacokinetic Results

F4 illustrates data for a single 2mg/kg intraperitoneal dose administration of resveratrol to rats (n = 3). The compound was rapidly absorbed. The blood concentration declined in a 2-exponential fashion. The k_{e1}, $t_{1/2}$, and AUC of resveratrol were 0.185 min^{-1}, 3.74 min, 9917 min·ng/ml respectively.

References

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3. *E. Celotti, R. Ferrarini, R. Zironi, L. S. Conte, J. Chromatogr. A 730 (1996) 47-52.*
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