

**Determination of (-)-Epigallocatechin Gallate in Rat Blood Liquid Chromatography
with Multi-Channel Electrochemical Detection and Automated Blood Sampler**

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PURPOSE

Determination of (-)-epigallocatechin gallate (EGCg) (F 1) in rat blood by a multi-channel electrochemical detector and evaluation of the pharmacokinetics of EGCg with an automated blood sampler. EGCg is an important tea catechin. The inhibitory activity of EGCg against tumorigenesis has been demonstrated in many animal models. As the part of bioanalytical core at Purdue-UAB Botanical Center, our efforts are focus on the bioavailability of tea catechins, and their absorption, biodistribution and metabolism.

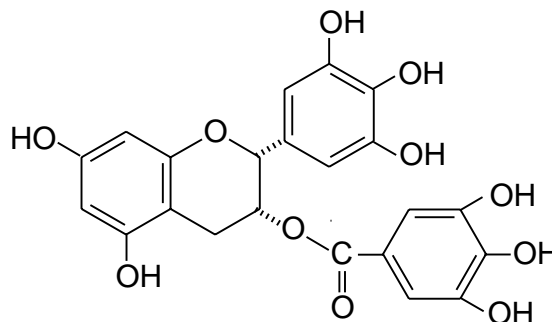


Figure 1. The structures of (-)-epigallocatechin gallate

METHODS

1. Conditions

LCEC System: BAS 490e chromatograph with a multi-channel amperometric detector (epsilon™, BAS) and ChromGraph version 2.00 software
Electrode: Four 2 mm glassy carbon working electrodes

Potential: +800, 700, 600, 500 mV vs. Ag/AgCl

Column: C18 5 mm column (Discovery 150 x 2.1 mm, Supelco)

Mobile phase: 10 mM sodium acetate, 1% acetic acid, pH 3.5, 20% methanol (v/v)

Flow rate: 0.4 ml/min

Blood collecting system: An automated blood sampler (Culex™, BAS), including a rat containment system (Raturn™, BAS) and a fraction collector (HoneyComb™, BAS)

2. Multi-channel electrochemical detection

LC with UV and chemiluminescence [1-4] have been used for the determination of EGCg in green tea and in biological samples, most of them with low selectivity and poor detection limit. Multi-channel electrochemical detection has been proved to be very useful in the identification and determination of antioxidant [5]. Four electrode detector experiments were performed. A depiction of the working electrode configuration of the four-channel electrochemical detector is shown in Figure 2. Radial and cross flow patterns were used, as both of them have an equal concentration of analyte passing over the four electrodes in the thin-layer channel.

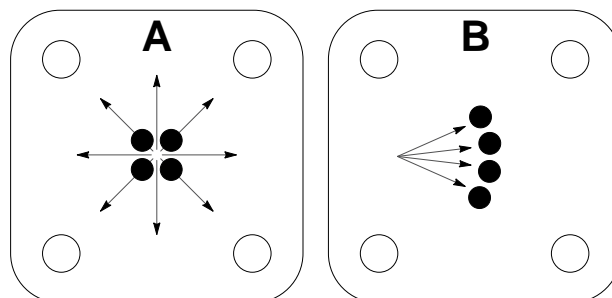


Figure 2. A depiction of the four working electrode configuration for four channel electrochemical detection, (A) radial flow, (B) cross flow

3. 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. After surgery, the rats were installed in the RaturTM, then allowed to recover for one day with free access to food and water. EGCg (1 mg) was dissolved in 0.1 ml glycerol then diluted with 0.4 ml saline. The rats were dosed intraperitoneally (i.p.) with a single dose of 2 mg/kg. The blood was automatically withdrawn from the jugular vein and followed by a heparin/saline flush using the automated blood sampler, CulexTM. A total 200 ml of blood and saline (1:1) was collected by the fraction collector.

RESULTS AND DISCUSSION

Multi-channel electrochemical detection provides a selective and sensitive approach for the determination of natural antioxidants in blood [6]. By monitoring four potentials simultaneously one can easily determine the optimum potential during method development and also verify peak purity by ratioing the response at different energies for both standards and samples. In this study, a multi-channel detector with four glassy carbon electrodes was used. Figure 3 shows a chromatogram for EGCg extracted from rat plasma.

Using this method it was possible to quantify the blood concentration following a single dose of EGCg to rats with good accuracy and precision. Thus the pharmacokinetic properties of EGCg can be examined for intraperitoneal, oral and intravenous dosing. After administration of EGCg, blood samples were periodically collected by the automated blood sampler (CulexTM).

The proposed method was used for the determination of EGCg in rat blood. Figure 4 shows chromatograms for blank plasma and a blood sample after 33 min of a single 2 mg/kg intraperitoneal administration of EGCg.

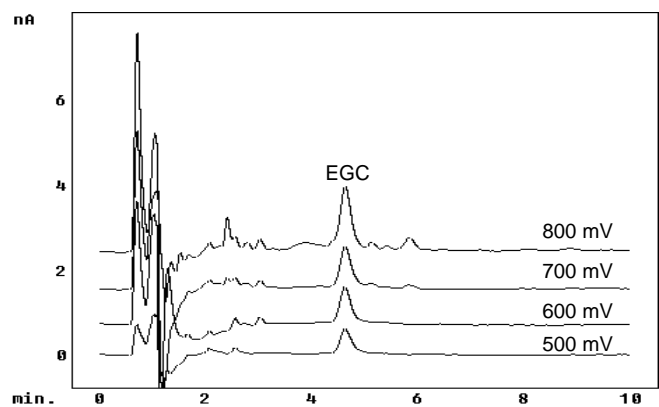


Figure 3. Four channel detection of rat plasma spiked with 100 ng/ml of EGCg

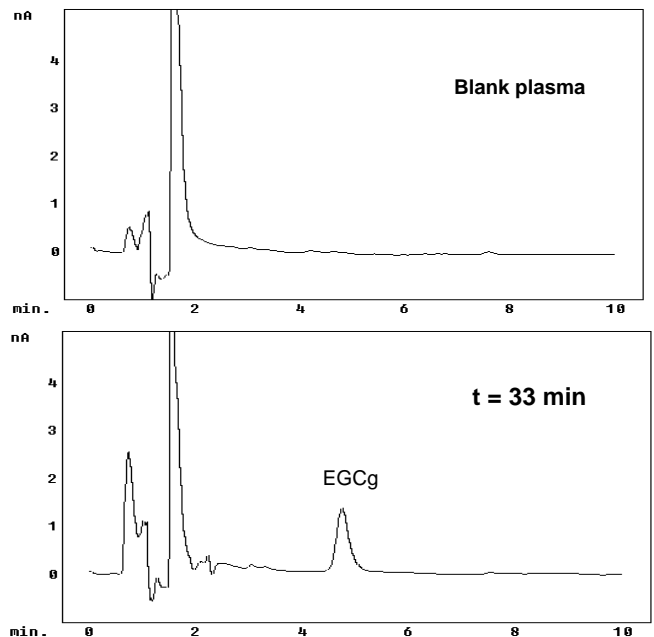


Figure 4. Chromatograms of blank plasma and blood sample (t = 33 min)

Figure 5 illustrates data for a single 2 mg/kg intraperitoneal dose of EGCg. The compound was rapidly absorbed to reach the maximum blood concentration. This method is being used to study EGCg kinetics using various routes of administration to better understand the potential role of this compound in human consumption of green tea.

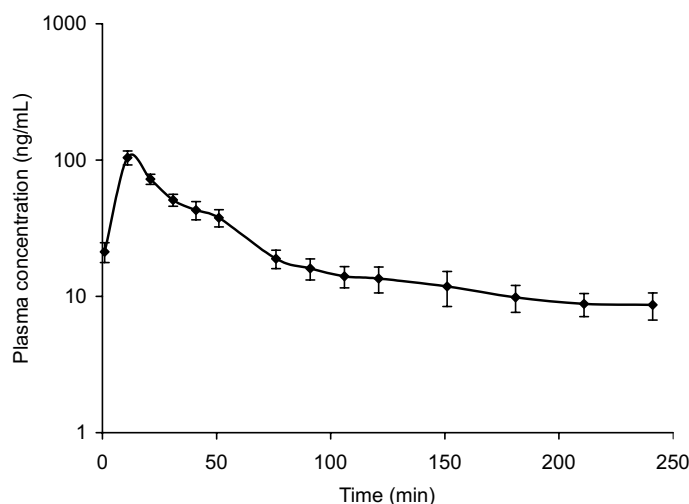


Figure 5. Time course of EGCg concentration in blood after intraperitoneal administration of 2 mg/kg of EGCg to rat (n = 3)

CONCLUSION

A LC procedure with multichannel electrochemical detector was developed for the determination of EGCg in rat blood. The automated blood sampling device and the reported method offer several advantages, such as very low animal stress, easy and accurate withdrawal of blood, a rapid and clean extraction scheme with a superior lower limit of quantitation.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support by NIH grant for Purdue-UAB Botanical Center.

REFERENCES

- [1] I. Sakata, M. Ikeuchi, I. Maruyama, T. Okuda, *Yakugaku Zasshi*, 111 (1991) 790-793.
- [2] L. Chen, M. J. Lee, H. Li, C. S. Yang, *Drug Metab. Dispos.*, 25(1997) 1045-1050.
- [3] K. Nakagawa, T. Miyazawa, *Anal. Biochem.*, 248 (1997) 41-49.
- [4] W. E. Bronner, G. R. Beecher, *J. Chromatogr. A*, 805 (1998) 137-142.
- [5] D. A. Roston, P. T. Kissinger, *Anal. Chem.*, 53 (1981) 1695-1699.
- [6] Y. Zhu, T. Huang, M. Cregor, H. Long, C. B. Kissinger, P. T. Kissinger, *J. Chromatogr. B*, 740 (2000) 129-133.