Pharmacokinetics of (−)-Epigallocatechin-3-gallate in Conscious and Freely Moving Rats and Its Brain Regional Distribution

LEI-CHWEN LIN,[†,‡] MENG-NAN WANG,[†] TING-YU TSENG,[†,§]
JUNG-SUNG SUNG,[#] AND TUNG-HU TSAI*[,†,⊥]

Institute of Traditional Medicine, School of Medicine, National Yang-Ming University, Taipei,
Taiwan; National Research Institute of Chinese Medicine, Taipei, Taiwan; and Yang-Ming Branch,
Ho-Ping Branch, and Department of Education and Research, Taipei City Hospital, Taipei, Taiwan

A liquid chromatography technique coupled with tandem mass spectrometry (LC-MS/MS) electrospray ionization was used to measure (−)-epigallocatechin-3-gallate (EGCG) in rat plasma. This method was applied to investigate the pharmacokinetics of EGCG in a conscious and freely moving rat by an automated blood sampling device. Multiple reaction monitoring (MRM) was used to monitor the transition of the deprotonated molecule *m/z* of 457 [M − H]⁻ to the product ion 169 for EGCG and the *m/z* of 187 to 164 for the internal standard. The limit of quantification (LOQ) of EGCG in rat plasma was determined to be 5 ng/mL, and the linear range was 5−5000 ng/mL. The protein binding of EGCG in rat plasma was 92.4 ± 2.5%. The brain distribution result indicated that EGCG may potentially penetrate through the blood−brain barrier at a lower rate. The disposition of EGCG in the rat blood was fitted well by the two-compartment model after intravenous administration (10 mg/ kg, iv). The elimination half-life of EGCG was 62 ± 11 and 48 ± 13 min for intravenous (10 mg/kg) and oral (100 mg/kg) administration, respectively. The pharmacokinetic data indicate that the oral bioavailability of EGCG in a conscious and freely moving rat was about 4.95%.

INTRODUCTION

(−)-Epigallocatechin-3-gallate (EGCG; **Figure 1**) is the major polyphenol in green tea, containing approximately 50% of the catechins (*1*). Polyphenolic compounds are known to possess various pharmacological effects, especially antioxidant activity. Moreover, a recent paper indicates that EGCG can reduce iron-regulated amyloid precursor protein and *β*-amyloid peptide in cell cultures implicated in Alzheimer's disease (*2*). Animal studies also show that green tea has preventive activity against cancer cells of the lungs, skin, intestine, colon, prostate, and other sites (*3*). Other studies with human cancer cell lines have shown that EGCG possesses a number of activities related to cancer prevention, such as inhibition of activator protein 1 and nuclear factor *κ*B transactivation and epidermal growth factor receptor signaling (*3, 4*). It has also been reported that EGCG possesses some pharmacological activities such as antibacterial (*5*) and anti-human immunodeficiency virus (*6*).

Because EGCG has attracted attention through studies of its numerous pharmacological effects, it is very important to obtain accurate pharmacokinetic information. Even though several papers have reported measuring the tea polyphenols in biological samples of rats and humans by liquid chromatography (LC) with electrochemical detection (*7, 8*), UV (*9*), chemiluminescence (*10*), and electrospray mass spectrometry (*11*), the use of LC-MS/MS to measure EGCG in the rat plasma of a conscious and freely moving rat has not yet been reported. Also, there are no studies reporting the brain distribution profile of EGCG in the six parts of the rat brain (brain stem, cerebellum, cortex, hippocampus, striatum, and the rest of the brain). We report, for the first time, an automated blood sampling system coupled with an LC-MS/MS method to detect EGCG in freely moving rats and its application to pharmacokinetics.

MATERIALS AND METHODS

Chemicals and Reagents. Commercial Taiwanese Woo-Long tea (a species of *Camellia sinensis*) (3 kg) was extracted with hot water (90 °C, 15 L × 3 times). The combined extract was concentrated by reduced pressure rotavapor to a volume of 1.5 L. The H₂O extract was then partitioned against EtOAc (1.5 L × 3 times). The EtOAc extract was subjected to Sephadex LH-20 column chromatography, eluted with EtOH, and monitored by TLC to give EGCG (purity = 98.1%, by HPLC). The stock solution of EGCG was dissolved in methanol at a concentration of 1 mg/mL in brown glass vials and stored at 4 °C for

---

* Address correspondence to this author at the Institute of Traditional Medicine, School of Medicine, National Yang-Ming University, 155 Li-Nong St. Section 2, Taipei 112, Taiwan [fax (886-2) 2822-5044; telephone (886-2) 2826-7115; e-mail thtsai@ym.edu.tw].
† National Yang-Ming University.
‡ National Research Institute of Chinese Medicine.
§ Yang-Ming Branch, Taipei City Hospital.
# Ho-Ping Branch, Taipei City Hospital.
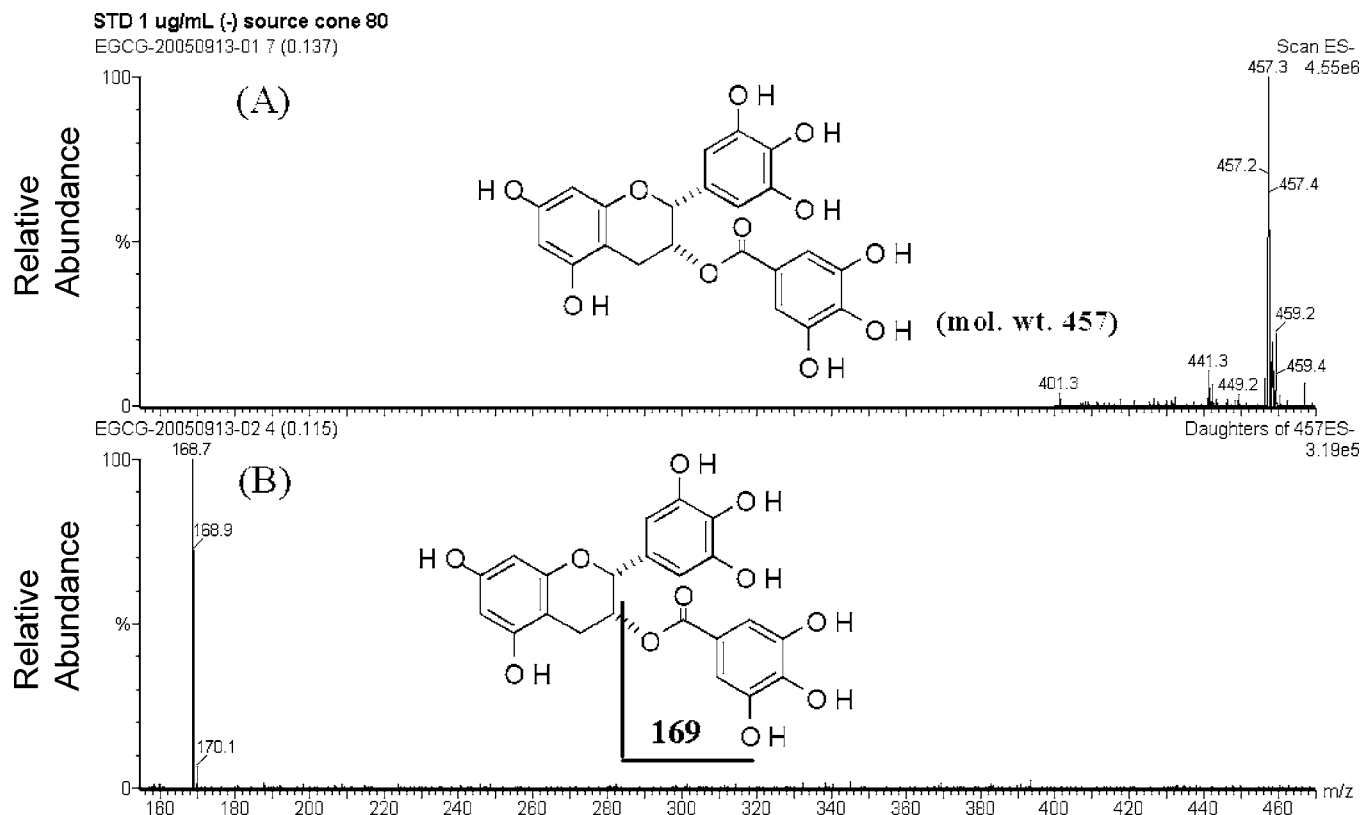⊥ Department of Education and Research, Taipei City Hospital.

Figure 1. Chemical structure of EGCG (molecular weight 457): (**A**) full-scan mass spectrum of EGCG and (**B**) its product ions in LC-MS/MS with electrospray negative-ion mode.

the assay of method validation. Liquid chromatographic grade solvents and reagents were obtained of HPLC grade, and chromatographic solvents were obtained from the Tedia Co., Inc. (Fairfield, OH). Triply deionized water (Millipore, Bedford, MA) was used for all preparations.

Experimental Animals. All experimental protocols involving animals were reviewed and approved by the institutional animal experimentation committee of the National Yang-Ming University. Male specific pathogen-free Sprague–Dawley rats were obtained from the Laboratory Animal Center of the National Yang-Ming University, Taipei. The animals had free access to food (laboratory rodent diet 5P14, PMI Feeds, Richmond, IN) and water until 18 h prior to being used in experiments, at which time only food was removed. Six Sprague–Dawley rats (280–320 g) were initially anesthetized with pentobarbital (50 mg/kg, ip) and remained anesthetized throughout the experimental period. To link together the experimental animal and the automated blood sampling device, a surgical operation was undertaken. Surgical sites were shaved and cleaned with 70% ethanol solution, and polyethylene tubes were implanted in the right jugular and right femoral veins for blood sampling and drug administration, respectively. In the oral administration group, only the right jugular was cannulated for sampling purpose. The cannulae were exteriorized, fixed in the dorsal neck region, and connected to the sampling system. The patency tubing was maintained by flushing with heparinized saline (15 units/mL). During the period of surgery, the body temperature of rats was maintained at 37 °C with a heating pad. After surgery, the rats were installed in the experimental cage and allowed to recover for 1 day.

LC-MS/MS. The LC-MS/MS analysis was performed using a Waters 2690 with a 996 photodiode assay detector together with an automatic liquid chromatographic sampler and an autoinjection system hyphenated to a Micromass Quattro Ultima tandem quadruple mass spectrometry (Micromass, Manchester, U.K.) equipped with an electrospray ionization source. Separation was achieved using a Zorbax Extend-C18 column (150 × 4.6 mm i.d.; 5 μm, Agilent, Palo Alto, CA), injection volume was 20 μL, mobile phase consisted of 40% methanol and 60% 1 mM CH₃COOH, and flow rate was set at 1 mL/min. After passing through the analytical column, the eluant was split, and 0.2 mL/min was delivered to the mass spectrometer.

For operation in the MS/MS mode, a mass spectrometer with an orthogonal Z-spray electrospray interface was used. The infusion experiment was performed using a mode 22 multiple-syringe pump (Harvard, Holliston, MA). During the analyses, the ESI parameters were set as follows: capillary voltage, 2.75 kV for negative mode; source temperature, 80 °C; desolvation temperature, 350 °C; cone gas flow, 120 L/h; desolvation gas flow, 530 L/h. The cone voltage of m/z 457 was adjusted to maximize the intensity of the deprotonated molecular ion (precursor) as 80 V, and the collision voltage was also adjusted to optimize the product ion signals as 17 eV for the EGCG analysis. The cone voltage of m/z 187 was 55 V, and the collision voltage was 20 eV for theophylline (I.S., internal standard) analysis. The MRM was used to monitor the transition of the deprotonated molecule with m/z 457 [M − H]⁻ to the product ion 169 for EGCG analysis and m/z 187 to 164 for internal standard analysis. All LC-MS/MS data were processed by the MassLynx version 3.5 NT Quattro data acquisition software.

Method Validation. The sample preparation for calibration curves was obtained by freshly spiked plasma samples or brain tissue homogenates with stock solution of EGCG at a concentration range of 5–5000 ng/mL. All linear curves were required to have a coefficient of estimation at least >0.995. The intra-assay and interassay variabilities were determined by quantitating six replicates at concentrations of 5, 10, 25, 100, 500, 1000, and 5000 ng/mL using the LC-MS/MS method described above on the same day and six consecutive days, respectively. The accuracy (bias %) was calculated from the mean value of observed concentration (C_{obsd}) and nominal concentration (C_{nom}) using the relationship accuracy (bias %) = [(C_{obsd} − C_{nom})/C_{nom}] × 100. The relative standard deviation (RSD) was calculated from the observed concentrations as precision (RSD%) = [standard deviation (SD)/C_{obsd}] × 100.

Blood Sampling and Sample Preparation. The automated blood sampling system DR-II (Eicom Corp., Kyoto, Japan) was applied to conscious and freely moving rats. EGCG was dissolved in normal saline at doses of 10 and 100 mg/kg for iv injection and oral administration, respectively. About 150 μL of blood sample was withdrawn from the jugular vein into a heparin-rinsed vial with a fraction collector according

to a programmed schedule at 0, 1, 10, 20, 30, 40, and 50 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, and 7 h. Each blood sample was centrifuged at 3000g for 10 min. The resulting plasma (50 μL) was vortex-mixed with 10 μL of internal standard (theophylline, 0.01 μg/mL) solution and 10 μL of preservative solution (20% ascorbic acid and 0.05% Na$_2$-EDTA) (*12*), and the mixture was extracted by ethyl acetate (1 mL) for 10 min and centrifuged for 10 min at 6000g. After centrifugation, 1 mL of the clear supernatant was transferred to another centrifuge tube, dried under nitrogen, and reconstituted with 100 μL of mobile phase. An aliquot (20 μL) of the solution was directly injected onto the LC-MS/MS for analysis. Data from these samples were used to construct the pharmacokinetic curve of EGCG. The same sample handling process was used for the determination of precision and accuracy. The plasma sample was diluted by mobile phase at a ratio of 1:1 (v/v) before analysis, if the EGCG concentration was >5000 ng/mL.

Protein Binding and Brain Regional Distribution. For protein binding of EGCG assay, the drug was given via iv bolus to rats at a dose of 10 mg/kg. Then, the blood sample (3 mL) was withdrawn by heart puncture at 15 min after injection. The rat blood sample was centrifuged at 3000g under 4 °C for 10 min. The plasma was divided into two parts, and 50 μL of plasma was used to measure the total form concentration of EGCG (C_t). The remaining plasma was transferred to an ultrafiltration tube (Centrifree, Millipore, Bedford, MA) for free form measurement (C_f). Measurement of EGCG was the same as the above description. The protein binding of EGCG was calculated by using the equation

$$[(C_t - C_f)/C_t] \times 100\%$$

For the brain distribution study, the animal was sacrificed at 15 min by decapitation after EGCG administration (50 mg/kg, iv). The brain stem (Bs), cerebellum (Cb), cortex (Cx), hippocampus (Hp), striatum (St), and the rest of the brain (Rb) were further dissected and weighed for the determination of regional distribution of EGCG. Each brain tissue was homogenized in 5-fold of its volume with 50% aqueous methanol. The homogenate was then centrifuged at 3000g for 10 min. An aliquot of 50 μL of the supernatant was vortex-mixed with 10 μL of I.S. solution. The following procedures and analyses of the brain tissue samples were the same as for the preparation of the plasma samples.

Matrix Effect and Recovery Evaluation. Three sets of extraction methods were prepared to evaluate the recovery and the matrix effect in the quantitative bioanalytical method.

Set 1. Three standard EGCG solutions (0.1, 0.5, and 1.0 μg/mL) were constructed using neat solutions of EGCG and I.S. in the mobile phase. The samples were prepared by placing 10 μL of the appropriate concentrations of EGCG, 10 μL of I.S. solution, and 80 μL of the mobile phase (total volume = 100 μL) into 1.5 mL centrifuge tubes. After mixing, the solutions were transferred into autosampler vials, and 20 μL was injected directly into the LC-MS/MS system.

Set 2. Three EGCG solutions spiked after extraction were constructed in three different lots of plasma or brain homogenate by placing 50 μL of plasma or brain homogenate in 1.5 mL centrifuge tubes followed by the addition of 20 μL of the mobile phase. The plasma or brain homogenate was extracted with 1 mL of ethyl acetate. After centrifugation (3000g for 10 min, at 4 °C), the ethyl acetate extracts were separated, dried by nitrogen gas, reconstituted with 80 μL of mobile phase, 10 μL of I.S. solution, and 10 μL of the appropriate concentrations of EGCG (0.1, 0.5, and 1.0 μg/mL), and filtered by a 0.45 μm syringe filter. Twenty microliters of the extract was used in LC-MS/MS analysis. In set 2, EGCG was spiked after extraction into different plasma or brain homogenate, whereas in set 3, EGCG were spiked into different plasma or brain homogenate before extraction.

Set 3. Three EGCG solutions spiked before extraction were constructed in three different lots of plasma or brain homogenate by placing 50 μL of plasma or brain homogenate in 1.5 mL centrifuge tubes to which 10 μL of the appropriate concentrations of EGCG (0.1, 0.5, and 1.0 μg/mL) and 10 μL of I.S. solution, both in the mobile phase, were added before extraction. The following preparation procedures were as the same manner as in set 2. The dry residue was

reconstituted in 100 μL of the mobile phase and filtered by a 0.45 μm syringe filter, and 20 μL was used in LC-MS/MS analysis.

By comparing the peak areas of the standard EGCG solutions (0.1, 0.5, and 1.0 μg/mL), EGCG solutions spiked before and after extraction into different lots of plasma or brain homogenate, and the peak area ratios of EGCG to the I.S. solution, the recovery and ion suppression or enhancement associated with a given lot of plasma or brain homogenate were assessed (*13*).

Pharmacokinetic Application. Pharmacokinetic calculations were performed on each individual set of data using the pharmacokinetic software WinNonlin Standard Edition, version 1.1 (Pharsight Corp., Mountain View, CA) by compartmental method and noncompartmental method for oral and intravenous administrations, respectively. The area under the concentration−time curve (AUC) is used as a measure of the total amount of unaltered drug that reaches the systemic circulation. The oral bioavailability (*F*) is defined as the fraction of unchanged drug reaching the systemic circulation followed by the oral administration route. The absolute oral bioavailability of a drug is generally measured by comparing the respective AUCs after oral and intravenous administration according to the following equation:

$$F = (\text{AUC}_{po}/\text{dose}_{po})/(\text{AUC}_{iv}/\text{dose}_{iv})$$

The clearance (Cl) was calculated as

$$\text{Cl} = \text{dose}/\text{AUC}$$

RESULTS AND DISCUSSION

Characterization of EGCG: brown amorphous power; IR (KBr)$_{max}$, v 3417, 1688, 1625, 1456, 1380, 1235, 1141, 1025 cm^{-1}; ^{1}H NMR (CD$_3$OD), δ 2.85 (1H, dd, J = 17.5, 2.5 Hz, H-4a), 2.99 (1H, dd, J = 17.5, 4.5 Hz, H-4b), 4.98 (1H, s, H-2), 5.54 (1H, H-3), 5.97 (2H, s, H-6, 8), 6.51 (2H, s, H-2′, 6′), 6.96 (2H, s, H-2″, 6″); ^{13}C NMR (CD$_3$OD), δ 26.5 (t, C-4), 69.7 (d, C-3), 78.1 (d, C-2), 95.8 (d, C-8), 96.4 (d, C-6), 99.4 (s, C-10), 106.8 (d, C-2′, 6′), 110.1 (d, C-2″, 6″), 121.2 (s, C-1″), 130.6 (s, C-1′), 133.4 (s, C-4′), 139.5 (s, C-4″), 145.9 (s, C-3″, 5″), 146.2 (s, C-3′, 5′), 156.8 (s, C-9), 157.1 (s, C-5), 157.3 (s, C-7); 167.5 (s, C-7″); APCIMS, m/z 459 [M + H]$^+$.

LC-MS/MS. In the analytical condition, the full scan in negative ion modes (scan range from m/z 200 to 500) was used to identify the analyte. With full-scan mass spectra for the determination of EGCG (precursor ion is 457 [M − H]$^-$) a cone voltage of 80 V was applied. Then, 17 eV collision energies were optimized to produce the main product ion at m/z 169, as shown in **Figure 1**. The analyte was detected in the negative ionization mode by monitoring the precursor−product combination in the MRM mode, and it provided high selectivity and sensitivity for the quantification assay. In the initial method development, a small signal was observed in the chromatograms when the mobile phase consisted of water/methanol (60:40, v/v) at a flow rate of 1.0 mL/min. To improve the signal, acetic acid (10 mM) was added to the mobile phase.

Panels **A** and **B** of **Figure 2** show the chromatogram of a drug-free plasma extract, with mass transitions of m/z 457→169 for EGCG and m/z 187→164 for internal standard, respectively, illustrating a clean baseline with no interference peaks eluted within 10 min. Panels **A**, **B**, and **C** of **Figure 3** show the chromatogram of a standard of EGCG (1000 ng/mL) and internal standard (10 ng/mL) spiked in plasma containing EGCG (984.3 ng/mL) collected from rat plasma after EGCG administration (10 mg/kg, iv). Each determination is completed within 10 min, and no carry-over peaks were detected in the subsequent chromatograms of plasma samples.

Method Validation. Linear least-squares regression analysis of the calibration graph on six different days demonstrated
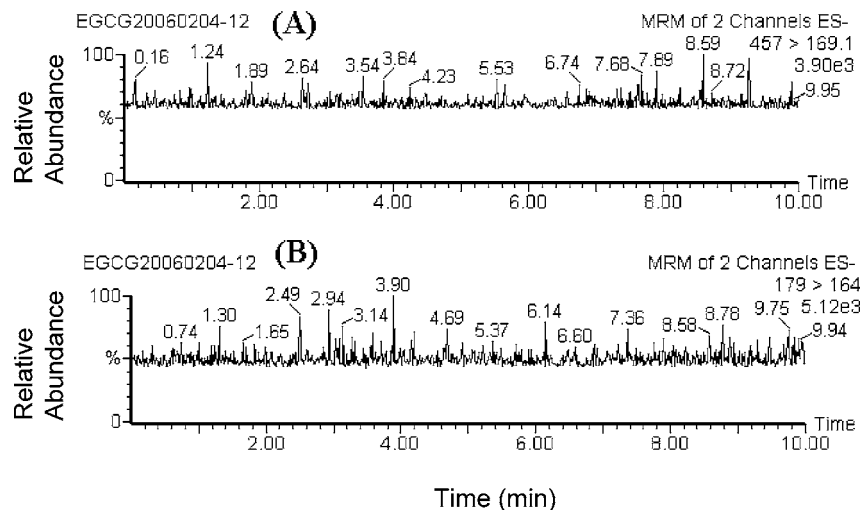
Figure 2. LC–tandem mass chromatogram showing the drug-free plasma extract with MRM: (**A**) the mass transitions were *m/z* 457→169 for EGCG and (**B**) *m/z* 187→164 for the internal standard (theophylline).
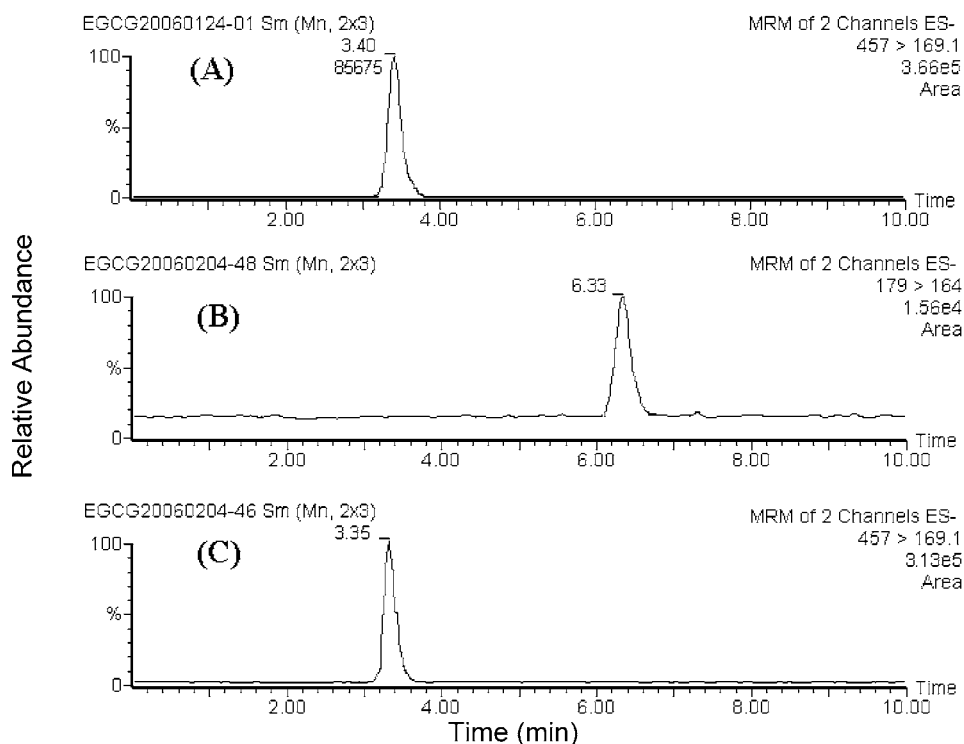

Figure 3. Representative MRM chromatogram showing the rat plasma sample spiked with (**A**) EGCG (1000 ng/mL) and (**B**) internal standard (theophylline, 10 ng/mL) and (**C**) containing EGCG (984.3 ng/mL) collected from a rat plasma after EGCG administration (10 mg/kg, iv).

linearity between the response and the nominal concentration of EGCG over the range of 5−5000 ng/mL. The results of linear regression analysis showed that the coefficient estimation of the standard curve was >0.995. The data showed excellent reproducibility. The lower limit of quantification (LLOQ) was 5 ng/mL, which was defined as the lowest concentration of the linear range. The intra- and interassay precision and accuracy values of EGCG in rat plasma are presented in **Table 1**, and all percent of bias and RSD values were within 17%.

A previous paper indicated that the linearity of the calibration curves was found to be 1.88−60 *μ*g/mL for the analysis of EGCG in the medicinal plant catechu (*Acacia catechu*), as detected by LC−electrospray ionization mass spectrometry (*14*). Moreover, several papers describe the measurement of EGCG

in rat plasma (*15*, *16*) and human serum (*17*, *18*). Masukawa et al. (*19*) provide the limit of detection (LOD) at 0.25 ng/mL for authentic EGCG by LC−electrospray ionization mass spectrometry. On the basis of the liquid−liquid extraction of this study, EGCG was spiked into rat plasma, and the LOD in rat plasma was determined to be 1 ng/mL at a signal-to-noise ratio of 3.

Matrix Effect and Recovery of Rat Plasma and Brain Homogenate. The results obtained in this manner were allowed to determine of the matrix effect (ME) and recovery (RE) of the extraction procedure. The peak areas obtained in neat standard solutions in set 1 were indicated as A, the corresponding peak areas for standards spiked after extraction into plasma or brain homogenate extracts as B (set 2), and peak areas for

Table 1. Intra-assay and Interassay Precision (RSD) and Accuracy (Bias) of the LC-MS/MS Method for the Determination of EGCG[a]

nominal concn (ng/mL)	obsd concn (ng/mL)	RSD (%)	bias (%)
	Intra-assay		
5	5.2 ± 0.9	17.0	−4.0
10	11.2 ± 1.5	13.0	−12.0
25	24.9 ± 1.0	4.0	0.4
50	47.3 ± 2.6	5.5	5.4
10	102.0 ± 3.6	3.5	−2.0
500	501.7 ± 7.9	1.6	−0.3
1000	998.9 ± 3.6	0.4	0.1
5000	4997.5 ± 4.1	0.08	0.05
	Interassay		
5	5.1 ± 0.8	15.6	−2.0
10	10.7 ± 1.2	11.2	−7.0
25	25.3 ± 1.4	5.5	−1.2
50	48.1 ± 2.8	5.8	3.8
100	102.7 ± 4.1	3.9	−2.7
500	501.2 ± 6.9	1.4	−0.3
1000	1001.2 ± 4.9	0.5	−0.1
5000	5003.2 ± 3.8	0.07	−0.06

[a] Data expressed as means ± SD ($n = 6$).

Table 2. Matrix Effect (ME) and Recovery (RE) Data for EGCG and I.S. (Theophylline) in Three Different Lots of Rat Plasma[a]

nominal concn (µg/mL)	EGCG			I.S.			ME[b]		RE[c]	
	set 1	set 2	set 3	set 1	set 2	set 3	EGCG	I.S.	EGCG	I.S.
0.01				7.30	7.76	6.85		106		88
0.1	1.41	1.42	1.11				100		78	
0.5	7.40	7.86	6.02				106		76	
1.0	14.82	15.61	11.84				105		76	
mean							103.6		76.6	

[a] Expressed as mean peak area at unit, × 10^4 ($n = 3$). [b] Matrix effect expressed as the ratio of the mean peak area of an analyte spiked postextraction (set 2) to the mean peak area of the same analyte standards (set 1) multiplied by 100. A value of >100% indicates ionization enhancement, and a value of <100% indicates ionization suppression. [c] Recovery calculated as the ratio of the mean peak area of an analyte spiked before extraction (set 3) to the mean peak area of an analyte spiked postextraction (set 2) multiplied by 100.

Table 3. Matrix Effect (ME) and Recovery (RE) Data for EGCG and I.S. (Theophylline) in Three Different Lots of Rat Brain Homogenate[a]

nominal concn (µg/mL)	EGCG			I.S.			ME[b]		RE[c]	
	set 1	set 2	set 3	set 1	set 2	set 3	EGCG	I.S.	EGCG	I.S.
0.01				7.69	7.76	6.52		101		84
0.1	1.74	1.86	1.38				107		74	
0.5	9.32	9.33	6.59				100		71	
1.0	18.36	19.28	13.98				105		73	
mean							104.0		72.6	

[a] Expressed as mean peak area at unit, × 10^4 ($n = 3$). [b] Matrix effect expressed as the ratio of the mean peak area of an analyte spiked postextraction (set 2) to the mean peak area of the same analyte standards (set 1) multiplied by 100. A value of >100% indicates ionization enhancement, and a value of <100% indicates ionization suppression. [c] Recovery calculated as the ratio of the mean peak area of an analyte spiked before extraction (set 3) to the mean peak area of an analyte spiked postextraction (set 2) multiplied by 100.

Table 4. Protein Binding of EGCG in Rat Plasma

exptl rat	protein binding (%)
1	89.3
2	94.9
3	92.5
4	91.2
5	95.7
6	90.6
av	92.4 ± 2.5

standards spiked before extraction as C (set 3); the ME and RE values can be calculated as follows (*13*):

$$ME\ (\%) = B/A \times 100$$

$$RE\ (\%) = C/B \times 100$$

A value of 100% ME indicated that the response in the mobile phase and in the plasma or brain homogenate extracts was the same and no matrix effect was observed (*13*). The results indicated that the mean matrix effects of EGCG and I.S. were 103.6 and 106% in plasma samples, respectively, and 104.0 and 101% in brain homogenates, respectively (**Tables 2** and **3**). Without significant difference of the sets A and B, the matrix effect in the quantitative analysis could be ignored. The mean recoveries for EGCG and I.S. were about 76.6 and 88% in plasma samples and about 72.6 and 84% in brain homogenates, respectively.

Protein Binding. Methods for evaluating drug protein binding ratio, including equilibrium dialysis (*20*) and ultrafiltration (*21*), all make use of a semipermeable membrane that separates the protein and protein-bound drug from the unbound drug. On the basis of the ultrafiltration method, the EGCG protein binding in rat plasma was 92.4 ± 3.1% (**Table 4**). Because a drug with a high protein binding ratio should be very important in clinical applications, any herb−drug interaction of EGCG due to protein binding displacement is possible. This concept is similar to the previous results of warfarin. The displacement of warfarin protein binding may affect the clotting time and lead to hemorrhage via drug−drug interaction (*22*). In a previous study, compound galloyl groups such as EGCG and ECG potentially bind to proteins or membrane surfaces by hydrogen bonding via phenolic terminals, particularly to the proteins (*23*), which may be similar to our findings.

Brain Regional Distribution. Panels **A**, **B**, and **C** of **Figure 4** and panels **A**, **B**, and **C** of **Figure 5** show the MRM chromatograms of cortex (6.23 ng/g), brain stem (3.76 ng/g; lower than LLOQ), hippocampus (4.18 ng/g), striatum (4.72 ng/g), cerebellum (7.13 ng/g), and the rest of the brain (1.31 ng/g; lower than LOD), respectively, at 15 min after EGCG administration (50 mg/kg, iv). The EGCG concentrations in the various brain regions were around the LLOQ (5 ng/mL). Although the lipophilicity of EGCG chemical groups suggests passive permeability across the blood−brain barrier (*24*), Nakagawa and Miyazawa (*16*) have detected an EGCG level of 0.5 nmol/g in rat brain tissue at 60 min after oral administration (500 mg/kg). This low brain distribution ratio may be due to its bipolar functional group, which might have difficulty in penetrating the blood−brain barrier. Another possibility is that the protein-bound drug is a large complex that cannot easily transverse cells or possibly even capillary membranes and, therefore, has a restricted distribution in the brain.

Pharmacokinetic Applications. This validated method was applied to measure the samples collected from a conscious and freely moving rat. **Figure 6** illustrates the concentration versus
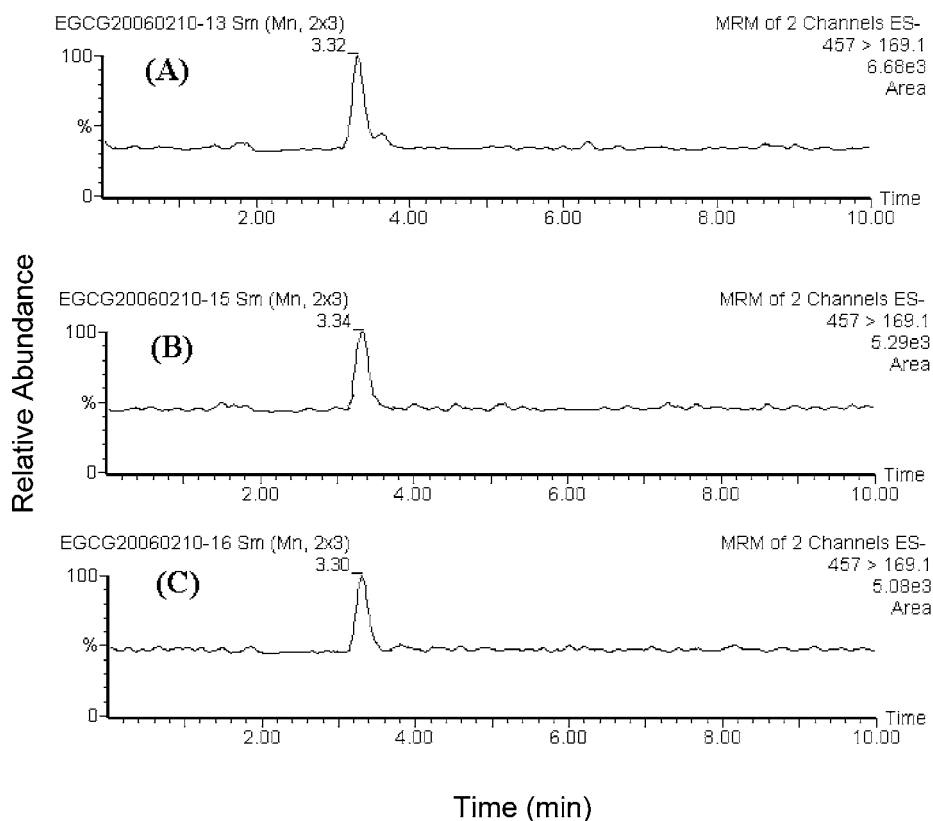
Figure 4. Representative MRM chromatogram showing EGCG in rat brain tissues at 15 min after EGCG administration (50 mg/kg, iv): (**A**) cortex; (**B**) brain stem; (**C**) hippocampus.
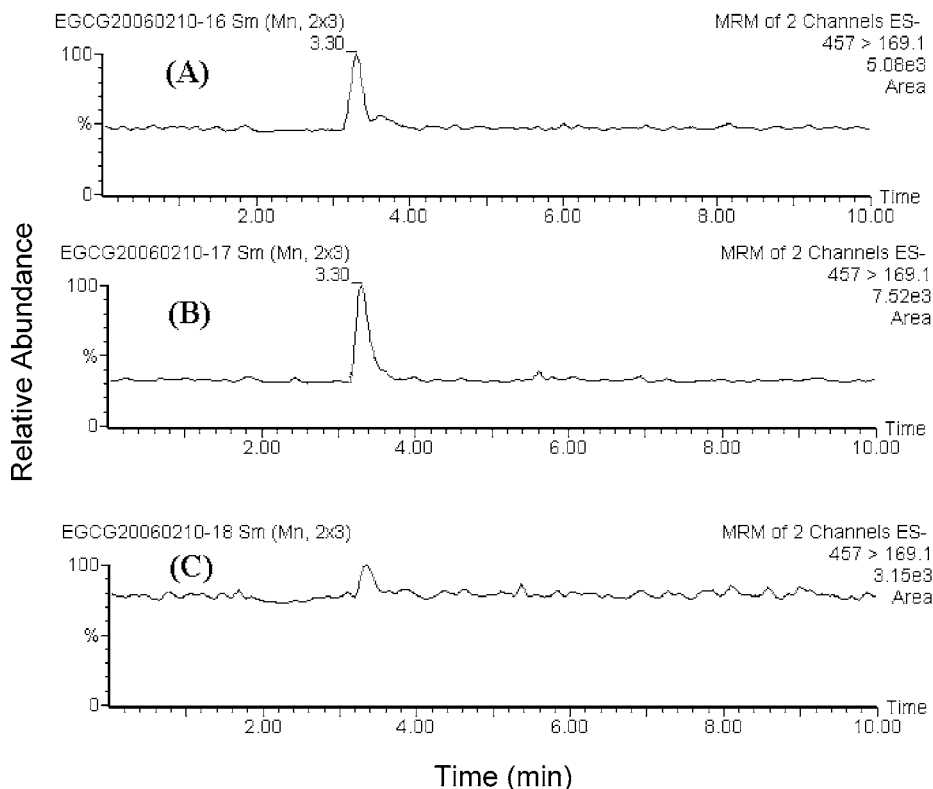

Figure 5. Representative MRM chromatogram showing EGCG in rat brain tissues at 15 min after EGCG administration (50 mg/kg, iv): (**A**) striatum; (**B**) cerebellum; (**C**) the rest of the brain.

time profiles of EGCG with a single intravenous and oral dose administration to six individual rats for each group. Plasma level versus time curves were fitted well into a two-compartment model after the iv administration. The pharmacokinetic data of AUCs were 161.20 ± 39.6 and 79.83 ± 16.5 min μg/mL for intravenous (10 mg/kg) and oral (100 mg/kg) doses, respectively

Pharmacokinetics of (−)-Epigallocatechin-3-gallate

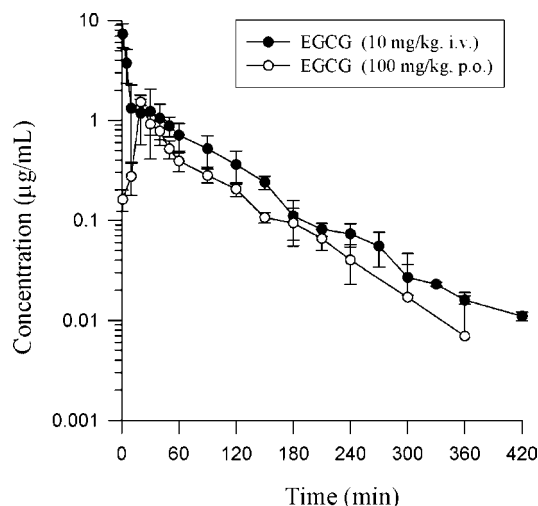*J. Agric. Food Chem.*, Vol. 55, No. 4, 2007 **1523**

Figure 6. Concentration versus time curves of EGCG after drug administration (100 mg/kg, po; and 10 mg/kg, iv) in rats.

Table 5. Pharmacokinetic Parameters of EGCG Administration (10 mg/kg, Intravenous; and 100 mg/kg, per Os) in Rats[a]

parameter	estimate (10 mg/kg)	estimate (100 mg/kg)
$t_{1/2}$ (min)	62 ± 11	48 ± 13
C_{max} (μg/mL)	8.92 ± 2.68	1.52 ± 0.11
AUC (min μg/mL)	161 ± 39	80 ± 16
T_{max} (min)		24 ± 7

[a] Data expressed as means ± SD ($n = 6$). $t_{1/2}$, elimination half-life; AUC, area under the concentration versus time curve; T_{max}, time to reach peak concentration.

(**Table 5**). The oral bioavailability $(AUC_{po}/dose_{po})/(AUC_{iv}/dose_{iv})$ of EGCG in a freely moving rat was about 4.95%.

The absorption of EGCG has been demonstrated by an oral administration to rats, and the analyte has been clearly measured in the blood of the portal vein by LC-MS (*25*). In addition, the oral bioavailability of EGCG was reported to be about 1.6% in rat (*12*) and 26.5% in mice (*26*). The absorption site of EGCG has been reported to be weakly absorbed by the intestine (*12*). Previous studies used methods either under anesthesia or conscious restraint technique, which may cause stress to the animal and affect the pharmacokinetic data. This automated blood sampling system provides a major advantage by allowing free movement, which can minimize the stress caused by the restraint or anesthesia. Stress may delay gastric emptying and slow the absorption of drugs in the gastrointestinal tract, and that may be the reason for the small difference in the oral bioavailability.

In summary, we have developed a sensitive, specific, and reliable LC-MS/MS assay for the determination of EGCG in rat plasma using an automated blood sampling system. The EGCG penetrates various brain regions at a dose of 50 mg/kg by iv injection. In addition, the method was applied in the pharmacokinetic study in freely moving rats, and its oral bioavailability was about 4.95%.

LITERATURE CITED

(1) Aucamp, J. P.; Hara, Y.; Apostolides, Z. Simultaneous analysis of tea catechins, caffeine, gallic acid, theanine and ascorbic acid by micellar electrokinetic capillary chromatography. *J. Chromatogr. A* **2000**, *876*, 235−242.

(2) Reznichenko, L.; Amit, T.; Zheng, H.; Avramovich-Tirosh, Y.; Youdim, M. B.; Weinreb, O.; Mandel, S. Reduction of iron-regulated amyloid precursor protein and β-amyloid peptide by (−)-epigallocatechin-3-gallate in cell cultures: implications for iron chelation in Alzheimer's disease. *J. Neurochem.* **2006**, *2*, 527−536.

(3) Yang, C. S.; Maliakal, P.; Meng, X. Inhibition of carcinogenesis by tea. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 25−54.

(4) Lambert, J. D.; Yang, C. S. Mechanisms of cancer prevention by tea constituents. *J. Nutr.* **2003**, *133*, 3262S−3267S.

(5) Hu, Z. Q.; Zhao, W. H.; Asano, N.; Yoda, Y.; Hara, Y.; Shimamura, T. Epigallocatechin gallate synergistically enhances the activity of carbapenems against methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **2002**, *46*, 558−560.

(6) Yamaguchi, K.; Honda, M.; Ikigai, H.; Hara, Y.; Shimamura, T. Inhibitory effects of (−)-epigallocatechin gallate on the life cycle of human immunodeficiency virus type 1 (HIV-1). *Antivir. Res.* **2002**, *53*, 19−34.

(7) Long, H.; Zhu, Y.; Cregor, M.; Tian, F.; Coury, L.; Kissinger, C. B.; Kissinger, P. T. Liquid chromatography with multi-channel electrochemical detection for the determination of epigallocatechin gallate in rat plasma utilizing an automated blood sampling device *J. Chromatogr. B* **2001**, *763*, 47−51.

(8) Sano, M.; Tabata, M.; Suzuki, M.; Degawa, M.; Miyase, T.; Maeda-Yamamoto, M. Simultaneous determination of twelve tea catechins by high-performance liquid chromatography with electrochemical detection. *Analyst* **2001**, *126*, 816−820.

(9) Lin, L. C.; Hung, L. C.; Tsai, T. H. Determination of (−)-epigallocatechin gallate in rat blood by microdialysis coupled with liquid chromatography. *J. Chromatogr. A* **2004**, *1032*, 125−128.

(10) Nakagawa, K.; Miyazawa, T. Chemiluminescence−high-performance liquid chromatographic determination of tea catechin, (−)-epigallocatechin 3-gallate, at picomole levels in rat and human plasma. *Anal. Biochem.* **1997**, *248*, 41−49.

(11) Dalluge, J. J.; Nelson, B. C.; Thomas, J. B.; Welch, M. J.; Sander, L. C. Capillary liquid chromatography/electrospray mass spectrometry for the separation and detection of catechins in green tea and human plasma. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1753−1756.

(12) Chen, L.; Lee, M. J.; Li, H.; Yang, C. S. Absorption, distribution, and elimination of tea polyphenols in rats. *Drug Metab. Dispos.* **1997**, *25*, 1045−1050.

(13) Matuszewski, B. K.; Constanzer, M. L.; Chavez-Eng, C. M. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal. Chem.* **2003**, *75*, 3019−3030.

(14) Shen, D.; Wu, Q.; Wang, M.; Yang, Y.; Lavoie, E. J.; Simon, J. E. Determination of the predominant catechins in *Acacia catechu* by liquid chromatography/electrospray ionization−mass spectrometry. *J. Agric. Food Chem.* **2006**, *54*, 3219−3224.

(15) Unno, T.; Takeo, T. Absorption of (−)-epigallocatechin gallate into the circulation system of rats. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1558−1559.

(16) Nakagawa, K.; Miyazawa, T. Absorption and distribution of tea catechin. *J. Nutr. Sci. Vitamin.* **1997**, *43*, 679−684.

(17) Unno, T.; Kondo, K.; Itakura, H.; Takeo, T. Analysis of (−)-epigallocatechin gallate in human serum obtained after ingesting green tea. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 2066−2068.

(18) Maiani, G.; Serafini, M.; Salucci, M.; Azzini, E.; Ferro-Luzzi, A. Application of a new high-performance liquid chromatographic method for measuring selected polyphenols in human plasma. *J. Chromatogr. B* **1997**, *692*, 311−317.

(19) Masukawa, Y.; Matsui, Y.; Shimizu, N.; Kondou, N.; Endou, H.; Kuzukawa, M.; Hase, T. Determination of green tea catechins in human plasma using liquid chromatography−electrospray ionization mass spectrometry. *J. Chromatogr. B* **2006**, *834*, 26−34.

1524 *J. Agric. Food Chem.,* Vol. 55, No. 4, 2007

Lin et al.

(20) Lin, Z. J.; Musiano, D.; Abbot, A.; Shum, L. In vitro plasma protein binding determination of flunarizine using equilibrium dialysis and liquid chromatography−tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2005**, *37*, 757−762.

(21) Peng, S. X.; Henson, C.; Wilson, L. J. Simultaneous determination of enantioselective plasma protein binding of aminohydantoins by ultrafiltration and chiral high-performance liquid chromatography. *J. Chromatogr. B* **1999**, *732*, 31−37.

(22) Levy, G.; Cheung, W. K. Pharmacological consequences of warfarin−protein binding. In *Protein Binding and Drug Transport*; Tillement, J. P., Lindenlaub, E., Eds.; Schattauer Verlag: Stuttgart, Germany, 1985; pp 445−457.

(23) Haslam, E. Natural polyphenols (vegetable tannins) as drugs: possible modes of action. *J. Nat. Prod.* **1996**, *59*, 205−215.

(24) Raje, S.; Cao, J.; Newman, A. H.; Gao, H.; Eddington, N. D. Evaluation of the blood−brain barrier transport, population pharmacokinetics, and brain distribution of benztropine analogs and cocaine using in vitro and in vivo techniques. *J. Pharmacol. Exp. Ther.* **2003**, *307*, 801−808.

(25) Okushio, K.; Matsumoto, N.; Suzuki, M.; Nanjo, F.; Hara, Y. Absorption of (−)-epigallocatechin gallate into rat portal vein. *Biol. Pharm. Bull.* **1995**, *18*, 190−191.

(26) Lambert, J. D.; Lee, M. J.; Lu, H.; Meng, X.; Hong, J. J.; Seril, D. N.; Sturgill, M. G.; Yang, C. S. Epigallocatechin-3-gallate is absorbed but extensively glucuronidated following oral administration to mice. *J. Nutr.* **2003**, *133*, 4172S−4177S.