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ORIGINAL CONTRIBUTION

Plasma pharmacokinetics of catechin metabolite 4′-O-Me-EGC in healthy humans

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Abstract
Background Tea is an infusion of the leaves of the *Camellia sinensis* plant and is the most widely consumed beverage in the world after water. Green tea contains significant amounts of polyphenol catechins and represents a promising dietary component to maintain health and well-being. Epidemiological studies indicate that polyphenol intake may have potential health benefits, such as, reducing the incidence of coronary heart disease, diabetes and cancer. While bioavailability of green tea bioactives is fairly well understood, some gaps still remain to be filled, especially the identification and quantification of conjugated metabolites in plasma, such as, sulphated, glucuronidated or methylated compounds.
Aim of the study In the present study, we aimed to quantify the appearance of green tea catechins in plasma with particular emphasis on their methylated forms.
Results After feeding 400 mL of green tea, 1.25% infusion to 9 healthy subjects, we found significant amounts of EC, EGC and EGCg in plasma as expected. EGC was the most bioavailable catechin, and its methylated form (4′-O-Me-EGC) was also present in quantifiable amounts. Its kinetics followed that of its parent compound. However, the relative amount of the methylated form of EGC was lower than that of the parent compound, an important aspect which, in the literature, has been controversial so far. The quantitative results presented in our study were confirmed by co-chromatography and accurate mass analysis of the respective standards. We show that the relative abundance of 4′-O-Me-EGC is ~40% compared to the parent EGC.
Conclusion 4′-O-Me-EGC is an important metabolite derived from catechin metabolism. Its presence in significant amounts should not be overlooked when assessing human bioavailability of green tea.

Keywords Green tea · Bioavailability · Catechins

Abbreviations

AUC	Area under the curve
BMI	Body mass index
C	Catechin
C_{max}	Maximum plasma concentration
T_{max}	Time needed to reach maximum plasma concentration
EC	(−)-Epicatechin
ECg	(−)-Epicatechin gallate
EGC	(−)-Epigallocatechin
EGCg	(−)-Epigallocatechin gallate
MS	Mass spectrometry
SE	standard error
IS	Internal standard

Background

Tea is an infusion of the leaves of the *Camellia sinensis* plant and is the most widely consumed beverage in the world after water [1]. Some biologically active components of tea include flavonoids (flavan-3-ols or catechins), caffeine, and fluoride. Human epidemiological studies indicate that polyphenol intake may have potential health benefits, for example to reduce incidence of coronary heart disease, diabetes and cancer [2]. Besides their antibacterial

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and antiviral activities [3], green tea catechins exert a potent inhibitory effect on LDL oxidation in vitro [4]. Furthermore, they were shown in epidemiological and intervention studies to improve cardiovascular and metabolic health [5, 6]. While several studies have provided substantial amount of information on the bioavailability[1] of green tea bioactives [7–17], there are still a number of open questions to be answered. Particularly, complete identification and quantification of colonic metabolites and conjugates in plasma are not yet fully reported, especially in humans. In general, the lack of appropriate standards of metabolites remains a bottleneck in the progression of elucidating the metabolism of polyphenols. Hence, most of the studies so far used enzymatic cleavage to identify and quantify catechins in their aglycone forms, and often methylated forms were not considered. Also in terms of biological activities, several questions remain. While the in vitro antioxidant activity of catechins is decreased by methylation [18], other bioactivities of metabolites are still largely unknown and require further investigations. For example, some studies report that methylation in general could help maintain or even improve the biological activities of polyphenols while increasing their metabolic stability due to blocking the sites of glucuronidation and sulfation hereby reducing their excretion rate [19–22]. EGCG and its methylated forms were reported to show allergy-alleviating effects including inhibition of histamine release, leukotriene release and cytokine production [23]. Furthermore, the same study revealed that methylated catechins were more potent anti-allergens than their non-methylated precursors.

Methylation of green tea catechins is known to occur via catechol-O-methyl transferase COMT, which is specific for the *ortho*-diphenolic structure of compounds [24]. Methylation of catechin and epicatechin has been reported in rats, after enzymatic digestion and LC–MS analysis of plasma and urine [25, 26]. Miketova et al. have used LC–ESI–MS/MS to characterize the constituents of green tea and to elucidate the structure of minor methylated compounds in green tea extract, not reported before [27]. In humans, Lee et al. have reported several times higher levels of Me-EGC in the circulation than EGC [28]. Their results were based on LC-Coulometric detection. More recently, glucuronide and sulphate conjugates of catechins in their aglycone and methylated forms have also been detected in urine and plasma after green tea ingestion [16, 17]. However, the quantification was done based on standards of commercially available aglycone forms. Proper quantification using appropriate standards of conjugates still remains a problem in understanding the metabolism of polyphenols.

The focus of the present study is to clarify the extent of methylation of green tea catechins in humans: an aspect on which controversial data have been reported in the literature [29, 30]. We have identified and quantified after enzymatic cleavage (by glucuronidase and sulfatase) the aglycone and methylated forms of catechins using corresponding commercially available standards. Concentration in plasma with time curves from 9 subjects were constructed and evaluated.

Materials and methods

Standards

Catechins standards (catechin, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, (−)-epigallocatechin gallate) were purchased from Extrasynthèse (Lyon, France) except methylated catechins (3′-O-Me-EGC and 4′-O-Me-EGC) which were purchased from Nacalai Tesque, Inc (Kyoto, Japan).

Subjects

Twelve healthy subjects were recruited for this study. Nine subjects (4 men, 5 women) completed the study. One subject dropped out and two others had an incomplete set of data (missing blood sample). The study was approved by the ethical committee of clinical research of the University of Lausanne, Switzerland (Protocol reference 136/07). Inclusion criteria included age 18–50 years, BMI 18–25, healthy and non-smokers.

Study design

The original protocol was a controlled 4-treatment cross-over study. Three of the treatments were considered for other objectives and will not be discussed in the present paper. One week prior to the first treatment, BMI was measured. Twenty-four hours prior to treatment until the end of the sampling period, the ingestion of coffee, tea, cola, alcohol, whole grain cereal (white bread allowed), certain fruits or vegetables or any medication was not allowed. Only water could be drunk ad libitum during the night and in the morning before the treatment. After giving their informed consent, subjects arrived fasted early in the morning at the metabolic unit. Baseline blood was sampled, and then subjects received 400 mL of 1.25% (w/v) green tea infusion (“green tea treatment”). Blood was collected at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 11 and 12 h after drinking the beverage. A standard lunch and dinner were

[1] Bioavailability: in the present study, bioavailability is limited to plasma appearance of polyphenols over time.

provided at the metabolic unit. Water was available ad libitum. Twenty-four hours after receiving the treatment, a final blood sample was taken to assess return to baseline.

Quantification of green tea beverage

Green tea was infused for 3 min in boiling water and allowed to cool down to 50 °C. Tea was then diluted 1:1 with methanol. Serial dilution (1:50, 1:250, 1:500) was done (independent triplicate was done for each concentration) from that mixture with water: formic acid: methanol (75:5:20). Quantification was done by spiking standards in the same final solution. Mean concentration was calculated based on the standard curve and dilution factors.

Plasma extraction

Extraction of green tea catechins from plasma after sulphatase and β-glucuronidase treatment has been described elsewhere in detail [31].

The performance of the cleaving enzymes is not known precisely since authentic standards of sulphated and glucuronidated EGC are not commercially available. Consequently, the efficacy of the enzymes to cleave conjugated forms is assumed to be 100%.

Quantification of green tea catechins in plasma

Analyses of catechins (except methylated forms) were performed via HPLC with electrochemical (coulometric) detection and have been already described in details elsewhere [31]. To take into account the analyte losses during sample preparation (recovery), all calibration curves were established in blank plasma matrix by measuring the response of known analyte enrichment.

Analyses of methylated forms were performed by liquid chromatography-mass spectrometry (LC–MS) on a Waters Acquity liquid chromatograph using a Waters Acquity BEH C18 column (1.7 µm, 150 × 2.1 mm). Eluents were acidified water (0.5% formic acid, solvent A) and methanol (solvent B). The following gradient was applied: 0–1 min isocratic at 82% A; 1–2 min linear gradient to 75% A; 2–7 min linear gradient to 70% A; 7–10 min linear gradient to 60% A; 10–13 min linear gradient to 30% A; 13–13.1 min linear gradient to 1% A; 13.1–16 min isocratic at 1% A; 16–16.1 min linear gradient to 82% A; 16.1–20 min equilibration at 82% A. A Waters Synapt HDMS mass spectrometer equipped with an electrospray ion source operated in negative ion mode was used for the detection. Plasma extracts obtained from 200 µL plasma were redissolved in 200 µL water : acetonitrile 7:3 acidified with 1% formic acid. 10 µL was injected into the LC–MS system. Identity of 4′-O-Me-EGC was confirmed by accurate mass (better than 5 ppm), fragmentation fingerprint and retention time data (co-chromatography). For quantification, m/z 0.05 wide ion traces were plotted, and peak integration was performed using the software Metabolynx V4.1 (SCN639).

Methylated catechins were quantified by comparing their peak area to that of epigallocatechin that served as an internal standard (already quantified by coulometric detection, see above). Relative response of the internal standard and methylated catechins was determined by analysing peaks areas of both compounds in mixtures with defined ratio. The relative response of EGC to 4′-O-Me-EGC was determined to be 1:1.69.

Statistical analyses

Participants were randomized to sequences issued from a 4 × 4 Williams Latin square. The areas under the available plasma curve (AUC) of green tea catechins were calculated over 12 h by the trapezoidal method (between the baseline set at 0 nM and the plasmatic curve). Additionally, area was also measured between the limit of quantification (LOQ) set for each compound and the curve itself.

Results

Beverage composition

Quantitative analyses of the green tea infusion (1.25% in 400 mL water) done via electrochemical detection showed that it contained 7 mg of C, 37 mg of EC, 39 mg of ECg, 81 mg EGC and 134 mg of EGCg. No methylated forms were found in the beverage.

Catechin plasma kinetics

We established a method [31] to measure C, ECg, EC, EGC and EGCg in plasma after green tea consumption. However, under in vivo conditions, only EC, EGC and EGCg could be detected. These compounds were absorbed very quickly with a C_{max} around 1–2 h after ingestion (Fig. 1; Table 1). Clearance from plasma was also rapid and was back to baseline 6–8 h after ingestion with a monophasic response. Using the LC–MS approach, EGC methylated at the 4′ position was detected and its kinetics followed a shape similar to its parent compound quantified here in its aglycone form after sulfatase and β-glucuronidase treatment (Fig. 1; Table 1). The mean area under the curve for plasma 4′-O-Me-EGC is about 40% that of the parent compound EGC. However, if the area is measured between the LOQ and the curve itself, 4′-O-Me-EGC is only 30% of the parent

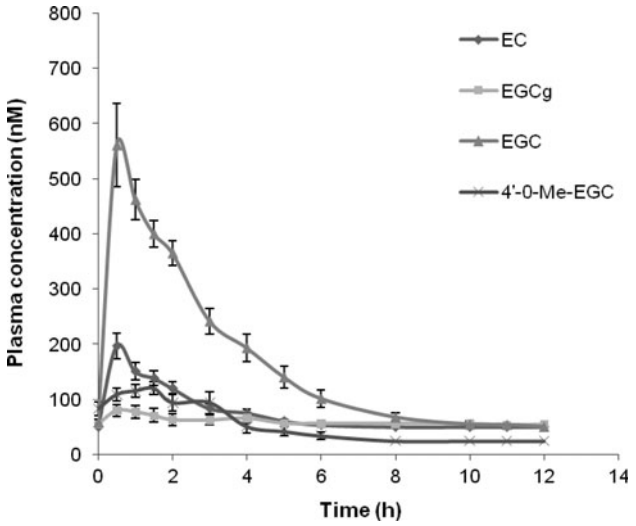

Fig. 1 Plasma kinetics of catechins after ingestion of green tea in healthy humans

compound EGC. Interestingly, the AUC of EGCg is about 35% of EGC, but when the curve is measured with LOQs and not 0 nM baseline level, the area of EGCg falls to about 10% of EGC.

Discussion

The present study focused on identifying and quantifying, using appropriate standards, catechins and methyl derivatives in human plasma samples after green tea ingestion.

Several human studies have described the bioavailability of catechins from green tea, using mainly green tea extracts, decaffeinated or not [7–17]. The ingested amounts of extracts varied between 1 and 4.5 g. EGC, EGCg and EC were the main compounds detected and studied in plasma due to their high amount in green tea. The bioavailability of ECg is poor, and catechin was never detected in plasma after green tea extract ingestion, probably due to the low amount of catechin in green tea. These data are in agreement with the results reported in the present study.

One of the early key studies to report plasma pharmacokinetics of green tea catechins was by Yang et al. [8]. Doses of 1.5, 3 or 4.5 g decaffeinated green tea extract were ingested by the volunteers. EGCg, EGC and EC appeared in plasma 30 min after ingestion. Their T_{max} were between 1 and 3 h depending on the compound but were not affected by the dose. For all 3 doses, EGC peaked at a much higher concentration than EGCg despite being less abundant in the original beverage. This result was in accordance with the results of the present study and by several other authors [9, 11, 13] showing that bioavailability of EGC is greater than that of EGCg.

Although polyphenols are often present as conjugates in plasma, a proportion of green tea catechin in plasma escapes this conjugation process and appears in plasma in the native form (unconjugated). After ingestion of green tea extract, more than 92% of EGCg measured in plasma was detected as the unconjugated form [12, 32]. However, Lee et al. found only 12–28% of EGCg in the unconjugated form [7]. Sulphation was the most abundant conjugation pathway for EGCg, representing about 58–72% of total EGCg, whereas the concentration of glucuronidated forms was minor (8–19%) [7]. By contrast, EGC and EC were present predominantly in plasma as conjugates [7, 11, 12]. Conjugated forms of EC were two-thirds as sulphate and one-third as glucuronide, while those of EGC were mostly as glucuronides (57–71%); the sulphates representing 23–36% of the total conjugates [7].

Lee et al. [11] fed 20 mg green tea solid/kg body weight to 8 subjects. They detected a methylated form of EGC (4′-O-Me-EGC) that was present in plasma at higher concentration than EGC. The amount of 4′-O-Me-EGC represented 8–31% of the ingested EGC and its T_{max} was measured at 1.7 ± 0.5 h [29]. In another study, a methylated form of EGCg, namely 4′,4″-DiMe-EGCg, was identified in plasma [30]. Its C_{max} reached 20.5 ± 7.7 nmol/L at 2 h after ingestion. In our study, we also found 4′-O-Me-EGC, but its AUC and C_{max} were consistently lower than those of EGC in contrast to that found by Meng [29].

More recently, Stalmach et al. [16] fed 500 mL of Choladi green tea containing 648 μmol of flavan-3-ols to 10 healthy subjects. They did not treat plasma samples with β-glucuronidase or sulfatase, and relative quantification was based on standard curves of parent compounds, as

Table 1 Plasmatic bioavailability parameters of catechins after ingestion of green tea in healthy humans

	EC	EGC	EGCg	4′Me-EGC
AUC (μM/h)[a,b]	53.8 ± 2.6	121.6 ± 7.7	43.7 ± 4.6	46.0 ± 6.2
Area between LOQ and curve (μM/h)[a,c]	17.8 ± 2.6	85.6 ± 7.7	8.6 ± 4.6	27.9 ± 6.2
C_{max} (nM)[a]	202.6 ± 21.1	572.1 ± 72.0	96.0 ± 11.2	137.2 ± 15.3
T_{max} (min)[a]	46.6 ± 11.3	43.3 ± 7.2	101.3 ± 30.2	127.5 ± 19.8

[a] Values are expressed as mean ± standard error

[b] AUC was measured as area between the baseline set at 0 nM and the curve

[c] Limit of quantifications (LOQ) were set at 50 nM for EC, EGC and EGCg and at 25 nM for 4′Me-EGC

commercial standards of those conjugated molecules were not available. They did find EGC to be the major metabolite, mostly in its glucuronidated form, but it peaked at 126 nM, much lower than the level found in the present study after full enzymatic deconjugation. They were also able to find 4′-O-Me-EGC both in sulphated (C_{max} 90 nM) and glucuronidated (46 nM) form. Here again, the AUC of 4′-O-Me-EGC expressed as aglycone appeared to be greater than the AUC of its parent compound. Del Rio et al. [17] fed 400 mL of ready to drink green tea containing 400 µmol of flavan-3-ols to 20 healthy subjects. They found data similar to Stalmach et al. [16]. Although both papers did an excellent job in improving the number of metabolites detected after green tea ingestion, one should be careful about the actual quantification of those metabolites. The MS responses of the conjugates may be different than those of the aglycone forms. Hence, there may be an over- or under-estimation of the plasma concentration of those molecules. In the present study, we used a commercially available standard to accurately determine the plasma concentrations of 4′-O-Me-EGC. Unambiguous identification of the isomeric structure of methylated EGC could also be performed, since complete chromatographic resolution of 3′-and 4′-isomers was achieved. According to this, the only observed circulating isomer was the 4′-O-Me-EGC.

We also looked for the presence of 3′-O-Me-EGC again using the appropriate standard but we could not detect this form.

Conclusions

Our aim was to quantify the appearance of green tea catechins in plasma with particular emphasis on their methylated forms. In addition to already known forms of catechins, we also detected significant amount of 4′-O-Me-EGC. Kinetics of this metabolite followed its parent compound, and the relative abundance of 4′-O-Me-EGC is ~40% compared to the parent EGC.

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References

1. Graham HN (1992) Green tea composition, consumption and polyphenol chemistry. Prev Med 21:334–350
2. Crozier A, Jaganath IB, Clifford MN (2009) Dietary phenolics: chemistry, bioavailability and effects on health. Nat Prod Rep 26:1001–1043
3. Friedman M (2007) Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Mol Nutr Food Res 51:116–134
4. Miura Y, Chiba T, Miura S, Tomita I, Umegaki K, Ikeda M, Tomita T (2000) Green tea polyphenols (flavan 3-ols) prevent oxidative modification of low density lipoproteins: an ex vivo study in humans. J Nutr Biochem 11:216–222
5. Wolfram S (2007) Effects of green tea and EGCG on cardiovascular and metabolic health. J Am Coll Nutr 26:373S–388S
6. Thielecke F, Boschmann M (2009) The potential role of green tea catechins in the prevention of the metabolic syndrome—a review. Phytochemistry 70:11–24
7. Lee MJ, Wang ZY, Li H et al (1995) Analysis of plasma and urinary tea polyphenols in human subjects. Cancer Epidemiol Biomarkers Prev 4:393–399
8. Yang CS, Chen L, Lee MJ et al (1998) Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. Cancer Epidemiol Biomarkers Prev 7:351–354
9. Chow HHS, Cai Y, Alberts DS et al (2001) Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and Polyphenon E1. Cancer Epidemiol Biomarkers Prev 10:53–58
10. Van Amelsvoort JMM, van Het Hof KH, Mathot JNJJ et al (2001) Plasma concentration of individual tea catechins after a single oral dose in humans. Xenobiotica 31:891–901
11. Lee MJ, Maliakal P, Chen L et al (2002) Pharmacokinetics of tea catechins after ingestion of green tea and (−)-Epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol Biomarkers Prev 11:1025–1032
12. Chow HHS, Cai Y, Hakim IA et al (2003) Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and Polyphenon E in healthy individuals. Clin Cancer Res 9:3312–3319
13. Henning SM, Niu Y, Lee NH et al (2004) Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement. Am J Clin Nutr 80:1558–1564
14. Chow HHS, Hakim IA, Vining DR et al (2005) Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. Clin Cancer Res 11:4627–4633
15. Henning SM, Niu Y, Liu Y et al (2005) Bioavailability and antioxidant effect of epigallocatechin gallate administered in purified form versus as green tea extract in healthy individuals. J Nutr Biochem 16:610–616
16. Stalmach A, Troufflard S, Serafini M et al (2009) Absorption, metabolism and excretion of Choladi green tea flavan-3-ols by humans. Mol Nutr Food Res 53:S44–S53
17. Del Rio D, Calani L, Cordero C, Salvatore S, Pellegrini N, Brighenti F (2010) Bioavailability and catabolism of green tea flavan-3-ols in humans. Nutrition Article in press
18. Harada M, Kan Y, Naoki H, Fukui Y, Kageyama N, Nakai M, Miki W, Kiso Y (1999) Identification of the major antioxidative metabolites in biological fluids of the rat with ingested (+)-catechin and (−)-epicatechin. Biosci Biotechnol Biochem 63:973–977
19. Walle T (2007) Methylation of dietary flavones greatly improves their hepatic metabolic stability and intestinal absorption. Mol Pharm 4:826–832
20. Walle T (2009) Methylation of dietary flavones increases their metabolic stability and chemopreventive effects. Int J Mol Sci 10:5002–5019

21. Wen X, Walle T (2006) Methylation protects dietary flavonoids from rapid hepatic metabolism. Xenobiotica 36:387–397
22. Wen X, Walle T (2006) Methylated flavonoids have greatly improved intestinal absorption and metabolic stability. Drug Metab Dispos 34:1786–1792
23. Mada-Yamamoto M, Inagaki N, Kitaura J, Chikumoto T, Kawahara H, Kawakami Y, Sano M, Miyase T, Tachibana H, Nagai H, Kawakami T (2004) O-methylated catechins from tea leaves inhibit multiple protein kinases in mast cells. J Immunol 172:4486–4492
24. Mateos R, Goya L, Bravo L (2006) Uptake and metabolism of hydroxycinnamic acids (chlorogenic, caffeic, and ferulic acids) by HepG2 cells as a model of the human liver. J Agric Food Chem 54 (23) 8724–8732
25. Baba S, Osakabe N, Natsume M, Yasuda A, Takizawa T, Nakamura T, Terao J (2000) Cocoa powder enhances the level of antioxidative activity in rat plasma. Br J Nutr 84:673–680
26. Baba S, Osakabe N, Natsume M, Muto Y, Takizawa T, Terao J (2001) In vivo comparison of the bioavailability of (+)-catechin, (−)-epicatechin and their mixture in orally administered rats. J Nutr 131:2885–2891
27. Miketova P, Schram KH, Whitney J, Li M, Huang R, Kerns E, Valcic S, Timmermann BN, Rourick R, Klohr S (2000) Tandem mass spectrometry studies of green tea catechins. Identification of three minor components in the polyphenolic extract of green tea. J Mass Spectrom 35:860–869
28. Lee MJ, Maliakal P, Chen L, Meng X, Bondoc FY, Prabhu S, Lambert G, Mohr S, Yang CS (2002) Pharmacokinetics of tea catechins after ingestion of green tea and (−)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol Biomarkers Prev 11:1025–1032
29. Meng X, Lee MJ, Li C, Sheng S, Zhu N, Sang S, Ho CT, Yang CS (2001) Formation and identification of 4′-O-Methyl-(−)-epigallocatechin in humans. Drug Metab Disp 29:789–793
30. Meng X, Sang S, Zhu N, Lu H, Sheng S, Lee MJ, Ho CT, Yang CS (2002) Identification and characterization of methylated and ring-fission metabolites of tea catechins formed in humans, mice, and rats. Chem Res Toxicol 15(8):1042–1050
31. Renouf M, Guy P, Marmet C, Longet K, Fraering AL, Moulin J, Barron D, Dionisi F, Cavin C, Steiling H, Williamson G (2010) Plasma appearance and correlation between coffee and green tea metabolites in humans. Br J Nutr 9:1–6
32. Kotani A, Miyashita N, Kusu F (2003) Determination of catechins in human plasma after commercial canned green tea ingestion by high-performance liquid chromatography with electrochemical detection using a microbore column. J Chromato B 788:269–275