Bioavailability and catabolism of green tea flavan-3-ols in humans

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Running title: Green tea flavanols bioavailability.

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Authorship: LC, NP and SS participated in requesting approval from the ethics committee, subject recruitment, collection and interpretation of the clinical data, and subjects' management; DDR was responsible of conception and design of the study; DDR & LC were the primary authors of the manuscript, and all other authors provided input and approved the final version of the manuscript; CC was involved in interpretation of laboratory data; FB was responsible for the study management and secured the funding.

Word count: 5120 words

Number of tables: 2

Number of figures: 4

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Acknowledgments: the study was supported by a research grant from Soremartec SpA, Alba, Italy.

Abstract

Objective: Aim of this study was to investigate green tea flavan-3-ol catabolism, plasma pharmacokinetic and urinary excretion by HPLC-MS/MS to evaluate their absolute bioavailability, by taking into account all the known and some unknown catabolites deriving from their interaction with the gastrointestinal tract and its hosted microflora.

Research Methods & Procedures: A feeding study was carried out on 20 healthy human volunteers who ingested 400mL of a ready to drink green tea containing approximately 400 μmoles of flavan-3-ols. Urine and plasma were collected for 4 and 24 hours, respectively and 39 relevant catabolites were identified in these biological fluids by means of tandem mass spectrometry.

Results: In biological fluids, 39 relevant flavan-3-ol catabolites were identificated. In plasma, (–)-epigallocatechin-3-gallate was the only unmetabolised compound and the highest in absolute concentration if compared to (–)-epigallocatechin and (–)-epicatechin conjugates. Colonic microflora derived polyhydroxyphenyl-γ-valerolactones were by far the main urinary catabolites, averagely 10 times more concentrated than flavan-3-ol conjugates. The calculated bioavailability was equal to 39% and it is interesting to notice the great variability in urinary excretion of colonic metabolites among participants, probably related to differences in their own colonic microflora.

Conclusions: this study demonstrates that green tea catechins are more bioavailable than previously observed when colonic ring fission metabolites are taken into consideration. Regular consumption of RTD green tea containing flavan-3-ols allows a non marginal exposition of the human body to their catabolites, somehow justifying the numerous beneficial actions described as linked to green tea intake.

Word count 247

Keywords: catechins, polyphenols, absorption, mass spectrometry.

Introduction

Green tea is one of the major dietary sources of dietary polyphenols, catechins or flavan-3-ols being the main polyphenolic subclass present in tea leaves. Epidemiological evidence is mounting describing the protective effects of green tea consumption against the risk of cardiovascular diseases and mortality. Moreover, specific mechanisms, such as vascular elasticity and protection against oxidative stress, have been extensively investigated and are good candidates to explain these protective effects [1,2].

Bioavailability studies with green tea or green tea extracts have shown very different and controversial results [3], with urinary excretion ranging from unquantifiable traces to values close to 10% of the ingested amount [4,5]. One study with only five volunteers, however, observed that two major phenolic catabolites, (–)-5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone (M4) and (-)-5-(3',4'-dihydroxyphenyl)-γ-valerolactone (M6) accounted for up to 40% of the amount of ingested pure (–)-epigallocatechin and (–)-epicatechin [6]. Similar differences could be observed when bioavalability studies were carried out with the second great source of flavan-3-ols, chocolate [3,7,8] or when single molecules, like (–)-epigallocatechin-gallate or (+)-catechin were introduced as supplements [9,10].

These contrasting values present in the literature mainly derive from the complex catabolism which flavan-3-ols undergo within the human body, making it very difficult to recognise and quantify every single metabolite appearing in plasma and urine after green tea ingestion. Analytical constraints have drastically limited the identification and characterisation of flavan-3-ol catabolites in the past [11], and the lack of synthesised pure standards have significantly decreased the quality of their quantification. However, with all the health benefits attributed to green tea intake, it is now necessary to define the absorption and catabolism of its bioactive components with greater clarity, with the help of more advanced analytical methodologies which are now readily available in most research laboratories. Therefore, in this study we used high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) to investigate green tea flavan-3-ol catabolism,

plasma pharmacokinetic and urinary excretion with the aim of estimating the bioavailability of these molecules in detail, by taking into account all the known and some unknown catabolites deriving from their interaction with several loci within the gastrointestinal tract.

Materials and Methods

Tea and chemicals

Two hundred mL bottles of ready-to-drink (RTD) green tea beverage were supplied by Soremartec Italia S.r.l. (Alba, CN, Italy). This product represents the 1,4% of the Italian market of RTD tea and the 21,6% of the Italian market of RTD green tea (Iri Infoscan, May 08/May 09) [12]. The product was industrially made from Sri Lanka tea leaves. The manufacturing process was based on infusion in hot water which reproduces the traditional tea preparation. The RTD tea is composed by tea infusion (water, tea), sugar, dextrose, lemon juice, ascorbic acid, flavour. Pure (–)-epicatechin, (–)-epigallocatechin, (–)-epigallocatechin-3-gallate, (–)-epicatechin-3-gallate and gallic acid standards were obtained from Sigma (St. Louis, MO, USA). All the solvents and reagents were purchased from Carlo Erba Reagenti (Milano, Italy).

Human feeding study

The feeding study was carried out on 20 healthy human volunteers selected according to exclusion criteria including diabetes mellitus, cardiovascular events, chronic liver diseases or nephropathies, cancer, organ failure and taking antioxidant or vitamin integrators. The volunteers were 26 ± 5 years old (mean ± SD) and with an average BMI of 23 ± 3 kg/m^2. Each volunteer signed an informed consent and the study protocol was approved by the Ethics Committee for Human Research of the University of Parma.

For two days prior to, and 24 h after the ingestion of tea, the subjects followed a diet almost deprived of flavonoids and phenolic compounds by avoiding fruit and fruit juices, chocolate, nuts, vegetables, tea and any kind of herbal tea, coffee, wine and dietary antioxidant supplements. To

check for compliance, the volunteers were asked to fill a 3-day weighed food record during the two days before the study and the study day. On the day of the study, after an overnight fast, each subject drank 400 mL of the tea beverage. Urine was collected before the volunteers drank the tea and in 0-4, 4-7, 7-10 and 10-24h collection periods after ingestion. The volume of urine collected during each period was measured and stored at –80ºC. Suitable aliquots of urine samples were filtered with 0.45µm nylon filter (Waters, Milford, MA, USA) and directly analyzed by HPLC-MS/MS without further processing. In 5 volunteers, blood was collected before and 1, 2, 3 and 4h after green tea intake. Flavan-3-ols metabolites were extracted as described by Mata-Bilbao et al. [13] with some modification. Two mL of plasma were mixed with 1 mL of antioxidant solution (containing 20 mg/mL ascorbic acid and 1 mg/mL EDTA) and 45 µL of *o*-phosphoric acid. After 2 minutes of vortex mixing, the samples were diluted with 9 mL of water. A solid phase extraction with Waters Oasis HLB (1cc – 30mg) (Milford, MA, USA) was applied to the mixture. Cartridge activation was achieved by adding 1 mL of methanol, 1 mL of bidistilled water, 1 mL of 70% (v/v) DMF containing 0.1% (v/v) formic acid and water, respectively. The cartridges were washed with 2 mL of water and 1 mL of 30% (v/v) methanol. Tea catechin metabolites were then eluted with 0.3 mL of 70% (v/v) DMF containing 0.1% (v/v) formic acid, and 30 µL of the eluted solution was injected into the HPLC-MS/MS system.

HPLC- ESI-MS/MS analysis

Flavan-ols and their metabolites in tea, plasma and urine were analysed using a Waters 2695 Alliance separation module equipped with a Micromass Quattro Micro Api mass spectrometer fitted with an electrospray interface (ESI) (Waters, Milford, MA, USA). Separations were performed using a Waters Atlantis dC18 3 µm (2,1 x 150 mm) reverse phase column (Waters, Milford, MA, USA). The mobile phase, pumped at a flow rate of 0.17 mL/min, was a 15-min linear gradient of 5 to 30 % acetonitrile in 1% aqueous formic acid. The tuning of the mass spectrometer was optimised by infusing a standard of (–)-epicatechin into the source along with 5% acetonitrile in 1% aqueous

formic acid, the initial HPLC mobile phase, at a flow rate of 30µL/min. The ESI source worked in negative ionisation mode. Source temperature was 120ºC, desolvation temperature was 350°C, capillary voltage was 2.8 kV, cone voltage was 35 V. The collision energy for MS/MS identifications was set at 25 eV. Following HPLC separation and MS/MS identification, flavan-3-ols and their metabolites were quantified using HPLC with the MS operating in the selected ion recording (SIR) mode. Unmetabolised flavan-3-ols were quantified using calibration curves of the appropriate standard compound while metabolites of epicatechin and epigallocatechin in urine and plasma were quantified using epicatechin and epigallocatechin, respectively. Glucuronide, methyl and sulphate metabolites of the ring-fission metabolites of tea catechins (namely, γ-valerolactones) were quantified as epicatechin equivalents.

Results

Analysis of tea

The flavan-3-ol content of 400 mL of RTD green tea (n = 12, each sample coming from a different pack, all packs from the same lot) was 106.2 ± 3.9 µmol (mean ± SD) (–)-epigallocatechin; 164.4 ± 5.5 µmol (–)-epigallocatechin-3-gallate; 39.6 ± 1.7 µmol (–)-epicatechin; 48.9 ± 1.7 µmol (–)-epicatechin-3-gallate; 19.5 ± 1.3 µmol (+)-gallocatechin; 9.8 ± 0.9 µmol (+)-catechin; 15.5 ± 0.6 µmol (+)-gallocatechin-3-gallate), making a total of 403.9 ± 9.4 µmol of total flavan-3-ols. The gallic acid content was 38.8 ± 0.8 µmols.

Identification of flavan-3-ols and their metabolites in plasma and urine.

Plasma and urine samples collected at different time points after the ingestion of 400 mL of tea were analysed by HPLC-MS/MS. Eight and 39 relevant compounds were tentatively identified in plasma and urine, respectively. The criteria of identification are mainly based on previously reported mass spectrometric analyses [14,15] and is reported in Table 1.

In particular, metabolites derived by the action of intestinal or hepatic UDP-glucuronosyltranferases, sulfotransferases and catechol-O-methyltransferase were identified with

the mass spectrometric detector through the loss of the conjugating groups (i.e. glucuronic acid and sulphate) as previously described by Stalmach and colleagues [5], to give the aglycone fragment ion (i.e. EC at m/z 289, EGC at m/z 305 and their methylated counterparts, at m/z 303 and 319, respectively).

The metabolites derived from colonic bacteria ring fission activity have been mainly identified as described by Sang and colleagues [15]. Briefly, the ring fission products (-)-5-(3',4'-dihydroxyphenyl)-γ-valerolactone (M6 and M6′) share a molecular weight of 208, whereas the (–)-5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone (M4) weighs 224 amu. In the absence of reference compounds, it was impossible to distinguish the M6 and M6′ valerolactones, which, therefore, are reported as M6/M6′. The main metabolites based on these structures and excreted in urine were again derived from the interaction of valerolactones with UDP-glucuronosyltranferases, sulfotransferases and catechol-O-methyltransferase and were identified for their loss of glucuronic acid and sulphate groups to generate the aglycone fragment (i.e. M4 at m/z 223 and M6/M6′ at m/z 207 or the methylated counterpart of M4 at m/z 237).

Examples of plasma and urine chromatographic profiles are reported in Figures 1, 2 and 3.

Quantitative analysis of catabolites in urine and plasma

After identification, plasma and urine samples were analysed by MS in the selected ion recording (SIR) mode to quantify metabolites.

The pharmacokinetics of some flavan-3-ol metabolites in plasma is represented in Figure 4 (EC-sulphate not shown as the lowest in concentration). Epicatechin-3-gallate was identified in plasma from some of the volunteers, but in unquantifiable amount. EGCG is the only unmetabolised compound and the highest in absolute concentration. EGC catabolites reach their peak plasma concentration after 2 hours from ingestion, whereas EC catabolites generally show their Tmax at 1 hour.

The excretion of tea-related components in urine are reported in table 3 together with the excretion percentage of each compound with respect to the total amount of ingested catechins.

Isomers have been grouped into one single excretion value for each time point. The identified molecules that do not appear in table 3 were present in unquantifiable amount or in very low amount in less than the 20% of our study group.

Among catechin conjugates, the main metabolites excreted with urine were epigallocatechin-O-glucuronide and its methoxy counterpart methyl-*O*-epigallocatechin-O-glucuronide. The main EC metabolite was methyl-epicatechin-sulphate, whereas the sulphate metabolite of epigallocatechin was almost negligible along all the 24 hours. The main class of colonic catabolites is represented by M6 (which can derive from all catechins) and M6′ valerolactones (which can derive solely, like M4, from catechins with three hydroxyl groups on the B ring, namely gallocatechins), of which the sulphated and the glucuronidated forms are the highest contributors to total phenolic excretion. These molecules are by far the main excreted metabolites and their urinary concentration is averagely 10 times higher than that of flavanols conjugates.

It is interesting to note that, contrarily to what previously reported, all the flavanols conjugates, with the only exception of methyl-EGC-sulphated-glucuronide, reach their peak excretion within the first 4 hours. A totally different behaviour is followed by valerolactones, in good agreement with their generation and absorption in the large intestine.

Bioavailability was calculated as a ratio between the total metabolite excretion and the total intake of flavan-3-ols. Flavan-3-ols bioavailability in this study was 39.5 ± 19.0%. Gallic acid is excreted as the 5.8 ± 2.6% of the ingested amount.

Discussion

The consumption of ready-to-drink teas, generally called iced tea, is increasing in the western countries and is often overcoming the intake of traditional hot tea [12]. Considering the evidence that tea is one of the most significant sources of polyphenols in the human diet and that tea consumption [16] and polyphenol intake in general [17] are strongly related to reduced risk for several chronic diseases, the aim of our investigation was to assess the actual exposure of human

volunteers to the most representive and bioavailable tea polyphenols (namely, flavan-3-ols) after acute consumption of a ready-to-drink green tea prepared by infusion of tea leaves.

Several works described bioavailability values for green tea flavan-3-ols ranging from 2 to 8.1% [3,5]. However, most of these researches failed to identify the whole pattern of molecules deriving from the catechins originally present in tea. As it is described in this work and elsewhere, polyphenols in green tea undergo many chemical modification along the gastrointestinal tract and inside the human body, before and after absorption. First of all, catechins can be conjugated by human enzymes at the small intestine level [18]. UDP-glucuronosyltranferases, sulfotransferases and catechol-*O*-methyltransferase promptly transform these polyphenols into their more hydrophilic conjugated forms. The absorbed fraction subsequently comes across a series of similar hepatic conjugating enzymes, and further transformation can occur in the kidneys [18,19]. The only exception to this effective enzymatic action is EGCG, which appears in plasma unconjugated, as previously reported possibly as a consequence of the presence of the 3-*O*-galloyl moiety. ECG was also identified as aglycone, but in unquantifiable amount in this study.

The fraction of flavan-3-ols not absorbed in the small intestine reaches the large intestine, where it can undergo several microbial processes finally leading to smaller molecules which can also be absorbed, reach the liver and, subsequently, the systemic circulation [15]. Based on the site of formation and absorption, the appearance in and excretion from the human body of these classes of metabolites is remarkably different. The conjugates deriving from human enzyme activity share a very fast kinetic in human plasma and are mainly excreted during the first 4 hours. The only exception is methyl-EGC-sulphated-glucuronide, which appears delayed in excretion leaving room to the hypothesis of a major involvement of hepatic enzymes in its formation. On the contrary, microflora-derived γ-valerolactones show a completely different excretive kinetic. They are generated in the colon, later in time if compared with catechin conjugates, and are therefore absorbed and excreted with a delay of several hours, maximum urinary concentration being at 24h. The long time excretion of these colonic metabolites was tested in one subject of this study and

lasted up to 54h (data not shown). None of these molecules was present in plasma up to the 4th hour, the last collection time in this study. They have, however, been described elsewhere at high concentrations in plasma collected after 12 hours [20,21]. None of the valerolactones was excreted as unconjugated metabolites, but it is not possible defining the origin of the enzymes involved in this process (colonic or hepatic).

The identification of most of these metabolites was possible thanks to the availability of the tandem mass spectrometric (MS/MS) detector, and this is probably the reason why several literature studies failed to pinpoint most flavan-3-ol derived molecules. Common in previous research was the treatment of samples with deconjugating enzymes, namely microbial glucuronidases and sulphatases, which allows the detection of aglycons in biological fluids [20, 6, 11] with non MS/MS detectors. However, this treatment does not allow to understand the metabolic processes undergone by dietary catechins and, above all, this kind of detection does not consider the methoxy derivative, which constitutes a notable fraction of the total excreted flavanols (up to 6% in this study). Several studies failed in identifying and quantifying γ-valerolactones, which alone constitute almost the 90% of the excreted flavan-3-ol metabolites.

Sang and colleagues [15] gave the most complete and detailed description of the human urinary metabolite profile of tea polyphenols using liquid chromatography with electrospray ionization tandem mass spectrometry with data-dependent acquisition, but they did not set up a bioavailability study. Moreover, contrarily to what observed by Sang and colleagues [15], urinary unconjugated valerolactones were not found in this study and some molecules with double conjugation were observed in urine which were never described before (namely, (Epi)gallocatechin-sulphate glucuronide, methyl-(Epi)gallocatechin-sulphate glucuronide, M6-/M6’-sulphate glucuronide, M6-/M6'- disulphate). This could partly explain why some isomers of EC and EGC conjugates identified in the cited report [15] were not observed in urine samples in this study.

The peak concentration (C_{max}) of circulating metabolites after drinking RTD green tea in this study reaches a total 0.2µmol/L, which is in touch with a putative biological effect. The highest

concentration is reached by EGCG after 1.4 hours from tea consumption, whereas the lowest was that of methyl-(epi)catechin-sulphate. This is only partially in touch with what observed before [5], demonstrating a strong variability among subjects.

Considering just non colonic metabolites, the bioavailability value observed in this study would be less than 4%, in touch with previous reports [5,22]. However, it must be noticed that the great majority of the excreted flavan-3-ol catabolites are microbial ring fission valerolactones, raising the bioavailability figure to almost 40%, and that it would be much more feasible for these compounds to be bioactive, based on their high concentration in biological fluids. Unfortunately, the literature lacks reports on their biological effects and the fact that they are present in conjugated forms makes even more difficult their investigation as putative protective agents. Moreover, it is interesting to notice the great variability in urinary excretion of these metabolites among study volunteers (from 7% to 51% of total ingested catechins for M6/M6'-sulphate in this study), probably related to different characteristics of their own colonic microflora. This raises the hypothesis of a different biological effect linked, somehow, to the presence or absence of certain strains of bacteria in the gut, being this also a possible explanation for strong variability in biological effects of tea consumption along the population [23]. Some similar observation has already been reported in the literature. The intestinal microbial transformation of daidzein into equol is subject to a wide inter-individual variability [24] and could be related to prevention of bone loss and fat accumulation [25]. However, it is clearly to early to associate a biological effect somehow related to the intestinal ability to transform catechins into specific breakdown products. *In vitro* studies should be performed to reveal the activities of the most common probiotic bacteria towards green tea catechins and to evaluate the bioactivity of γ-valerolactones. Moreover, intervention studies should be run to investigate possible differences in the biological effect of green tea catechins as related to different colon catabolite production.

A main limitation of this study was related to quantification of metabolites. In fact, due to the lack of commercially available standards, the quantification of conjugates was performed in

Selected Ion Recording with the related aglycone (EC or EGC) as a standard. The γ-valerolactones were all quantified in SIR in EC equivalents. This approach, however, is conservative, as the MS/MS detector was tuned for epicatechin, being therefore extremely sensitive to this molecule and letting hypothesise a marginal underestimation of the bioavailability values. Moreover, the excretion profile of colonic valerolactones obviously continues after the 24^{th} hour, and urinary concentration of these catabolites is detectable up to the 54th hour after ingestion. This observation is consistent with a further underestimation of the true bioavailability value for flavan-3-ols as consumed through ready to drink green tea.

Conclusion

This study demonstrates that green tea catechins are more bioavailable than previously observed when colonic ring fission metabolites are taken into consideration (39% of the amount ingested). Regular consumption of RTD green tea containing flavan-3-ols allows a non marginal exposition of the human body to their catabolites, somehow justifying the numerous beneficial actions described as linked to green tea intake.

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Table 1: MS/MS identification and location of flavan-3-ols catabolites.

Table 2. Quantification of principal flavan-3-ols metabolites excreted in urine at different collection time, after consumption of 400 mL of ready to drink green tea by twenty human volunteers (mean values in μmol ± SD) and excretion percentage of total flava-3-ols ingested with tea. n.d. – not detected.

Figure 1. Multiple reaction monitoring (MRM) chromatograms of a plasma sample two hours after green tea consumption. A: (Epi)gallocatechin-glucuronide (m/z 481→305); B: (Epi)catechin-glucuronide (m/z 465→289); C: (-)-Epigallocatechin-3-gallate (m/z 457→169); D: (-)-Epicatechin-3-gallate (m/z 441→169)

Figure 2. Multiple reaction monitoring (MRM) chromatograms of a urine sample 4 hours after green tea consumption. A: (Epi)gallocatechin-glucuronide (m/z 481→305); B: (Epi)catechin-glucuronide (m/z 465→289); C: (Epi)catechin-sulphate (m/z 369→289); D: Methyl-(Epi)gallocatechin-sulphate glucuronide (m/z 575→399)

Figure 3. Multiple reaction monitoring (MRM) chromatograms of a urine sample 10-24 hours after green tea consumption. A: M4-sulphate (m/z 303→223); B: M6-/M6'-sulphate-glucuronide (m/z 463→287); C: M4-glucuronide (m/z 399→223); D: Methyl-M4-sulphate (m/z 317→237)

Figure 4. Four hour pharmacokinetic, C_{max} and T_{max} of plasma flavan-3-ols and their metabolites in five human volunteers after consumption of 400 mL of ready to drink green tea (Mean values in nmol/L ± standard error of the mean). **A**: Methyl-(Epi)gallocatechin-glucuronide; **B**: (Epi)gallocatechin-glucuronide; **C**: (Epi)catechin-glucuronide; **D**: (-)-Epigallocatechin-3-gallate; **E**: Methyl-(Epi)gallocatechin-sulphate; **F**: Methyl-(Epi)catechin-sulphate.

C_{max} = peak plasma concentration and T_{max} = time of peak plasma concentration (Mean values ± standard deviation).

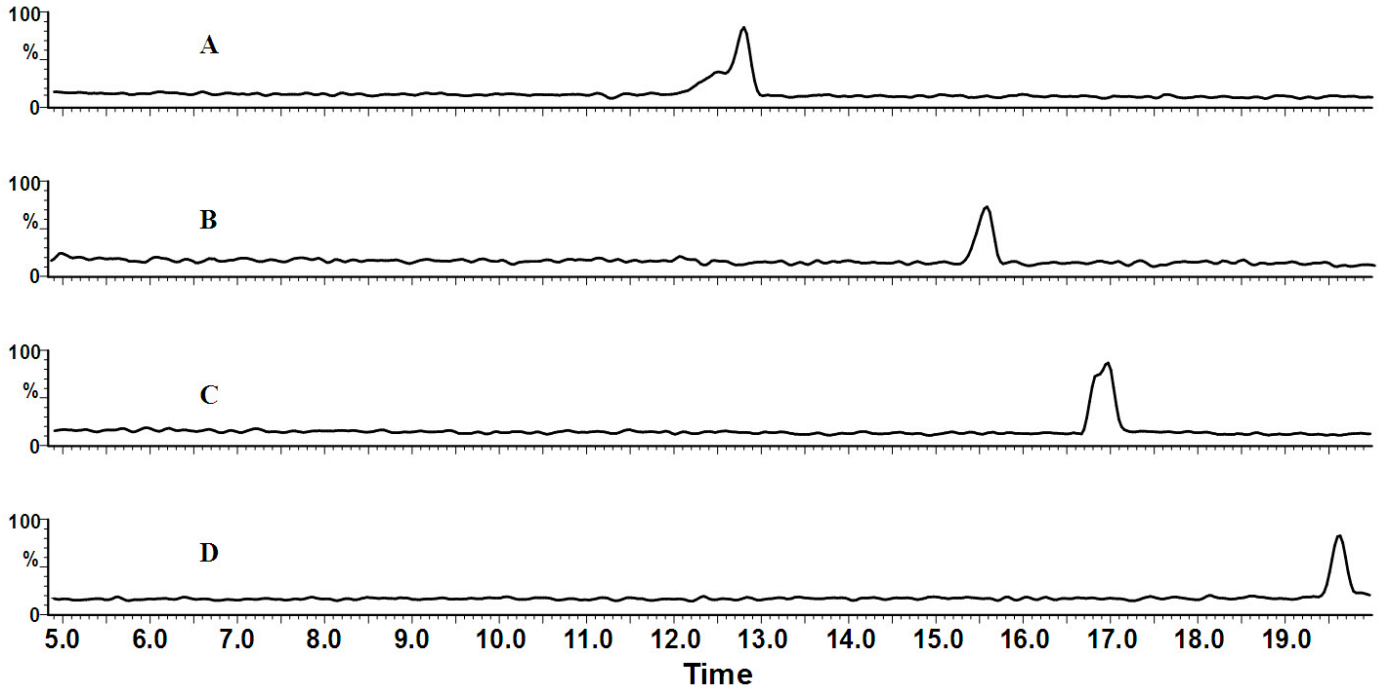
Table 1

	Molecule	$[M-H]^-$ (*m/z*)	MS^2 fragments (*m/z*)	LOCATION
1	(Epi)gallocatechin-sulphate glucuronide	561	385, 481, 305	Urine
2	M4-glucuronide	399	223	Urine
3	M4-sulphate	303	223	Urine
4	M6-/M6'-glucuronide	383	207	Urine
5	Methyl-(Epi)gallocatechin-sulphate glucuronide	575	495, 399, 319	Urine
6	M6-/M6'- sulphate glucuronide	463	287, 207	Urine
7	(Epi)gallocatechin-glucuronide	481	305	Plasma, urine
8	Methyl-(Epi)gallocatechin-sulphate glucuronide	575	495, 399, 319	Urine
9	(Epi)catechin-sulphate glucuronide	545	465, 369, 289	Urine
10	M6-/M6'- disulphate	367	287, 207	Urine
11	M6-/M6'- sulphate	287	207	Urine
12	(Epi)gallocatechin-sulphate	385	305	Urine
13	M6-/M6'- sulphate	287	287, 207	Urine
14	Methyl-(Epi)gallocatechin-glucuronide	495	319	Plasma, urine
15	(Epi)catechin-sulphate glucuronide	545	465, 369, 289	Urine
16	Methyl-M4-sulphate	317	237	Urine
17	(Epi)catechin-sulphate	369	289	Urine
18	(Epi)catechin-sulphate	369	289	Urine
19	(Epi)catechin-sulphate glucuronide	545	465, 369, 289	Urine
20	M4-sulphate	303	223	Urine
21	M6-/M6'-glucuronide	383	207	Urine
22	M4-glucuronide	399	223	Urine
23	Methyl-(Epi)gallocatechin-sulphate	399	319	Plasma, urine
24	(Epi)catechin-glucuronide	465	289	Plasma, urine
25	Methyl-(Epi)gallocatechin-sulphate	399	319	Plasma, urine
26	Methyl-(Epi)catechin-sulphate glucuronide	559	479, 383	Urine
27	Methyl-M4-sulphate	317	237	Urine
28	M6-/M6'-glucuronide	383	207	Urine
29	M6-/M6'- sulphate	287	207	Urine
30	Methyl-(Epi)catechin-glucuronide	479	303	Urine
31	(Epi)catechin-sulphate	369	289	Plasma, urine
32	Methyl-(Epi)catechin-sulphate glucuronide	559	479, 383	Urine
33	Methyl-(Epi)catechin-sulphate	383	303	Plasma, urine
34	Methyl-(Epi)catechin-glucuronide	479	303	Urine
35	(-)-Epigallocatechin-3-gallate	457	169	Plasma
36	Methyl-(Epi)catechin-sulphate	383	303	Plasma, urine
37	Methyl-(Epi)catechin-sulphate	383	303	Plasma, urine
38	Methyl-(Epi)catechin-sulphate	383	303	Plasma, urine
39	(-)-Epicatechin-3-gallate	441	289, 169	Plasma

Table 2

Catabolite	m/z	0-4 h	4-7 h	7-10 h	10-24 h	tot	% of intake
(Epi)catechin-sulphate	369	0.92 ± 0.51	0.37 ± 0.20	0.07 ± 0.08	0.08 ± 0.14	1.44 ± 0.60	0.36
Methyl-(Epi)catechin-sulphate	383	1.19 ± 0.47	0.52 ± 0.35	0.15 ± 0.09	0.13 ± 0.12	1.99 ± 0.57	0.49
(Epi)catechin-glucuronide	465	0.85 ± 0.39	0.27 ± 0.08	0.13 ± 0.07	0.04 ± 0.07	1.29 ± 0.51	0.32
(Epi)catechin-sulphate glucuronide	545	0.12 ± 0.07	0.07 ± 0.04	0.01 ± 0.03	n.d.	0.20 ± 0.10	0.05
(Epi)gallocatechin-sulphate	385	0.06 ± 0.10	0.01 ± 0.02	0.01 ± 0.02	n.d.	0.08 ± 0.11	0.02
Methyl-(Epi)gallocatechin-sulphate	399	1.06 ± 0.78	0.56 ± 0.58	0.01 ± 0.06	n.d.	1.63 ± 1.07	0.40
(Epi)gallocatechin-glucuronide	481	2.81 ± 1.17	1.00 ± 0.41	0.35 ± 0.17	0.23 ± 0.13	4.39 ± 1.35	1.09
Methyl-(Epi)gallocatechin-glucuronide	495	1.48 ± 0.60	0.82 ± 0.34	0.35 ± 0.13	0.28 ± 0.20	2.93 ± 0.87	0.73
Methyl-(Epi)gallocatechin-sulphate-glucuronide	575	0.12 ± 0.07	0.14 ± 0.07	0.08 ± 0.06	0.03 ± 0.05	0.37 ± 0.20	0.09
M6-/M6′-sulphate	287	0.62 ± 1.52	8.75 ± 12.92	16.21± 15.36	65.63 ± 33.62	91.21 ± 51.03	22.58
M6-/M6′-disulphate	367	n.d.	n.d.	0.01 ± 0.05	n.d.	0.01 ± 0.05	0.00
M6-/M6′-glucuronide	383	0.61 ± 0.31	2.02 ± 3.28	4.37 ± 4.30	12.52 ± 6.84	19.52 ± 12.10	4.83
M6-/M6′-sulphate glucuronide	463	n.d.	0.12 ± 0.17	0.26 ± 0.24	0.69 ± 0.41	1.07 ± 0.64	0.26
M4-sulphate	303	0.05 ± 0.20	1.39 ± 1.96	2.98 ± 4.44	5.26 ± 6.82	9.68 ± 12.12	2.40
Methyl-M4-sulphate	317	0.10 ± 0.25	2.34 ± 3.83	5.83 ± 8.12	10.78 ± 12.74	19.05 ± 22.43	4.72
M4-glucuronide	399	n.d.	0.57 ± 1.07	1.84 ± 2.38	2.40 ± 3.95	4.81 ± 6,00	1.19

Figure 1


100
%
0
A
B
C
D
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
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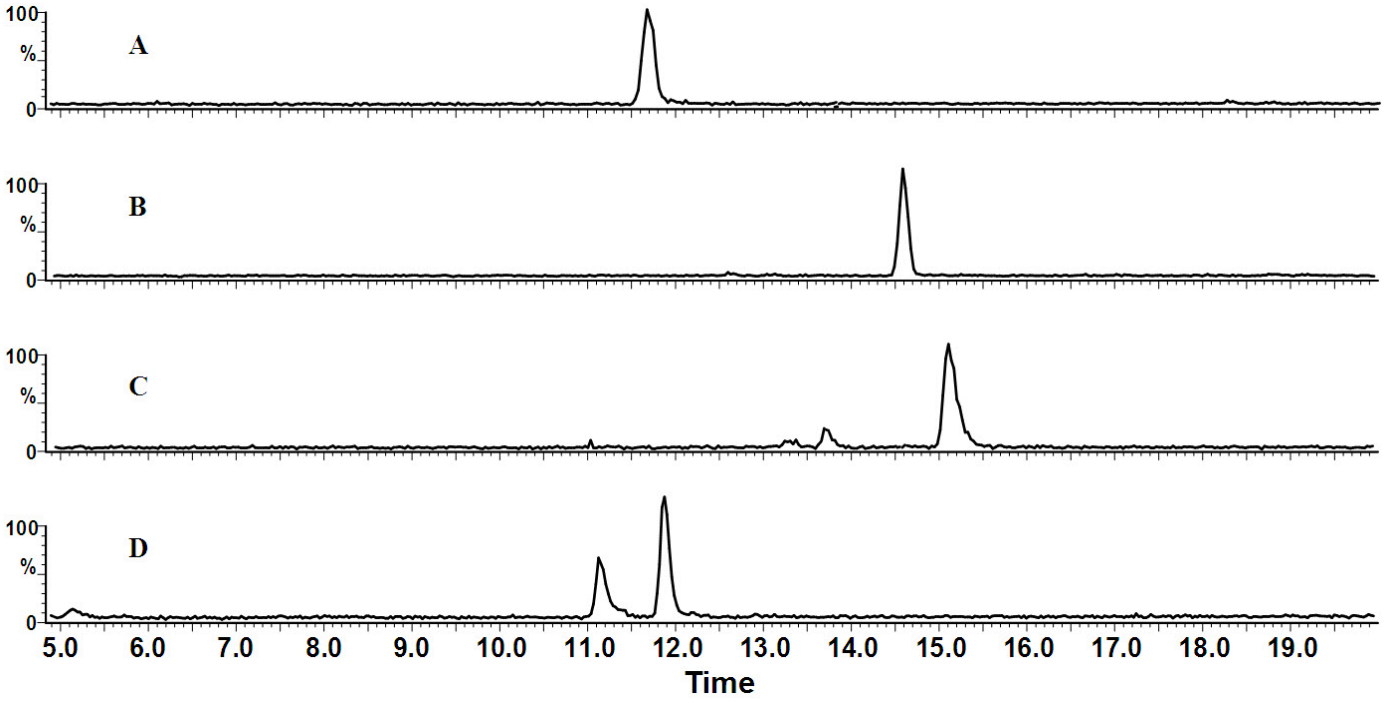
17.0
18.0
19.0
Time

Figure 2


100
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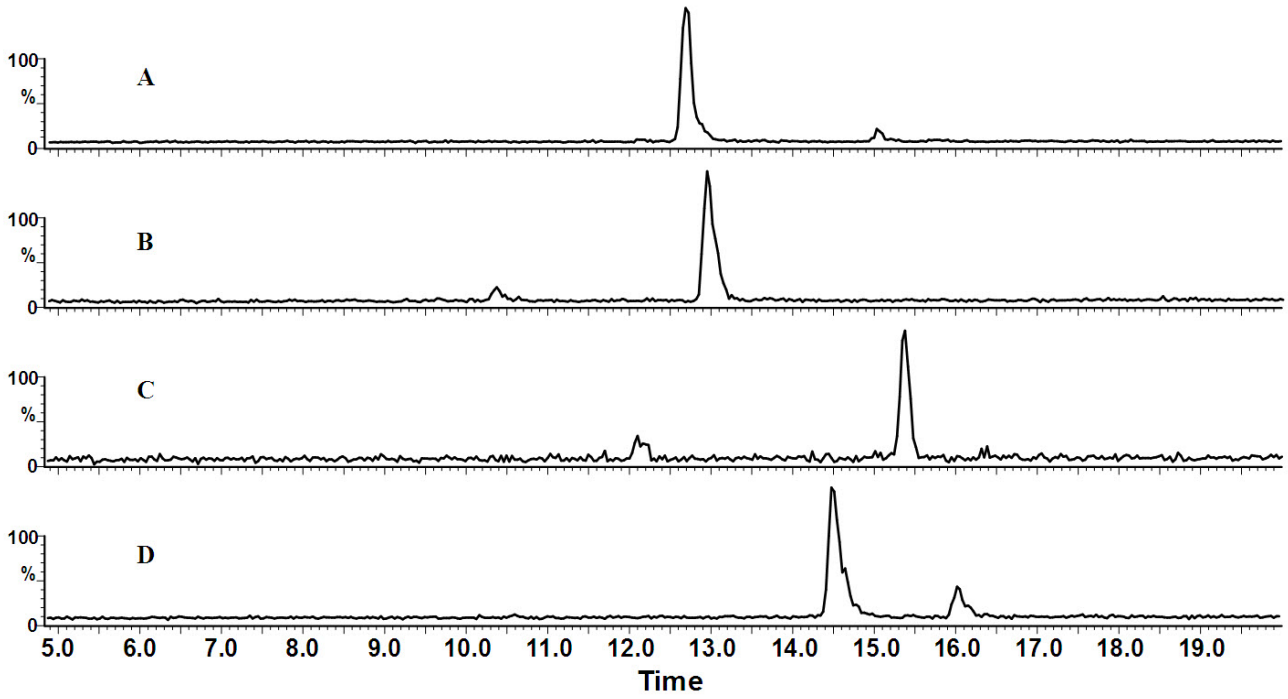
15.0
16.0
17.0
18.0
19.0
Time

Figure 3


A
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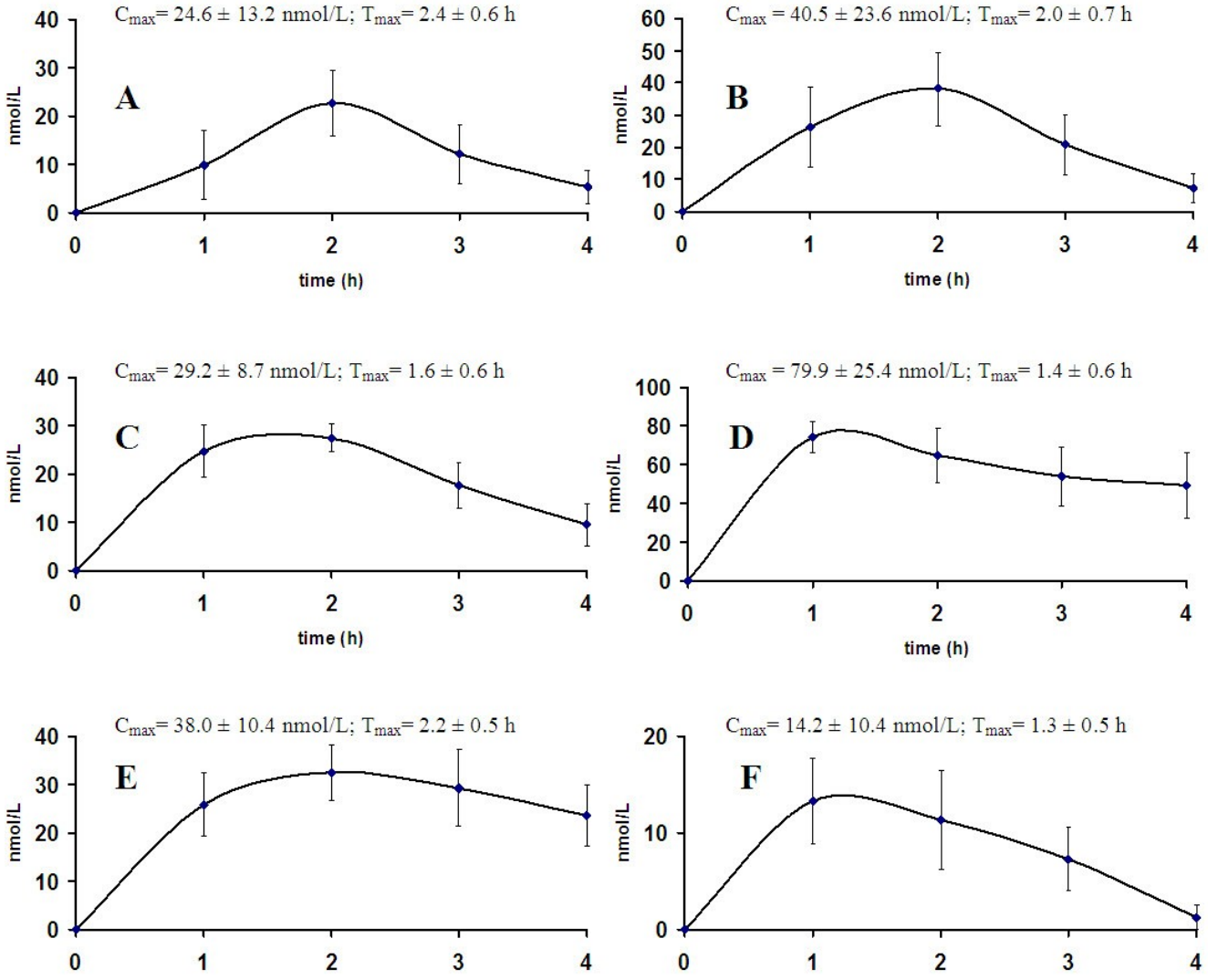
Time

Figure 4


C_{max}= 24.6 ± 13.2 nmol/L; T_{max}= 2.4 ± 0.6 h
A
C_{max} = 40.5 ± 23.6 nmol/L; T_{max}= 2.0 ± 0.7 h
B
C_{max}= 29.2 ± 8.7 nmol/L; T_{max}= 1.6 ± 0.6 h
C
C_{max} = 79.9 ± 25.4 nmol/L; T_{max}= 1.4 ± 0.6 h
D
C_{max}= 38.0 ± 10.4 nmol/L; T_{max}= 2.2 ± 0.5 h
E
C_{max}= 14.2 ± 10.4 nmol/L; T_{max}= 1.3 ± 0.5 h
F
nmol/L
time (h)