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Journal of Chromatography B, 727 (1999) 179–189

JOURNAL OF CHROMATOGRAPHY B

Validated method for the quantitation of quercetin from human plasma using high-performance liquid chromatography with electrochemical detection

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Received 5 August 1998; received in revised form 11 December 1998; accepted 8 January 1999

Abstract

A validated method for the quantitation of trace levels of quercetin from human plasma to be used in pharmacokinetic and biomarker studies is presented. Quercetin conjugates were hydrolysed enzymatically, plasma proteins were removed using a Bond Elut C18 extraction column and additional interferences were removed by extracting them into a toluene–dichloromethane mixture. The HPLC system consisted of an Inertsil ODS-3 column (250×4.0 mm) and a mobile phase with 59% methanol in phosphate buffer (pH 2.4). High selectivity and a low quantitation limit (0.63 μg/l) were achieved by using electrochemical detection at a low potential. The method has excellent reproducibility: R.S.D. values of peak-heights were 2% and 7.9%, respectively, for within-day and between-day precision. The method was applied to a small scale study of quercetin pharmacokinetics and quercetin was shown to be absorbed from a 20 mg dose. No free quercetin was detected in plasma and no evidence of significant amounts of quercetin glycosides in plasma was found.

Keywords: Quercetin

1. Introduction

The flavonoids are a group of polyphenols widely occurring in the plant kingdom. One of the flavonoid subclasses, the flavonols, and particularly quercetin, has received much attention during the past few years. Quercetin has been reported to have many biological activities, such as antioxidant, antithrombotic, anti-inflammatory and anticarcinogenic activities [1]. Epidemiological studies suggest that dietary flavonols, including quercetin, could prevent coronary heart disease [2] and cancer [3]. Quercetin is one of the most abundant flavonoids in the diet with an intake estimated as 2.6–38.2 mg per day [4]. The compound is mainly present as glycosides in foods, with the aglycone form being quite rare. The structures of quercetin aglycone and quercetin glycosides present in onions (spireaoside) and tea (rutin), are shown in Fig. 1.

Although in vitro studies and animal experiments show that quercetin and other flavonoids are bio-

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0378-4347/99/$ – see front matter
PII: S0378-4347(99)00049-3

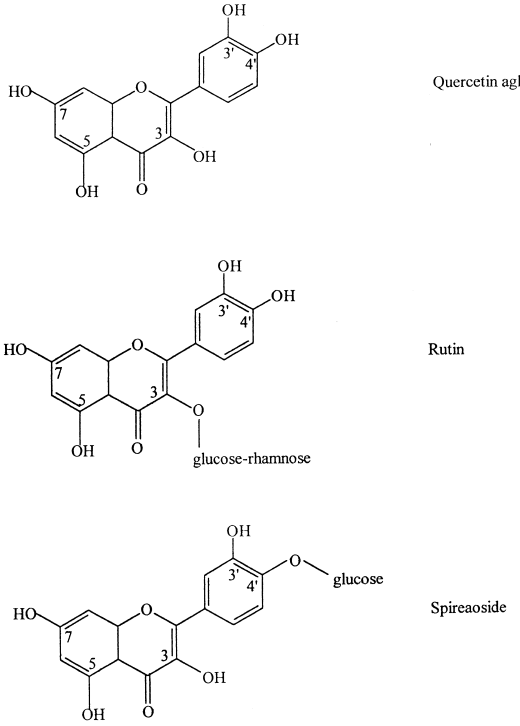

Fig. 1. Structures of quercetin, rutin and spireaoside.

logically active in many respects, it has been difficult to draw any definite conclusions about their relevance to human health. Analytical methods suitable for the measurement of quercetin from human tissues have only been available for a few years. They have mainly been based on HPLC with UV-detection [5–9], which lacks both the selectivity and sensitivity required, when analyzing low levels of quercetin in plasma. Recently, analytical methods with lower detection limits, based on HPLC with fluorometric [10] or electrochemical [11,12] detection, have been introduced. Little validation data, however, or data about the stability of quercetin during the different steps of analysis, have been presented. Previous reports [13–15] and preliminary experiments in our laboratory have shown that quercetin is unstable under various conditions.

Information about the different forms of quercetin present in plasma is scarce. This information is relevant, because conjugation can considerably affect the biological activity of a compound. It has been assumed that quercetin, as many other compounds, is conjugated with glucuronide and/or sulfate groups in the liver [16]. Quercetin could also be present in plasma as glycosides, as proposed by Hollman et al. [17]. In one study [18], large amounts of rutin and other quercetin glycosides were found in plasma of unsupplemented subjects, by using HPLC with diode-array detection, and UV-spectra for identification.

This paper describes a reproducible, sensitive and selective method for the quantitation of trace levels of quercetin aglycone in plasma. The method can be used in supplementation studies and when analyzing quercetin in plasma of unsupplemented subjects. In this report, the method was applied to a small scale study of quercetin pharmacokinetics. We also attempted to assess whether unconjugated quercetin or quercetin glycosides are present in plasma.

2. Experimental

2.1. Reagents and chemicals

Quercetin, rutin, epicatechin, (+)-catechin, tert.-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), dithiothreitol (DTT), ubiquinone (Q10), β-glucosidase, β-glucuronidases (type HP-2, B and IX-A) and sulfatase (type V) were obtained from Sigma (St. Louis, MA, USA). Spireaoside was purchased from Extrasynthese (Genay, France). Trolox, (R)-(+)-trolox and (S)-(−)-trolox were obtained from Aldrich Chem. Co. (Milwaukee, WI, USA). Ascorbic acid, oxalic acid, potassium dihydrogen phosphate and sodium acetate were purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade. Solvents were from BDH (Poole, UK), and of HPLC grade, except for ortho-phosphoric acid, toluene and dichloromethane, which were ARISTAR grade. Distilled and deionized water was used for the preparation of all solutions.

2.2. Sample collection

A high-quercetin pool was prepared by combining plasma obtained from 15 healthy subjects, male and female, who had been asked to consume 3 onions, 5 cups of tea or 200 g of lingonberries on the day before, and on the morning of sample collection. A

low-quercetin pool was obtained by combining plasma from 5 subjects who had been following a strict quercetin-free diet for 5 days. Fasting blood samples were collected into EDTA tubes and centrifuged at 1000 *g* for 15 min at room temperature. The pooled plasma samples were immediately frozen and stored in −70°C. The plasma pools were used in the validation of the method and later as references in the analysis of samples from supplementation studies.

2.3. Hydrolysis and extraction procedures

Quercetin conjugates were hydrolysed by incubating 1 ml of EDTA plasma with 110 μl of 0.78 *M* sodium acetate buffer (pH 4.8), 100 μl of 0.1 *M* ascorbic acid and 40 μl of a crude preparation from *Helix pomatia*, containing 4000 U β-glucuronidase and 200 U sulfatase activity (type HP-2, Sigma), for 17 h at 37°C.

After incubation, the sample was diluted with 2 ml of phosphate buffer (70 m*M*, pH 2.4) and added to a Bond Elut C18 solid phase extraction column, preconditioned with 6 ml of methanol and 6 ml of phosphate buffer. The column was washed with 9 ml of phosphate buffer and 0.5 ml of water. The column was dried in nitrogen atmosphere by suction and quercetin was eluted into a conical glass tube with 2 ml of methanol. The methanol eluate was dried under a gentle stream of nitrogen at 50°C using an N-evap evaporator (Organomation, Berlin, Ma, USA). When the tube was completely dry, 1 ml toluene–dichloromethane (80:20, v/v) and 200 μl of 5.3 *M* acetic acid–32 m*M* oxalic acid (80:20, v/v) (pH 2.4) were added. The tubes were shaken with a multi-tube vortex for one minute and centrifuged for 15 min at 1000 *g*. Of the lower phase,150 μl was transferred into 200 μl vials (Chromacol, Welwyn Garden City, UK) for HPLC analysis.

2.4. Chromatographic conditions

Chromatographic analysis was performed with a system consisting of an HP 1090 liquid chromatograph, an HP 3396 II integrator with a 9122 C/D disc drive (Hewlett-Packard, Palo Alto, CA, USA), a Coulochem 5100A electrochemical detector with a model 5011 analytical cell (ESA inc., Chelmsford, Ma, USA) and an Inertsil ODS-3 analytical HPLC column (250×4.0 mm I.D., 5 μm) (GL Sciences, Tokyo, Japan). The HPLC column was protected by a 0.5 μm precolumn filter (Rheodyne, Cotati, CA, USA). The mobile phase consisted of 59% of methanol in phosphate buffer (70 m*M*, pH 2.4). The flow-rate of the eluent was 1 ml/min and the injection volume was 30 μl. The detector was set to 100 mV.

2.5. Optimization

The conditions described in Sections 2.3 and 2.4 were chosen for routine analysis. However, many different analytical techniques were tested and optimization of the different steps of analysis was performed before the optimal conditions were found.

The recovery of quercetin from serum, or plasma prepared by using different anticoagulants, was investigated. Venoject® tubes (Oriola, Espoo, Finland) containing the following anticoagulants were used: K_3-EDTA, Li-heparin and Na-fluoride-K_2-oxalate. Both glass and plastic tubes containing EDTA were tested.

The following Bond Elut solid phase extraction (SPE) columns were tested: C18, CN, NH4, silica, diol, PBA (Varian, Harbour City, CA, USA). The following SPE columns from IST were tested: C18, CN, C8 and ENV (polystyrene divinyl benzene) (International Solvent Technology, Hengoed, UK). For further clean-up of the SPE extract, different liquid–liquid extraction methods were also tested, including the following solvents and buffers: hexane, toluene, ethyl acetate, butyl-acetate, tert-butyl methyl ether, dichloromethane, acetic acid, ortho-phosphoric acid, hydrochloric acid, perchloric acid and oxalic acid.

To find the optimal enzyme or enzyme combination for the hydrolysis of quercetin conjugates, we tested β-glucuronidases and sulfatases purified from several sources. According to the supplier, the preparations from *Helix pomatia* contained both β-glucuronidase and sulfatase activity, while preparations from bovine liver and *E. coli* contained β-glucuronidase activity and preparations from Patella vulgata contained sulfatase activity only. Briefly, 1 ml of plasma was incubated with 110 μl 0.78 *M* sodium acetate buffer (pH 4.8), 100 μl 0.1 *M*

Table 1
Relative recovery of endogenous quercetin using different enzymes[a,b]

Enzyme	Relative recovery (%)
β-glucuronidase (Helix pomatia)[c]	100[d]
β-glucuronidase (bovine liver)[e]	21
β-glucuronidase (*E. coli*)[e]	20
Sulfatase (Patella vulgata)[f]	10
β-glucuronidase (bovine liver)+ sulfatase (Patella vulgata)[g]	18
No enzyme	0

[a] 1 ml of high-quercetin plasma (80.0 μg/l) was incubated with 110 μl 0.78 *M* sodium acetate buffer (pH 4.8), 100 μl 0.1 *M* ascorbic acid and the indicated amount of enzyme for 17 h at 37°C.
[b] The results are means of duplicate samples.
[c] 4000 U β-glucuronidase+200 U sulfatase.
[d] Value set at 100%.
[e] 4000 U β-glucuronidase.
[f] 150 U sulfatase.
[g] 4000 U β-glucuronidase+150 U sulfatase.

ascorbic acid and enzyme for 17 h at 37°C (see Table 1). Each enzyme or enzyme combination was tested at pH 4.8 and pH 6.4. In this experiment, an incubation time considerably longer than one usually needed for maximal hydrolysis was chosen. The time-course of hydrolysis for the enzyme chosen for routine analysis, Helix Pomatia type 2, was studied by incubating high-quercetin plasma with this enzyme ($n=2$) for 0, 1, 2, 3, 4, 6, 8, 12, 14, 16, 18, 20, 23, 26 or 29 hours.

The ability of β-glucuronidase–sulfatase from Helix pomatia, and β-glucosidase from almonds, to cleave glycosides (rutin or spireaoside) added to low-quercetin plasma was also examined. The results were compared with the ability of the enzymes to cleave endogenous quercetin conjugates in high-quercetin plasma.

2.6. Quantitation and standards

The plasma quercetin peak was quantitated using the standard additions method. Three plasma standards were prepared in duplicate by addition of up to 30 μl of quercetin standard in methanol to 1 ml aliquots of low-quercetin plasma to obtain final concentrations of 3.5, 33.5 and 103.5 μg/l. The standards were treated exactly the same way as samples, i.e., they were subjected to hydrolysis and extraction procedures. Standard curves were obtained by plotting peak height versus quercetin concentration.

The quercetin stock standard (1 mg/ml methanol) was stored at −20°C. The stability of the standard was regularly checked by UV-spectroscopy. The exact concentration of the standard was calculated using the molar absorptivity value of 22 300 l mol^{-1} cm^{-1} at 370 nm [19].

2.7. Linearity and limit of quantitation

The linearity of the assay was evaluated by plotting the peak height of standards in the range of 3.5–320 μg/l against the corresponding concentration. The standards were made by spiking low-quercetin plasma and they were treated like the other samples.

Since plasma containing less quercetin than 3.5 μg/l could not be obtained, a theoretical quantitation limit was calculated. This was done by analyzing spiked low-quercetin plasma ($n=6$), which contained all the same ingredients, and underwent the same extraction procedures as normal samples, but did not undergo the 17 h hydrolysis. The limit of quantitation was defined as the lowest concentration of quercetin that can be measured with an acceptable accuracy and precision (R.S.D<10%) and a signal to noise ratio greater than three.

2.8. Precision, accuracy and recovery

The intra-assay precision of the method was assessed by analyzing spiked and non-spiked low-quercetin plasma, and high-quercetin plasma ($n=6$ per group). Inter-assay precision was determined by analyzing high-quercetin plasma ($n=6$) on 7 separate days.

Recovery was determined by comparing the peak height of hydrolyzed ($n=6$) spiked low-quercetin plasma ($n=6$) to the peak height of standards ($n=6$). The height of the quercetin peak in non-spiked low-quercetin plasma was subtracted. Samples were prepared by addition of 60 or 200 ng of quercetin to 1 ml aliquots of plasma. Also, the recovery of quercetin from rutin and spireaoside added to plasma was assessed ($n=6$).

2.9. Identification of the quercetin peak

The quercetin peaks in low-quercetin and high-quercetin plasma were identified by comparing their retention times and hydrodynamic voltammograms with those of a quercetin standard. The voltammograms were generated by oxidizing the standard and the plasma extracts at 30–500 mV.

2.10. Stability

The stability of quercetin in blood collected into EDTA tubes was studied by incubating the tubes for 0, 1, 2, 3, 4 or 6 h in 37°C ($n=2$) before separation of plasma. The stability of quercetin in plasma stored at +4°C was evaluated by comparing the quercetin concentration of high-quercetin plasma analyzed immediately after thawing from −70°C ($n=4$) with that of thawed plasma stored at +4°C for 0, 1 or 3 days ($n=4$). The effect of freezing and repeated thawing of plasma samples on quercetin was studied by analyzing the quercetin concentration of plasma samples directly after separation of plasma, after freezing of the samples once (−70°C) and after freezing and thawing the samples two or four times.

The quercetin sparing activity of several antioxidants during hydrolysis was evaluated. The tested antioxidants were: trolox, ascorbic acid, BHA, BHT, DTT, catechin, epicatechin, myricetin, ubiquinone and TBHQ. Since quercetin in the final extraction step was extracted into an acidic aqueous phase, where it could be subject to degradation, the stability of quercetin in the different acids mentioned in Section 2.5 was evaluated. The stability of quercetin in plasma extracts in the acid–antioxidant combination chosen for the final method was tested by storing the extracts at room temperature for 0, 1 and 4 days ($n=6$).

2.11. Application

To study the pharmacokinetic disposition of quercetin, 3 healthy volunteers were administered 20 mg of quercetin in gelatin capsules. The capsule was given after an over-night fast and the participants were not allowed to eat before the 4 h sampling. The participants followed a low-quercetin diet for two days prior to the study and during the study day. Venous blood was taken into EDTA tubes at 0, 1, 2, 4, 8 and 24 h. Blood was centrifuged at 1000 *g* for 15 min at room temperature and plasma was stored at −70°C.

3. Results and discussion

3.1. Chromatography

Representative chromatograms of low-quercetin and high-quercetin plasma extracts are shown in Fig. 2. The retention time of quercetin was 7.8 min. The HPLC column used, Inertsil ODS-3, was superior to the other HPLC columns tested, giving a quercetin peak with least tailing.

The detector was set to +100 mV, although the maximum response for a quercetin standard was achieved at much higher potentials. When the oxida-

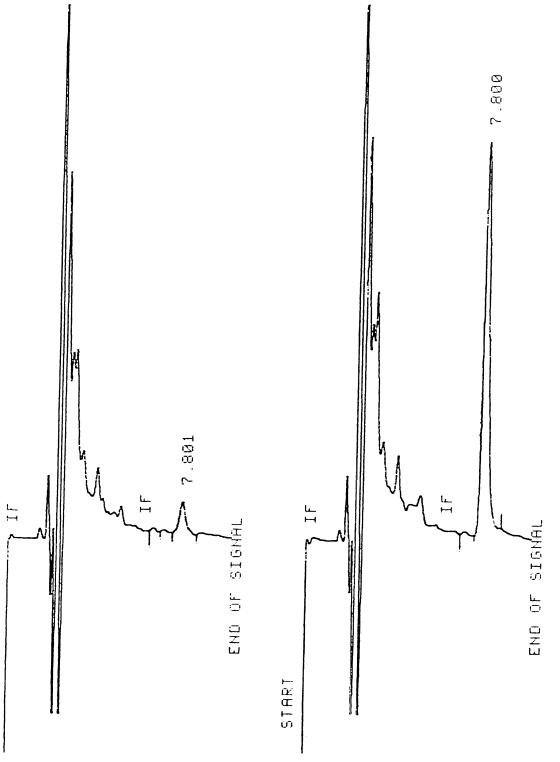

Fig. 2. Chromatograms of (A) low-quercetin plasma and (B) high-quercetin plasma. The plasma samples had been subjected to hydrolysis and extraction procedures described in Section 2.3.

tion was done at a potential this low, the method was extremely selective and there was no interference from other compounds present in the plasma extract. Also, the use of the coulometric detector was effortless with short stabilization periods. As compared to UV detection, the improvement in quantitation limit and selectivity was substantial.

3.2. Optimization

Equal amounts of quercetin were recovered when blood samples were collected into vacutainers containing EDTA, heparin or no anticoagulant (serum). The recovery of quercetin when using oxalate as anticoagulant was 13% compared to the recovery when using the other anticoagulants. This could be due to interference on the liquid–liquid extraction step.

Hydrolysis of quercetin glucuronides and sulfates was carried out enzymatically. The recoveries of quercetin when using β-glucuronidase from bovine liver or *E. coli* (containing no sulfatase activity), β-glucuronidase from bovine liver or *E. coli* and sulfatase from Patella vulgata, or a crude extract of Helix pomatia containing both enzyme activities, are shown in Table 1. The recovery of quercetin when using β-glucuronidase from *E. coli* or bovine liver was 20 and 21%, respectively, compared to the recovery with β-glucuronidase–sulfatase from Helix pomatia. Addition of sulfatase from Patella vulgata to any of the β-glucuronidase preparations did not increase the recovery. Also, no increase in recovery was seen when the hydrolysis was performed at a higher pH (6.4) (data not shown). The differences in hydrolizing efficiency between the different enzyme preparations is probably due to differences in the specificities of these enzymes towards different substrates. No quercetin was detected when the incubation was performed in the absence of enzyme. A time-course curve illustrating the efficiency of hydrolysis when using β-glucuronidase–sulfatase from Helix Pomatia (type 2) is shown in Fig. 3. Maximal results were obtained after 8 h of hydrolysis. However, since quercetin was stable under the conditions used for a considerably longer time and since a longer incubation time is more practical, an incubation time of 17 h was chosen for routine analysis.

Quercetin glycosides (spireaoside and rutin) added to plasma were completely cleaved by β-glucuronidase–sulfatase from Helix pomatia. Cleavage of added glycosides by β-glucosidase from almonds

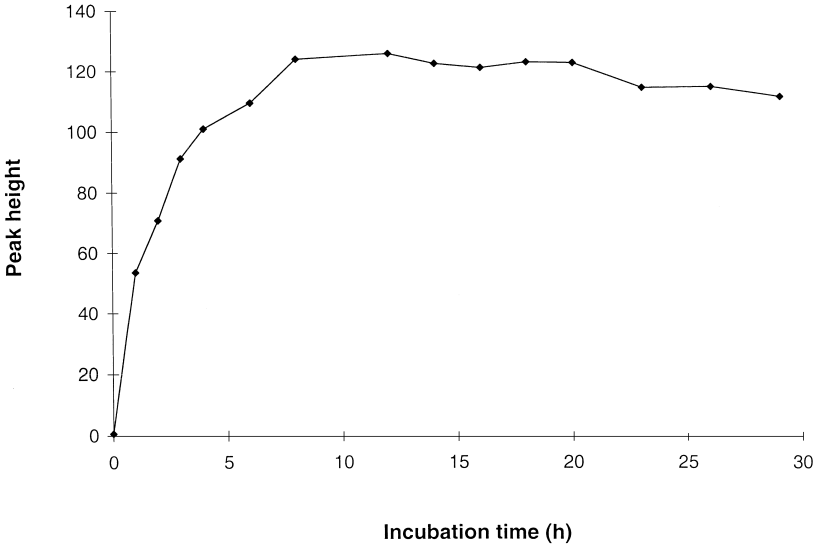

Fig. 3. Time-course of hydrolysis using Helix pomatia beta-glucuronidase–sulfatase (type 2). Hydrolysis and extraction procedures are described in Section 2.3.

was also efficient. The recovery of quercetin from high-quercetin plasma after incubation with β-glucosidase, however, was only 17% compared to the recovery after incubation with β-glucuronidase–sulfatase from Helix pomatia. From this data it can not be concluded that 17% of the quercetin found in this sample is in glycoside form, because some unspecific cleavage could also occur. The results merely suggest that the percentage of quercetin present in glycoside form in the high-quercetin pool sample fell between 0 and 17%.

The need for antioxidants during hydrolysis was evident. Recovery of quercetin, when antioxidants were omitted, was low (7–51%) and varied considerably between days. Of all the antioxidants examined, ascorbic acid, myricetin, epicatechin, trolox and TBHQ were the most effective in protecting quercetin from oxidation or degradation. In the final method, ascorbic acid was used because of interfering compounds present in some of the other antioxidant standards (trolox, myricetin) or because of coelution (TBHQ).

Of all the tested SPE columns, the best extraction efficiencies were obtained with Bond Elut C18 and IST ENV, giving recoveries of 98.8 and 97.1%, respectively. Bond Elut C18 was chosen for routine analysis, because smaller elution volumes of methanol were required. Acidification of the SPE-column and a wash-step with an acidic buffer were found to be necessary for release of quercetin from proteins. Extensive binding of quercetin to plasma proteins [20], possibly through hydrogen bonding or hydrophobic interactions [21], has been reported. In addition to the first extraction step, a second, more selective extraction step was conducted to acquire a cleaner sample. The best results were obtained by dissolving the dried methanol eluate in acid, and extracting interfering compounds into a mixture of toluene and dichloromethane (80:20, v/v). Quercetin recovery in the acid phase was complete when using acetic acid, but only 35% when using ortho-phosphoric acid or oxalic acid. Since quercetin was unstable in all the tested acids, except for the two latter, different mixtures of oxalic acid or ortho-phosphoric acid and acetic acid were examined. A mixture of 80% of 5.3 *M* acetic acid and 20% of 32 m*M* oxalic acid yielded a 100% recovery of added quercetin and good stability.

The second extraction step can be omitted when analyzing plasma samples with higher quercetin concentrations. However, in our experience, use of both extraction steps is to be recommended, especially when analyzing large amounts of samples. Extraction of less polar compounds into dichloromethane–toluene allows shorter HPLC runs and frequent washes of less polar compounds accumulating on the HPLC column can be avoided. Also, interfering peaks originating from some lots of Helix pomatia enzyme preparations can be separated from the quercetin peak. If the second extraction step is omitted, the dried methanol eluate from the SPE column should be dissolved in a larger amount of methanol and acid. The sample should also be centrifuged prior to injection into the HPLC system, to avoid clogging.

3.3. Identification

The low-quercetin plasma pool was obtained from 5 adults who had been on a strict quercetin-free diet for 5 days. The pool contained a compound with the same retention time as quercetin, and which could not be distinguished from quercetin by using ion-pairs or another type of HPLC column. A series of blank tests were done to ensure that this peak did not originate from reagents or the enzyme. For instance, extraction of non-hydrolyzed low-quercetin plasma, containing all reagents used in hydrolysis, did not yield a quercetin peak, while hydrolyzed samples did.

To identify the quercetin peak, hydrodynamic voltammograms of quercetin in high-quercetin and low-quercetin plasma, and a quercetin standard, were created. The voltammograms were identical (Fig. 4). From this data it is concluded that plasma from subjects following a quercetin-free diet for 5 days still contains quercetin. The use of voltammograms is an excellent identification method for electroactive compounds, like flavonoids [22,23]. Diode-array detection lacks the sensitivity required for identifying quercetin at these low levels. Also, even at higher concentrations, absorption spectra are not necessarily specific enough to be used for identification of flavonoids in plasma. A mass-spectrum for quercetin in this concentration range is not attainable.

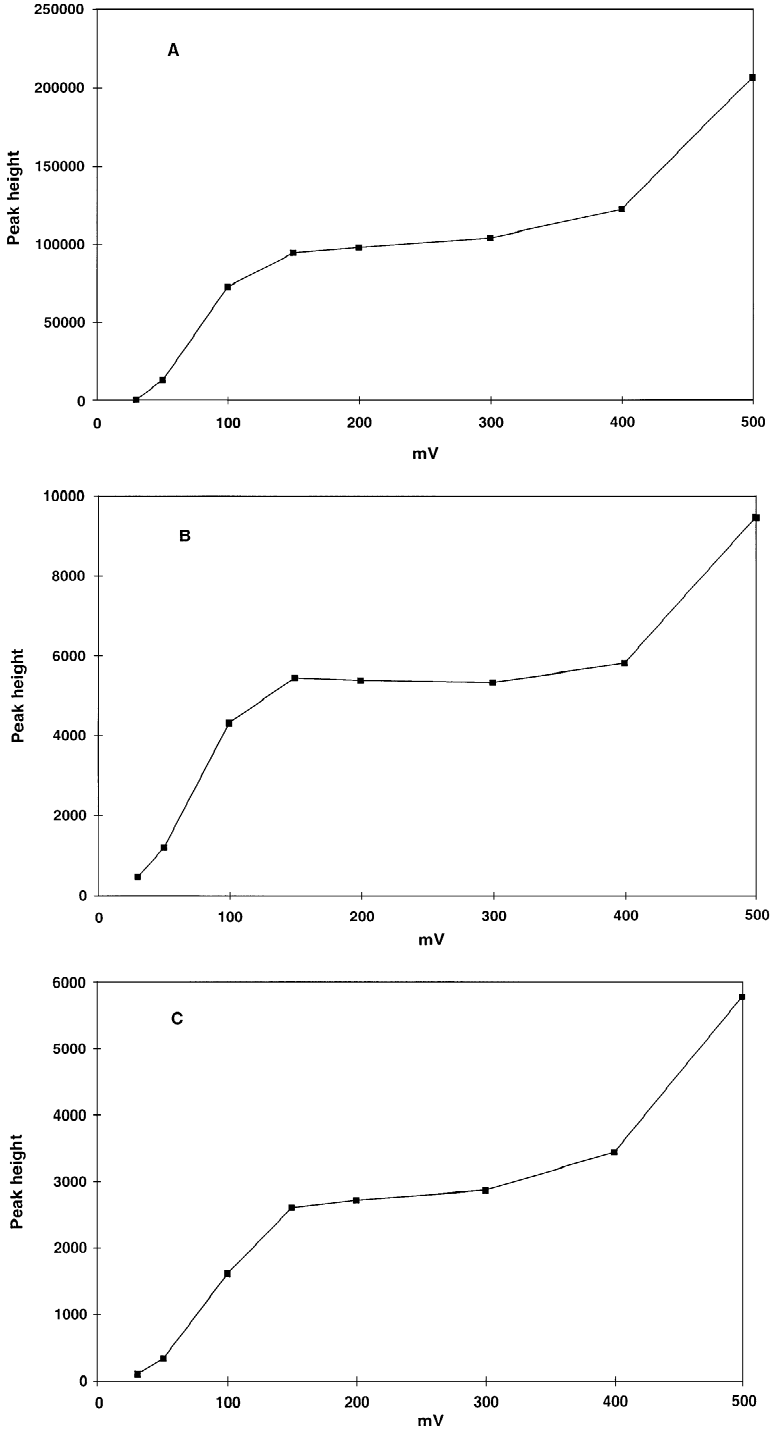

Fig. 4. Hydrodynamic voltammograms of (A) high-quercetin plasma, (B) low-quercetin plasma and (C) a quercetin standard.

3.4. Validation

Validation was conducted with high-quercetin plasma (80 μg/l), and spiked and non-spiked low-quercetin plasma. The concentration of quercetin in low-quercetin plasma was estimated from the calibration curve through extrapolation, and was found to be 3.5 μg/l. The calibration curve was linear in the examined concentration range (3.5–320 μg/l), with a correlation coefficient of 0.999 and $y=4.57x+16.2$. The limit of quantitation in non-hydrolyzed plasma was 0.63 μg/l (R.S.D. 6.6%, $n=6$). Plasma containing less than 3.5 μg/l quercetin could, however, not be obtained. Detection limits of 100 μg/l when using UV-detection [5] and 5 μg/l using fluorescence detection [10] have been reported. Quantitation limits for these methods were not reported, but it is usually considerably larger than the detection limit.

The recovery of quercetin from hydrolyzed low-quercetin plasma spiked with 60 or 200 ng quercetin was 70% and 71%, respectively. The intra-assay precision for high-quercetin and low-quercetin plasma was 2.0% and 2.6%, respectively. The intra-assay precision of low-quercetin plasma spiked with 60 or 200 ng quercetin was 4.3% and 4.1%, respectively. Inter-assay precision of high-quercetin plasma ($n=6$) analyzed on 7 separate days was 7.9%.

3.5. Stability

Endogenous quercetin was stable in blood incubated at 37°C for 0–6 h before separation of EDTA plasma. Quercetin was also stable in plasma samples maintained at +4°C for 1 or 3 days (Table 2). Furthermore, there was no loss of recovery in plasma extracts kept at room temperature for 1 or 4 days (Table 2). No loss of quercetin was detected in samples frozen once (−70°C), as compared to samples analyzed immediately after separation of plasma. Also, no degradation of quercetin occurred after 2 or 4 repeated freeze–thaw cycles

The stability of quercetin in plasma was also studied by Liu et al. [5]. The authors found that over 90% of the initial amount of quercetin added to plasma could be recovered after 5 h at +37°C. A decrease of 40% in recovery was discovered after 10 h. The compound was stable for at least 2 months at −20°C. The stability tests were performed with high concentrations of quercetin (5 mg/l).

3.6. Application

Fig. 5 shows the plasma quercetin concentration vs. time profile after a single dose of 20 mg of quercetin taken orally. Plasma quercetin concentrations increased significantly in all three subjects

Table 2
Stability of endogenous quercetin in plasma and plasma extracts

Matrix	Temperature (°C)	Time (days)	*n*	Relative recovery (%)	
				High-quercetin[a] plasma	Low-quercetin[b] plasma
Plasma[c]	+4	0	4	100[d]	100[d]
		1	4	100	99
		3	4	98	101
Plasma extract[e]	+27	0	6	100[d]	100[d]
		1	6	99	101
		4	6	100	97

[a] Quercetin concentration 80.0 μg/l.
[b] Quercetin concentration 3.5 μg/l.
[c] Frozen plasma was thawed and analyzed immediately or kept at +4°C for indicated time. Plasma samples were subjected to hydrolysis and extraction procedures described in Section 2.3.
[d] Value set at 100%.
[e] Frozen plasma was thawed and subjected to hydrolysis and extraction procedures. Plasma extracts were analyzed by HPLC immediately after extraction or kept at 27°C for indicated time.

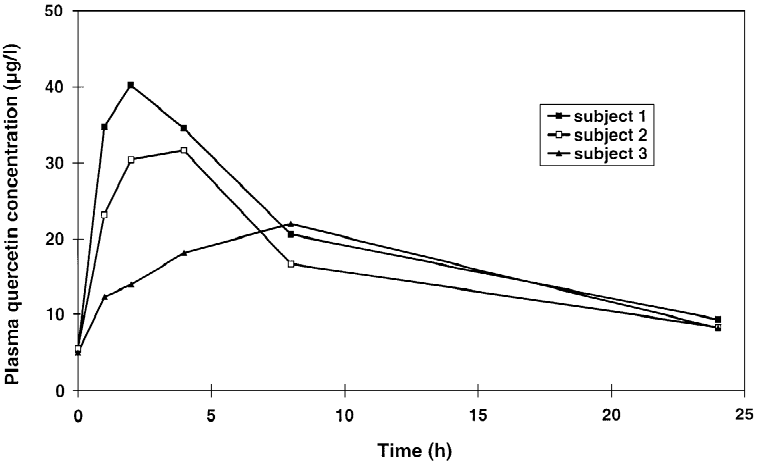

Fig. 5. Plasma quercetin concentrations at 0, 1, 2, 4, 8 and 24 h after oral administration of 20 mg of quercetin. Each line corresponds to one subject.

within one hour after ingestion of the capsule. Peak concentrations ranged between 20–44 μg/l. The response was about 6–13-fold compared to baseline. Although the study was done with a very small number of subjects, it clearly indicates that quercetin is absorbed from a small dose. To our knowledge, this is the first report of plasma concentrations of quercetin, after supplementation with quercetin aglycone. In previous supplementation studies, where plasma quercetin concentrations have been measured, quercetin glycosides [6,17] or foods containing mostly quercetin glycosides [17], have been used.

4. Conclusions

This paper describes a validated method for the quantitation of trace levels of quercetin in human plasma. The method has excellent precision, reproducibility and sensitivity. Special attention has been paid to the stability of quercetin during the different steps of analysis. The method can be used when measuring quercetin in plasma of non-supplemented and supplemented subjects, and is therefore suitable for both supplementation studies and biomarker studies.

The method was applied to a small scale study of quercetin pharmacokinetics. The results showed absorption of quercetin aglycone from a dose within the range of normal intake. An attempt was also made to evaluate whether free quercetin or quercetin glycosides are present in plasma. No free quercetin was found in a plasma pool collected from 15 adults following a quercetin rich diet for one day. According to our results, it is more likely that the compound is conjugated with glucuronide and sulfate groups in the liver, rather than absorbed in its glycoside form. The sites and the degree of conjugation, however, remain unknown.

Acknowledgements

The excellent technical assistance of Eva Kammiovirta is acknowledged. The authors also want to thank the volunteers participating in the study. This work was supported by the Finnish Heart Research Foundation, Juho Vainio Foundation, Yrjö Jahnsson Foundation and the project ‘Nutritional quality and safety of Finnish foods’.

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